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Electrophysiology of Opioids*†

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* The word opioid (noun or adjective) has been used to describe *both* the plant alkaloids related to morphine (and the large number of synthetic analogues that mimic their effects) *and* the endogenous animal peptides of the three major families that share the common N-terminal (Tyr-Gly-Gly-Phe...). The word opiate is used when it is wished to distinguish the first group of opioids; this is generally in the context of pharmacological actions of the exogenous drugs. The term opioid peptides is used to refer to the second group as a whole, usually in the context of elucidating their physiological role.

† This article is the third of a series of reviews on various aspects of opioid pharmacology which has been arranged with the help of Hans W. Kosterlitz, Aberdeen, and Eric J. Simon, New York, acting as Consulting Editors. The Editor and Associate Editors are grateful for their assistance. The first two articles of this series, "Animal Models for Dependence" by W. L. Woolverton and C. R. Schuster and "Opioid Receptors: Autoradiography" by J. K. Wamsley, appeared in the first issue of this volume. The fourth article in the series, "Pharmacology of the Opioids" by W. R. Martin, follows herewith in this issue. Other topics will follow.

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I. Introduction

ELECTROPHYSIOLOGICAL analysis of the effects of opioids on the nervous system has been performed at several levels, and by using a variety of preparations, recording techniques, and methods of drug administration. Two general approaches have been used. The first has been the measurement of the change in properties of single neurones or groups of neurones during application of opioids directly to them. This has included studies of passive membrane properties, electrical excitability (action potential generation and propagation), chemical excitability (synaptic potentials and their propagation), and neurosecretory properties. The second approach has been the study of how opioid-induced changes in the function of certain neurones affect other areas of the nervous system. For example, such studies have recorded activity of neurones at different levels of the neuraxis in response to a cutaneous noxious stimulus, and studied the effects of opioids applied at different sites. Experiments of the first kind have been performed in vivo and in tissue removed from an animal and maintained in vitro. Experiments of the second kind must be performed in vivo.

A. *In Vitro Preparations*

An important advantage of in vitro preparations is the ability to perfuse the tissue with opioid agonists and antagonists at known concentrations. Provided that the organization of the tissue is relatively simple, then effects may be interpreted in terms of receptors located on a known structure. For example, in the myenteric plexus preparation, most afferent inputs to the neurones can be disrupted by the method of preparation, and any effects of opioids can be attributed to a direct interaction with receptors on the neurones from which electrical activity is recorded. With in vitro preparations of central nervous tissue (slices and cultures), the complex organization of these tissues does not necessarily permit this assumption; however, perfusion with solutions with reduced calcium ion concentration has often been used to isolate a neurone from its synaptic connections.

The ability to visualize neurones is a particular advantage of in vitro preparations. Intracellular recording is more readily performed and it may be possible to insert two microelectrodes into a neurone permitting the use of the voltage clamp technique. In addition, drugs may be administered from micropipettes at discrete sites on cell processes. In tissue culture preparations it has been possible to study drug effects on synaptic transmission by impaling the postsynaptic neurone with a microelectrode and stimulating a single afferent fibre. The ionic environment of cells can be altered by changes in the perfusion fluid. Clearly, in vitro preparations offer many advantages in studying how opioids affect the function of single neurones.

B. *In Vivo Studies*

The best evidence that an effect on neurones is relevant to the pharmacological or therapeutic action of an opiate remains its demonstration in vivo. In vivo experiments permit the study of identified neurones with intact inputs which, in certain circumstances, can be defined. In vivo preparations present problems by virtue of anaesthesia, maintenance of stable recording conditions, and ignorance of accurate drug concentrations. In many parts of the central nervous system, anaesthesia can be avoided by the use of decerebrate animals. In vivo experiments are obviously the only ones in which natural stimuli can be used to excite or inhibit central neurones and in which changes in the function of separate areas of the central nervous system can be simultaneously studied. With both in vitro and in vivo preparations, the results obtained are very much dependent on the methods of drug administration and the concentrations attained at the receptor site. This requires separate consideration.

C. *Methods of Opioid Administration*

1. Systemic Administration. The combination of systemic (usually intravenous) administration of opiates while recording the firing of neurones either with microelectrodes or surface electrodes has yielded much information on which areas of the nervous system are affected by particular doses of these drugs. Such studies, however, are limited in providing knowledge of how these changes come about. It is rarely possible to attribute changes in the firing of a particular central neurone following systemic administration of an opiate to an action at receptors on that neurone. Systemic drugs could affect the firing of a neurone by changing the blood flow through the central nervous system; by changing the sensitivity of peripheral sensory receptors; by altering conduction in afferent nerve fibres; by changing the release or action of transmitters released from afferent fibres; by a multitude of possible effects on the numerous neurones with which it is connected; or, finally, by altering membrane properties as a direct consequence of an action at receptors on that neurone.

2. Microelectrophoretic (Iontophoretic) Administration. By administering drugs electrophoretically from micropipettes with tips positioned near single neurones, it is possible to exclude many of the problems inherent in interpreting the effects of systemically administered substances. When multibarrel micropipettes are used for this technique, the most commonly used arrangement is one in which the centre barrel is used to obtain extracellular recordings while drugs are ejected from the outer barrels. The sites of recording and drug administration are essentially the same, and probably close to the soma or large processes of the neurone studied. Micropipette assemblies have been used for extracellular recording in which the recording electrode protrudes some 50 to 80

μm beyond the tip of the drug-administering micropipettes (434, 524). For intracellular recording this protrusion is essential and usually of the order of 60 to 150 μm.

When drugs are administered in this way concentrations attained near receptors are unknown. Thus, an effect of an opioid, even one that is antagonized by naloxone, is not necessarily relevant to the actions of systemically administered substances. When using the microelectrophoretic technique it is not possible to be certain that any observed effect will also occur when analgesic doses of opiates are administered systemically or when opioid peptides are released by neighbouring neurones. The likelihood that an observed effect will occur during systemic opiate administration can sometimes be assessed by consideration of the following factors.

First, a range of opiate agonists and antagonists should show appropriate activity with a rank order of potencies similar to those found with systemic administration. The non-opiate isomers of such compounds should not exhibit activity. Second, the activity of the opiate when applied by microelectrophoresis should mimic in some detail that following systemic administration. For example, if the systemically administered opiate is known to interfere with activation of a neurone from one source but not from another, then a similar selective effect should be observed when it is administered from a micropipette. This type of result is relatively rare but has been of particular use in the spinal cord.

Third, opiate binding sites should be demonstrable in the area of electrophoretic administration. This is probably the least useful in deciding the relevance of effects observed in microelectrophoretic experiments. Binding of ligands to brain homogenates and the autoradiographic distribution of radiolabelled ligands are relatively coarse techniques in revealing the presence of receptors on neurones of a particular area. Nevertheless, a correspondence among results from all methods does increase the probability than an observed effect is relevant to the action of systemically administered opiates. Fourth, intravenous naloxone should be used. If the event produced by an opiate administered from micropipettes is reversed by doses of intravenous naloxone just adequate to block the action of analgesic doses of the same opiate in that species, then the probability is increased that the event is relevant. Ideally, the effect of intravenous naloxone before administration of the opiate should be known since apparent reversal of the effects of the exogenous opiate could result from antagonism of the action of a tonically released endogenous opioid peptide at a remote site.

3. Pressure Ejection from Micropipettes. Application of high pressure to a micropipette containing opioids has been used to expel small quantities close to the neurone under study. This technique suffers from many of the difficulties associated with microelectrophoresis and it is particularly difficult to measure the volumes ejected. In the central nervous system, tips readily occlude and the high pressures necessary to eject solutions may result in a sudden clearance and consequent ejection of a large volume of solution. Pressure ejection works well with in vitro preparations; effects produced by high concentrations often used in electrophoretic experiments can be avoided by the use of dilute solutions.

4. Perfusion of Tissues in Vitro. Perfusion permits administration of opioids in known concentrations. With an opiate this method permits good assessment of the relevance of an observed effect to the in vivo situation following systemic administration. With the endogenous opioid peptides, the simultaneous activation of all receptors on a neurone or tissue is probably an unphysiological condition if these substances are neurotransmitters normally released at discrete sites, and such results should be interpreted with due caution.

5. Microinjection of Opiates. A few investigators have injected relatively large volumes of opiates from needles implanted in certain areas of the nervous system, and recorded the firing of neurones in other sites. As with microelectrophoretic administration, the concentrations at receptors mediating changes in cell firing are unknown and the observed effects are equally difficult to relate to the actions of systemically administered opiates.

Clearly, there are limitations to all methods of administration. Any assessment of a transmitter role for opioid peptides or an understanding of how systemically administered opiates produce their effects on the nervous system requires consideration of data from a variety of techniques.

II. Central Nervous System

In the discussion that follows it should be remembered that there are two separate questions that are being reviewed. The first concerns how therapeutic doses of opiates produce their effects on the central nervous system. The second deals with the physiology of opioid peptides. The latter were discovered through similarity of action to opiates but it is now clear that subpopulations of receptors exist with different ligand preferences. It is becoming clear that differences exist between the effects of opiates and opioid peptides on a particular system, and among the various members of these two classes. These differences will be frequently referred to throughout the text. Despite this, it has been considered more appropriate to discuss the central nervous system by regions, and within regions by physiological subdivisions, rather than to consider the opiates and opioid peptides separately.

A. Spinal Cord

The majority of neurophysiological studies of opiate actions on the central nervous system of mammals have dealt with the spinal cord. This preference probably derives from the relatively large amount of data available on the structure and function of the spinal cord when compared with other parts of the central nervous system.

1. Spinal Reflexes. The measurement of reflexes by

neurophysiological techniques consists of recording efferent spinal activity (usually by electrodes placed on cut ventral roots) in response to electrical stimulation of primary afferent fibres. When dorsal roots are stimulated it is possible to differentiate monosynaptic reflexes to impulses in large fibres of muscle origin, but little else. Stimulation is better done peripherally with muscle and cutaneous nerves separately. With muscle nerves, those from flexor or extensor muscles should be studied separately. The only monosynaptic reflex that can be studied readily is that to Ia primary afferents, and the polysynaptic discharges to impulses in these fibres tend to be intermingled with those produced by higher strength peripheral nerve stimuli. Nevertheless, it may be possible to correlate the appearance of a discharge in the ventral roots with the use of a stimulus of particular strength and, hence, to ascribe it to impulses in fibres other than Ia afferents. When a cutaneous nerve is stimulated, it may be possible to distinguish a monosynaptic reflex, but in addition peaks produced by mixed mono- and polysnaptic discharges to group II (Aα,β), group III (Aδ), and C fibres can often be differentiated. It is particularly difficult to assess the significance of results in which reflex discharges are described as being either monosynaptic or polysynaptic without stating the probable fibre types excited by the stimulus strengths used and the latencies of the potentials recorded. In much of the literature that deals with opiates and spinal reflexes the use of the term "polysynaptic reflexes" is thus confusing.

The older literature on reflexes (289) paid particular attention to the enhanced spinal reflexes observed with toxic doses of morphine. This action probably results from blockade of the spinal inhibitory transmitter, glycine (104), but does not contribute to opiate analgesia. Opiates, however, do have significant effects on spinal reflexes in analgesic doses. These alone will be discussed. A previous review (334) has also dealt with these effects.

a. OPIATES AND SPINAL REFLEXES. i. **Frog.** Morphine sulphate (100 μM) reduced the monosynaptic ventral root response elicited by stimulating the lateral spinal cord 80 ms after a dorsal root stimulus (529). This effect was not seen in the presence of naloxone (100 μM), although the antagonist had no effect of its own on the reflex. Interestingly, an elevation of the calcium concentration of the perfusing solution also reduced the effect of morphine (see below, section III). The effective concentrations of morphine were high, but there is a paucity of knowledge regarding the concentration of morphine that may be pharmacologically relevant in the frog. It is possible that this action of morphine is due to a hyperpolarization of the dorsal root fibres of the frog cord (168, 362, 363) leading to a block of impulse propagation.

ii. **Rat.** Morphine (2 mg/kg) did not affect the reflex ventral root discharge resulting from stimulation of the dorsal roots of the anesthetized rat (199). The stimuli used appear to have activated only myelinated fibres. Morphine did, however, depress the α-motoneurone discharge that had been facilitated by subthreshold conditioning stimulation of the dorsal roots. The facilitation that follows repetitive stimulation was considered to be a better simulation of a noxious input to the spinal cord but the methods did not differentiate between flexor and extensor motoneurones and did not define the afferent fibres excited. This effect of morphine was not blocked by naloxone (259). A similar facilitation of α-motoneurone reflex activity could be induced by stimulation of the substantia nigra and this was blocked by naloxone (1 mg/kg), suggesting involvement of an endogenous opioid in its mediation (261). Seeber et al. (444) found that the depolarization of α-motoneurones (recorded in the ventral root) induced by tetanic stimulation of Aα,β (but not Aδ or C) fibres in the ipsilateral common peroneal nerve was reduced by morphine (0.5 to 3 mg/kg). Levorphanol, but not dextrorphan, had a similar effect and in this study naloxone (0.5 mg/kg) antagonized the action of morphine. This action of morphine was limited to flexor motoneurones—it was not seen in the spinalized animal and may therefore be assumed to reflect changes in descending control of the flexor motoneurone pool due to an action of morphine at a supraspinal level. Spinal reflexes to noxious cutaneous stimuli, such as heating the tail of the rat (116), are extensively used as tests of analgesia. This reflex, however, has been little investigated by neurophysiological techniques.

iii. **Cat.** There is a general agreement that, in spinal cats, morphine and related narcotics depress reflexes to impulses in small diameter primary afferents of both cutaneous and muscular origin. Thus, Koll et al. (276) showed that electrical stimulation of the superficial peroneal nerve produced reflexes to group II (Aα,β) Aδ, post Aδ, and to C fibres, and that intravenous morphine (0.3 to 0.4 mg/kg) preferentially reduced the firing of motoneurones to the post Aδ and C fibre afferents. In the experiments of Bell and Martin (32), morphine (3.0 mg/kg) and the purported κ agonist WIN 35,197-2 (0.3 mg/kg) both reduced reflexes to stimulation of C fibre afferents of the superficial peroneal nerve of the spinal decerebrate cat. Other reflexes were not studied. The effect on C reflexes in this preparation was further studied by microinjecting both morphine (10 μg) and [Met]enkephalin (5 μg) in the dorsal and ventral horns (33). Both compounds depressed the C reflex when injected in the dorsal horn and this was prevented by pretreatment with naltrexone (2 mg/kg). When injected in the ventral horn, both compounds increased C reflexes, an effect not modified by naltrexone. It was suggested that opioids act at an unknown site in the dorsal horn to reduce C reflexes.

There are several reports that analgesic doses of opiates reduce flexor reflexes in the cat (49, 91, 491). Mixed muscle and cutaneous afferents were stimulated in these experiments and it is not possible to determine the fibre types excited. Flexor reflex afferents include high threshold muscle afferents, but it is not necessary to excite unmyelinated fibres to elicit flexor reflexes (324).

There is a dispute in the literature on the effect of morphine on monosynaptic reflexes to impulses in large

myelinated muscle afferents. Several workers have found that analgesic doses of morphine have no effect on monosynaptic reflexes in extensor muscles (49, 91, 125, 295, 491, 494). Krivoy et al. (292), however, found that intravenous morphine (0.5 to 12.5 mg/kg) depressed both the mono- and polysynaptic reflexes recorded in ventral roots by stimulating dorsal roots. The stimulus parameters are not given so that contribution of various fibre types to the polysynaptic reflex cannot be determined. They emphasized that the greatest depression of monosynaptic reflexes by morphine occurred when the dorsal root was stimulated at a relatively high rate (12.5 Hz). The actions of morphine on both mono- and polysynaptic reflexes were reversed by intravenous naloxone (1.0 mg/kg).

The suggestions by Krivoy et al. (292) that the effect of morphine was related to the frequency of stimulation supports the findings of Jurna and his collaborators. Jurna and Schafer (262) reported that when the popliteal nerve of the decerebrate cat was stimulated at 0.1 Hz the resultant monosynaptic reflex was not reduced by intravenous morphine (5 mg/kg). The increase in the reflex produced by tetanizing the nerve for 1 s at 300 Hz was, however, reduced by this dose of morphine. Jurna (254) found that the discharge of α-motoneurones of the decerebrate cats to sustained muscle stretching was reduced by intravenous morphine and pethidine (1 to 2 mg/kg). Goldfarb and Hu (195) also found that both morphine and oxymorphine in doses of 0.1 to 10 mg/kg decreased monosynaptic reflexes in both flexor and extensor muscles of the spinal decerebrate cat, but the frequency of afferent nerve stimulation in these particular experiments is not clear.

Stimulation of large diameter (group I) afferents, derived from muscle, activates many interneurones in the spinal cord apart from motoneurones responsible for the relevant monosynaptic reflex. The evidence from the experiments cited indicates that if a monosynaptic reflex is elicited at a slow rate, such that each reflex is uninfluenced by the activity of interneurones excited or inhibited by the prior stimulus, then the effect of analgesic doses of opiates is negligible. With repetitive activation, which is probably more relevant to physiological states, monosynaptic reflex activation of motoneurones by impulses in group I afferents is depressed by opiates. Investigations on the mechanisms by which these changes occur will be considered when discussing the effects of opiates on motoneurones and the excitability of the central terminations of primary afferent fibres.

Nearly all of the experiments just discussed have been on spinal animals and indicate that opiates directly affect the function of spinal neurones. In the cat, as in the rat (444), some experiments have been interpreted to suggest that the effect of morphine on a particular reflex has been mediated primarily at supraspinal sites. Thus, Takagi et al. (470) found little depression by morphine of reflexes in spinal animals but a significant depression of reflexes in cats with intact spinal cords. This was interpreted as an enhancement of descending inhibition by morphine. This finding is difficult to reconcile with the reports of many other investigators that opiates have effects on spinal reflexes in both acute and chronic spinal animals. The question of whether opiates increase descending inhibition will be discussed below when considering the effects of these drugs on neurones of the dorsal horn.

In cats, morphine reduces both direct and recurrent inhibition of monosynaptic reflexes to impulses in Ia muscle afferents. Recurrent inhibition of motoneurones is produced by Renshaw cells when excited by axon collaterals of adjacent motoneurones (158); direct inhibition is produced by interneurones at the base of the dorsal horn when monosynaptically excited by impulses in Ia afferents from a muscle antagonistic to that innervated (159). Both Renshaw cells and Ia inhibitory interneurones are believed to release glycine (109). Kruglov (295) found that morphine (5 to 10 mg/kg) reduced recurrent but not indirect inhibition of monosynaptic reflexes in the cat. This was confirmed by Curtis and Duggan (104) but higher doses of morphine (20 mg/kg) did reduce direct inhibition. Morphine blocks the action of glycine (104) and this may be responsible for the effects of morphine on spinal inhibition, but the differential action on direct and recurrent inhibition has not been satisfactorily explained (see also section II A).

iv. **Dog.** The chronic spinal dog has been the subject of numerous investigations since the classic studies by Wikler's group (494). In these experiments, and those described below, reflexes were elicited by natural stimuli such as tendon stretch and measured by strength of muscle contraction; thus, they are not directly comparable with reflexes elicited and measured by neurophysiological methods. The results, however, are probably more relevant to the effects of opiates under physiological conditions. Houde et al. (238) showed that morphine had little effect on the monosynaptic stretch reflex (knee jerk) but depressed the flexor and crossed extensor reflexes. Martin and collaborators (331, 332) showed that several analgesics had these effects, and that there was a good correlation between ability to depress spinal reflexes and analgesic activity in man. The relative ability of these various narcotics to inhibit the reflexes of the chronic spinal dog, along with other aspects of their pharmacological profile, formed the basis for the first suggestions that opiates may act on three distinct types of receptor (333). In addition to reversing the effects of opiate agonists, naloxone (327) and naltrexone (333) enhanced the reflex when given alone, this action being similar to that described subsequently for the cat.

v. **Man.** Intravenous morphine (1 mg/kg) depressed the evoked potential recorded with a lumbar epidural electrode in response to tibial nerve stimulation (335). That part of the potential, which is believed to correspond to primary afferent depolarizations, was particu-

larly depressed. Naloxone (0.1 mg/kg) reversed this effect, and often increased the size of the reflex above the pre-morphine control level.

In four volunteers with chronic paraplegia, low doses (0.2 to 0.3 mg/kg) of morphine completely suppressed polysynaptic reflex activity in the tibialis anterior evoked by sural nerve stimulation (considered a nociceptive reflex) (496); these doses had no detectable effect on a monosynaptic (H) reflex (497). Intravenous naloxone (20 μg/kg) completely reversed within 1 min the depressant action of morphine, returning the reflex response to a level significantly greater than the pre-morphine control. The results support an important spinal action of opiates in blocking nociceptive reflexes when administered in usual therapeutic doses.

vi. **Summary.** A large body of evidence indicates that opiates reduce reflexes through the lumbar spinal cord to small diameter primary afferents whilst only affecting monosynaptic reflexes to large diameter muscle afferents that are facilitated by repetitive conditioning stimuli, and that this is due to a direct action of opiates at the spinal level. The effects on reflexes to other cutaneous and muscle afferents have not been adequately studied.

b. OPIOID PEPTIDES AND SPINAL REFLEXES. Perfusing the spinal cord with opioid peptides has been shown to reduce behavioral reflexes to peripheral noxious stimuli (512) but such experiments are outside the scope of this review. Bell et al. (33) microinjected [Met]enkephalin (5 μg) into the dorsal and ventral horn of decerebrate cats while studying reflexes in the ventral roots to stimulation of C primary afferents. In the dorsal horn, the peptide reduced the reflex and systemic naltrexone reduced this effect. In the ventral horn [Met]enkephalin enhanced the reflex and naltrexone did not antagonize this action. With microinjection, concentrations are unknown and the naltrexone reversible action is more likely to be relevant to a physiological role of opioid peptides. That endogenous opioids do influence spinal reflexes has been suggested by experiments with opioid antagonists and these are discussed subsequently when considering the function of opioid peptides in the spinal cord.

2. *Intravenous Opiates and the Firing of Spinal Neurones.* From the work on opiates and spinal reflexes it is clear that these drugs have effects on the spinal cord that are independent of supraspinal structures. The first step in the understanding of how these changes in reflexes are produced is to study the firing of single spinal neurones during systemic administration of opiates. Dorsal horn neurones, motoneurones, and Renshaw cells have all been studied in this way. This first section deals with the effects of opiates in spinal animals. The question of changes, by opiates, of supraspinal controls of neurones of the dorsal horn is discussed separately.

a. DORSAL HORN NEURONES. Since the first synapse in the transmission of nociceptive information from periphery to brain occurs in the dorsal horn, the effects of opiates on central neurones have been studied extensively in this area. The connections between primary afferents and the neurones of the superficial dorsal horn have been extensively investigated by using established silver techniques (68, 192, 193, 437, 467) and more recently by transport of radiolabelled amino acids (299) or horseradish peroxidase (62, 63, 315). The synaptic arrangements within the upper dorsal horn have been examined with the electron microscope with or without prior sectioning of dorsal roots (412). The diagrams contained within figure 1 summarize some of these findings. Nociceptive primary afferents terminate mainly within the superficial laminae (I and II) of the dorsal horn. The small myelinated (Aδ) nociceptive afferents terminate principally in lamina I while lamina II (the substantia gelatinosa) receives nearly all of the unmyelinated nociceptive afferents. Large myelinated afferents from cutaneous mechanoreceptors of many different functional types terminate mainly in laminae III and IV. Many of the fibres enter these laminae from below (ventrally) and there are few penetrations into laminae II. Intracellular injections of horseradish peroxidase have enabled the study of the processes of neurones of these laminae. As shown in figure 1, the dendrites of neurones of laminae I and II mainly arborize within the same laminae but deeper neurones have a more complex arrangement (62, 76). Many have dorsally directed dendrites with some penetrating the substantia gelatinosa (186). Other have mainly radially directed dendrites.

Functionally, many neurones in the dorsal horn are excited by noxious cutaneous stimuli. Some respond only to such stimuli. The anatomical studies of Gobel (192, 193) suggest that such neurones should be relatively abundant in lamina I and Christensen and Perl (86) did observe them. However, many neurones in several laminae of the dorsal horn respond to several cutaneous stimuli both noxious and non-noxious, and these include cells shown to project to supraspinal areas (77, 148, 213, 297, 503). It has been suggested (324) that the information transmitted supraspinally by many of these multireceptive neurones is not primarily concerned with the perception of sensory information but rather is informing supraspinal regions, important in movement control, of the level of excitability of neurones involved in spinal reflexes. This creates problems in attempting to relate the effects of opioids on neurones to alterations in the perception of pain and there is no completely satisfactory answer to this problem. In some instances, however, the opioids have proved to be selective in their effects, reducing nociceptive responses without altering responses to other modalities; in these cases it is not unreasonable to relate this effect to analgesia. The functional consequences of a nonselective depression of all responses of a neurone, both noxious and non-noxious is uncertain; this is further considered in relation to the function of opioid peptides in the spinal cord.

i. **Spontaneous Firing.** Kitahata et al. (273) found that, in decerebrate spinal cats, morphine administered

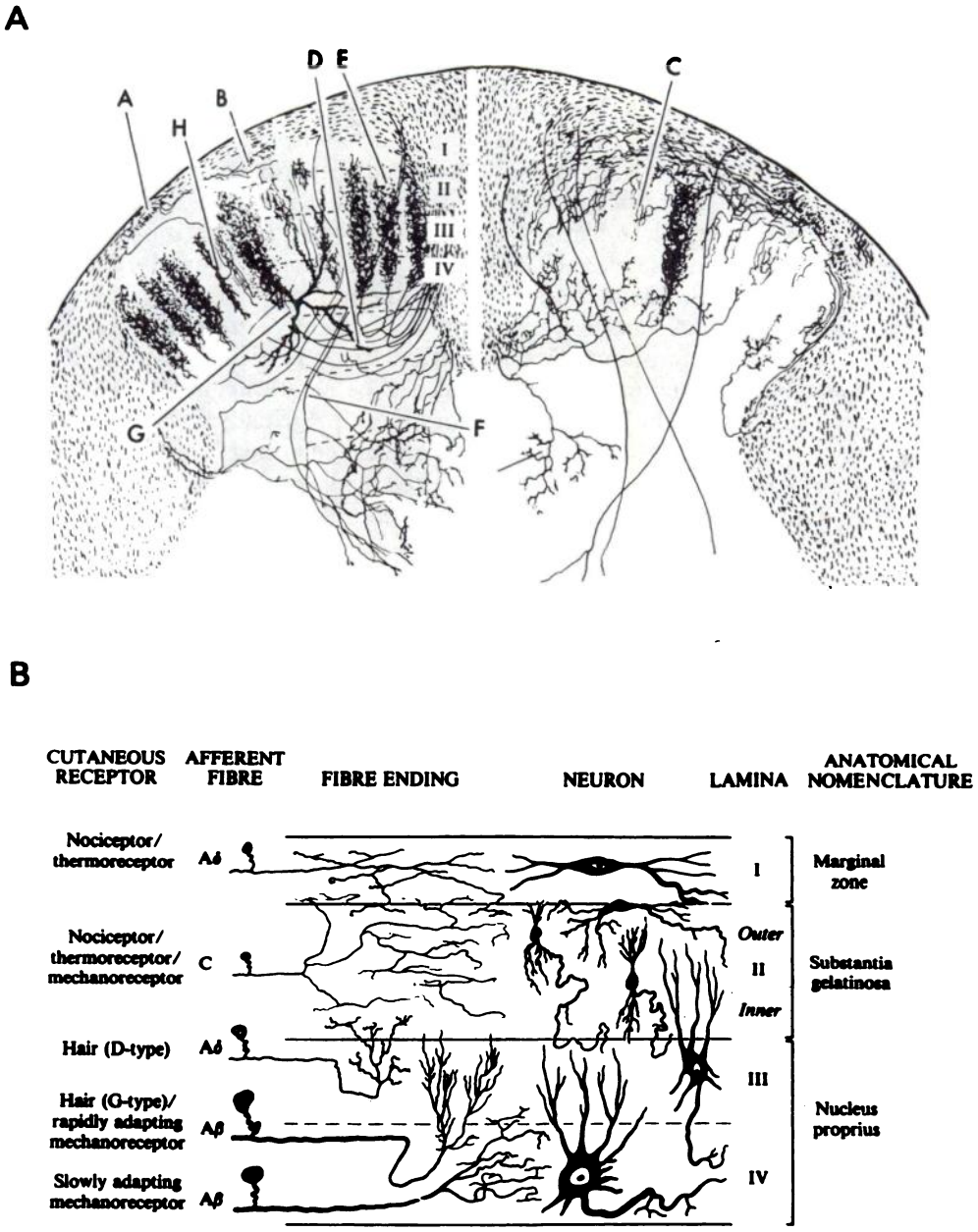

FIG. 1. Anatomy of the upper dorsal horn. A, a Golgi preparation of a transverse section of the dorsal half of the spinal cord. Small diameter afferents A, B, and C are shown entering the cord and transversing the top of the grey matter before penetrating lamina I and aborizing in lamina II. D and E are large diameter afferents that enter the grey matter deep in dorsal horn from its medial aspect and then travel dorsally before aborizing in laminae III and IV. A large diameter afferent, F, penetrates the dorsal horn from above and aborizes deep in the dorsal horn. Two neurones are labeled; H in lamina III and G in lamina IV. [Relabelled and reproduced with permission from Scheibel and Scheibel (437)]. B, A diagrammatic scheme of the connections between primary afferents and neurones of the upper dorsal horn. [Reprodued with permission from Cervero and Iggo (76)].

intravenously in doses of up to 2 mg/kg inhibited the spontaneous firing of neurones of spinal laminae I and V but had little activity on the firing of neurones of laminae IV and VI. The results supported the findings of others that neurones of lamina I and V were excited by mechanical noxious skin stimuli whereas those in lamina IV and VI were unresponsive (405). Thus, the lamina selective effects of morphine were considered relevant to analgesia. The spontaneous firing of neurones of laminae VII was also reduced by intravenous morphine and this effect was antagonized by naloxone (475). The cells studied were excited by a variety of noxious skin stimuli and predominantly inhibited by innocuous stimuli. Recent reports indicate, however, that the laminar classification of neurones needs to be modified. Several groups have reported that a proportion of neurones of lamina IV are excited by noxious heating of the skin (77, 148, 213) and it is therefore unwise to infer a relationship to analgesia solely by laminar location of neurones.

ii. **Excitation of Dorsal Horn Neurones by Impulses in Primary Afferents.** If a spinal action of morphine is important in producing analgesia, then it is likely that an excitation of dorsal horn neurones by nociceptive primary afferents will be reduced by the

appropriate doses of systemic morphine. Several groups have examined this possibility. Electrical stimulation of peripheral nerve, noxious mechanical or thermal cutaneous stimuli, or intra-arterial administration of bradykinin have all been used to activate dorsal horn neurones.

When peripheral cutaneous nerves are stimulated electrically the firing of dorsal horn neurones can be interpreted in terms of responses to impulses in groupings of primary afferent fibres. Aα,β primary afferents have peripheral conduction velocities of 25 to 80 m/s, Aδ conduct at 5 to 25 m/s, and C fibres at less than 2 m/s (167). These groupings of fibres cut across several sensory modalities. Thus, while impulses from most mechanoreceptors in the cat are probably in the Aα,β fibres, some occur within the C fibre group (64). Impulses from nociceptors are carried mainly by C fibres and Aδ fibres (29, 42). Apart from this limitation, following excitation by impulses in fast conducting fibres, a neurone may be inhibited by impulses along polysynaptic pathways and this may interfere with excitation by subsequent impulses in slowly conducting primary afferents. Electrical stimulation of peripheral nerve does, however, offer one significant advantage over the use of natural stimulation; changes in peripheral circulation produced by drugs, or procedures such as sectioning the spinal cord, do not alter the number of afferent fibres excited by each stimulus. These procedures can have significant effects on the firing of nociceptors by natural stimuli, as will be subsequently discussed.

Intravenous morphine (2.0 mg/kg) (145, 307, 308), phenoperidine (0.2 mg/kg) (41), and fentanyl (20 to 40 μg/kg) (249) depressed the excitation of dorsal horn neurones of the cat by electrical stimulation of slowly conducting (Aδ and C) fibres, with little effect on excitation by Aα,β afferents. These actions were reversed by naloxone or nalorphine. Le Bars et al. (308) noted that naloxone after morphine increased firing to levels greater than those present before morphine. Figure 2 illustrates these actions of morphine and naloxone.

It was not known in the experiments just cited whether the neurones studied projected to supraspinal sites. Jurna and Grossman (257) studied the firing of anterolateral tract axons of the cat in spinal segments cephalic to the site of entry of dorsal root fibers conveying impulses produced by electrical stimulation of peripheral nerve. Most of the neurones studied thus probably projected to supraspinal areas, although some may have been propriospinal. They also observed a reduction of firing by impulses in Aδ and C fibre primary afferents after intravenous morphine (0.5 mg/kg) and this was reversed by naloxone. Axons excited by impulses in large (Aα,β) primary afferents were little affected by morphine. In a similar study in the rat it was found that morphine (0.5 mg/kg), pethidine (1 mg/kg), and levorphanol (0.5 mg/kg) produced naloxone reversible reductions in excitation of anterolateral tract axons by impulses in unmyelinated primary afferents (260). High doses of all three drugs were needed to depress activity evoked by Aδ primary

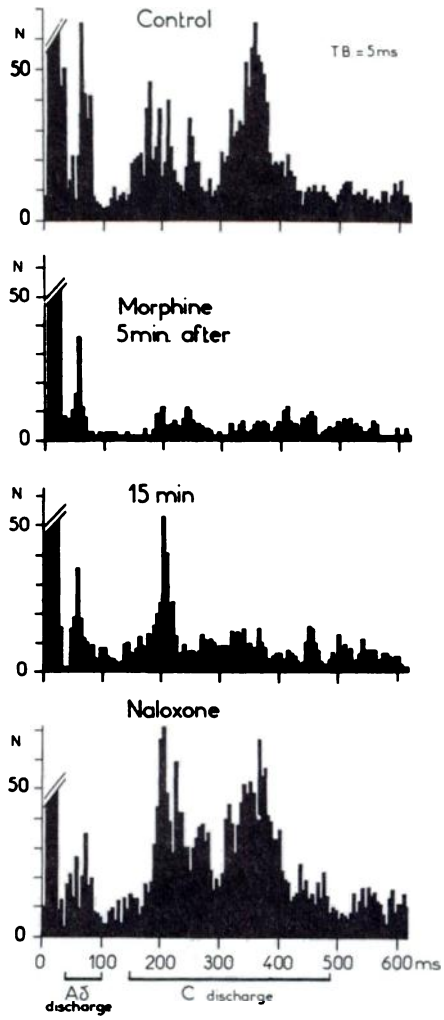

FIG. 2. Reduction by intravenous morphine (2 mg/kg) of the excitation of a lamina V neurone of the cat by impulses in slowly conducting primary afferents. Each histogram is the sum of 50 responses to electrical stimulation of the sural nerve (16 V, 1 ms pulses). Memory address dwell time 5 ms. The column produced by cell firing to impulses in A$\alpha\beta$ fibers is truncated. Naloxone (0.2 mg/kg i.v.) was administered 20 min after morphine. [Reproduced, with permission, from Le Bars et al. (307)].

afferents and only the action of morphine was reversed by naloxone.

A variety of techniques has been used to study the effects of intravenous opiates on the responses of dorsal horn neurones to natural stimuli. Cutaneous nociceptors have been activated by mechanical deformation of the skin, by noxious heating, and by the intra-arterial injection of bradykinin. For quantitative purposes, radiant heat is a convenient stimulus and, in the cat, there is evidence that raising the glabrous skin surface above 45°C results in afferent impulses almost exclusively from nociceptors (29). Bradykinin appears the least satisfactory stimulus, as its effects may correlate poorly with those of mechanical and heat stimuli (456). The influence of all of these stimuli on sensory receptors is affected by changes in skin circulation. With radiant heat, it has been shown that a decrease in skin circulation produces increased firing of nociceptors to a previously stable stimulus, presumably as a consequence of increased tem-

peratures being attained in the deeper layers of the skin (143). The distribution of bradykinin is obviously related to skin circulation. Changes in the patency of arteriovenous anastomoses, known to be in part under spinal control (210), will affect the proportion of injected bradykinin shunted away from nociceptors. Since morphine affects flow through peripheral vessels (439), caution is needed in interpreting changes in cell firing since these may in part result from artifactual changes in afferent input.

Zieglgansberger and Bayerl (524) found that intravenous morphine (2 mg/kg) reduced the excitation of dorsal horn neurones by constant indentation of the skin by a stylus. The pressure used was painful to the experimenters. Etorphine (1 to 10 μg/kg) also reduced the excitation of dorsal horn neurones evoked by pinching the skin in decerebrate spinal cats (510). Etorphine was not completely selective in that the excitation of neurones by light touch of the skin was also slightly reduced. It was noted that the size of the receptive fields of some neurones to touching the skin was reduced by etorphine.

In spinal decerebrate cats, intravenous morphine (1 mg/kg) reduced the excitation of lamina VII neurones by noxious heating of the skin and this reduction was antagonized by naloxone (476). A reduction of such responses by intravenous morphine (0.8 to 2.5 mg/kg) and meperidine (10 to 15 mg/kg) was also observed in chloralose-anaesthetized cats (70). Einspahr and Piercey (164) also observed a reduction by intravenous morphine sulphate (1.0 to 2.0 mg/kg), of the excitation of dorsal horn neurones by noxious heating of the skin, but this effect was not selective. Some neurones were also excited by deflection of cutaneous hairs by puffs of air; these responses were also reduced concomitantly with those to noxious heat and spontaneous firing. The responses of neurones excited only by hair deflection were unaffected by similar doses of morphine.

The results obtained in spinal animals when intra-arterial bradykinin has been used as the noxious stimulus have not supported those obtained when using other forms of noxious stimulation. In the spinal rabbit (432, 471) and cat (398), morphine had little effect on the excitation of dorsal horn neurones by intra-arterial bradykinin. In the latter study, it was noted that, on the same neurones, both spontaneous firing and the short and long latency action potentials produced by electrical stimulation of peripheral nerve were reduced by morphine. The failure of these results with bradykinin to agree with those obtained with pinch and noxious heat may result from problems associated with receptor types activated by bradykinin (456) and the effects of morphine on peripheral circulation. Before these results can be interpreted solely in terms of central effects it needs to be demonstrated that the firing of C fibres in peripheral nerves produced by intra-arterial bradykinin is unchanged after intravenous morphine.

Collectively, these experiments on dorsal horn neurones in spinal animals indicate that systemic morphine depresses the response of these neurons to impulses in fine diameter afferents and this therefore may be an important component of opiate analgesia. The selectivity of morphine, however, is not absolute since some investigators have reported significant effects on responses of neurones to innocuous skin stimuli. This latter action, however, has occurred concomitantly with reductions in spontaneous firing, and this could be of nociceptive origin in animals subjected to extensive surgery. A reduction in this basal level of excitability alone could reduce the responses of neurones to a variety of inputs. The problem of whether the effect of morphine on dorsal horn neurones is produced solely by activity at opiate receptors in the spinal cord or by effects on supraspinal areas controlling spinal neurones will be subsequently discussed.

b. MOTONEURONES. Several studies have shown effects on the firing of motoneurones following intravenous opiates but it has been inferred that these resulted from primary actions on other neurones and not from activation of opioid receptors on motoneurones. Morphine (2 mg/kg) reduced polysynaptic depolarizing potentials evoked in cat motoneurones by stimulation of dorsal roots and this was reversed by naloxone (259). No effects were observed on monosynaptically induced potentials. Because a reduction occurred in the repetitive firing of interneurons adjacent to motoneurones, it was considered that changes in the responses of these cells mediated the effects on motoneurones. In the rat, morphine (0.5 to 3 mg/kg) reduced the activation of flexor but not extensor motorneurones following repetitive activation of large diameter primary afferents (444). Since this did not occur after spinal transection, the primary site of action of morphine was not on motoneurones. The firing of some extensor motoneurones was increased after morphine (298) and this was related to the "lead pipe" rigidity of the limbs of rats after systemic opiates.

c. RENSHAW CELLS. Renshaw cells are interneurones located in the ventromedial aspect of the spinal grey matter which are excited by impulses in cholinergic axon collaterals of motoneurones (158) and which in turn inhibit motoneurones, probably by the release of glycine (109). These cells have been studied with intravenous opiates mainly in relationship to the effects of morphine on spinal inhibition. Morphine more readily reduced inhibition of motoneurones by Renshaw cells (recurrent inhibition) than by inhibitory interneurones excited by impulses in Ia primary afferents (direct inhibition) (104, 295). Morphine probably blocks spinal inhibition through antagonism of the postsynaptic action of glycine (see section 4A) but it was considered possible that the greater effect on recurrent inhibition resulted from a reduced excitation of Renshaw cells by impulses in motoneurone axon collaterals. This has not been found experimentally. In doses of 0.5 to 2.0 mg/kg intravenous morphine did not depress the excitation of Renshaw cells

evoked by ventral root stimulation (140, 169). Indeed, Duggan et al. (141) found that intravenous morphine increased the ventral-root-evoked firing of one third of the Renshaw cells studied with no effect on the remainder. Thus, the differential sensitivity of direct and recurrent inhibition of motoneurones to intravenous morphine has not been satisfactorily explained.

3. Intravenous Opiates and Supraspinal Inhibition of Dorsal Horn Spinal Neurones. It has been proposed that the important actions of systemically administered morphine on the spinal cord do not result from a direct effect on spinal neurones but rather from activation of descending pathways that ultimately inhibit the spinal transmission of nociceptive information. There is abundant evidence that electrical stimulation at certain brain stem sites can inhibit the excitation of spinal neurones by peripheral noxious stimuli. This has been demonstrated for stimulation in the periaqueductal grey matter (PAG) of the midbrain (74, 153, 314, 383) and the raphe nuclei of the medulla (143, 171, 203, 503, 519). Inhibition of neurones of the trigeminal nucleus has been shown following stimulation of the PAG (447, 520) and medullary raphe (322). This inhibition can be selective; with spinal neurones excited by both noxious and non-noxious cutaneous stimuli, stimulation near the medullary raphe mainly reduced excitation by noxious stimuli (143, 383).

Not surprisingly, it has been suggested that the descending fibres purportedly activated by morphine and other opiates come from neurones located in sites that produce analgesia when electrically stimulated. The evidence that bears upon this hypothesis falls into five categories which will be discussed in turn.

a. EFFECT OF SUPRASPINAL AND LIMITED SPINAL LESIONS ON MORPHINE ANALGESIA. These studies have been of two types. Either a direct surgical or electrolytic lesion has been made in an area, or a selective interference with certain pathways has been made by the use of compounds such as 5,6-dihydroxytryptamine or 6-hydroxydopamine. When these compounds are administered systemically they interfere with the functioning of 5-hydroxytryptamine (5-HT)- or noradrenaline (NA)-containing fibres in many areas of the central nervous system; it cannot be concluded that effects on descending spinal fibres necessarily explain changes in the effectiveness of morphine. Relatively limited lesions have been produced by local administration of these neurotoxins, however, and these alone will be discussed.

In some experiments animals have been allowed to recover from a surgical lesion and the effectiveness of morphine as an analgesic has been compared before and after the lesion; these studies have been interpreted in terms of simple absence of function of the destroyed neurones. There is evidence, however, that neuronal changes can occur after central lesions and this can complicate the interpretation of data. After transection of the spinal cord of the rat, the latency of movement of the tail to noxious heat was reduced within 24 hours of the operation (40, 507, 523). This result is compatible with release of spinal neurones from tonically present descending inhibition and therefore agrees with results obtained in anaesthetized or decerebrate cats (61, 150, 213, 483). In the experiments of Berge (40), this shortened latency persisted, but in those of Zelman et al. (523) it returned to control values within 6 days implying reorganization or changed responses of neurones within the spinal cord. This observation suggests that changes in the effectiveness of morphine in prolonging response latencies when the test is performed at 5 to 7 days postsection (and when base-line latencies may not be different from prelesion values) cannot necessarily be related solely to lack of function of descending fibres.

Midline surgical lesions, which included the nucleus raphe magnus of the rat, reduced morphine analgesia, as measured by the latency to tail movement following noxious heat (78, 411, 513). This nucleus contains cells of origin of descending 5-HT-containing fibres and it was suggested that morphine activated these neurones, which in turn inhibited the spinal transmission of nociceptive information. In these three series of experiments animals were tested 7 to 14 days after nucleus raphe magnus lesions. However, Proudfit (409, 410) found that, in the period up to 7 days after such lesions, the effectiveness of morphine as an analgesic was unchanged even though animals were hyperalgesic. Injection of a local anaesthetic in the region of the raphe magnus also produced hyperalgesia but again there was no change in the effect of systemic morphine (410). It was concluded that the nucleus raphe magnus was not necessary for the action of morphine in prolonging the tail flick latency of rats.

In rats, lesions of the dorsolateral funiculus of the spinal cord reduced the effectiveness of morphine as an analgesic (26, 28, 214). All of these studies examined morphine both before and 9 to 10 days after such lesions, and found no shift in base-line latencies to noxious heating of the tail with lesions of any quadrant of the spinal cord. As with complete transection of the cord, therefore, it is possible that some central reorganization had occurred, and lack of function of descending dorsolateral fibres may not be the reason for the relative ineffectiveness of morphine.

5,7-Dihydroxytryptamine has been injected into the upper dorsal horn in an attempt to produce a selective destruction of descending 5-HT-containing fibres (122). By tail flick testing these animals were indistinguishable from control subjects but the effectiveness of morphine was reduced at 7 days after the injection. Collectively, the data from lesion experiments in rats do not provide convincing evidence that opiates activate descending inhibition of spinal neurones. The important period immediately after a lesion needs to be re-examined with most of the experiments cited.

b. COMPARISONS OF THE ACTIONS OF OPIATES ON DORSAL HORN NEURONES IN SPINAL ANIMALS WITH

THOSE WITH INTACT CENTRAL NERVOUS SYSTEM. These studies have been of two types. Either an effect of morphine on a spinal event has been measured and the spinal cord then acutely transacted, or a comparison has been made of data collected from preparations of the two types.

Intravenous morphine (2.0 mg/kg) reduced the amplitude of a short latency field potential recorded in the ventrolateral funiculus of the spinal cord of the barbiturate-anesthetized cat after electrical stimulation of a splanchnic nerve (433). The reduction was reversed by sectioning of the spinal cord at C1. This result was interpreted in terms of an activation by morphine of descending inhibition of spinal neurones. These experiments did not measure inhibition before morphine administration although it was reported that, in preliminary experiments, sectioning the cord had no effect on the amplitude of the recorded potential. But supraspinal inhibition of many spinal neurones is tonically present in barbiturate-anaesthetized cats (150, 213, 483), so that the conclusion of Satoh and Takagi (433) is probably not justified. Supraspinal inhibition needs to be measured before and after morphine administration. Satoh et al. (432) found that morphine (up to 2.0 mg/kg) reduced the excitation of dorsal horn neurones of the unanaesthetized rabbit by intra-arterial bradykinin and was less effective when the spinal cord was transected. Such a result favours the hypothesis that morphine inhibits these cells by a supraspinal action. As discussed previously, however, modifications of the distribution of bradykinin by morphine and particularly by cord transection make it difficult to interpret these experiments soley in terms of central effects of morphine.

Hanaoka et al. (212) compared morphine effects between animals with intact and those with sectioned spinal cords. The collected data from many decerebrate cats indicated that morphine (0.5 to 2.0 mg/kg) had a greater effect in reducing excitation of spinal neurones in those animals with intact spinal cords than in those with the cord transected (212). Most neurones studied were in lamina V and both spontaneous firing and that produced by pinching of the skin were analyzed. This result is the opposite of that reported by Le Bars et al. (308); they found that intravenous morphine (2.0 mg/kg) had little effect on the firing of spinal neurones in decerebrate cats with the cord intact, a finding that contrasted with their previous reports of the effectiveness of morphine on dorsal horn neurones in spinal animals. This relative ineffectiveness of morphine was attributed to a masking of its action by hyperactive supraspinal inhibition, which had been shown for spinal transmission of flexor reflex afferents in the decerebrate cat (324). Le Bars et al. (305) extended their studies to spinal and cord-intact halothane-anaesthetized rats. Dose-response curves of the effect of morphine on depressing the excitation of dosal horn neurones by impulses in unmyelinated primary afferents were similar in the two preparations. This result suggests that the spinal actions of morphine in intact animals are not dependent upon supraspinal areas. There is no obvious explanation for the differences in results obtained by Le Bars et al. (306, 308) and Hanaoka et al. (212). It should be noted, however, that in experiments in which tonic descending inhibition was measured directly, a greater depression of the firing of dorsal horn neurones by analgesic doses of morphine was found in spinal animals than in animals with intact cords (145; see below).

c. MORPHINE AND THE FIRING OF RAPHE NEURONES. If morphine increases descending inhibition of spinal transmission of nociceptive information then analgesic doses of morphine should influence the firing of the cells of origin of the appropriate descending fibres. Experiments to test this have largely been directed at the medullary raphe. Electrical stimulation of this region inhibits the firing of spinal neurones (143, 171, 203) and projections from raphe neurones to the spinal cord have been shown (27, 52, 115, 384).

Inhibition of spinal neurones could be produced in several ways. The descending fibres could establish direct connections with the cells inhibited, could excite spinal inhibitory interneurones, or could inhibit a particular afferent input to the dorsal horn presynaptically by axo-axonic contacts. It is equally possible that the appropriate descending fibres could be tonically active, and excite spinal neurones on nociceptive pathways. If morphine depressed the firing of these supraspinal neurones, inhibition of nociceptive transmission in the spinal cord would result. What is to be emphasized by outlining these possible modes of action is that any change in the firing of supraspinal neurones cannot be interpreted unless the mechanism of descending inhibition is known.

Experiments studying the firing of raphe neurones when morphine has been administered systemically or microelectrophoretically are described in section B 6. These experiments have not shown consistent effects on cell firing following morphine and as such have not supported an increase in descending inhibition as being necessary to the spinal actions of opiates.

d. MICROINJECTION OF MORPHINE IN THE BRAIN STEM AND RESPONSES OF LAMINA V SPINAL NEURONES. Injection of morphine (40 μg) in the region of nucleus reticularis gigantocellularis (NRGC) inhibited the excitation of six of 10 dorsal horn neurones by intra-arterial bradykinin (469). Similarly administered naloxone (40 μg) reversed this action of morphine. When microinjected into the PAG of rats, morphine (4 to 16 μg) and etorphine (0.25 to 0.5 μg) reduced the excitation by noxious heating of the skin of 55% and 80% respectively of dorsal horn neurones studied (36). These effects were reversed by intraperitoneal naloxone (1 mg/kg). By contrast, Le Bars et al. (305) failed to observe inhibition of dorsal horn neurones of the rat when morphine (5 μg) was injected near the nucleus raphe magnus. Indeed, one half of neurones was excited. In these experiments, this dose of

morphine produced naloxone-reversible analgesia (tail flick test) when injected near the nucleus raphe magnus of unanaesthetized rats. Concentrations after microinjection are unknown. Hence, even the demonstration of inhibition of spinal neurones after microinjection of opiates in a particular area does not necessarily mean that such inhibition occurs with analgesic doses of systemic morphine.

There are some puzzling but relevant observations from experiments in which *analgesia* (hot plate and tail flick tests) has been produced by systemic morphine and attempts have been made to reverse this by microinjecting naloxone at different brain sites. Firstly, it was shown that spinal intrathecal naloxone in the rat abolished analgesia by systemic morphine (up to 75 mg/kg). With high doses of morphine, spinal naloxone was less effective (513a). This suggests that the important site of action of systemic morphine in producing analgesia is the spinal cord. Subsequently, however, it was shown that injecting naloxone into the third ventricle had a similar effect (517). In both studies evidence was presented that naloxone did not diffuse widely and that its action was relatively restricted. It was also shown that microinjecting naloxone near the nucleus raphe magnus reversed analgesia by low but not high doses of morphine (128). In explanation of these seemingly paradoxical results, Yeung and Rudy (518) proposed a multiplicative action of morphine at spinal and supraspinal sites such that interference with the action at one site would drastically impair the combined action. The important point for the present discussion is that experiments of this type are not crucial in deciding whether opiates inhibit nociceptive transmission at the spinal level by increasing descending inhibition. What needs to be shown is whether naloxone injected in a variety of supraspinal sites reverses depression of nociceptive responses of single dorsal horn neurones by systemic morphine.

e. MEASUREMENTS OF DESCENDING INHIBITION OF SPINAL NEURONES. Tonically present descending influences on spinal neurones can be reversibly removed by cooling the spinal cord above the area being studied. Several investigators have found that such a block of spinal conduction increases the excitation of many spinal neurones by peripheral noxious stimuli (77, 145, 150, 213, 483). The descending inhibition revealed in this way is relatively selective in that responses of dorsal horn neurones to deflection of hairs are little changed (150). Because of the circulatory effects of both systemic morphine and spinal cold block, these experiments are best performed by exciting dorsal horn neurones by electrical stimulation of nerve.

In decerebrate cats, Jurna and Grossman (257) studied the excitation of axons in the anterolateral tract of the spinal cord by electrical stimulation of peripheral nerve. These axons are derived from dorsal horn neurones and presumably convey nociceptive information to supraspinal areas. Analgesic doses of morphine (0.5 to 2.0 mg/kg) not only reduced the firing of these axons by impulses in C fibre afferents but also reduced descending inhibition of such excitation as revealed by spinal cold block. The decerebrate preparation, whilst being unanaesthetized, has exaggerated supraspinal inhibition of spinal transmission of flexor reflex afferents (324). If the tonic supraspinal inhibition of nociceptive transmission is also exaggerated then there are limitations in applying results from decerebrate preparations to conscious animals. In cats that were only lightly anaesthetized (with α-chloralose or pentobarbitone sodium), intravenous morphine (0.5 to 1.0 mg/kg) also reduced descending inhibition of dorsal horn neurone excitation by impulses in C fibre primary afferents (145). These experiments showed that the direct spinal action of morphine on the excitation of dorsal horn neurones was not enhanced but *reduced* by its supraspinal effects. This supports the findings of Jurna and Grossman (257). Both the spinal and supraspinal actions of morphine were reversed by naloxone (0.1 to 0.3 mg/kg). Figure 3 illustrates these results.

Although these experiments deny any increase of descending inhibition as being of importance to the effects of morphine on dorsal horn neurones in the decerebrate or anaesthetized cat, the important unknown is to what extent is descending inhibition tonically present in conscious animals? This cannot be answered definitively, but the hyperalgesia produced by brain stem or spinal cord lesions in rats (40, 411, 507, 523), cats (270), and monkeys (481) suggests that such tonic inhibition exists in conscious animals.

The effects of morphine on descending inhibition of spinal neurones after electrical stimulation of the brain stem raphe has also been studied. Both morphine (0.5 to 16 mg/kg) and meperidine reduced this inhibition in the decerebrate cat. Nalorphine antagonized the action of morphine in three of seven experiments but had no effect on the action of meperidine (453). Thus, in three series of experiments in which descending inhibition was measured electrophysiologically, in no case was this increased by morphine.

A differing effect of cyclazocine was obtained by Kawajiri and Satoh (269). In unanaesthetized rabbits the firing of lamina V neurones to the peripheral intra-arterial injection of bradykinin was reduced by systemic cyclazocine (0.5 mg/kg). Measurements of descending inhibition by the cold block technique showed that this was increased and accounted for the decreased firing of the spinal neurones studied. Supporting this conclusion is the inactivity of cyclazocine in tests of analgesia when administered near the spinal cord by the intrathecal catheter technique (514). This experiment does suggest differences between morphine and cyclazocine, but the use of bradykinin with the cold block technique leads to problems in interpretation because of possible changes in afferent input (discussed previously), and these experiments should be repeated with electrical stimulation of peripheral nerve.

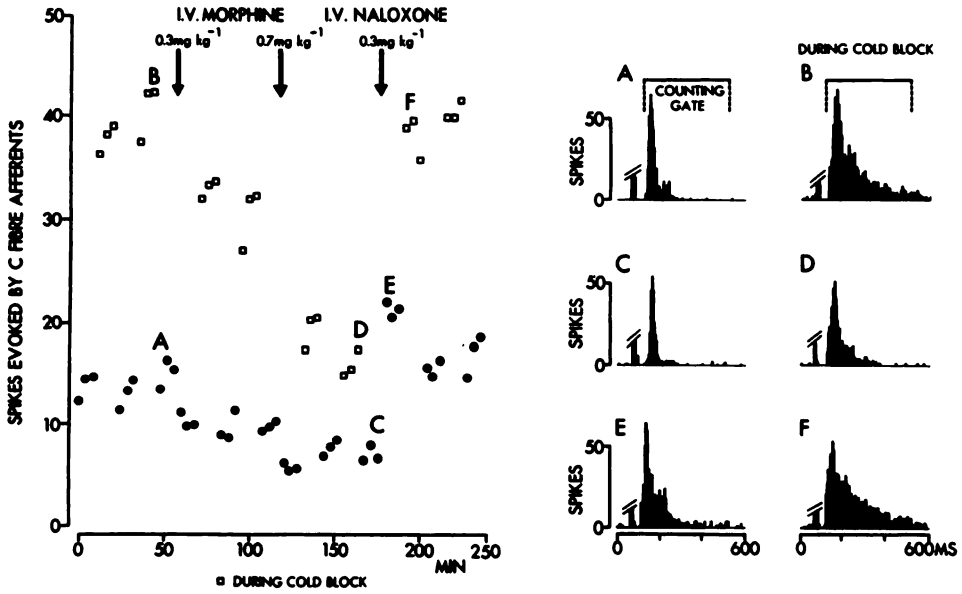

FIG. 3. Intravenous morphine reduces the excitation of a lamina V neurone by impulses in unmyelinated primary afferents, and also reduces descending inhibition. Each histogram is the sum of 16 responses to electrical stimulation of the tibial nerve with a stimulus strength 200 times threshold for the fastest conducting fibers. The columns representing responses to impulses in A fibers have been truncated. With each stimulus the number of action potentials to impulses in unmyelinated afferents was determined by gating an electronic counter (period of gate indicated above the histograms). The means for 16 responses are plotted on the left. ●, observations made with the spinal cord intact and descending inhibition present; □, observations made, on the same neurone, during periods when descending inhibition was temporarily removed by cooling the spinal cord several segments rostral to the recording site. The letters A to F indicate the times at which the histograms were compiled. Note that morphine sulphate (total dose 1.0 mg/kg) markedly reduced the increase in responses produced by temporary cold block. This indicates a reduction in descending inhibition. [Repoduced, with permission, from Duggan et al. (145)].

The previous experiments have assumed that the firing rate of projecting neurones of the dorsal horn is sufficient to encode nociceptive information for supraspinal transmission. Le Bars et al. (302) have recently proposed a different mechanism which requires that morphine *reduce* one form of descending inhibition of spinal neurones to produce analgesia. They observed that, when studying multireceptive neurones of the dorsal horn of the rat, noxious stimuli to many areas remote to the receptive field of the neurone being studied inhibited both the spontaneous firing of the neurone and its responses to cutaneous stimuli (304). It was proposed that a noxious stimulus would thus excite one group of neurones in the spinal cord and widely inhibit others, and that pain recognition depended on this contrast in firing. Doses of morphine lower than those affecting cell excitation in response to a peripheral noxious stimulus reduced this diffuse noxious inhibitory control (DNIC) from remote areas. Removal of contrast by reducing DNIC was proposed as a means of analgesia. Since DNIC depends upon supraspinal structures it was also suggested that this amounts to a reduction of descending inhibition by morphine. This is an ingenious proposal which merits further experimentation. As outlined in the introduction to this section on the spinal cord, the encoding of nociceptive information in the spinal cord is poorly understood and perhaps it is an oversimplification to expect that reduced firing of projecting dorsal horn neurones in response to cutaneous noxious stimuli is an adequate basis for analgesia.

In the case of morphine, it appears improbable that an increase of descending inhibition is responsible for its spinal actions. Indeed the opposite seems more probable: the actions of morphine on spinal neurones are reduced by its effects on supraspinal inhibition. This has interesting implications for the use of epidural and subdural morphine for the clinical relief of pain. As well as localizing the effect of morphine, the effect on the spinal cord (135) is probably greater than that of comparable concentrations produced by systemic morphine, since there is no diminution in the effect of the locally administered drug by a reduction of descending inhibition. This unexpected bonus may be a major factor in the effectiveness of spinally administered morphine.

4. Microelectrophoresis of Opioids and the Firing of Spinal Neurones. Electrophysiological studies with intravenous opiates have defined groups of neurones the firing of which is altered by analgesic doses of these drugs; but such studies cannot decide the structures primarily affected by the drugs. The microelectrophoretic technique permits the study of interactions between opioids and single neurones.

a. OPIOIDS AND PUTATIVE TRANSMITTERS IN THE SPINAL CORD. Possible antagonism of the postsynaptic action of a neurotransmitter is a necessary investigation when studying the central effects of any drug. The opioids have been extensively investigated from this aspect over the past decade.

i. Amino Acids. There is a large body of evidence that glycine and γ-aminobutyric acid (GABA) are inhib-

itory transmitters in the spinal cord (109). Morphine blocked the inhibitory action of glycine on spinal interneurones and Renshaw cells (104, 137) but this action was not antagonized by naloxone (141) and therefore is unlikely to have any relevance to opiate analgesia. Similar results have been obtained in experiments on cultured neonatal dorsal horn neurones of the mouse (202, 487).

In many species high doses of morphine produce convulsions (289), an effect that may be explained by a blockade of spinal inhibition at glycine-mediated synapses. Higher concentrations of morphine than those affecting glycine reduced the depressant action of GABA (137, 487). The high concentration of morphine needed to reduce the action of GABA makes it unlikely that this effect is relevant to the action of analgesic doses of morphine.

The amino acids L-glutamate and L-aspartate may be excitatory transmitters in the spinal cord (109). In studying interaction between excitants and a particular substance it is important to distinguish between a depression of cell excitability with an associated reduced effectiveness of *any* excitant, and a selective antagonism of the postsynaptic action of a *particular* excitant. Often the only completely satisfactory way to demonstrate the latter is to study a neurone that is excited by two classes of compound, such as cholinomimetics and amino acids, and show a selective reduction in excitation by one class. Several investigators have found that morphine reduces excitation of spinal neurones by L-glutamate (69, 137, 141, 524). There is considerable disagreement on the relevance of this finding to the effects of systemic opiates.

Zieglgansberger and Bayerl (524) found, with extracellular recording and microelectrophoresis of drugs, that simultaneous administration of naloxone prevented the reduction of L-glutamate excitation by morphine. Intracellular studies on motoneurones and interneurones, mainly of lamina V, showed that morphine reduced the depolarizing action of L-glutamate without itself producing detectable changes in membrane potential or conductance. Naloxone ejected before or with morphine prevented these actions. By contrast, both Dostrovsky and Pomeranz (138) and Duggan et al. (141) found that naloxone did not reverse the reduction by morphine of L-glutamate-induced excitation. Whereas Zieglgansberger and Bayerl (524) found dextrorphan to be without effect on spinal neurones, Dostrovsky and Pomeranz (138) found this non-opiate enantiomer to depress excitation of spinal neurones by L-glutamate. This latter effect was also reported by Duggan et al. (141) who found dextrorphan to be more potent than morphine in this respect. Morphine has been also reported to *enhance* the excitation by L-glutamate of a proportion of dorsal horn neurones, an action also not antagonized by naloxone (141, 397).

As with opiates, Zieglgansberger and Tulloch (528) found that [Met]- and [Leu]enkephalin reduced the excitation of dorsal horn neurones by L-glutamate. They used intracellular recording from dorsal horn cells and from motoneurones, and administered both the enkephalins and L-glutamate some 100 to 180 μm dorsal to cell bodies; they failed to detect any effect on cell membrane potential or resistance by the same applications of the enkephalins that reduced depolarization by L-glutamate.

On cultured spinal neurones (discussed later), studies of the interactions of opioids with depolarization by L-glutamate have not given uniform results. [Leu]enkephalin has been variously reported to reduce depolarization by L-glutamate (24, 25), or to have no effect (202). High concentrations (1.5 mM) of morphine did not reduce the action of L-glutamate on these neurones (487).

L-Glutamate is generally considered to depolarize neurones in vivo by a direct postsynaptic action. A reduction of L-glutamate-induced depolarization would thus suggest that a compound is acting postsynaptically. The discordant results with both opiates and opioid peptides are puzzling but do suggest that this is not a consistent effect of these compounds. As will be subsequently discussed, the bulk of evidence indicates an activation of membrane potassium conductance as an important postsynaptic action of opioids on neurones. There are several ways in which such an action could depress responses to L-glutamate without necessarily causing hyperpolarization of the cell body [see below, and North (367)].

ii. Acetylcholine. Connections between motoneurone axon collaterals and Renshaw cells are perhaps the best documented central cholinergic synapses in mammals and have therefore been studied with opiates because of the well known effects of these drugs on peripheral cholinergic synapses (388).

Morphine and levorphanol (but not dextrorphan) excited Renshaw cells of the cat (119, 140, 141) and rat (117), and this excitation was reduced by naloxone. Since naloxone also reduced excitation by acetylcholine but not by an amino acid it was considered that the simplest explanation of these results was that the opiates were acting as agonists at the nicotinic receptors on Renshaw cells (141). When Renshaw cells were excited by alternate ejection of acetylcholine and an amino acid, morphine enhanced the action of both substances in the cat (141) but preferentially increased the effect of acetylcholine in the rat (319). This selective action of morphine was not reproduced by levorphanol (141) or etorphine (319), both of which reduced the excitation of Renshaw cells by acetylcholine and amino acids. Both [Met]- and [Leu]enkephalin, however, excited Renshaw cells of the cat (118) and this excitation was abolished by naloxone.

These actions of morphine may be relevant to the total pharmacological effects of morphine on the spinal cord, since intravenous morphine does excite some Renshaw cells (141), but it is very improbable that they have any relationship to opiate analgesia. In the spinal cord, few neurones other than Renshaw cells are excited by acetylcholine (111). It should be noted that no group has reported that opiates reduce the excitation of Renshaw cells by L-glutamate.

iii. Substance P. Neurochemical studies have suggested that substance P may be a transmitter released by small diameter primary afferent fibres (234, 472). A possible explanation for the relatively selective action of opiates on spinal transmission of impulses in these afferents is a postsynaptic block of the action of the relevant transmitter. This has not been observed. Administered near the dorsal horn neurones, morphine either had no effect on, or potentiated, excitation by substance P (397).

iv. Monoamines. There are no reports of interactions between opiates and monoamines administered from micropipettes. Administered in the substantia gelatinosa, naloxone did not modify the actions of noradrenaline or 5-HT (215).

b. MICROELECTROPHORETICALLY ADMINISTERED OPIATES AND SPONTANEOUS FIRING AND SYNAPTIC EXCITATION OF DORSAL HORN NEURONES. Because of the analgesic actions of morphine, microelectrophoretic studies of opiates and spinal neurones have concentrated on dorsal horn neurones activated either by noxious stimuli or by impulses in unmyelinated fibres. Difficulties arise in comparing the work of different groups because of the varied use of the term "nociceptive neurone." All use this term to describe neurones excited by noxious cutaneous stimuli but frequently it is not stated what proportion of such units was excited only by noxious stimuli, and what proportion was excited also by non-noxious stimuli. Many dorsal horn neurones in several laminae are excited by both noxious and non-noxious stimuli (77, 149, 211, 213). The implication that depression of the spontaneous firing of a nociceptive neurone is necessarily related to analgesia is unwarranted if that neurone is also excited by innocuous skin stimuli. Since the role that a particular neurone plays in the transmission and ultimate perception of cutaneous sensations is usually unknown, it is probably better to define the stimuli to which the neurone responds and the manner in which opiates affect both spontaneous firing and its excitation from all known sources.

i. Administration Near Cell Bodies. Calvillo et al. (69) found that morphine reduced the excitation of dorsal horn neurones by noxious heating of the skin. Spontaneous firing and the firing in response to administration of L-glutamate were also reduced, suggesting a postsynaptic site of action. The firing of units unresponsive to noxious stimuli was unaffected by morphine. The effects of morphine were reversed by naloxone administered at the same site in only two of seven instances. A subsequent report (70) confirmed this action of morphine but found that excitation of both nociceptive and non-nociceptive units by L-glutamate was reduced. It was not reported whether naloxone reversed this effect. Zieglgansberger and Bayerl (524) found that microelectrophoretic morphine and levorphanol depressed spontaneous and L-glutamate-induced firing of lamina IV and V cells. With both types of cell, firing by stylus indentation of the skin was also reduced by the opiates. All of these actions were reversed by prior or simultaneous administration of naloxone.

By contrast, several investigators failed to observed naloxone reversible depression of the firing of dorsal horn neurones when morphine has been administered near the somata of these neurones (118, 138, 141, 148, 397). In these studies morphine frequently excited dorsal horn neurones and, when both noxious and non-noxious cutaneous stimuli were used, responses to both were enhanced. Naloxone did not reverse excitation by morphine. When depression of cell firing was produced by morphine this also was not antagonized by naloxone. In another study (31), morphine predominantly depressed the firing of nociceptive units and excited non-nociceptive units, but it was the latter action that was antagonized by naloxone. It was proposed that the neurones excited by morphine inhibited the responses of other neurones to noxious peripheral stimuli and that this action was relevant to the effects of systemic morphine. The logical test of this hypothesis is to look for such neurones following systemic morphine but this appears not to have been done. In addition, Piercey et al. (397) have shown that excitation of dorsal horn neurones by morphine is produced by higher concentrations than those attained by systemic analgesic doses and therefore probably has little relevance to analgesia. There is also dispute as to the effects of dextrorphan ejected near the bodies of dorsal horn neurones. This compound has been found variously to have no effect on these cells (524) or to be a depressant of cell firing at least as potent as levorphanol (138, 141).

Can a postsynaptic action of opiates explain the known effects of intravenous morphine on dorsal horn neurones and spinal reflexes? Since most reports have emphasized a preferential effect of opiates on spinal transmission of impulses in fine diameter afferents (see previous sections), for a postsynaptic effect to be an adequate basis either the relevant receptors should be restricted to neurones that are excited mainly by impulses in small diameter primary afferents (and little affected by large diameter afferents) or some feature of the effect of morphine on postsynaptic membranes should result in a greater reduction in excitation by small diameter fibres. No study has shown that depression by opiates is restricted to neurones excited only by noxious cutaneous stimuli. When morphine has been reported to depress "nociceptive neurones" the sample would almost certainly include a proportion of cells excited by both noxious and non-noxious stimuli and data on the effects of morphine on the excitation of such neurones by non-noxious stimuli are limited. If there is a population of small neurones not normally sampled by multibarreled micropipettes, and which are specifically nociceptive and depressed by opiates, they have not been revealed by autoradiographic studies of opiate binding in the dorsal horn (14). The possibility cannot be excluded, however,

until covalently bound ligands permit the study of opiate binding sites with the electron microscope.

Intracellular recording, combined with the administration of opiates 100 to 180 μm dorsal to the tip of the recording electrode, led Zieglgansberger and Bayerl (524) to propose that the postsynaptic action of opiates could explain the selectivity observed after systemic administration by a mechanism not dependent upon cell types. Microelectrophoretic morphine slowed the rate of rise of excitatory postsynaptic potentials (EPSPs) produced by electrical stimulation of peripheral nerve and reduced depolarizations by L-glutamate. This occurred in the absence of detectable changes in membrane potential or conductance. Most results were obtained from motoneurones but it was stated that dorsal horn cells responded similarly. Figure 4 illustrates the results of such an experiment. It was proposed that a reduction in the EPSP rate of rise would have little effect on spike initiation by fast rising EPSPs but would reduce firing by slowly rising polysynaptic EPSPs.

In these experiments, glued parallel electrode assemblies were used to apply drugs at a distance from the recording site. There is uncertainty in the relationship of the tip of the drug-administering pipette to the soma and dendrites of the recorded neurone. It is not uncommon to observe a reduction in EPSPs when applying an amino acid such as glycine or GABA without detecting any change in membrane potential of the soma. Indeed, for years GABA was considered an unlikely inhibitory transmitter because of an inability to show a membrane hyperpolarization of motoneurones despite clear decreases in EPSPs (110). Thus, it cannot be excluded that morphine reduced EPSPs and L-glutamate depolarization by a membrane potential or conductance change not detectable at the soma.

There are other difficulties with the hypothesis of Zieglgansberger and Bayerl. The most important is that other investigators using extracellular recordings have failed to observe a consistent depression by opiates of the excitation of dorsal horn neurones by substances considered as possible excitatory transmitters such as L-glutamate and substance P (141, 397); when such depression did occur it was not antagonized by naloxone (138, 141). Even though a slowing in the rate of rise of EPSPs may be an adequate explanation for differential effects of opiates on responses to electrical stimulation of peripheral nerve, it has not been shown experimentally that this suffices when dealing with natural cutaneous stimuli. With electrical stimulation of peripheral nerve, the EPSP induced in dorsal horn neurones by the arrival of a synchronous volley in large diameter afferents is rapidly rising and of large amplitude when compared with the depolarization produced by the arrival of the temporally dispersed impulses in unmyelinated afferents (408). It has not been shown, moreover, that depolarization from stroking the skin innocuously differs temporally from that produced by burning or cutting the skin. With continued sensory stimulation, both are likely to result in a continuous barrage of afferent impulses. But analgesic doses of morphine are virtually without effect on touch perception (288, 491) and, furthermore, many papers cited in this review have shown selective effects in the dorsal horn when natural cutaneous stimuli are used.

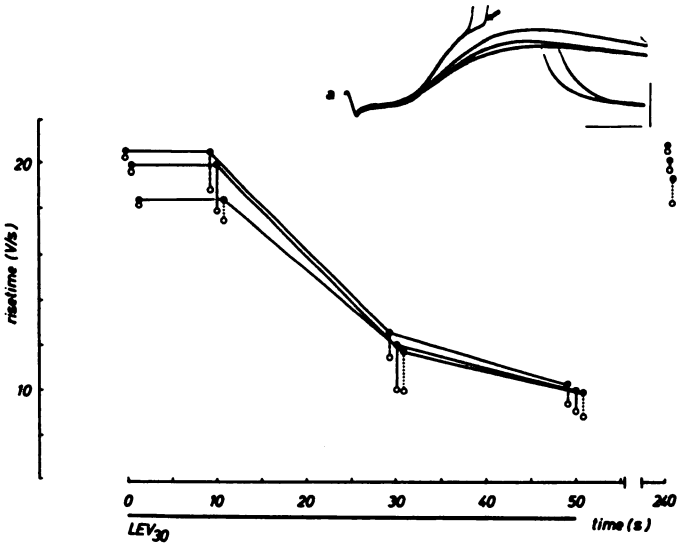

FIG. 4. Actions of morphine, levorphanol and naloxone on the rate of rise of excitatory postsynaptic potentials (EPSPs) in spinal motoneurones. The graph illustrates the effect of levorphanol (30 nA) on the rate of rise of EPSPs evoked in three different motoneurones by electrical stimulation of a dorsal rootlet of the same segment (pulse duration 0.5 ms, amplitude 1.0 V). The measurements of rate of rise were made at two times after the onset of the EPSP. (●, 0.25 ms; ○, 0.5 ms.) Note depression by levorphanol microelectrophoresis, and recovery 4 min later. The upper records are superimposed intracellular recordings of membrane potential, to illustrate the progressive changes in a monosynaptic EPSP in a biceps-semitendinosus motoneurone. Microelectrophoretic morphine (50 nA, 30 s) depressed the EPSP and this was reversed by naloxone (50 nA). Control trace and trace after naloxone give rise to action potentials (arrow). [Reproduced with permission from Zieglgansberger and Bayerl (524)].

ii. Administration in the Substantia Gelatinosa. The lack of uniformity of results obtained when morphine has been administered near cell bodies may result from a limitation of the microelectrophoretic technique. If receptors for a particular ligand are sparsely present near the cell body, then activation of receptors more distally placed may only occur at the expense of relatively high concentrations near cell bodies; these high concentrations may have effects not produced by the levels attained by systemic administration.

To explore the possibility that important opiate receptors might be located at sites away from the somata of dorsal horn neurones, Duggan et al. (147, 148) used two micropipette assemblies. One recorded the firing of neurones and administered drugs near cell bodies, while the other was positioned dorsal to the recording electrode with its tip in the substantia gelatinosa. Figure 5A illustrates this arrangement of electrodes. This latter location

was chosen because there is anatomical evidence that some of the dendrites of neurones of spinal laminae IV and V project dorsally and establish connections with incoming primary afferents in the substantia gelatinosa (407, 436, 467). Administered in the substantia gelatinosa, morphine selectively reduced the excitation of dorsal horn neurones by noxious heating of the skin while responses to hair deflection were relatively unaffected. The action of morphine was long lasting, but was reversed by naloxone administered either at the same sites as morphine or when given intravenously in doses as low as 0.1 mg/kg. Figure 5B illustrates these actions of morphine and naloxone. Although the concentrations attained in the substantia gelatinosa were unknown, the selective effects correlate well with those of systemic morphine. Moreover, naloxone administered in the substantia gelatinosa fully reversed the effects of systemic morphine on dorsal horn neurones (252).

The substantia gelatinosa is an anatomically complex area. In the experiments of Duggan et al. (148), excitant and depressant amino acids such as L-glutamate, glycine, and GABA failed to mimic the actions of morphine; this might suggest that the relevant opiate receptors were not located on the intrinsic neurones of the substantia gela-

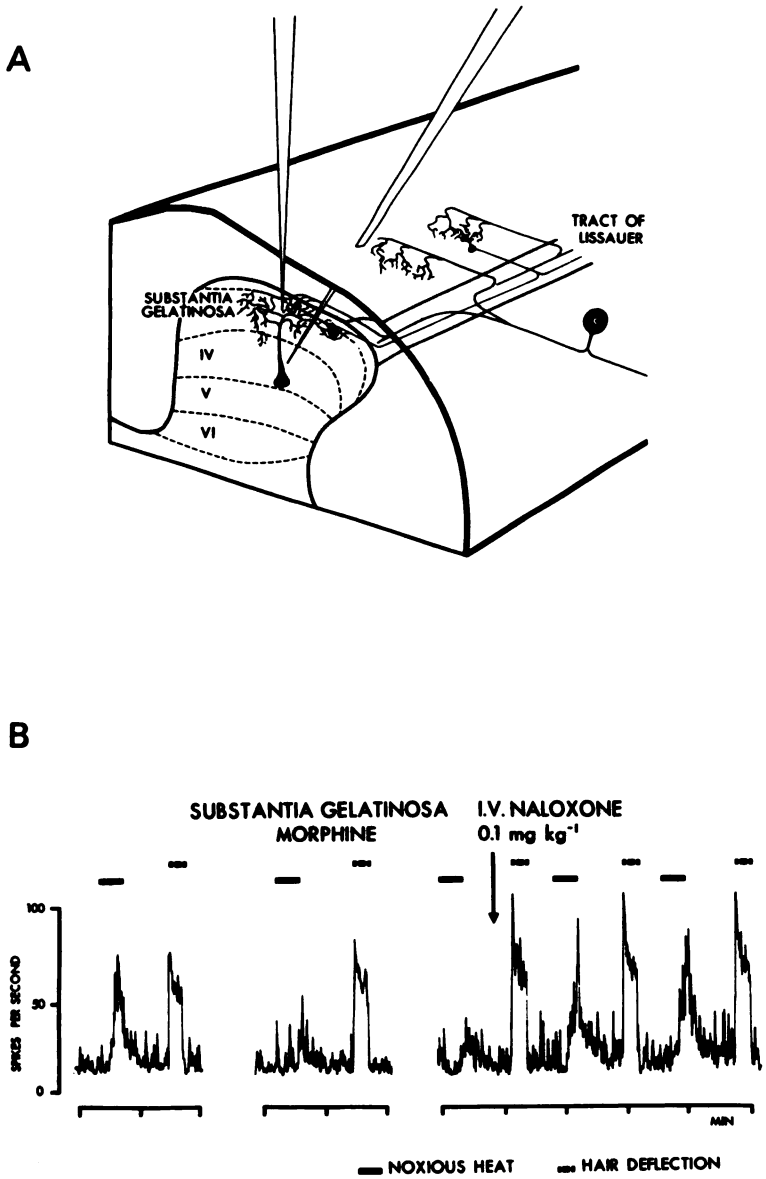

FIG. 5. Effects of morphine administered electrophoretically in the substantia gelatinosa. A, the arrangement of micropipette assemblies used to administer drugs in the substantia gelatinosa while recording from neurones of laminae IV, V, and VI. B, a lamina IV neurone was alternately excited by noxious heat to the third digital pad of the left hind limb, and by deflection of hairs close to the digital pad. Morphine (100 nA) ejected into the substantia gelatinosa (230 μm dorsal to the site of the recording) reduced the response to noxious heat and this was reversed by intravenous naloxone (0.1 mg/kg). [Reproduced, with permission, from Duggan et al. (148)].

tinosa nor on the dendrites of the deeper neurones from which action potentials were being recorded. It was suggested that morphine interfered with either the release or postsynaptic action of the transmitter released from unmyelinated primary afferent fibres.

Sastry and Goh (427) performed similar experiments to those of Duggan et al. (148), except that a glued parallel pipette assembly (single plus a three-barrel) was used to record the activity of neurones of the substantia gelatinosa as well as administer drugs in this area. Both morphine and [Met]enkephalinamide depressed the excitation of neurones of laminae IV and V by noxious heating of the skin but at the same time similar responses of neurones of the substantia gelatinosa were increased. It was proposed that these neurones excited by opioids were ultimately inhibitory to the nociceptive responses of deeper neurones, a suggestion that does not imply any direct action of morphine on primary afferent fibres. Experiments with intracellular recording from rat substantia gelatinosa neurones in vitro (discussed below) have not found any cells that were excited by opioids.

Evidence from other techniques points to a location of opiate receptors on primary afferent fibres. Autoradiographic studies of the distribution of systemically administered etorphine found a high density of binding sites in the substantia gelatinosa (14, 393). Receptor binding techniques have found high levels of opiate binding sites in the upper dorsal horn of the monkey (301). Dorsal root section produced a fall in the number of binding sites, suggesting that some were on primary afferents. The possibility that the fall was produced by transsynaptic degeneration of spinal neurones could not be excluded. Experiments on cultured dorsal root ganglion cells and spinal cord explants (cited below) also have indicated that opiate receptors are located on the terminals of primary afferent fibres.

If opiates indeed interfere with the release of transmitters by impulses invading the terminals of unmyelinated primary afferent fibres, this interference could come by at least two mechanisms. Either action potentials could be prevented from invading the terminals, or terminal membranes could be affected so that incoming action potentials release less transmitter. Depolarization of terminals has been proposed as the basis of presynaptic inhibition of transmission of impulses in myelinated afferents in the spinal cord (438). Presynaptic inhibition has a structural basis in axo-axonic synapses and activity of opioid peptides at axo-axonic synapses in the substantia gelatinosa has been proposed (148, 250). Further discussions of the mechanism by which opiates may interfere with the function of primary afferent fibres will be postponed until after consideration of results obtained when opioid peptides have been administered microelectrophoretically in the dorsal horn.

c. MICROELECTROPHORETICALLY ADMINISTERED OPIOID PEPTIDES AND SPONTANEOUS FIRING AND SYNAPTIC EXCITATION OF DORSAL HORN NEURONES. **i. Administration Near Cell Bodies.** Duggan et al. (149) found that [Met]enkephalinamide administered near the bodies of neurones of laminae IV and V reduced the excitation of these cells by both noxious and non-noxious cutaneous stimuli. This action was reduced by naloxone administered from micropipettes but not by naloxone given intravenously in doses of 0.1 to 0.3 mg/kg. Zieglgansberger and Tulloch (528) also observed this nonselectivity of action with [Met]- and [Leu]enkephalin administered from micropipettes; the drugs were administered some 40 to 60 μm away from the site of extracellular recording. Since excitation by L-glutamate was also reduced by the enkephalins, these experiments and those of Duggan et al. (149) are most simply explained by a postsynaptic location of enkephalin receptors on the neurones studied. Although these results can be explained by a hyperpolarization of postsynaptic membranes, this was not observed in the intracellular studies of Zieglgansberger and Tulloch (528). Amounts of enkephalin adequate to reduce the changes in membrane resistance and potential by L-glutamate had no effect on resting potential or resistance. It should be noted, however, that the hypothesis proposed by Zieglgansberger and Bayerl (524) to explain selectivity in the postsynaptic action of opiates (slowing the rate of rise of EPSPs) cannot be applied to the opioid peptides since the enkephalins readily reduced the excitation of neurones by noxious and non-noxious cutaneous stimuli (149, 528).

Selectivity of action of opioid peptides on dorsal horn neurones have been found in cats (413) and rabbits (431). Enkephalins depressed the spontaneous firing and the firing evoked by noxious cutaneous stimuli but did not depress the firing of neurones excited only by non-noxious stimulation of the skin. Depression by the enkephalins was antagonized by naloxone. It is not clear what proportion of the neurones studied was excited by both types of stimuli and, in these cases, whether responses to both stimuli were reduced concurrently.

It should be noted that in contrast to results with morphine, all investigators agree that the effect of enkephalins administered near cell bodies are reversed by naloxone. Differences between receptors for opioid peptides and opiates have been described in experiments with peripheral tissues (320), homogenates of brain (282, 283), and dorsal roots and spinal cord (172). In the latter study, whereas [^{3}H]morphine binding was equally distributed between dorsal roots and the dorsal horn, [^{3}H]-[D-Ala^2,D-Leu^5]enkephalin binding was higher in the dorsal horn. It is possible, therefore, that the receptors present in the substantia gelatinosa differ from those present near the somata of dorsal horn neurones. This could underlie the uniformity of naloxone reversible effects observed with microelectrophoretic administration of opioids in the substantia gelatinosa, and the difficulties and inconsistencies attaching to naloxone reversal of opioid (particularly morphine) effects in the deeper laminae. The substantia gelatinosa receptor may dis-

criminate between morphine and the enkephalins tested less well than the receptor in the region of the cell bodies. Electron microscope studies have found that immunoreactive enkephalin is contained in terminals in the spinal cord which establish mainly axo-dendritic synapses with dorsal horn neurones (12, 242, 300, 421). This distribution, when coupled with the results of administration from micropipettes, suggests that opioid peptides could function as inhibitory transmitters at axo-somatic or axo-dendritic synapses on spinal neurones.

ii. Administration in the Substantia Gelatinosa. Both [Met]enkephalinamide (147, 149) and [D-Ala^2,D-Leu^5]enkephalinamide (118) reduced the excitation of neurones of spinal laminae IV and V by peripheral noxious stimuli. This action was relatively selective in that responses to hair deflection were little affected, but differed from the action of morphine since spontaneous firing was usually reduced concomitantly with excitation by noxious heat. As with morphine, naloxone readily reversed these actions of the enkephalins when administered intravenously or in the substantia gelatinosa. Figure 6 illustrates these actions of [Met]enkephalinamide and their reversal by naloxone. Unlike those of morphine, the effects of [Met]enkephalinamide were relatively brief; responses returned to control levels within 10 min of the end of ejection. In contradistinction to the effects of administration near cell bodies, both morphine and the enkephalins gave similar effects in the substantia gelatinosa. Neither [Met]enkephalinamide nor morphine are particularly selective ligands (281) and these effects observed in the substantia gelatinosa cannot be assumed to result from interactions at either μ or δ or κ receptors. In ligand binding experiments, both μ- and δ-type binding sites were found on dorsal roots (172).

Duggan et al. (152) explored differences between morphine and [Met]enkephalinamide further by administering both compounds not only in the substantia gelatinosa but at progressively decreasing distances from the cell body in lamina IV or V; firing of the cell body was recorded extracellularly. Morphine depressed nociceptive responses only when applied in the substantia gelatinosa and was virtually inactive at more ventral positions. By contrast naloxone reversible depression was produced by [Met]enkephalinamide not only in the substantia gelatinosa but at nearly all sites down to the cell body of the recorded neurone. These results suggest that there is a population of receptors affected by enkephalin ventral to the substantia gelatinosa which, while probably rele-

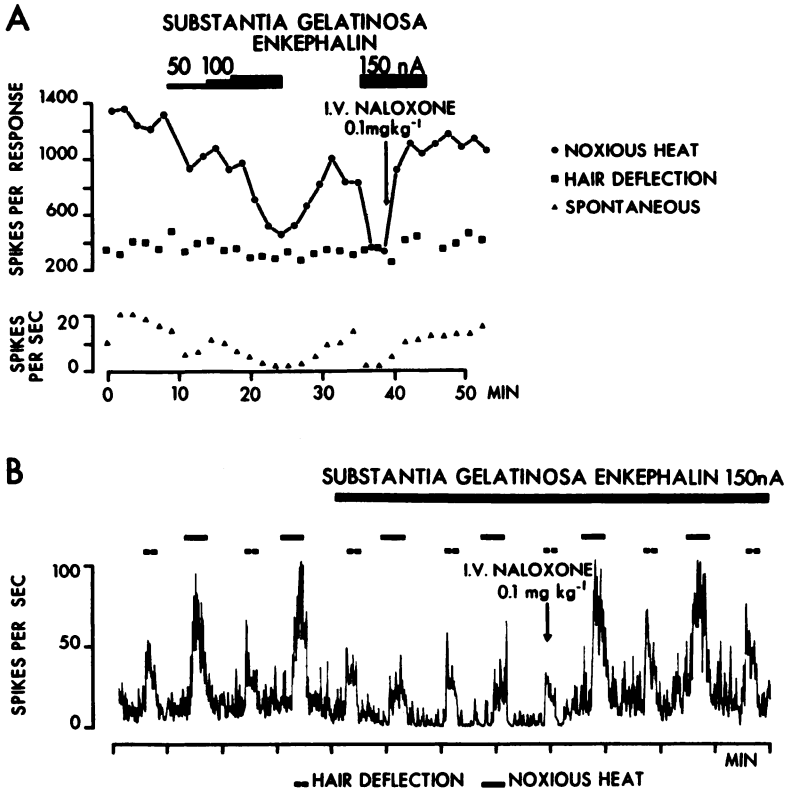

FIG. 6. Effects of [Met]enkephalinamide administered in the substantia gelatinosa. A neurone in lamina IV was excited by alternate noxious heat to the fourth digital pad of the left hind limb and by deflection of hairs. Ratemeter records of cell firing to these two sensory stimuli are shown in part B while the time integrals of these responses are plotted in part A. A, ●, responses to noxious heat; ■, responses to hair deflection; ▲, spontaneous firing. [Met]enkephalinamide (50 to 100 nA) was ejected 220 μm dorsal to the recording site (see fig. 5) in the substantia gelatinosa. This reversibly depressed nociceptive responses. This depression was repeated with a larger microelectrophoretic current (150 nA) and this effect was rapidly reversed by intravenous naloxone (0.1 mg/kg). [Reproduced, with permission, from Duggan et al. (149)].

vant to the physiology of opioid peptides in the spinal cord, may not contribute to the effects of systemic morphine.

[Met]enkephalinamide was active at many sites in the substantia gelatinosa where substance P had no effect (144). This finding has implications for the hypothesis that opioid peptides and morphine may interfere with the release of transmitter from primary afferent fibres. Thus, if enkephalins are released at axo-axonic synapses on substance-P-releasing terminals within the substantia gelatinosa (250), there should be a correspondence between sites at which enkephalin and substance P influence the firing of deeper neurones. This correspondence was not observed and substance P was only active at sites where excitant amino acids also excited deeper neurones, suggesting a location near the dendrites of these cells. It is possible that opioids can block transmission of nociceptive information at sites on primary afferents remote from their terminations. Such a block of propagation by opioids has been demonstrated on myenteric neurones (347, 370).

5. Opioid Actions on Spinal Neurones in Vitro. The limitations that attach to the interpretation of membrane actions of opioids studied in vivo have been discussed above. Recently, it has become possible to maintain and record from the spinal cord preparation in vitro. The frog spinal cord lives well in vitro even when left intact; satisfactory recordings can be obtained from rat and cat spinal cord for up to 24 hr when slices are perfused with oxygenated physiological saline solution.

A. FROG. [Met]enkephalin and [D-Ala^2,D-Leu^5]enkephalin hyperpolarized frog motoneurones when applied in concentrations of 5 to 10 μM (362). The hyperpolarization was recorded from the ventral roots by the sucrose gap technique. After addition of tetrodotoxin (TTX), spontaneous synaptic potentials were abolished but the hyperpolarization remained. The hyperpolarization was blocked by naloxone (1 μM). Evans and Hill (168) made a similar finding. The opioid peptides did not block depolarizing responses to glutamate or hyperpolarizing responses to GABA or glycine.

These effects of opioid peptides were not mimicked by morphine. Morphine (10 to 200 μM) produced no hyperpolarization and did not reduce glutamate depolarizations. Morphine had strychnine-like actions in depressing glycine responses and with the higher concentrations (200 μM) also depressed GABA responses. These effects on glycine and GABA responses were not blocked by naloxone and are consonant with those found in vivo in the cat spinal cord (362).

Enkephalins also hyperpolarized the dorsal roots of the isolated spinal cord of the frog, and this action occurred at concentrations significantly lower than those needed to hyperpolarize motoneurones ([D-Ala^2,D-Ala^5] enkephalin, 10 nM) (362, 168). This action of enkephalin persisted in solutions that block synaptic transmission, indicating that it results from a direct action on the primary afferent fibres.

B. RAT. The spinal cord of the newborn rat has been studied by recording from dorsal root fibres (466). [D-Ala^2,Met^5]enkephalinamide (3 μM) depressed or abolished the dorsal root potential evoked by stimulating an adjacent root. This action, which was blocked by naloxone (500 nM), was accompanied by a hyperpolarization of the dorsal root fibres.

Murase et al. (355) found that [Met]enkephalin and [D-Ala^2,D-Leu^5]enkephalin caused small hyperpolarizations in 21 of 24 unidentified dorsal horn neurones. They recorded intracellularly from a slice cut from the spinal cord of a young (8 to 19 days) rat. The effective concentrations were 100 nM to 100 μM but anatagonism by naloxone was not adequately demonstrated.

Yoshimura and North (521) recently reported the effects of opioids on neurones in the substantia gelatinosa of the rat spinal cord. They used a 300 μm slice cut from the spinal cord of an adult rat; the slice preparation survived in vitro for 24 hr and intracellular recordings were made for several hours from single neurones. Both morphine and [Met]enkephalin caused concentration-dependent hyperpolarizations that persisted in calcium-free, high magnesium solutions. The effective concentrations were 30 nM to 10 μM. More significantly, naloxone reduced the opioid effect at concentrations of 3 nM, implying that the receptor involved had a high affinity for naloxone. The opioid hyperpolarization was associated with a large increase in membrane conductance, and could be reversed to a depolarization by artificially hyperpolarizing the cell membrane. The reversal potential shifted as a Nernstian function of the extracellular potassium concentration, indicating that opioids increase the potassium conductance of rat substantia gelatinosa neurones (fig. 7). Interestingly, only about one half of all the cells tested were hyperpolarized by opioids. This contrasted with the higher proportion (about 80%) of cells hyperpolarized by noradrenaline (522).

In summary, in vitro experimentation has provided direct support that both the actions of opioids predicted from in vivo studies do in fact occur. One is a hyperpolarization of some substantia gelatinosa cells, due to an increase in potassium conductance. Another is a hyperpolarization of some primary afferent fibres. Whereas the in vitro techniques cannot address the issue of which of these actions underlie morphine analgesia, they do provide a way to control the drug concentrations applied, which is unfortunately lacking in electrophoretic experiments. This should enable the underlying receptor types to be elucidated on the various elements of the dorsal horn.

6. Opioids and Primary Afferent Terminals. The hypothesis that opioids modify transmitter release from primary afferent terminals can be investigated experimentally. With myelinated primary afferents there is

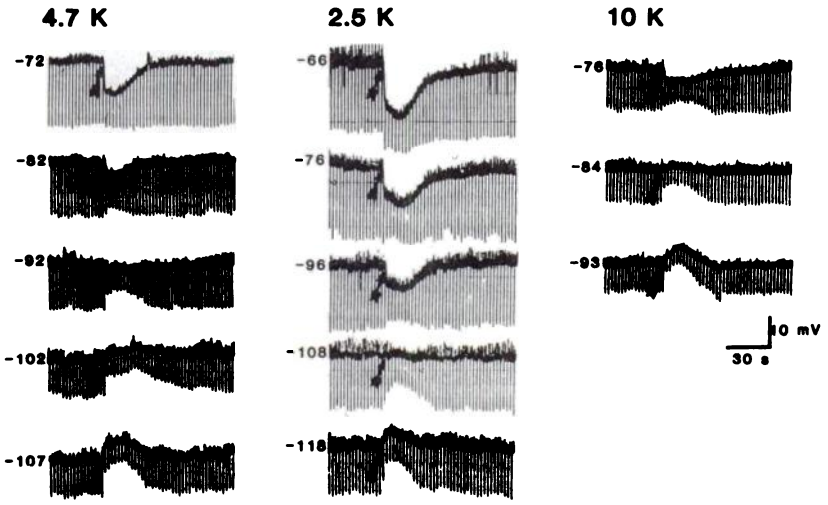

FIG. 7. Opiates increase potassium conductance in a substantia gelatinosa neurone. The traces are intracellular recordings from a single neurone in a transverse slice cut from the spinal cord of an adult rat and superfused in vitro with physiological saline solution. The downward deflections are hyperpolarizing electrotonic potentials resulting from passing constant amplitude current pulses across the neurone membrane; their amplitude gives an estimate of cell membrane resistance. [Met]enkephalin was applied at the arrows [three brief pulses of pressure (7 kPa, 25 ms) were applied to a micropipette containing [Met]enkephalin (10 mM) positioned in the solution just above the slice]. Left, in control solution (4.7 mM potassium) caused a membrane hyperpolarization accompanied by a fall in resistance. The hyperpolarization reversed at about −92 mV. Centre, in lower potassium; right, in higher potassium. The reversal potential for the response to enkephalin shifted as expected for an increase in potassium conductance. Note the apparent lack of voltage sensitivity in the enkephalin-induced conductance increase. This hyperpolarization was reversibly abolished by naloxone (100 nM) (not shown). (Unpublished observations of Megumu Yoshimura.)

evidence that presynaptic inhibition of transmission of impulses from these fibres to dorsal horn neurones occurs by the release of GABA at axo-axonic synapses onto the terminals of primary afferents (102, 438). Release of GABA depolarizes the terminals with a resultant reduction in the potential changes by invading impulses and a reduced release of transmitter. The methods used to investigate presynaptic inhibition of myelinated afferents include the measurement of dorsal root potentials (DRP) and tests of excitability of primary afferent terminals by electrical stimulation. These techniques have been used in investigations with opioids.

a. DORSAL ROOT POTENTIALS. When a dorsal root is placed on a pair of electrodes, a prolonged negativity at the central recording electrode can be recorded following the passage of a volley of impulses. This is termed the dorsal root potential V (DRP V) (317) and is interpreted as depolarization of the central endings of primary afferent fibres. When measured in this way, it is probably depolarization of the terminals of larger myelinated fibres which mainly contribute to the recorded potential.

In the decerebrate cat, the DRP V produced by electrical stimulation of a dorsal root was reduced by morphine (1.5 to 10 mg/kg), meperidine (20 mg/kg), and methadone (5 mg/kg) (291). Nalorphine (2 mg/kg) reversed the action of all compounds. Decreases in DRP V following electrical stimulation of peripheral nerve were produced by morphine (0.15 to 1.0 mg/kg) in anaesthetized cats (83) and rats (495). In the former study, meperidine increased DRP V in doses of 0.15 to 0.63 mg/kg but decreased this potential at doses of 1.0 to 5.0 mg/kg. Also in cats, the potent enkephalin analogue [D-Ala^2,MePhe^4,Met(O)^5]enkephalin-ol (FK 33,824) reduced DRP V produced by peripheral nerve stimulation (404). This is therefore similar to the action of opioids on primary afferents of the perfused spinal cord of immature rats (466) and frogs (362) mentioned above. Studies of this type do not permit distinction between an action on primary afferents (and the possible mechanisms are multiple) and an effect on interneurones forming axo-axonic synapses with primary afferents. In addition, it is difficult to relate the reduction in DRP to function since, in the experiments cited, many fibre types would contribute to the generation of this potential.

b. ELECTRICAL STIMULATION OF TERMINALS OF PRIMARY AFFERENTS. In these experiments a microelectrode in the spinal cord is used to stimulate nerve terminals electrically, and antidromically conducted action potentials are recorded in the periphery. Earlier studies (482) recorded changes in the amplitude of the compound potential from activity in many axons but most recent studies have measured the stimulus intensity necessary to produce firing of a single fibre in 50% of tests (i.e., a stimulus threshold). A decrease in threshold is usually interpreted as primary afferent depolarization (PAD) and it is this depolarization that has traditionally been believed to result in reduced release of transmitter. A major problem is whether the microelectrode is indeed stimulating the terminals of primary afferents or a preterminal segment. Curtis (103) has recently suggested criteria for deciding between these possibilities.

Jurna et al. (259) studied the effect of intravenous morphine on PAD. He measured the amplitude of a peripherally recorded compound action potential in a

muscular and a cutaneous nerve after stimulating its central endings. PAD was evoked by prior tetanic stimulation of the same nerve. Whereas morphine (2.0 mg/kg) abolished PAD in the muscle nerve, it reduced the duration but not the maximal amplitude of that in the cutaneous nerve. The condition velocities of the fibre group studied are not given, nor is it clear whether morphine affected basal excitability. Carstens et al. (73) studied the terminal excitability of single A and C primary afferents and found that intravenous morphine (1 mg/kg) had no consistent effect on A fibres but decreased the excitability of C fibres. Reversal by naloxone (1.0 mg/kg) was observed with only two of six fibres; with two a further decrease in excitability followed naloxone administration. Unlike the observations of Jurna et al. (259), no effects on PAD were seen. The circulatory effects of morphine (particularly when naloxone is given after morphine) create great difficulties in these experiments, since minor changes in separation of the electrode tip and the terminal being studied can produce significant changes in fibre threshold.

Sastry (424–426) administered morphine and [Met] enkephalin microelectrophoretically near the terminals of cutaneous A and C fibres in the superficial laminae of the dorsal horn. Both substances produced a naloxone-reversible decrease in terminal excitability of A δ and C fibres associated with an increase in the PAD produced by stimulation of small diameter afferents of the superficial peroneal nerve. Large ejecting currents produced decreases in terminal excitability which were not reversed by naloxone. No consistent effects were observed with large myelinated afferents. Intravenous morphine (2.0 mg/kg) and meperidine (0.5 mg/kg) also decreased the excitability of Aδ and C primary afferents (424). Sastry has proposed that morphine decreases the transmission of impulses from small diameter primary afferents to dorsal horn neurones by an enhancement of the presynaptic inhibition that these fibres exert on each other. The decrease in excitability during morphine was interpreted as a hyperpolarization of terminals and this was considered responsible for the observed increases in PAD.

All of the experiments cited above basically sought to investigate aspects of presynaptic inhibition in the spinal cord and its modification by either systemic opiates or locally applied opioids. The interpretation of many of the experiments is equivocal for the following reasons. Even if the fibre type that is depolarized by a well-defined stimulus is known (and this was not so for many of the experiments quoted), a reduction in PAD of that fibre could have two causes. One cause is a depression of the activation of the interneurones producing PAD or block of the action of the transmitter released by these cells. In this case the basal excitability of the fibre depolarized by the stimulus should remain unchanged, unless the interneurones were tonically active in which case excitability would increase. The second cause is a direct action by the opioid on the fibre depolarized by the stimulus. There is more than one possibility here. 1) If the opioid were to depolarize the terminal with a conductance increase, this would have the effect of reducing monosynaptic transmission through the synapse but reduce the changes induced by activity at axo-axonic synapses upon it. The basal excitability of the fibre, however, would increase. 2) If the opioid were to hyperpolarize the terminal with a conductance increase, the situation would be similar to 1) but the basal excitability would be decreased. A large hyperpolarization could prevent the terminal being invaded, in which case transmission would cease.

From these considerations it follows that investigations on opioids and presynaptic inhibition of muscle afferents have not been adequate. Thus it is not possible to say whether the effects of opioids on spinal reflexes discussed previously derive wholly or in part from effects at primary afferent terminals. In particular, data are lacking on changes in basal terminal excitability following systemic opiates. With studies of cutaneous afferents, investigations have indicated a preferential effect on small diameter afferents and a decrease in their basal excitability. The findings of Sastry (424) that PAD was increased is consistent with a conductance *decrease* accompanied by a hyperpolarization. Such an action of opioid peptides would be quite different from that proposed for GABA, and from that observed directly with intracellular recording from other neurones.

A primary afferent fibre branches extensively on entering the spinal cord, but the proportion of the hundreds of terminals normally invaded by the action potential or whether controls exist to vary this proportion is unknown. Such a control could occur near points of branching or at varicose release sites; opioids may influence the ability of branches to follow repetitive incoming impulses. All of the studies cited above attempted to study effects by opioids at *terminals* and hence have not provided data on this alternative mechanism. Some of the results from the peripheral nervous system, which are discussed later in this review, indicate that opiates block action potential propagation in varicose fibres; such an action could contribute to reduced transmitter release from primary afferent fibres. In later sections, the further possibility will be raised that opioids inhibit transmitter release by reducing the amount of calcium entering the nerve terminal during the action potential. Such a mechanism would not necessarily cause any changes in excitability or PAD. For example, in locus coeruleus neurones (see section II B 7), the reduction in calcium entry occurs secondarily as a result of the increased membrane conductance to potassium ions (376). Thus, an increase in potassium conductance could underlie the excitability changes in dorsal horn terminals, and also cause a reduction in the release of primary afferent transmitters either by blocking propagation or by such secondary modulation of calcium entry.

7. Opioids and Adenylate Cyclase. It has been proposed that opioids produce their effects on neurones through inhibiting the synthesis of adenosine-3′,5′-cyclic monophosphate (cyclic AMP). Opiates inhibited prostaglandin-stimulated synthesis of cyclic AMP in rat brain homogenates, and this was blocked by opiate antagonists such as naloxone (90, 92). Both opiates (477) and opioid peptides (197) reduced prostaglandin-stimulated synthesis of cyclic AMP by cultured neuroblastoma × glioma hybrid cells. Administration of relatively large doses of methylxanthines to rats produced signs that were likened to those of morphine withdrawal in dependent animals (89, 175). The inhibition of phosphodiesterase by methylxanthines was suggested to be responsible for these results, and, by analogy, elevated levels of cyclic AMP in neurones were considered to be responsible for the signs of opiate withdrawal. Morphine-induced analgesia in mice was reversed by cyclic AMP administered intracerebroventricularly, and even when given intravenously in doses of 10 mg/kg (232); the xanthines, caffeine and theophylline, lower the vocalization threshold for presumed painful electrical stimulation of the tail of the rat (386).

The proposal that opioid inhibition of adenylate cyclase is an intermediate step in the inhibition of cell firing has been investigated in the central nervous system by microelectrophoretic techniques. Cyclic AMP does not readily penetrate cell membranes and therefore attempts have been made to reverse an effect of morphine by administering dibutyryl cyclic AMP extracellularly. In the substantia gelatinosa of the spinal cord of the cat, this compound had no effect on the morphine depression of the spinal transmission of nociceptive impulses (142). Isobutylmethylxanthine, an inhibitor of cyclic AMP phosphodiesterase, also had no influence on the action of morphine in the substantia gelatinosa (142). In the rat, intravenous aminophylline also failed to modify the effects of intravenous morphine on excitation of projecting spinal neurones by impulses in unmyelinated primary afferents (256).

On neurones of the mesencephalic reticular formation of the rat, dibutyryl cyclic AMP reversed the depression by morphine of the excitation of neurones by painful cutaneous stimuli (236). Dibutyryl cyclic AMP alone frequently enhanced the synaptic response.

8. Function of Opioid Peptides in the Spinal Cord. a. ADEQUACY AND SPECIFICITY OF OPIOID ANTAGONISTS. Electrophysiologists can infer that opioids have a physiological role either through finding similarities between the actions of exogenous opioids and a particular physiological event, or through reduction of such an event by an opioid antagonist. Since most of the evidence has related to antagonism by naloxone, it is necessary to discuss the specificty of this compound as an antagonist of the opioid peptides. This question has been recently reviewed (151, 436) and the present discussion will be restricted to interactions of naloxone with substances administered near single central neurones.

Naloxone has prevented or reversed the effects of opiates and opioid peptides on neurones in many areas of the central nervous system. The specificity of this antagonism, however, has not been rigorously tested. In the substantia gelatinosa of the spinal cord, concentrations of naloxone adequate to block the actions of morphine were without effect on similar actions of NA or 5-HT (215). Amino acids were not tested in this area for antagonism by naloxone since they usually had no effect of their own. Administered near the bodies of spinal neurones, naloxone had no effect on depression of firing by glycine and GABA (141).

Several actions of microelectrophoretic naloxone, apparently not related to opiate antagonism, have been reported. Depression of cell firing has been observed in the spinal cord (141) and thalamus (146, 181). Excitation by acetylcholine of Renshaw cells and ventrobasal thalamic neurones was reduced by naloxone (141, 146). On Renshaw cells, morphine excitation was reduced concurrently with excitation by acetylcholine. With neurones of the rat olfactory tubercle-nucleus accumbens region, naloxone reduced the depression of cell firing by GABA without affecting that by morphine (134). This interaction between naloxone and GABA has been further studied by intracellular recordings from cultured spinal neurones of the mouse (201). Both (+)- and (−)-naloxone were equieffective in depressing GABA-induced depolarizations (KCl-filled electrodes were used) when administered microelectrophoretically. A similar result was obtained when the naloxone isomers were added to the perfusing fluid, but concentrations of 100 nM to 1 μM were required. Lack of stereospecificity was also apparent in the naloxone inhibition of GABA binding to a synaptic membrane fraction of human cerebellum. Similar inhibition was shown by morphine, levorphanol, and its non-opiate isomer dextrorphan (134). The IC50 for naloxone was 308 μM, approximately 50 times greater than that of bicuculline.

To what extent do these actions of naloxone interfere with its usefulness in identifying physiological events that may involve release of opioid peptides? In microelectrophoretic experiments, it is clear that if a particular synaptic inhibition is reduced by naloxone administered from a micropipette, it is necessary to test for antagonism of the inhibition by substances such as bicuculline, a GABA antagonist (105, 106), and strychnine, a glycine antagonist (107). If an excitation is reduced by naloxone then acetylcholine antagonists such as dihydro-β-erythroidine and atropine should be tested. Both (+)- and (−)-naloxone should be tested in either situation although present constraints on the availability of (+)-naloxone make this a counsel of perfection.

With systemic administration, dose considerations and stereospecificity are important. Since intravenous doses

of bicuculline of 0.2 to 0.6 mg/kg are required to reduce presumed GABA-mediated inhibition in the spinal cord, cerebellum, and cerebral cortex of the cat (106), reduction of an inhibition by similar or lower doses of naloxone is unlikely to result from GABA antagonism; this is because, as cited previously, the affinity of naloxone for GABA binding sites was 50 times lower than that of bicuculline (134). Within this dose range, effects on Renshaw-mediated inhibition in the spinal cord are unlikely to be of importance since naloxone (0.1 to 0.5 mg/kg) had no effect on cholinergic activation from ventral root stimuli. Provided that a physiological event can be reduced by naloxone in doses no greater than 0.1 mg/kg (for the cat, this will be different for other species), and provided that the event is also affected similarly by other opioid antagonists yet not affected by non-antagonist isomersof these compounds, then it is reasonable to infer that opioid peptides are involved.

The description of multiple opioid receptors and the possibility that different opioid peptides could act as ligands at each of these sites has seriously complicated the interpretation of the effects of opioid antagonists on physiological events. Naloxone binds to all three of the principal subtypes of opiate receptors, μ, δ, and κ, although with quite different affinities (283, 320). It appears improbable therefore that naloxone should fail to reveal an opioid-peptide-mediated event provided an adequate dose is given. With systemic administration the important question is what dose should be given. No definitive answer can be given to this. In fact, the situation is particularly complicated because ligand binding studies in vitro are generally allowed to come to equilibrium; in vivo naloxone may be antagonizing the action of an opioid peptide that is present near the receptor in high concentration for a relatively short period of time. As implied previously, doses of the order of 0.1 mg/kg (for the cat) are unlikely to produce effects through nonspecific interactions with GABA and acetylcholine; this dose antagonizes the effects of [Met]enkephalinamide on the firing of neurones of the dorsal horn (149). Whether doses of this order would be adequate to reveal an event mediated, for example, by dynorphin acting on a κ-receptor (81, 240) is not known.

The second corollary to this consideration relates to the following—the action of which substance is being revealed by an opioid antagonist. Although naloxone has different affinities for the different opiate receptors, the ligand binding data cannot be extrapolated to the in vivo situation; the type of ligand antagonized cannot be inferred reliably from the dose of naloxone reducing a particular event. There is good evidence that some newer compounds bind irreversibly with one subtype of opiate receptor. Thus the furmarate methyl ester derivative of naltrexone (βFNA) appears to preferentially bind to μ-receptors (473) while a diallyl derivative of [Leu]enkephalin (ICI 154, 129) has an affinity predominantly for δ-receptor sites (448). These compounds have yet been little used in studying the electrophysiology of opioid peptides in vivo. On the basis of their use in peripheral tissues (473, 448) and central neurones in vitro (377), one may be optimistic that they will be useful in ascribing receptor identities in electrophysiological experiments in vivo. For the present, it is only possible to speak of naloxone-reversible events. Provided the dose and stereospecific considerations enable the elimination of the involvement of GABA and acetylcholine, then one can reasonably infer the involvement of an endogenous opioid peptide.

b. OPIOID ANTAGONISTS AND SPINAL REFLEXES. Earlier studies in both the spinal cat (292) and dog (327) found that naloxone had no effect on a variety of spinal reflexes when given in the dose range 0.2 to 1.0 mg/kg. Large doses of naloxone (20 mg/kg) increased flexor reflexes in the spinal dog (327). Goldfarb et al. (195, 196) reported that, in decerebrate spinal cats, low doses of naloxone (0.1 to 2.0 mg/kg) increased monosynaptic reflexes in both flexor and extensor muscles, and also increased polysynaptic reflexes to impulses in large diameter cutaneous afferents. Bell and Martin (32) examined reflexes to C fibre afferents in decerebrate spinal cats and found these reflexes to be increased by naloxone.

These effects of naloxone on reflexes in the decerebrate cat have been confirmed in the barbiturate-anaesthetized spinal cat. Duggan et al. (154) found that naloxone (0.05 to 0.1 mg/kg) increased monosynaptic reflexes of flexor and extensor muscles, reflexes to impulses in large diameter cutaneous afferents, and reflexes to impulses in C primary afferents. The effect was stereospecific since only the (−)-isomer of furylmethylnormetazocine (FMN), which is an opioid antagonist with little agonist action (296), increased spinal reflexes. The (+)-isomer of FMN is not an opioid antagonist and did not increase spinal reflexes.

In paraplegic man, naloxone (10 to 30 μg/kg) had no effect on the monosynaptic (H) reflex, a polysnaptic tactile reflex, nor on a reflex evoked by a noxious stimulus (497). Similar doses in normal humans produced a small increase in the monosynaptic (H) reflex (51).

Enhancement of a monosynaptic reflex could result from an increased release of transmitter from primary afferent terminals or an increased excitability of motoneurones. In either case this could result from a direct excitant action of the opioid antagonists or reduction in the effectiveness of an inhibition. It is not possible to exclude completely an excitant action of naloxone and FMN, unrelated to antagonism of the action of opioid peptides, as being responsible for the increase in spinal reflexes. However, excitation by naloxone is rare when it is administered electrophoretically from micropipettes (141, 148, 524). It is more probable, therefore, that a reduction of an inhibition is responsible for the effects of opioid antagonists on spinal reflexes.

In the case of a monosynaptic reflex, the inhibition must be tonically present. This is because transmission of the reflex is complete before any inhibitory interneurones also activated by the peripheral nerve stimulus can exert their effects on either primary afferent terminals or motoneurone excitability. This conclusion requires that the nerve stimulus be repeated at such a low frequency that each reflex is uninfluenced by interneurones activated by the prior nerve stimuli. In the experiments of Duggan et al. (154) the stimulus frequency was 0.2 to 1.0 Hz, making it probable that interference with tonic inhibition was responsible for the effects of opioid antagonists.

From the considerations of dose discussed previously, it is unlikely that the effects of naloxone on spinal reflexes in doses of 0.05 to 0.1 mg/kg can be attributed to activity at receptors for acetylcholine and GABA. The stereospecificity observed with FMN, when considered together with the low dose of naloxone, make it likely that the effects of the opioid antagonists on spinal reflexes occurred through antagonism of the action of an opioid peptide.

Morton et al. (349) obtained intracellular recordings from motoneurones during naloxone administration. EPSPs were increased by naloxone in two thirds of neurones in the absence of changes in membrane potential or conductance. The same doses potentiated the spinal reflexes. In addition, EPSPs from several primary afferents, both muscular and cutaneous, were all increased in a single motoneurone. Such a result is most readily explained by block of a somatic inhibition but the failure to detect changes in somatic conductance and membrane potential argue against this. Thus, the mechanism of the tonic inhibition revealed by opioid antagonists is unknown. It is not clear whether opioid peptides affect motoneurones or primary afferent terminals directly, or control the excitability of interneurones acting on these structures.

The most intriguing question to be answered is: What is the functional significance of inhibition of motoneurones involving opioid peptides? Since most investigations have attempted to link opiates and opioid peptides to control of the perception of pain, at first sight it seems curious that these compounds appear to inhibit monosynaptic reflexes in decerebrate and anaesthetized spinal cats. Since the inhibition revealed by naloxone was present with both flexor and extensor motoneurones, and the responses of these neurones to a variety of primary afferents were similarly inhibited, the functional consequence of this inhibition may be a relative immobility of limbs. The significance of this inhibition would be clear if the stimulus to the release of opioid peptides were known. Release of immunoreactive enkephalin from the perfused spinal cord of the anaesthetized cat was measured by Yaksh and Elde (511). Basal release was unaltered by stimulation of large diameter afferents of peripheral nerves, but increased approximately threefold when unmyelinated afferents were stimulated. In view of this result, it is possible that the surgery necessary to prepare animals for neurophysiological recording is an adequate stimulus for release of opioid peptides and that this is responsible for the inhibition of spinal reflexes so readily detected in these preparations. This suggestion finds support from the effects of naloxone on respiration in the cat. In animals acutely prepared for measurements of respiratory function, naloxone (0.4 mg/kg) increased ventilation (401). After recovery from similar surgery, however, naloxone had no effect on respiratory rate or minute ventilation (490).

Restriction of movements is not likely to be of relevance to analgesia. Such a response has been observed in animals some time after the inflicting of injury and it may be linked to the commencement of healing (484). Opioid peptides may play a role in this process. The effects of opioid antagonists on animal behavior after injury have yet to be examined.

c. NALOXONE AND DORSAL HORN NEURONES. Since inhibition of firing is the predominant effect of opioid peptides, naloxone has been tested for effects on inhibition of spinal neurones from a variety of sources. The predominant supposition behind nearly all of the experiments cited below is that opioid peptides have a role to play in the spinal transmission of nociceptive information.

Inhibition could be produced by tonically released opioid peptides and this would be revealed by an increase in spontaneous firing with naloxone administration. In the cat, the effects of naloxone administration have been variable. Inconsistent increases in spontaneous firing with naloxone (up to 2.0 mg/kg) have been the main finding in barbiturate and α-chloralose-anaesthetized cats (149, 150, 454, 528). Henry (220), however, observed increased spontaneous firing of 22 of 30 dorsal horn neurones in chloralose-anaesthetized cats following intravenous (0.05 to 0.15 mg/kg) but not microelectrophoretic administration of naloxone. On 13 of 15 neurones, naloxone also increased the responses of neurones to noxious heating of the skin. In a prior study with the cat (150), this occurred with only two of 13 neurones. Henry (221) found that naloxone (0.1 to 0.6 mg/kg) more consistently increased the firing of neurones of the dorsal horn of the cat by noxious heating of the skin when given in the day or early evening. Late night injections were relatively ineffective. He proposed that a circulating factor was responsible and suggested β-endorphin as a possible candidate. The obvious experiment to test this is to inject β-endorphin systemically.

In the chloralose-anaesthetized rat, intravenous naloxone (0.2 mg/kg) increased the firing of 12 of 19 dorsal horn neurones evoked by impulses in unmyelinated primary afferents (417); in decerebrate spinal rats, naloxone (0.2 to 1.0 mg/kg) increased the excitation of 11 of 15 neurones of laminae IV and V by impulses in unmyelinated primary afferents. By contrast, the responses of 13

of 17 neurones of the substantia gelatinosa were inhibited (173). The (+)-isomer of naloxone was without effect in similar doses.

Tonically present inhibition of spinal neurones could be of local or supraspinal origin. In unanaesthetized cats, tonic supraspinal inhibition can be measured by reversibly blocking spinal conduction in a segment cephalic to the recording site. Naloxone (up to 3.2 mg/kg) partially reduced this inhibition in only one of eight experiments (150), or had no effect (454).

Inhibition of dorsal horn neurones by electrical stimulation of fibre tracts or brain stem nuclei has also been tested for antagonism by naloxone. Wall and Yaksh (485) found that stimulation of Lissauer's tract produced inhibition of cat polysynaptic ventral root reflexes and reduced the inhibition produced from stimulation of Lissauer's tract. Since this tract contains axons of cells of the substantia gelatinosa, this result might be interpreted to indicate release of opioid peptides from these neurones. Immunohistochemical techniques have indicated that intrinsic neurones of the substantia gelatinosa contain enkephalin (233, 234). Dorsal column stimulation in the cat inhibited the excitation of dorsal horn neurones by noxious heating of the skin but naloxone (up to 2.0 mg/kg) did not reduce this inhibition (454).

Prolonged inhibition of the responses of rat lumbar dorsal horn neurones to impulses in both A and C primary afferents can be produced by a distal noxious stimulus such as immersing the tail in hot water. Naloxone (0.3 mg/kg) reduced this inhibition by approximately 50%, the effect lasting 30 minutes (303). Both in this work and that of Fitzgerald and Woolf (173) (vide supra) the effect of naloxone sometimes required up to 10 min to occur, a result difficult to reconcile with the rapid effect of intravenous naloxone in reversing the action of morphine.

Some (3, 330, 384, 530), but not all (515), behavioural studies of analgesia produced by electrical stimulation of the midbrain and medullary raphe nuclei have found a reduction of this analgesia by systemic naloxone. Electrophysiological studies of inhibition of dorsal horn neurones by stimulation of these areas have also given variable results. Both Carstens et al. (72) and Duggan and Griersmith (142) found that, in the cat, such inhibition following electrical stimulation of the PAG was unaffected by naloxone (up to 3 mg/kg). A similar result was obtained in the decerebrate rat (255). Duggan and Griersmith (142) also found that inhibition from electrical stimulation near the medullary raphe was not reduced by naloxone. The inhibition by raphe stimulation of the firing of rat dorsal horn neurones by C fibre primary afferents was reduced by intravenous naloxone (0.2 mg/kg) (417). With most of these neurones the number of uninhibited spikes was increased by naloxone and, since the effect of raphe stimulation was measured by the *percentage* decrease in number of action potentials, some of the apparent ineffectiveness of raphe stimulation after naloxone may be related to the increase in spontaneous firing.

Pomeranz et al. (403) reported that, in chloralose-anaesthetized cats, electroacupuncture reduced the excitation of dorsal horn neurones by cutaneous noxious stimulation were unaffected. This effect of acupuncture was reduced by spinal transection, hypophysectomy, and naloxone (0.3 mg/kg) (402, 403). It was suggested that an opioid was released by the pituitary during acupuncture rather than from inhibitory neurones of the dorsal horn.

From this collection of non-uniform data it is not possible to come to definitive conclusions on the function of opioid peptides in the spinal cord. Although it seems that species differences may be important, this is not an adequate explanation since naloxone failed to reduce some inhibitions in the rat (255) and reduced others in the cat (403, 485). The reports of effects by naloxone are sufficiently numerous to indicate that opioid peptides do indeed have a physiological role in spinal cord function, and the variability in results between experimenters probably results from our lack of knowledge of the processes controlling their release. The underlying assumption in most of these investigations is that opioid peptides are released in response to pain and that their function is to suppress pain. The former belief has an experimental basis in the experiments of Yaksh and Elde (511) discussed earlier. But such function of this spinal release of opioid peptides is actually conjectural. The effects of opioid antagonists on spinal reflexes suggest that opioid peptides have a role other than or in addition to suppression of the transmission of nociceptive information. As suggested previously, one such role may be to restrict movements of a damaged area of the body possibly as part of the repair process.

It is possible that some of the inconsistencies in the reported effects of opioid antagonists on spinal neurones result from zealous attempts to relate these to the control of nociceptive transmission. A significant part of this review has described experiments showing that opioid peptides have effects consistent with such a role. Nevertheless, differences between opiates and opioid peptides have been demonstrated and it has been pointed out that the functional significance of some of the effects of opioid peptides on single neurones is not clear. For example, the nonselective depression of all responses of a multireceptive dorsal horn neurone may not be related to nociception. It is not unreasonable to conclude that our knowledge of the functions of opioid peptides in the spinal cord, much less in the brain, is in its infancy.

9. Summary of Effects of Opioids on Spinal Neurones. Collectively, the evidence suggests that there are significant differences in the effects of opiates and opioid peptides in the dorsal horn. For morphine there is a large body of evidence that an important site of action is the substantia gelatinosa but the exact mechanism remains obscure. Both the selectivity of its action and the distri-

bution of binding sites favour an action by morphine near the terminals of unmyelinated primary afferents and/or on the cell bodies of neurones intrinsic to the substantia gelatinosa.

It is uncertain whether the first action results in a block of impulse propagation into terminals or reduces transmitter release by another mechanism. In either case it is equivalent to presynaptic inhibition of transmission of primary afferent impulses. The physiological role of these receptors for opiates on primary afferents is uncertain. They are unlikely to be acted upon by enkephalin since anatomical studies of immunoreactive enkephalin do not find this peptide within terminals making synaptic contacts with primary afferents. Either the correct endogenous ligand for the receptors has not been studied or the receptors are activated by a process different to the operation of most synapses, such as wide diffusion of locally released or blood-borne agonists.

The above conclusions are based largely on the action of morphine. With the description of multiple opiate receptors and knowledge that different opioids bind differently to these subtypes, it is clear that there are many deficiencies in our understanding of how opioids affect the spinal cord. For example, it is not known whether drugs of the agonist-antagonist type have effects in the substantia gelatinosa. Cyclazocine is a poor analgesic when administered spinally by intrathecal catheter, yet it reduces spinal reflexes of several types in the chronic spinal dog. Thus, while the effects of morphine on primary afferents and/or substantia gelatinosa neurones may be an adequate explanation for both the reduction of nociceptive reflexes and for spinal analgesia, this cannot be the reason for the reduction of non-nociceptive reflexes, including monosynaptic muscle reflexes, by a variety of opioids. Activity at opioid receptors other than those affected by morphine may be responsible for these actions, but there is no direct evidence for this.

For the opioid peptides, there is substantial evidence for a postsynaptic action on spinal neurones. Both the anatomy and the effect of enkephalins administered near neurones favour this conclusion. Some of the physiological data suggest a presynaptic action similar to that of morphine but anatomical studies do not provide a basis for this. In this respect it needs to be pointed out that perfusing the spinal cord with enkephalin analogues (512) or injecting them intracerebroventricularly has given only limited information on the function of these compounds. Because concentrations are unknown, ligands of limited selectivity could interact with more than one receptor subtype. The combined data from increasingly selective agonists and antagonists may be rewarding in the future.

The functional significance of opioid peptides in the spinal cord appears to be much wider than originally envisaged. Thus, the expectancy that the physiology of opioid peptides would be similar to the pharmacology of opiates does not appear to be adequate. There is evidence that opioid peptides do have a role in controlling the spinal transmission of nociceptive information, but results of experiments on motoneurones suggest an additional function not related to the perception of pain but possibly to behaviour after injury.

Finally, it appears that while the spinal cord offers many advantages in studying the action of opioids on the central nervous system, its contribution to analgesia from systemic morphine may be quite modest. The relatively powerful effects of morphine on the spinal cord appear to be partially offset by its decrease of descending inhibition.

B. Brain Stem

Of the heterogeneous group of nuclei and fibre tracts that comprise the brain stem, those which on anatomical and physiological grounds have a role to play in the transmission of nociceptive information have been most extensively studied with opioids. These include the trigeminal nuclei, midbrain, and medullary reticular formation, and the monoamine-containing neurones of the raphe and locus coeruleus. In addition, there have been limited studies on the autonomic disturbances produced by opiates such as changes in respiration, blood circulation and pupillary size.

1. Trigeminal Nuclei. Afferents from the teeth project to the trigeminal nuclei and, in man, there is evidence that pain is the predominant sensation resulting from stimulation of the pulp and dentine with a variety of techniques (10, 80). Electrical stimulation of the tooth pulp of animals therefore has been widely used as a noxious stimulus and has been used in quantitative studies of opiate-produced analgesia. It should be noted that, like cutaneous stimuli, this stimulus is not free of artifacts. In man, adrenaline was more potent than morphine in suppressing the jaw jerk produced by electrical stimulation of the tooth pulp (248), a finding that may have resulted from changes in tissue impedance following vasoconstriction rather than any true analgesic properties of adrenaline.

a. INTRAVENOUS OPIATES AND TOOTH PULP STIMULATION. Intravenous morphine increased the threshold necessary to produce a jaw jerk by tooth pulp stimulation in the cat (463) and this was abolished by transection of the spinal cord at the first cervical segment (416). This latter observation suggested an action by morphine on neurones of the trigeminal nucleus caudalis. In a study of field potentials evoked in the brain stem by tooth pulp stimulation, however, Chin and Domino (84) and Nakamura and Mitchell (359) found no consistent effect by morphine on recordings obtained near the descending trigeminal tract. In the unanaesthetized rabbit, evoked potentials in the regions of the nucleus caudalis from tooth pulp stimulation were unchanged by morphine (2.0 to 4.0 mg/kg), pentazocine (5.0 to 10.0 mg/kg), and cyclazocine (0.5 to 1.0 mg/kg) (174).

A decrease in short latency but not long latency poten-

tials recorded deep in the nucleus caudalis of the cat was produced by fentanyl (365). A similar decrease in short latency action potentials evoked by tooth pulp stimulation was observed with neurones located at the junction of the nucleus caudalis and adjacent reticular formation. Such neurones were considered analogous to those of lamina V of the spinal cord. Field potentials are large during periods of near synchronous activity of neurones and this may be responsible for the relative lack of effects of opiates on tooth-pulp-evoked field potentials. The later asynchronous, synaptic, and action potentials of neurones are those most likely to be altered by any pharmacological agent and these are not necessarily represented in field potentials.

b. OPIATES ADMINISTERED FROM MICROPIPETTES. Administered microelectrophoretically near the somata of trigeminal neurones, morphine produced effects not antagonized by naloxone in nucleus oralis but had more selective effects on neurones of nucleus caudalis (7). Morphine depressed the spontaneous firing of these neurones and naloxone prevented this depression. A similar depression was observed with [Met]enkephalin (7, 8). When cells were excited by both tooth pulp (noxious) and infra-orbital nerve (non-noxious) stimulation, responses to the noxious stimulus were reduced more by administration of [Met]enkephalin. This selectivity led Andersen et al. (8) to suggest an action by enkephalin on structures presynaptic to the neurones studied, possibly on primary afferent fibres. Biochemical studies have found that opiates inhibit the high potassium-induced release of substance P from slices of trigeminal nuclei, a finding consistent with an action of opiates on primary afferent fibres (250).

c. FUNCTION OF OPIOID PEPTIDES IN THE TRIGEMINAL NUCLEI. Opiate binding sites and immunohistochemical enkephalin reactivity are found in the substantia gelatinosa of the trigeminal nuclei (15, 234). There are no reports of naloxone increasing the spontaneous firing of trigeminal neurones; that is, there is no suggestion that opioid peptides are tonically released.

In the cat, naloxone (0.4 mg/kg) prevented inhibition of the digastric reflex to tooth pulp stimulation which resulted from stimulation in the PAG (447). Stimulation of the PAG also inhibited reflexes produced by infraorbital nerve stimulation, but this action was not modified by naloxone. When single neurones of the trigeminal nuclei were studied, naloxone reversed the effects of PAG stimulation on excitation by tooth pulp stimulation more frequently in nucleus oralis than in nucleus caudalis. Both Jessell and Iversen (250) and Andersen et al. (8) had suggested that opioid peptides have a presynaptic effect on the terminals of trigeminal primary afferents. Changes in terminal excitability consistent with terminal depolarization can be induced in tooth pulp afferents by stimulation of brain stem sites. Hu et al. (239) found that such changes were induced by stimulation in the PAG, contralateral ventrobasal thalamus, and the medullary nucleus raphe magnus of the chloralose-anaesthetized cat. Naloxone (0.4 mg/kg) had no effect on this. Lovick et al. (321) found that systemic naloxone (2.0 to 5.0 mg/kg) had no effect on the increased excitability of terminals of tooth pulp afferents of the decerebrate cat produced by stimulation of the medullary raphe. Thus, the mechanism whereby naloxone influences PAG inhibition of the digastric reflex is unknown.

Opiates relieve toothache and one of the more famous cases of physical dependence on opiates resulted from this use of the drug (125). The studies cited suggest that some of this action occurs in the trigeminal nuclei, but compared to the spinal cord, this advantageous area of the brain stem has been studied relatively little with opioids.

2. Lateral Vestibular Nucleus. In the barbituate-anaesthetized rabbit, [Met]- and [Leu]enkephalin administered microelectrophoretically depressed the spontaneous firing of neurones of the lateral vestibular nucleus of Deiter (79). No opioid antagonists were examined. These investigations were prompted by the demonstration of the coexistence of enkephalins and GABA within Purkinje neurones of the cerebellum, some of which have monosynaptic projections to Deiter's nucleus.

3. Brain Stem Reticular Formation. Since the reticular formation consists of those diffuse areas of the brain stem not easily distinguishable as nuclear groups or fibre tracts (406), it is particularly difficult to compare the results of different laboratories on reticular neurones and to attempt to relate such results to effects of opiates, such as analgesia, on the whole animal. Thus, reticular neurones can often be activated from a variety of cutaneous receptors as well as from the cochlea and eye (350). Nevertheless, certain evidence suggests that the reticular formation may be an important site of action of opiates in producing analgesia.

Many ascending extralemniscal fibres terminate in the bulbar reticular formation (5, 53) and noxious cutaneous stimuli excite many reticular neurones (35, 65). In conscious cats, neurones of the NRGC were observed to fire before the onset of escape behaviour following a noxious cutaneous stimulus (75). Microinjections of opiates in several areas of the brain stem, including reticular sites, have produced analgesia in the rat, cat, and monkey, although there is dispute over the most sensitive areas [reviewed by Yaksh and Rudy (514)]. Gebhart (189) has recently reviewed the effects of opiates given both systemically and locally on brain stem reticular neurones.

a. INTRAVENOUS OPIATES AND THE FIRING OF RETICULAR NEURONES. In several studies, intravenous opiates reduced the excitation of reticular neurones by noxious skin stimuli. This has been shown for mesencephalic reticular neurones of the rat excited by noxious mechanical skin stimuli (207), neurones of NRGC of the cat excited by electrical stimulation of the saphenous nerve with a stimulus presumably adequate to excite C fibres (465), and lateral medullary reticular neurones of the rat

excited by noxious heating of the tail (338). Systemic naloxone reversed the depression of nociceptive responses by opiates. Most mesencephalic neurones of the rat were not excited by non-noxious skin stimuli and, with the few that were, excitation by this means was not modified by morphine (207). In these studies with intravenous morphine, it was not possible to decide whether the effects observed resulted from activity at receptors on the neurones studied or on other neurones projecting to the reticular formation.

b. MICROELECTROPHORETICALLY ADMINISTERED OPIOIDS. **i. Opiates.** Several effects have been observed when morphine and other opiates have been administered electrophoretically near the cell bodies of brain stem reticular neurones. Bradley and Dray (57, 58) found that morphine excited approximately half of the rat reticular neurones sampled and depressed the spontaneous firing of about one quqarter. A similarly variable effect of morphine occurred on the responses of neurones to NA, acetylcholine, and 5-HT. Naloxone reduced depression but not excitation of neurones by morphine (60). Both levorphanol and dextrorphan excited or depressed medullary reticular neurones but only depression by levorphanol was reversed by naloxone (55). Since this early report, different results have been obtained by other groups. In the decerebrate cat, morphine depressed the spontaneous firing of reticular neurones, excitation rarely being observed (505). Davies and Dray (118) found that morphine excited 55 of 65 rat brain stem neurones and that this excitation was blocked by naloxone administered from micropipettes. Excitation was also the most common effect of microelectrophoretic morphine applied to medial reticular neurones of the barbiturate-anaesthetized rat (18, 334). Neither action was antagonized by naloxone.

The experiments just cited were on unidentified reticular neurones and possible effects on synaptic activation were not studied. Noxious mechanical skin stimuli were used to activate rat mesencephalic reticular neurones in the experiments of Haigler (207) and microelectrophoretic morphine reduced the responses of cells to this stimulus. Such depression was not necessarily associated with a depression of spontaneous firing. Methadone and oxymorphone acted similarly to morphine and the actions of all three opiates were reversed by naloxone administered either from micropipettes or intravenously. An inconsistent finding in this report is that levorphanol frequently excited neurones. These microelectrophoretic studies have not really determined whether the depression of nociceptive responses of many reticular neurones following administration of systemic opiates is secondary to effects at other areas (such as the spinal cord) or derives in part from a direct effect on the neurones studied. The work of Haigler (207) suggests that there is a direct effect and it is unfortunate that several other investigators did not synaptically activate reticular neurones when studying the effects of opiates.

Denavit-Saubie et al. (124) investigated the depression of respiration produced by analgesic doses of opiates. Morphine, levorphanol, and dextrorphan were administered microelectrophoretically near those medullary neurones of the cat that fired with the rhythm of efferent activity in the phrenic nere. Such neurones were near the nucleus of the solitary tract, nucleus ambiguus, and the pontine nucleus parabrachialis medialis. Morphine and levorphanol, but not dextrorphan, reduced the amplitude of the respiratory related bursts of firing of reticular neurones without necessarily reducing firing between bursts. Excitation by L-glutamate was also reduced and both effects were antagonized by prior administration of naloxone.

ii. Opioid Peptides. Depression of the spontaneous firing of reticular neurones was the predominant effect of [Met]enkephalin in both the rat (56, 228) and cat (180). Excitation was rarely observed. Depression of firing was readily blocked by naloxone in the rat but was not observed in the cat (190). Davies and Dray (118) found that [Met]enkephalin more frequently excited than depressed brain stem neurones of the rat and that naloxone antagonized such excitation. The reasons for these differences are not apparent.

In all of these studies, the effect of the enkephalin rarely outlasted the period of administration by more than 2 min. A much longer depression of firing (up to 10 min) was observed when the more protease-stable enkephalin derivatives, [D-Ala^2,Met^5]enkephalin and [D-Ala^2,D-Leu^5]enkephalin, were administered near reticular neurones of the rat (59). With β-endorphin such depression outlasted the period of administration by up to 1 hr (323).

The studies above examined the spontaneous firing of unidentified reticular neurones. A few studies have examined synaptic responses. Both [Met]- and [Leu]enkephalin depressed the respiratory-related firing of reticular neurones of the cat (124, 420). Naloxone reduced the action of both peptides. In the rat, [Met]enkephalin reduced the activation of mesencephalic reticular neurones by painful mechanical stimulation of the skin (236). This study emphasized that morphine and the opioid peptide frequently differed in their effects on the same neurone suggesting perhaps the presence of different receptor types and an unequal distribution of these among neurones. With medial reticular neurones of the decerebrate cat both [Met]enkephalin and β-endorphin depressed synaptic excitation by stimulation of the tooth pulp (323).

Four studies have attempted to assign physiological significance to these effects of opioid peptides. Sessle et al. (446) studied inhibition of interneurones adjacent to the nucleus of the tractus solitarius and the inhibition of their activation from the superior laryngeal nerve by electrical stimulation of the PAG and nucleus raphe magnus (NRM). Naloxone (0.4 mg/kg) reduced this inhibition with three of five neurones. It is not stated

whether naloxone increased the uninhibited responses of neurones. The same doses of naloxone reduced PAG and NRM inhibition of coughing and swallowing reflexes in two cats. These numbers are really too small to come to conclusions on the possible involvement of opioid peptides in these inhibitions. Lovick and Wolstencroft (323) found that stimulation of the NRM inhibited the excitation of medial reticular neurones by tooth pulp stimulation in the decerebrate cat. Intravenous naloxone (0.15 to 1.0 mg/kg) failed to reduce this inhibition; it was, however, reduced by administering bicuculline microelectrophoretically near the inhibited neurones. Mohrland and Gebhart (343) studied the excitation of medial reticular neurones of the anaesthetized rat by pinching the skin; in many cells it was inhibited by electrical stimulation of the PAG. Systemic (intraperitoneal) naloxone (1 mg/kg) blocked this inhibition. Hill et al. (225) found that PAG stimulation inhibited the firing of reticular neurones in the rat caudal medulla and that this was blocked by systemic naloxone in 11 of 16 cells.

c. MICROINJECTION OF OPIATES IN THE PERIAQUEDUCTAL GREY (PAG) MATTER. Microinjection of opiates in the PAG produces analgesia in rats (514). There is evidence (27, 36) that this analgesia results from activation of descending pathways that inhibit the transmission of nociceptive information in the dorsal horn. There are few direct projections from the PAG to the spinal cord and interposed reticular neurones have been proposed. Mohrland and Gebhart (343) examined the firing of neurones of the medial reticular formation of the medulla of the chloral-hydrate-anaesthetized rat during microinjections of morphine in the PAG; the same dose of morphine by microinjection previously had produced analgesia in each animal. With both the nucleus gigantocellularis and paragigantocellularis, morphine administered in the PAG excited approximately half of neurones tested. This result is compatible with the hypothesis outlined above. Both electrical stimulation of the PAG and microinjection of morphine inhibited the excitation of about half of neurones tested. Both actions were reduced by systemic naloxone (1 mg/kg, intraperitoneally). It is possible that this is the significant result and that morphine in the PAG produces analgesia in part by inhibiting the responses of reticular neurones important in ascending pathways. This highlights one of the difficulties inherent in studies of reticular neurones; the absence of knowledge of the function of the neurones studied renders it difficult if not impossible to attach significance to the changes in firing.

Because of the complexity of the reticular formation and the diversity of the results presented, it is a daunting task to draw conclusions from this section. The results of opioids administered microelectrophoretically are not uniform and in particular the significance of excitation by morphine is uncertain. While a depression of firing probably occcurs with many reticular neurones following analgesic doses of opiates, the contribution of this to analgesia and the other effects of these drugs remains obscure. Opioid peptides may play a role in brain stem function but electrophysiological evidence for this is sparse.

4. Periaqueductal Grey Matter. It was mentioned above that electrical stimulation or microinjection of morphine in the PAG produces analgesia in the rat and cat (337, 383, 515). Since electrical stimulation is generally considered to excite neurones, this similarity between morphine and stimulation could mean that morphine excites many neurones of the PAG. This has not been found with microelectrophoretic administration. Depression of firing was the main effect of morphine and [Met]enkephalin (180, 442, 505) when administered from micropipettes near neurones of the PAG. Excitation of neurones (particularly by morphine) was occasionally observed in these experiments. Naloxone reversed the depression of cell firing by both morphine and [Met] enkephalin in the rat (180) but failed to modify the action of either compound in the cat (505). It has been proposed that both morphine and the enkephalins mimic electrical stimulation by inhibiting the firing of tonically active inhibitory interneurones (180, 516). No evidence substantiates this hypothesis.

5. Inferior Olivary Nucleus. Both [Leu]enkephalin and [Met]enkephalinamide depressed the spontaneous and amino acid firing of neurones of the inferior olive of the anaesthetized rat (440). Both drugs were administered iontophoretically and by pressure ejection but the reversibility of the effects by naloxone was not examined. Neurones of the inferior olive have a prolonged falling phase in their action potentials which is associated with an inward calcium current (316). Action potential duration was shortened by the enkephalins but only when cell firing has been depressed. This result has similarities to the effects of opioids on calcium currents in locus coeruleus and primary afferent neurones discussed later.

6. Nucleus of Edinger-Westphal and Pretectal Area. Morphine produces meiosis in man (176). In dogs anaesthetized with nitrous oxide, morphine (0.2 mg/kg) increased the spontaneous firing of neurones of the Edinger-Westphal nucleus but reduced the excitation of these neurones by light (311). The increased spontaneous firing was correlated with a decrease in pupil diameter. The spontaneous firing and excitation by light of pretectal neurones was decreased by these same doses of morphine. Levallorphan (0.05 mg/kg) abolished all these actions of morphine. Opiates have not been administered from micropipettes near single neurones of these areas, but it should be noted that the preoptic and accessory optic areas of the rat are rich in sites that bind radiolabelled etrophine (16, 17).

7. Brain Stem Raphe Neurones. This group of cells includes the dorsal raphe group of the midbrain and the more caudal group of the pons-medulla such as the NRM and pallidus of the rat and nucleus centralis inferior of the cat. Since it is probably only the caudal nuclei that

projects to the spinal cord (115), and it is this group that has been proposed to be important in the descending control of spinal neurones, the midbrain and the pons-medulla raphe nuclei will be discussed separately.

a. RAPHE NUCLEI OF THE PONS-MEDULLA. As outlined in section II A 3, several studies have examined the firing of raphe neurones after opiate administration in attempts to test the hypothesis that opiates produce analgesia by increasing descending inhibition to the spinal transmission of nociceptive information.

Intravenous morphine has not produced consistent effects on the firing of raphe neurones of the pons-medulla of anaesthetized animals. Thus, of 30 raphe magnus neurones in the anaesthetized rat, morphine (0.5 to 10 mg/kg) excited eight, inhibited 14, and had no effect on the spontaneous firing of eight (121). Haigler (208) found that of nine brain stem raphe neurones of the rat, four were excited and two were depressed by intravenous morphine (12 mg/kg). In the decerebrate or anaesthetized cat it has been reported that intravenous morphine excited neurones of the NRM (9), at least in those experiments in which morphine and naloxone did not change blood pressure; only six neurones were acceptable by this criterion and of these only four were excited by morphine and subsequently depressed by naloxone. Further data from the cat were obtained by Fields and Andersen (170). Most of this work was concerned with effects of microinjection of opiates in the PAG and is further discussed below. Raphe-spinal neurones were identified by antidromic activation. The spontaneous firing of 16 raphe spinal neurones was studied during systemic administration of morphine (1 to 3 mg/kg); excitation was observed with seven, but in only four was it reversed by naloxone. This low proportion of affected cells does not strongly enhance the proposition that systemic opiates inhibit spinal neurones through increased activity of raphe spinal neurones.

An increase in multiple unit activity in the region of NRM of the rat following intravenous morphine was reported by Oleson et al. (382). These rats were partially restrained but conscious, and the recording electrodes were relatively coarse. This result is further discussed when dealing with dorsal raphe neurones of the midbrain.

Administered microelectrophoretically near medullary raphe neurones of the cat, morphine has been variously reported to have no effect (318), or to depress the firing of approximately half of the neurones studied (505). In the latter study [Met]enkephalin depressed approximately 80% of neurones in the region of the NRM but this was not antagonized by naloxone. Studies in the rat have shown either no effect by microelectrophoretic morphine on medullary raphe neurones (280) or excitation (430). In the latter experiments, excitation of raphe neurones by both morphine and [Leu]enkephalin was antagonized by intravenous naloxone (0.5 mg/kg). Since recovery from the effects of naloxone was not observed, these results would be more conclusive if excitation by an amino acid such as L-glutamate had been shown to be unaffected by naloxone concurrently with a reduction in the effect of morphine or enkephalin. These results contrast with those of Haigler (208) in which electrophoretic morphine excited only four of 38 brain stem raphe neurones, having no effect on the remainder.

Microinjection of opiates in the PAG produces analgesia (515) and it has been proposed that spinal inhibition from increased activity in raphe-spinal fibres is an important component [reviewed by Yaksh and Rudy (514) and Gebhart (189)]. This mechanism would predict that raphe-spinal neurones should be excited by microinjection of opiates in the PAG. In urethane-anaesthetized rats the results were variable, with approximately equal numbers of raphe neurones being excited, inhibited, or unaffected after morphine injection in the PAG (30). Also studying the rat, Mohrland and Gebhart (344) found one-third of medullary raphe neurones to be excited by morphine injected in the PAG. In the cat, Fields and Anderson (170) found that 10 identified raphe-spinal neurones out of a total of 33 studied showed a naloxone reversible increase in firing after microinjection of etorphine, morphine, [D-Ala^2,D-Met^5]enkephalin, or [Met]enkephalin in the PAG.

These experiments permit several interpretations. Ideally, they should study raphe neurones known to produce spinal inhibition of transmission of nociceptive information. Since there is no way of identifying such cells even among identified raphe-spinal cells, the significance of the reported increases in firing is obscure. If a high proportion of cells had shown such increases after systemic opiates then the case would be strengthened that this was related to inhibition in the cord; but the reported changes are relatively small and variable, and thus do not support the hypothesis that systemic opiates activate raphe neurones and thus enhance descending inhibition of spinal neurones.

b. DORSAL RAPHE NEURONES OF THE MIDBRAIN. As with raphe neurones of the pons-medulla, intravenous morphine has had variable effects on the firing of dorsal raphe neurones. In anaesthetized rats, intravenous morphine (2 to 4 mg/kg) had no effect on the spontaneous firing of dorsal raphe neurones (207, 280). In restrained, locally anaesthetized rats, intravenous morphine (2 to 14 mg/kg) reduced the firing of three of seven raphe neurones (208). These studies used microelectrodes and recorded the firing of single neurones. Different results have been obtained when relatively coarse electrodes have been used to record the activity of many units simultaneously. In unrestrained rats with chronically implanted electrodes, morphine increased multiple unit activity in the dorsal raphe nucleus concurrently with a reduction in the increase in firing evoked by noxious skin stimuli (382). This result was confirmed by Urca and Liebeskind (479), who emphasized that such increases were not seen in the firing of single dorsal raphe neurones recorded with microelectrodes. They also re-

ported that while increases in multiple unit activity occurred following morphine in unanaesthetized rats, a decrease in firing was present in urethane-anaesthetized animals. They inferred that the population of cells activated by morphine and important in the production of analgesia are not normally sampled in miroelectrode studies. There are difficulties with multiple unit recordings. Systemic morphine produces swelling or contraction of the brain through effects on circulation. Multiple unit recordings may thus sample different populations of neurones before and after morphine administration. Raphe nuclei are part of the reticular formation and indeed Urca and Liebeskind (479) noted a correlation between changes in multiple unit activity and the electroencephalogram. The increase in multiple unit activity was considered by Urca and Liebeskind to support the hypothesis that morphine inhibits the excitation of dorsal horn neurones by noxious cutaneous stimuli because it increases descending inhibition of these neurones from the raphe nuclei; but the observed increases in multiple unit activity may be more relevant to the effects of morphine on cerebral function (493).

The most complete study of the effects of microelectrophoretically administered opiates on dorsal raphe neurones of the rat was that of Haigler (208). Although morphine and levorphanol inhibited the firing of a proportion of neurones of this nucleus, this was also seen with dextrorphan, and naloxone was not a reliable antagonist of the opiates. Similar results were obtained on neurones in areas that receive a projection from the dorsal raphe nuclei such as the amygdala, optic tectum, and subiculum. Thus, this study provided no support for the proposed role of dorsal raphe cells in opiate analgesia.

8. Locus Coeruleus. The locus coeruleus is composed almost entirely of catecholamine-containing neurones (115). It is an area of dense labelling after systemic administration of radiolabelled etorphine (15), and enkephalin-containing axo-dendritic synapses are found on neurones of this area by immunohistochemical techniques (396). The remarkable homogeneity of the neurones of the locus coeruleus in terms of catecholamine content makes them particularly attractive for electrophysiological studies in vitro where the projections of a given cell can often not be established.

a. IN VIVO STUDIES. In rats, intravenous morphine (2 to 4 mg/kg) inhibited the excitation of neurones of the locus coeruleus by cutaneous noxious stimuli and this was reversed by naloxone (280). The firing of adjacent neurones of the dorsal raphe nucleus was unaffected by morphine. Strahlendorf et al. (462) also observed a depression of spontaneous firing of cat locus coeruleus neurones after intravenous morphine (0.25 to 1.0 mg/kg).

Both morphine (45) and [Met]enkephalin (443) depressed the firing of neurones of the locus coeruleus of the rat when administered from micropipettes. The action of morphine was relatively prolonged when compared with that of [Met]enkephalin but inhibition by both compounds was prevented or reversed by antagonists such as naloxone or levallorphan. Levorphanol, but not naloxone, also depressed the spontaneous firing of neurones of the locus coeruleus. Guyenet and Aghajanian (204) also found that [Met]enkephalin depressed the firing of neurones of locus coeruleus of the rat. This action was blocked by naloxone and it was noted that these concentrations of naloxone were without effect on depression of cell firing by GABA (see Ref. 134).

The depression of cell firing by systemic morphine is less when it is administered to rats previously exposed to morphine for several days. It is uncertain whether this tolerance is due to changes in neurones of the locus coeruleus interconnected areas but the former is probable in view of the action of opioids on neurones of the locus coeruleus. Naloxone increased the firing rate of locus coeruleus neurones when administered to rats chronically treated with morphine, but had no effect when given to naive rats (1). However, the excitation by naloxone was not great and may have been due to a simple reversal of an ongoing depression by the morphine still present in the circulation, without necessarily indicating differences between naive and morphine-dependent animals. A local administration of naloxone is necessary to exclude the possibility that the locus coeruleus neurones were excited secondarily to a primary action of naloxone at a site afferent to the nucleus. There is considerable importance attaching to these studies of opiates on the locus coeruleus neurones for two reasons. The first is that they are the cell bodies of neurones that account for more than half of the NA content of the brain (6)—the widespread projections of the cells could translate local opiate action into a multitude of distant effects. Second, the withdrawal syndrome produced by naloxone in dependent animals (and man) has several similarities to the effects of stimulating the locus coeruleus. These similarities formed the rationale for the use of the α_2-adrenoceptor agonist clonidine in the treatment of opiate withdrawal (194). Clonidine hyperpolarizes locus coeruleus neurones in vivo (2) and reduces the excitation of these neurones by naloxone in morphine dependent rats (1).

As the cell bodies of the locus coeruleus neurones themselves have opiate receptors, these may also be expressed on the other parts of the neuronal membrane. The action of opiates on the terminal regions of locus coeruleus fibres in the rat cortex was investigated by Nakamura et al. (360). A stimulating electrode was placed in the frontal cortex of the urethane-anaesthetized rat, and the current adjusted so that a single locus coeruleus neurone was antidromically activated with 50% of the stimuli. Morphine (1 to 40 μM), [D-Ala^2,D-Leu^5] enkephalin, and [Met]enkephalinamide raised the threshold for antidromic activation when they were applied by local infusion from an implanted cannula; the effects were blocked by naloxone (10 μM). These exper-

iments suffer from many of the limitations of interpretation previously discussed for the excitability testing of primary afferent terminals in the spinal cord such as uncertainty that the opioids are acting *directly* on the membrane of the locus coeruleus axons and the possibility that preterminal and not terminal regions were tested for excitability. The simplest explanation, however, is that the terminal regions of these cells are hyperpolarized by opioids (378). As will be mentioned below, such an action of opioids has been characterized in detail at the level of the cell body.

The locus coeruleus receives afferent input from many sources (6). Enkephalin-like immunoreactivity is contained in profiles making synaptic contacts with locus coeruleus neurones (396). The nucleus also lies close to the caudal extent of the projection of a β-endorphin/ACTH fibre system originating from cell bodies in the arcuate nucleus of the hypothalamus. Strahlendorf et al. (462) found that electrical stimulation in the cat arcuate nucleus led to an inhibition of firing of locus coeruleus cells. Intravenous naloxone (5 mg/kg) partially reduced the inhibition. Naloxone alone increased the spontaneous firing of many cells but since these doses of intravenous naloxone are high, it cannot be concluded that the naloxone blocked β-endorphin action at the level of the locus coeruleus. Local application of naloxone would be helpful.

Collectively, these data suggest that actions on neurones of the locus coeruleus may contribute to the effects of systemic morphine. Since locus coeruleus activity is generally associated with an enhancement of awareness of the environmental stimuli (13), it is possible that a reduction of this activity by opiates contributes to the affective component of their action. Additionally, the locus coeruleus had been implicated in stimulation-produced analgesia (42); lesions of the locus coeruleus impair the ability of morphine to suppress responses to electrical stimuli of the rat hind paw (423).

b. IN VITRO STUDIES. Satisfactory recordings can be obtained from locus coeruleus neurones for at least a day in a superfused slice maintained at 37°C in vitro. With such a preparation, Pepper and Henderson (389) found that opioids hyperpolarized guinea-pig neurones, and this action was reversed by naloxone. The opioid action was shown to be a direct effect on the impaled neurone because it persisted in solutions that blocked synaptic transmission. The hyperpolarization was associated with a decrease in cell input resistance.

Similar results were obtained in the rat locus coeruleus by Williams et al. (498). Two further findings were made in these experiments. First, the opioids hyperpolarized the neurones through an increase in the membrane potassium conductance. The evidence for this was the Nernstian dependence of the opioid reversal potential on the extracellular potassium concentration (498) and the prevention of opioid hyperpolarizations by intracellular caesium or extracellular barium, both of which block potassium channels (376). Second, the actions of opioids occurred in the concentration range of 30 nM to 10 μM. Naloxone antagonized opioid action competitively with a K_e (determined by the pA_2 method) of about 2 nM (fig. 8). This K_e was the same whether morphine or [D-Ala^2,D-Leu^5]enkephalin was the agonist.

N,N-bisallyl-Tyr-Gly-Gly-ψ(CH_2S)-Phe-Leu-OH (ICI 154,129), which is a relatively selective δ-receptor antagonist (448), also blocked opioid actions but only at concentrations appropriate to an action on μ receptors (377). β-FNA, a selective and irreversible μ-receptor antagonist (473), blocked equally well the hyperpolarization evoked by morphine, [D-Ala^2,D-Leu^5]enkephalin, ethylketazocine, and [D-Ser^2,Leu^5]enkephalin-Thr. All these experiments suggest that locus coeruleus neurones are hyperpolarized by opioids as a result of their occupation of μ-type receptors (473, 498). The agreement was good between the naloxone K_e determined in binding studies (at μ-binding sites) and on single locus coeruleus neurones. However, estimates of agonist equilibrium constants by the method of partial irreversible receptor blockade gave values of 5 to 15 μM (380). This indicates both that opioids hyperpolarize locus coeruleus neurones with a small proportion of space receptors (460) (the ED50 is about 1 μM), and that agonist binding sites determined biochemically are not those responsible for inhibition.

The actions of opioids on calcium entry were also studied in rat locus coeruleus neurones in the expectation that events occurring on the cell bodies may reflect similar actions at sites from which transmitter is released (e.g. axon terminal varicosities). Locus coeruleus neurones have calcium action potentials in the presence of TTX (376). The rate of rise and duration of the calcium action potential was reduced by morphine, [Met]enkephalin, and [D-Ala^2,D-Leu^5]enkephalin. However, this was apparently not a direct action on the calcium conductance, because it was prevented in circumstances in which opioids no longer increased potassium conductance (intracellular caesium or extracellular barium). Thus, opioids indirectly modulated calcium entry into locus coeruleus neurones by increasing the membrane potassium conductance. This is analogous, but inverse, to the indirect modulation of calcium entry first described by Kandel (265) in *Aplysia*. In that case, 5-HT increased action potential duration by reducing potassium conductance; the increased action potential duration occurs in a presynaptic cell, and as a result it releases more transmitter.

The nucleus locus coeruleus has given considerable insight into how opioids affect one type of central neurone. The results have been remarkably uniform; every neurone is hyperpolarized by morphine, and to approximately the same extent by a given concentration. In summary, the experiments have indicated that opioids interact with a single receptor type having an affinity for naloxone characteristic of a μ-binding site. This interaction results in an increase in potassium conductance,

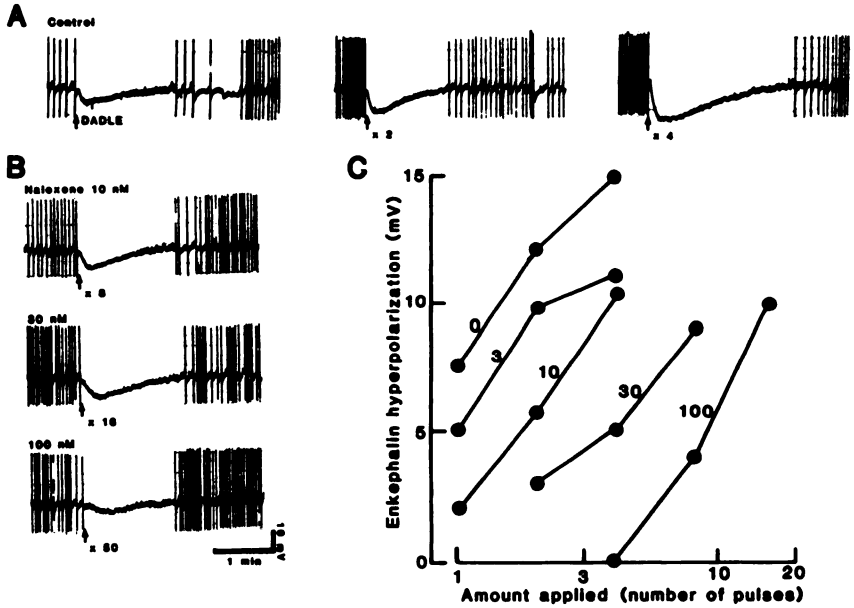

FIG. 8. Enkephalin interacts with a naloxone-sensitive receptor on locus coeruleus neurones. A and B are intracellular recordings from a spontaneously firing locus coeruleus neurone in a tissue slice cut from rat pons and maintained in vitro. Upward deflections are action potentials (truncated); downward deflections are action potential afterhyperpolarizations. [D-Ala^2, D-Leu^5]enkephalin was administered at arrows by applying various numbers of pressure pulses (55 kPa, 24 ms, number indicated) to a micropipette containing the peptide positioned above the slice. A, control responses. B, selected records taken in the presence of different amounts of naloxone (added to the superfusing solution, concentration indicated). C, dose-response curves to [D-Ala^2, D-Leu^5]enkephalin at the concentration of naloxone indicated (nM). [Reproduced, with permission, from Williams et al. (498)].

hyperpolarization, and inhibition of cell firing. The increase in potassium conductance, by shunting the calcium spike, could also impair NA release (378, 379).

C. *Thalamus*

1. Ventrobasal Thalamic Nuclei. In mammals, the thalamic nuclei ventralis posteromedialis, and ventralis posterolateralis are major sites of termination of the medial lemniscus and spinothalamic and secondary trigeminal tracts (11, 54, 87). Most studies of ventrobasal neurones have emphasized the response of these neurons to non-noxious stimuli (339, 400) but responses to noxious stimuli have been observed (450). In addition, many spinothalamic neurones convey nociceptive as well as other information (502) and establish connection with neurones of the ventrobasal thalamus.

In the cat, intravenous morphine (up to 10 mg/kg) had no effect on the amplitude and latency of potentials evoked in the ventrobasal thalamic nuclei by stimulation of the sciatic nerve (455). A similar result was observed in the rat (226). Indeed the short latency excitation of ventrobasal thalamic neurones by subcutaneous electrical stimulation was occasionally increased (226). A depressant action by systemic morphine on ventrobasal neurones has been observed in the rat. Shigenaga and Inoki (450) found that morphine (10 mg/kg) injected intraperitoneally reduced the excitation by tooth pulp stimulation of one third of the ventrobasal neurones studied.

Morphine was an excitant when administered microelectrophoretically near the cell bodies of ventrobasal neurones, in that the responses of these neurones to acetylcholine and DL-homocysteic acid were increased (141). Naloxone not only antagonized this effect but reduced excitation by acetylcholine (141).

Collectively, these results suggest that the ventrobasal thalamus is not an area where significant changes in function are produced by analgesic doses of opiates. Relatively few binding sites were found in the ventrobasal thalamus after systemic administration of radiolabelled etorphine (16).

2. Medial and Posterior Thalamic Nuclei. Anatomical and physiological studies indicate that neurones of the medial and posterior thalamic nuclei play a role in the transmission of nociceptive information (4, 53, 264, 504). The medial thalamus is an area that binds radiolabelled etrophine (16) and microinjection of opiates at medial thalamic sites produces analgesia in rats (516). The nuclei studied with opiates include the centre-median parafascicularis complex, the lateralis posterior and, in the rat, lateralis anterior.

In the cat, intravenous morphine (0.5 to 1.0 mg/kg) reduced the excitation of neurones of the centre-median parafascicularis area by L-glutamate; this action, which was antagonized by naloxone (0.3 mg/kg), was not necessarily accompanied by changes in spontaneous firing, suggesting a direct action on the neurones studied (146). In the rat, morphine (0.25 to 1.17 mg/kg) reduced the excitation of neurones of the nucleus lateralis anterior without changes in spontaneous firing or excitation by L-glutamate (227). This latter observation suggests that morphine did not act at receptors located on the neurones

studied. A comparative study in the rat found that less intravenous morphine was required to inhibit the nociceptive excitation of thalamic than of medullary dorsal horn neurones (0.7 ± 0.18 mg/kg, n = 13 vs. 2.5 ± 0.72 mg/kg, n = 16) (229).

Emmers (166) has described two areas of the rat thalamus, SI and SII, with ipsilateral and bilateral representation of the body surface. Within SII, neurones were found that were excited by noxious skin stimuli. These neurones were partly in the ventrobasal complex and partly more posterior. Electrical stimulation of the sciatic nerve produced early and late groups of action potentials in such neurones and morphine (4 mg/kg) reduced or abolished the late peaks. The histograms of cell firing were very similar to those recorded in the rat cortex by Biscoe et al. (46) where intravenous etorphine (2 to 12 μg/kg) abolished the later peaks of firing. The proposal by Emmers (166) that pain is encoded in the afterdischarges of thalamic neurones does not accord with the reports of others and hence the significance of the suppression of afterdischarges by opiates is uncertain.

Nakahama et al. (357) found selective depression of the nociceptive responses of neurones of the nuclei centralis lateralis, ventralis lateralis, and dorsalis medialis of the locally anaesthetized cat following administration of morphine (1 mg/kg) and pentazocine (2 mg/kg). Most neurones were excited by both noxious and non-noxious cutaneous stimuli and the selective effects of the opiates are similar to those observed in the spinal cord. What is not known is how many of the effects observed in the medial thalamus can be accounted for by spinal actions or whether there is a progressive reduction in nociceptive transmission at several sites between.

These studies with systemic opiates have been further investigated by administering compounds from micropipettes. Duggan and Hall (146) found that both morphine and naloxone depressed the spontaneous firing of neurones of the centre-median parafascicularis region of the cat. No antagonistic action of naloxone was observed and, following administration of either, neurones were hyperexcitable to L-glutamate for up to 43 min. This failure of naloxone to antagonize depression of cell firing by morphine was also observed in the rat thalamus (226). Another study in the urethane-anaesthetized rat found a mixture of excitation and depression by microelectrophoretic morphine. Only depression of firing was reversed by naloxone and the synaptic excitation of neurones was not studied (415). Both [Met]enkephalin and [D-Ala^2,D-Leu^5]enkephalin reduced the excitation of rat lateralis anterior neurones by noxious skin stimuli (227). Naloxone antagonized the action of [D-Ala^2,D-Leu^5]enkephalin on two of three neurones. In a study primarily aimed at differences between naive and morphine-dependent rats, naloxone alone depressed 23% and excited 13% of the medial thalamic neurones sampled (181). These results have similarities to those obtained with spinal neurones; namely, that naloxone-reversible effects were observed with opioid peptides but not with morphine. Therefore, it is possible that a significant number of receptors important to the effects of systemic opiates are located at sites other than the cell bodies of medial thalamic neurones. No studies have explored this possibility.

D. Hypothalamus

When administered systemically or intracerebroventricularly, morphine has produced many effects consistent with a disturbance of hypothalamic functions. These include changes in body temperature regulation (21, 22), blood glucose levels (345), and the secretion of anterior (191) and posterior (123, 209) pituitary hormones. Although the hypothalamus did not show a high density of binding sites to radiolabelled diprenorphine in the original studies of Atweh and Kuhar (16), this area has proved to be relatively rich in immunoreactive opioid peptides (233, 419).

Morphine (3 to 5 mg/kg) slowed the spontaneous firing of nine of 12 anterior hypothalamic neurones of the urethane-anaesthetized rat (163). Naloxone reversed this action of morphine but had no effect of its own. In the areas of the hypothalamus concerned with feeding behaviour, Kerr et al. (271) found that, in the rat, morphine (10 mg/kg) consistently increased the firing rate of neurones of the ventromedial nucleus while inhibiting that of neurones of the lateral hypothalamic area. These authors have proposed that it is the effects of opiates on neurones of these areas that produce the drug-seeking behaviour associated with physical dependence. Dafny et al. (113) obtained multiunit recordings from the anterior hypothalamic area and ventromedial nucleus of freely moving rats. Morphine (0.5 to 30 mg/kg) injected intraperitoneally produced variable effects with increases or decreases in firing in approximately equal numbers of neurones. In a subsequent study (112) only depression of spontaneous multi-unit activity was reversed by naloxone. The more frequently observed increases after systemic morphine (10 mg/kg) were unchanged or even increased by naloxone (1.0 mg/kg).

Systemic morphine reduces the secretion of oxytocin in the rat in response to suckling (209). Clarke et al. (88) have shown that this probably results from an action near the terminals of paraventricular neurosecretory neurones. Intracerebroventricular morphine (2 to 4 μg) reduced the periodic rises in intramammary pressure produced by suckling in urethane-anaesthetized rats, but did not change the bursts of firing of paraventricular neurones that precede these rises. They also found that naloxone alone increased the rises in mammary pressure and suggested that opioids were tonically active in this system. However, it was observed that intravenous injection of the enkephalin analogue [D-Ala^2,Me Phe^4, Met(O)^5]enkephalin-l (FK 33,824) (1 μg) increased intramammary pressure (19). This was associated with a decrease in the compound action potential recorded in

the hypothalamo-hypophyseal tract following distal stimulation. A decrease in this potential was interpreted as resulting from collision between orthodromically and antidromically conducted impulses and hence as indicating increased firing of neurones releasing neurohypophyseal hormones. It is difficult to reconcile this result with that of Clarke et al. (88), although one group studied basal release of oxytocin and the other, increases in release from natural stimulation.

Ono et al. (385) found a correspondence between the effects of microelectrophoretic opioids and those of glucose on neurones of the hypothalamus of chloralose-urethane-anaesthetized rats. Inhibition was the predominant effect of glucose, morphine, and [Met]enkephalin on neurones of the lateral hypothalamic area, whereas all three compounds excited cells of the ventromedial nucleus. Both naloxone and levallorphan reduced the effect of the opioids but not that of glucose. The responses to microelectrophoretic morphine parallel the findings of Kerr et al. (271) with systemic morphine and suggest that the effects of opiates on blood glucose levels stem in part from a direct action on hypothalamic neurones.

In an investigation of the effects of morphine on thermoregulation, Baldino et al. (21) implanted bilateral thermodes near the hypothalamus of the rat and identified neurones responsive to variations of hypothalamic temperature. A naloxone reversible excitation by microelectrophoretic morphine was observed with neurones excited by warming the hypothalamus whereas a depression of cell firing occurred with neurones excited by cooling the hypothalamus. In this species, morphine has dose-dependent effects on body temperature, low doses (5 to 8 mg/kg) producing hyperthermia and higher doses giving hypothermia. The observations of Baldino et al. (21) may be relevant to morphine-induced hypothermia. A subsequent paper from this laboratory (20) has emphasized some of the nonspecific effects of naloxone administered from micropipettes near hypothalamic neurones.

The analogues [D-Ala^2,MePhe^4,Met(O)^5]enkephalin-ol and [MeTyr^1,D-Ala^2,MePhe^4,Met(O)^5]enkephalin-ol (10 to 100 nM) reduced the spontaneous firing of paraventricular neurones of slices of the hypothalamus of rats (353). Naloxone (1 μM) reversed this inhibition of firing. Morphine (1 to 100 μM) reduced the spontaneous firing of half of the cells tested but it is not clear whether naloxone reversed this effect. It should be noted, however, that the experiments of Clarke et al. (88) suggested that doses of morphine adequate to reduce evoked release of oxytocin, do so without changing the firing of paraventricular neurones. In the urethane-anaesthetized guinea-pig both [Met]- and [Leu]enkephalin reduced the firing of tubero-infundibular and adjacent unidentified neurones when administered microelectrophoretically (71). Reversal by naloxone was not consistently observed and, although it was implied that the results have relevance for the control of release of anterior pituitary hormones, the effects of naloxone alone on induced changes in cell firing do not appear to have been examined.

These few reports suggest that opioid peptides have a functional role in the hypothalamus. Beyond the scope of the present review are the many accounts that opioid antagonists influence the secretion of anterior pituitary hormones presumably through controlling the secretion of hypothalamic-releasing factors [reviewed by Grossman and Rees (200)]. This is potentially a rich area for neurophysiological investigation.

E. Basal Ganglia

The basal ganglia contain a high density of opiate binding sites (16) and one of them, the globus pallidus, has the most dense immunohistochemical network of enkephalin-containing fibres in the brain (233). Despite this, studies on the effects of opioids on neurones of the basal ganglia have been relatively few and restricted to the caudate nucleus.

1. Intravenous Opiates. In barbiturate-anesthetized rats, intra-arterial morphine (3 to 6 mg/kg) depressed the spontaneous firing rate of all caudate neurones studied (312). This was not considered a direct effect on caudate neurones but rather to result from excitation of nigrostriatal dopamine-releasing neurones, since pretreatment with haloperidol or pimozide prevented the action of morphine. Systemic morphine (20 mg/kg) also depressed the firing of neurones in the mouse striatum and this effect was reversed by naloxone (2 mg/kg) (43).

Dafny et al. (114) studied the effects of systemic opiates on multi-unit activity in the caudate nucleus in both naive and morphine-dependent rats that were freely moving, having had electrodes previously implanted. Intravenous morphine (0.5 to 6 mg/kg) increased firing in the naive animals, and only caused inhibition at the very high dose of 30 mg/kg. It was reported that this effect was reversed by naloxone (1 mg/kg). In this study, multi-unit activity in other brain regions (medial thalamus, PAG, mesencephalic reticular formation) was inhibited. It is of interest that the inhibition of caudate neurones reported by other investigators to occur in anaesthetized animals was not seen in freely moving rats; the excitations reported presumably reflect actions of systemic morphine at sites other than the caudate nucleus.

2. Microelectrophoretic Opioids. Morphine and [Met]enkephalin applied microelectrophoretically inhibited the excitant amino-acid-induced firing of about 50% of neurones in the rat caudate nucleus (188). The inhibition was reversed by concomitant application of naloxone, which failed to block depression by dextrorphan. Dopamine inhibited the firing of almost all the caudate neurones studied, but these effects were blocked by α-flupenthixol and not by naloxone. Zieglgansberger and Fry (526) and Frederickson and Norris (180) also found that spontaneously firing caudate neurones of the rat were inhibited by application of enkephalin. Zieglgansberger

and Fry (526) noted that tachyphylaxis appeared to develop rapidly to this inhibitory action when the [Met] enkephalin application was repeated at short intervals. It has been proposed that in the rat striatum, inhibition by morphine results from a release of adenosine from nerve terminals (390). In these experiments, however, the effects of morphine were poorly or not antagonized by naloxone and hence it is improbable that they have relevance to activity at opiate receptors.

Nicoll et al. (364) found that microelectrophoretic application of [Met]enkephalin, β-endorphin, and normorphine inhibited the firing of almost all neurones in the rat caudate—remarking that this area contained one of the highest percentages of opiate sensitive neurones of all brain regions that they examined. Naloxone blocked these effects when applied microelectrophoretically or systemically (2 mg/kg). The inhibitory action of the opiates and opioid peptides were not significantly different in rats that had been pretreated with 6-hydroxydopamine so as to destroy the nigrostriatal pathway. This result contrasts with that of Lee et al. (312) who used systemic morphine. It also differs in one respect from the finding of Zieglgansberger and Fry; no tachyphylaxis was observed when opiates were applied repeatedly for up to 1 hr. The globus pallidus is rich in fibres containing immunoreactive enkephalin, most being derived from the striatum (101). In anaesthetized rats, microelectrophoretic morphine depressed the spontaneous and L-glutamate-evoked firing of neurones of the globus pallidus (241). Naloxone blocked inhibition by morphine but not by dopamine.

Microelectrophoretic application of naloxone near caudate neurones has had no effects on cell firing other than a blockade of the inhibition caused by opiates. On the other hand, naloxone excited a proportion of striatal neurones in rats that had previously been chronically exposed to morphine by pellet implantation (184). These findings are similar to those reported for myenteric neurones of the guinea-pig ileum (see section III A 1), and indicate that the effect of naloxone on neurones chronically exposed to morphine is opposite in direction to the acute action of opiates on the same neurones in naive animals. Such a demonstration of "dependence" at the single neurone level has been paralleled to a degree by evidence of tolerance. In rats made tolerant to morphine by prolonged treatment, enkephalin had almost no inhibitory effects on neuronal firing (similar results have been described for the cerebral cortex, section II G).

These studies on caudate neurones suffer more than most from a complete lack of functional identification of the cells from which activity is recorded. None have studied opiate effects in the globus pallidus, a region which is exceedingly dense in enkephalin-immunoreactive fibres, some of which appear to form a striopallidal pathway (101), and which is also thought to receive inhibitory input from the neostriatum. The results almost uniformly indicate that opioids inhibit the firing of caudate neurones, whether this is spontaneous or evoked by DL-homocysteate or L-glutamate.

In only one study (183) were synaptically evoked responses examined. It was found that [Met]enkephalin reduced the late synaptic response of the rat striatum evoked by stimulating the ipsilateral cerebral cortex. This was not associated with any change in the early synaptic response. Unlike enkephalin, GABA reduced both the early and late response. This prompted the authors to suggest that opiates selectively reduce the postsynaptic action of the transmitter that mediates the late response, although a presynaptic action on the release of such a transmitter would be an equally plausible explanation.

Until single units are identified on the basis of their physiological inputs or projections, the contribution of these actions of microelectrophoretically applied opiates to the well known effects on locomotion of systemic opiates will remain mysterious. In similar vein, knowledge of the effects of enkephalin on unidentified striatal neurones offers but limited insight into its physiological role in the basal ganglia. These limitations are particularly relevant in view of the patchy or "clustered" distribution of opiate binding sites in the striatum (394).

F. Nucleus Accumbens and Nucleus of the Stria Terminalis

The firing of neurones in the rat nucleus accumbens septi was inhibited by microelectrophoretic application of [Leu]enkephalin, [Met]enkephalin, and morphine (326). These effects were reversed by naloxone. The neurones were not identified physiologically and were continuously excited by microelectrophoretic application of DL-homocysteate. Dingledine et al. (134) confirmed that microelectrophoretic application of morphine inhibited cell firing in the region of the nucleus accumbens/olfactory tubercle of the rat. However, this was reduced by naloxone in only two of 14 cells. The nucleus accumbens appears to receive a rich innervation of fibres immunoreactive to enkephalin (165, 235). This inhibition of firing therefore represents another site at which the effect may reflect a physiological role of enkephalin. It has been suggested by Dill and Costa (129) that the nucleus accumbens may be a more important site than the caudate in producing catalepsy and analgesia. Indeed, injection of morphine into the nucleus accumbens caused a marked increase in spontaneous motor activity in rats (391).

The stria terminalis contains fibres from the amygdala to basal forebrain structures including the bed nucleus of the stria terminalis (287). In a slice preparation of this nucleus, which included the terminal part of the stria terminalis, stimulation of the latter produced a negative potential that was suppressed by [D-Ala^2,Met^5]enkephalinamide (1 μM). Naloxone (1 μM) blocked the action of the opioid peptide (435). Naloxone also reduced an inhibition of neurones (four of 11 tested) that followed

their excitation by stimulation near the stria terminalis. This action was not reversible despite repeated washing of the tissue. Although the concentration of naloxone was less than that interfering with GABA effects on other neurones, this action needs to be reexamined with isomers of naloxone and other opioid antagonists. It is not possible to assign a functional significance to the inhibition reduced by naloxone.

G. Cerebral Cortex

Effects of opioids on cerebral cortical neurones may be relevant to the subjective changes that follow opiate administration. Probably because of the relative ease with which the electroencephalogram (EEG) can be recorded, the cerebral cortex was one of the first areas of the brain studied for changes in recorded potentials following opiate administration.

1. Intravenous Opiates. Both in the rat (66) and dog (492) intravenous morphine produced a sleeplike pattern in the EEG without the animals showing signs of drowsiness. In the dog, this "behavioural dissociation" effect was most marked with low doses of morphine (5 mg/kg). A similar action has been reported for man (493), rabbits, and cats (361). Subsequent studies on cortical neurones have not attempted to explain this readily observed action of morphine. It could, for example, result from a primary action of opiates on thalamic or reticular neurones.

The short latency primary-evoked response recorded from the surface of the cerebral cortex in response to a peripheral nerve stimulus is not reduced by analgesic doses of opiates. This has been shown for tooth pulp stimulation in the dog (84), stimulation of the sciatic nerve in the cat (455) and rat (46), and stimulation of the radial nerve in the rat (263). In the last study, morphine (2 mg/kg) increased the amplitude of the primary evoked response. Both intravenous etorphine (46) and pentazocine (5 to 10 mg/kg) (351) increased the latency of the primary evoked response recorded from the cerebral cortex without changing its amplitude. This effect was reversed by opioid antagonists. The regularly recurring potential waves that follow the primary evoked response were reduced by opiates. In the rat, intravenous etorphine (2 to 12 μg/kg) abolished these afterdischarges to sciatic nerve stimulation, an action which was completely reversed by intravenous diprenorphine (6 to 37.5 μg/kg). However, this effect of etorphine was not mimicked by intravenous morphine administration (2 to 4 mg/kg).

2. Microelectrophoretic Opioids. Krnjević (293) included morphine in his list of compounds that inhibited the activity of neurones in the neocortex of the cat. In a further investigation of the abolition by etorphine of the late cortical potentials following sciatic nerve stimulation, Biscoe et al. (46) administered etorphine near neurones of layer V of areas 2 and 3 of the rat cerebral cortex. Etorphine reduced the afterdischarges of nine of the 11 cells studied but no studies were performed on the possible reversal of this effect by diprenorphine. It was noted that the currents of etorphine used were without effect on excitation of cortical neurones by L-glutamate and acetylcholine. These results led Biscoe et al. (46) to propose that the effects of systemic etorphine on late cortical evoked potentials resulted from an action on transmitter release from intracortical terminals and not on subcortical areas.

Bioulac et al. (44) studied cells in the cerebral cortex of the decerebrate cat. They classified neurones on the basis of sensitivity to acetylcholine. Those which were excited by acetylcholine were almost all excited by morphine (50 nA, 3 to 9 min); this was not blocked by naloxone but it was blocked by methylatropine. It is possible, therefore, that cholinesterase inhibition by morphine contributed to this effect.

More uniform responses to microelectrophoretic application of morphine were found in the anaesthetized rat by Satoh et al. (434). About two thirds of cells in the sensorimotor cortex were inhibited by microelectrophoretically applied morphine. The effect reached its maximum within 10 to 20 s, and recovered within 10 to 30 s after termination of the microelectrophoretic current. About half of the cells inhibited showed a transient increase in firing when the current was terminated. The degree of the inhibition was related to the microelectrophoretic currents, at least up to 100 nA. Higher morphine currents often produced a slowly developing excitation that was also seen with high currents of naloxone. By contrast, lower currents of microelectrophoretically applied naloxone blocked the inhibitory effects of morphine. A diminished response to successive ejections of morphine was observed but, to be interpreted, such experiments require alternating morphine ejection with another depressant such as GABA. In addition, morphine should be contained within more than one barrel of the microelectrode to ensure that a diminishing transport number is not responsible for the fading of responses (526). Levorphanol, but not dextrorphan, also inhibited cell firing. When morphine was applied to neurones in the cortex of rats made tolerant to and dependent on this opiate, only three of 48 cells were inhibited.

Enkephalins produced a depression of firing similar to that caused by morphine in the sensorimotor (527) and frontal (180) regions of the cerebral cortex of the rat. Cells in the "posterior" cortex (180) were not inhibited by enkephalins.

In the rat, a progressive reduction of inhibition of cortical neurones has been observed with opioid peptides as well as with morphine (500). With continued (20 to 30 min) administration of morphine the inhibition faded; the neurones were still normally inhibited by [D-Ala^2,D-Leu^5]enkephalin and [Met]enkephalin. After continued ejection of [Met]enkephalin, however, cells became insensitive both to the peptide and morphine. The authors interpreted this result in terms of a one-way lack of

cross-desensitization, and that more than one opioid receptor was present on the neurones studied. The conclusion may be correct, but cannot be considered proven by the results obtained. The lack of knowledge of concentrations and tissue disposition of the ejected drugs make conclusions on receptor subtypes most suspect.

The most thorough study of the action of enkephalin analogues on cortical neurones was made by Palmer et al. (387). They applied [Met]enkephalin, [D-Ala^2,Met^5] enkephalinamide, and acetyl[Met]enkephalin by microelectrophoresis to neurones in either the frontal or the parietal cortex of the anaesthetized rat. Both the spontaneous firing and the firing evoked by acetylcholine or L-glutamate were inhibited by [Met]enkephalin. A correlation was noted between excitation by acetylcholine and inhibition by [Met]enkephalin, but it should be noted that most cells studied were cholinoceptive [compare with the findings of Bioulac et al. (44) with morphine in the cat, vide supra]. Similar results were obtained in both frontal and anterior parietal cortex; in frontal cortex [D-Ala^2,Met^5]enkephalin was equipotent with [Met]enkephalin whereas in parietal cortex it was significantly more potent, suggesting perhaps a greater abundance of enkephalinase or other peptidase activity in the former site. This is of some interest in view of the finding that the frontal cortex is significantly more dense than other cortical regions in opiate binding sites (230, 395) and that the distribution of enkephalinase parallels rather closely that of stereospecific opiate binding (329). The inhibition by the enkephalins could not be mimicked by the N-acetyl analogue, a substance that is inactive in opiate binding and isolated tissue bioassays. Palmer et al. (387) stated that in three rats in which the medullary pyramids were electrically stimulated, a high proportion of cells studied were antidromically activated by this stimulus. Moreover, almost all neurones studied were in the deeper cortical layers, more than 500 μm from the surface. This supports their surmise that these cells are most likely to be deep pyramidal cells—the extracellular electrodes would more readily record activity from these larger cells than from smaller intrinsic cortical neurones, and in the rat the pyramidal neurones are commonly cholinoceptive (461). It is of interest that [^{3}H]diprenorphine accumulates in the deeper layers of the cortex after systemic administration (394). The interpretation that neocortical pyramidal cells appear to be directly inhibited by opioids is different from the conclusion reached for the paleocortex (vide infra), where inhibition of nonprojecting cells appears to result in an excitation of the principal output neurones. Intracellular recording from pyramidal cells of the cerebral cortex may be useful in substantiating this direct action although, as in the case of the hippocampus, the substantial electrical load of the neuronal dendrites may make interpretation difficult.

H. Hippocampal Formation

1. In Vivo Experiments. The important effects of opiates on mood and emotions have prompted several studies of their interactions with neurones in the limbic system. In addition, the production of epileptiform discharges in the hippocampus by opioids has stimulated research into the possible relevance of this effect to human convulsive disorders. Some earlier studies used coarse electrodes to record hippocampal potentials evoked by stimuli in other brain regions. Systemic morphine depressed the responses to stimulation of the tibial (328) or radial (358) nerve, but had no effect on the responses that followed stimulation of the septum, cortex, and contralateral hippocampus (328, 358). Morphine also did not affect the threshold or duration of the afterdischarge (recorded with a surface electrode) that follows hippocampal stimulation (136). These findings allow few conclusions to be drawn about the site of this opiate action except that it is unlikely to involve the hippocampus directly. Recent evidence, however, indicates that opiates have direct effects in the hippocampus. Opiates and opioid peptides produce epileptiform-like activity in EEG recordings from hippocampus (limbic seizures) when they are applied into the lateral ventricle (218).

Recordings from single neurones in the hippocampus in vivo have been largely limited to the larger pyramidal cells in the CA1 and CA3 regions. Since the interpretation of recent results from the hippocampus requires a knowledge of interactions between pyramidal cells, inhibitory interneurones, and extrinsic afferent fibres, figure 9 has been included to illustrate these points. Most workers agree that pyramidal neurones are excited by systemic or microelectrophoretic opioids although there has been confusion regarding the pharmacological relevance and specificity of this excitation. Systemic opiates increased the firing rate of neurones both in the hippocampus and amygdala of the cat (85). These effects occurred with quite low doses of morphine (0.5 to 2 mg/kg) and they were reversed completely in the hippocampus and partially in the amygdala by naloxone (0.2 to 0.4 mg/kg).

Nicoll et al. (364), Segal (445), Hill et al. (224), Fry et al. (182), and Dunwiddie et al. (157) agree that some neurones in the rat hippocampus are excited by microelectrophoretic application of opiates and opioid peptides, but there is considerable disagreement as to the proportion of cells affected and the naloxone reversibility of the effects. Nicoll et al. (364) stated that the excitation occurred with 12 of 12 cells with normorphine, 12 of 14 cells with β-endorphin and 19 of 20 cells with [Met] enkephalin. The effects were usually reversed by microelectrophoretic or systemic administration of naloxone (2 mg/kg). They studied only spontaneously firing neurones. Dunwiddie et al. (157) found that six of 25 cells were excited by [D-Ala^2,Met^5]enkephalinamide. These excitations were not mimicked by N-acetyl[Met]enkephalin, and were blocked by microelectrophoretic or systemic naloxone. Picrotoxin excited all of the cells excited by enkephalin, but this action was not affected by naloxone. Enkephalin also increased the amplitude of the

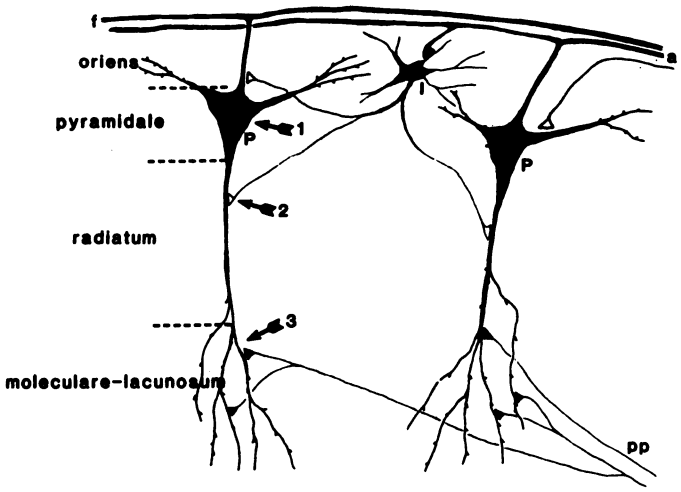

FIG. 9. Schematic representation of the neuronal organization of the CA1 region of the hippocampus. P, pyramidal cell; I, inhibitory interneurone; a, alveus; p, input from perforant path; f, fiber projection to fornix. The axons of the pyramidal cells are shown projecting upwards and dividing in the alveus, before giving off collaterals that reach the inhibitory interneurone cells. These cells release γ-aminobutyric acid (GABA) which increases the conductance of the pyramidal cell membrane. △, inhibitory synapses; ▲, excitatory synapses. Major electrophysiological findings with opioids are: 1) spontaneous firing of pyramidal cells is increased by opiates, and also by agents that antagonize the effects of GABA; 2) spontaneous inhibitory postsynaptic potentials (IPSPs) are abolished by opiates, as well as by GABA antagonists; 3) the excitatory postsynaptic potential (EPSP) which is generated on the distal part of the apical dendrites of the pyramidal cells (in stratum lacunosum-moleculare) is increased in amplitude by morphine. (See text for further details.)

population spike evoked by commissural stimulation, and caused the appearance of a secondary population spike. The population spike mainly represents the synchronized firing of pyramidal cells to an efferent volley. These effects were not mimicked by the N-acetyl analogue, but were blocked by microelectrophoretically applied naloxone.

Hill et al. (224) reported that [Met]enkephalin, [Leu]enkephalin, and [D-Ala^2,D-Leu^5]enkephalin all had similar effects on unidentified hippocampal cells—exciting one third of cells, inhibiting one third, and not affecting one third. The excitations were blocked by an unstated amount (microelectrophoretic current) of naloxone. In some of the cells in which spontaneous firing rate was increased by enkephalin, the excitation evoked by glutamate was reduced; this suggests that the peptides may have inhibitory or excitatory effects depending on the initial level of neuronal activity. The important control of increasing spontaneous firing with a non-opioid compound appears not to have been performed.

Segal (445) found no effect of microelectrophoretic morphine on the spontaneous activity of 84 of 105 CA1 cells, the remainder being either excited (10) or inhibited (11). On the other hand, the activity induced by microelectrophoretic application of glutamate, acetylcholine, aspartate, or NA was reduced in most experiments by concomitant application of morphine. This action of morphine was not affected by simultaneous application of naloxone at similar microelectrophoretic currents (50 to 100 nA) suggesting an action other than at opiate receptors. Enkephalin had effects similar to those of normorphine, although a higher proportion of cells was excited. Naloxone was not tested on the enkephalin excitation.

Fry et al. (182) agreed with Segal (445) that the excitatory effects of morphine and [Met]enkephalin on rat hippocampal neurones occurred only in a relatively small proportion of cells and were "nonspecific"; that is, they were not blocked by prior or concurrent application of naloxone. More significantly both levorphanol and dextrorphan excited neurones at similar electrophoretic currents. The same workers later showed that a few cells were inhibited by morphine and enkephalin, and that some of these inhibitions were sensitive to naloxone blockade (185).

These authors maintained that the low levels of opiate binding sites that exist in the hippocampus render the structure particularly unlikely to contain a large number of cells showing specific opiate effects. However, autoradiographic studies suggest that, although most of the hippocampal formation shows a low grain density of tritiated diprenorphine binding, some parts have moderate to high levels (16, 155). A slightly increased grain density occurs in the stratum oriens, which contains the cell bodies of the hippocampal basket cells, as well as the stratum lacunosum-moleculare, where the pyramidal cell apical dendrites ramify and receive the perforant path input. The amygdala contains a particularly high density of grains. The significance of this distribution will be mentioned again below.

More recent studies, both in vivo and in vitro, of the effects of locally applied opiates and opioid peptides on hippocampal neurones have provided evidence that some excitations are "specific," and are probably due to disinhibition of tonically active inhibitory neurones. The first evidence for this came from in vivo studies, in which it was found that the excitation of the pyramidal cells was blocked by concurrent microelectrophoretic application of magnesium ions (presumed to prevent local transmitter release) or the GABA antagonist bicuculline (219, 525). It is known that the relatively small number of basket cells in the hippocampus exert a significant inhibition of pyramidal cell activity, probably by releasing GABA (108). Further support for this mechanism of excitation by disinhibition was the finding (525) that the period of inhibition that follows transcallosal stimulation was reduced by [Met]enkephalin. This inhibition is due to activation of basket cells both directly (feed-forward), and recurrently from the pyramidal cells (feed-back), and these in turn inhibit the pyramidal cells by releasing GABA. During enkephalin-induced basket cell inhibition, the recurrent inhibitory mechanism is eliminated.

2. In Vitro Experiments. In vitro experiments on the rat hippocampal slice preparation largely support the interpretation that pyramidal cell excitation is the result

of disinhibition (157, 310, 354, 363). Only inhibitory effects of microelectrophoretically applied enkephalins could be found during extracellular recording from pyramidal cells and these were not stereospecific and not prevented by naloxone. On the other hand, when added to the perfusate, [Met]enkephalin (1 to 150 μM) increased the amplitude of the field population spike response of the pyramidal cells; a similar effect was produced by picrotoxin (309). This increase in field potential was also observed by Corrigall and Linseman (93) when morphine (500 nM to 50 μM) was applied to the hippocampal slice preparation. This action of morphine was also produced by levorphanol, but not by dextrorphan, and was reversed by naloxone (100 nM to 10 μM). Valentino and Dingledine (480) studied the effects of a variety of opioid analogues on the potentiation of the field population spike as a function of the stimulus voltage. They found the following order of potency: [D-Ala^2,D-Leu^5]enkephalin > [D-Ser^2,Leu^5]enkephalin-Thr > β-endorphin > morphiceptin ≫ morphine ≫ ethylketazocine. They drew the appropriate conclusion that the receptor type involved could not be identified at present. Autoradiographic studies suggest a μ-binding site in striatum pyramidale and oriens (198, 222).

Dunwiddie et al. (157) subjected brain slices to varying degrees of hypoxia in an attempt to destroy selectively the interneurones. Such treatment eliminated the effect of enkephalin whilst not significantly altering the field potentials recorded from slices. In further experiments (310), they identified, electrophysiologically, interneurones located close to the border of stratum pyramidale and stratum oriens. This is a part of the hippocampus that shows a relative abundance of autoradiographic binding sites. The extracellularly recorded firing of these cells was depressed by [D-Ala^2,Met^5]enkephalinamide though not by N-acetyl[Met]enkephalin and this action was reversed by naloxone. Deadwyler and Robinson (120, 418) reported that morphine sulphate (1 mM) caused a depolarization and discharge of CA1 neurones that was partially blocked by naloxone (100 μM). They considered that these changes could result from a reduction in tonic inhibitory synaptic inputs; unfortunately the concentrations used were so high that useful conclusions cannot be drawn.

Gahwiler (187) recorded intracellularly from pyramidal cells taken from newborn rats and maintained in culture. Low concentrations (10 to 100 nM) of the opioid peptides [D-Ala^2,MePhe^4,Met(O)^5]enkephalin-ol, [Met]enkephalin, and [Leu]enkephalin and higher concentrations (1 to 10 μM) of opiates (fentanyl and morphine) all caused an increase in the number and amplitude of spontaneous EPSPs, and increased the frequency of action potential firing. There was no detectable change in postsynaptic membrane properties and all the compounds tested caused the appearance of occasional paroxysmal depolarizing shifts. The calcium action potentials that could be induced in the presence of TTX were not affected by [D-Ala^2,MePhe^4,Met(O)^5]enkephalin-ol (1 μM) (205). The actions of opioid peptides were prevented by naloxone (10 nM to 10 μM). Bicuculline mimicked the effects of the opioid peptides, but the action of bicuculline was not blocked by naloxone.

If opioid peptides excite hippocampal pyramidal cells through disinhibition, then spontaneous inhibitory postsynaptic potentials (IPSPs) should be decreased with peptide application. At normal resting potentials spontaneous IPSPs are difficult to detect, but they are made more apparent by converting them to depolarizing potentials by the use of KCl-containing electrodes and by adding barbiturate to the perfusate. Nicoll et al. (363) recorded from CA1 cells of the rat with intracellular electrodes. The spontaneous and evoked IPSPs were reversibly abolished by the addition of [D-Ala^2,D-Met^5]enkephalinamide (10 nM to 5 μM) or morphine (1 μM) to the perfusate. These IPSPs were blocked by picrotoxin or bicuculline, evidence which strongly suggests that they were due to the release of GABA from spontaneously active interneurones. The response of the CA1 cells to exogenous application of GABA was not affected by the enkephalin analogue, which implies that the main action of the opioid is to inhibit firing of, or transmitter release from the inhibitory interneurones. Naloxone (2 μM) reversed the effect of the enkephalin analogue. Essentially similar though less complete studies have been made by others (451, 336).

The conclusion that pyramidal cell excitation by opioids is entirely due to inhibition of inhibitory interneurones was disputed by Haas and Ryall (206) and Dingledine (130), who used both extracellular and intracellular recording. They found that enkephalin and [D-Ala^2,MePhe^4,Met(O)^5]enkephalinol caused no change in the IPSP recorded from CA1 pyramidal cells, but increased the amplitude of the EPSP that followed stimulation of Schaffer collaterals. This effect occurred without detectable change in the soma membrane properties. This action is not necessarily incompatible with the disinhibition proposed on the basis of extracellular studies. Some inhibitory interneurones synapse not only onto cell bodies but also the dendrites of the pyramidal cells. GABA released at synapses on pyramidal cell dendrites may not cause any detectable change in the properties of the pyramidal cell soma membrane, but could be effective in shunting out the EPSP near, or more proximal to, its site of generation in the stratum radiatum (130). Inhibition of the inhibitory cells would reduce this tonic effect and thereby increase the amplitude of the EPSP. Haas and Ryall (206) presented some evidence against this; namely, that enkephalin did not change the depolarization caused by microelectrophoretic application of DL-homocysteic acid to the dendritic region of the pyramidal cells, which is the site of generation of the EPSP. This suggests that enkephalins increase the size

of the EPSP by presynaptic facilitation. It is possible, however, that GABA released from inhibitory cells acts tonically to reduce release of the transmitter that is responsible for the EPSP; GABA mediates both pre- and postsynaptic inhibition at other sites. If this were the case, removal of this inhibition by enkephalin would indirectly enhance release of excitatory transmitter. The finding that the GABA antagonist bicuculline produces an increase in the amplitude of the EPSP closely similar to that caused by morphine and enkephalin, is compatible with such an interpretation.

Tentative conclusions may be made regarding opioid actions in the hippocampus (fig. 9). First, the membrane potential and conductance of the soma of pyramidal cells are unaffected by opioids. Second, opioids excite pyramidal cells. The mechanism of this seems to be an increased electrotonic spread of EPSPs generated in the dendrites, so that they more readily reach the spike initiation region in the soma. This increased electrotonus probably derives from an increased resistance of the dendritic membrane, which occurs secondarily as a result of removal of a tonic action of GABA. The GABA is released from inhibitory interneurones intrinsic to the hippocampus which makes synaptic contact at least in part on electrotonically distant dendrites of pyramidal cells. The influence of such an interneurone must be reduced by opioids, presumably by hyperpolarization and/or inhibition of GABA release. The challenge remains to record from such cells, identify them, and apply opioids.

In all the experiments discussed, the majority of neurones lie in the stratum pyramidale (CA1 or CA3) of the hippocampus, with a few cells in the dentate fascia (206). The only other structure in the hippocampal formation that has received attention is the amygdaloid nucleus. This is of interest because parts of the amygdala contain a particularly high density of opiate binding sites (16). Neurones in the cat amygdala were excited by systemic administration of opiates (85) but the mechanism of this action was not studied.

I. Cerebellum

The action of opioids on neurones of the cerebellum has been neglected by electrophysiologists probably because of its negligible content of opiate binding sites (395). A small proportion (about 20%) of cerebellar Purkinje cells was inhibited by [Met]enkephalin, β-endorphin, and normorphine when these substances were administered electrophoretically. A similar proportion of neurones was excited, and it was not stated whether either of these actions was reversed by naloxone (364).

III. Peripheral Nervous System

The peripheral nervous system of vertebrates has been the subject of numerous electrophysiological studies of opiate action, both in the hope that these tissues may provide a useful model system for the study of central effects, and in test of the hypothesis that opiates may have effects on peripheral structures relevant to analgesia.

A. Effects on Neuronal Properties

1. Extracellular Recording. a. COMPOUND ACTION POTENTIALS. In the frog sciatic nerve, a period of tetanic stimulation is followed by a positive afterpotential which reflects a hyperpolarization of nerve fibres (290). This was increased by morphine in concentrations less than 1 μM, and this effect was not seen when both morphine and a 1000-fold higher concentration of levallorphan was used. A similar augmentation of the afterhyperpolarization was observed in the cat sural nerve in vitro, although rather high morphine concentrations were required (50 to 100 μM) (258). This prolonged afterhyperpolarization probably contributed to the increased refractoriness that was observed. Similarly high concentrations of naloxone (20 μM) partially reversed this effect of morphine. When studied in vivo, the cat sural nerve responded differently to morphine administration. Doses of 2 mg/kg (intra-arterial or intravenous) resulted in a reduction in the Aδ and C fibre compound action potential amplitudes. These effects were not seen on the sural nerve in vitro and thus may have occurred near the relevant sensory receptors. In the same study, the guinea-pig and rabbit vagus nerves showed a reduction in the size of the afterhyperpolarization in the presence of morphine (100 to 500 μM). These concentrations far exceed those required to act on neurones in some other parts of the peripheral nervous system (see below). Naloxone was an effective antagonist in only some experiments despite its application in a concentration (20 μM) which is more than 1000 times greater than its affinity for opiate binding sites in the brain (395). Myelinated fibres are unaffected by opiates unless the concentration is raised to toxic levels (342).

Cairnie and Kosterlitz (67) and Kosterlitz and Wallis (284) studied the effects of morphine on impulse propagation in the hypogastric nerve and the nerve to the nicitating membrane of the anaesthetized cat. They also made extracellular recordings by the sucrose gap technique from the isolated vagus nerve of the rabbit and the isolated hypogastric nerve of the cat. In these in vitro experiments, morphine (up to 30 μM) did not affect the resting potential, the A or C fibre compound action potential, or the afterhyperpolarization following the C fibre compound action potential. The membrane potential of frog sympathetic ganglion cells has also been studied by the sucrose gap technique (508, 509). [Met]enkephalin (1 μM), [D-Ala^2,Met^5]enkephalinamide, and morphine (5 μM) each hyperpolarized the ganglion cells, and these effects were blocked by naloxone (1 μM). The slow IPSP was also reduced by morphine and it was concluded that this was due to a hyperpolarization of preganglionic nerve terminals. Minker et al. (341) recently reported that synaptic transmission was blocked by morphine in the isolated sympathetic ganglia of frog

and rat. Very high concentrations of morphine were applied (200 μM to 800 μM) but antagonism by naloxone (10 nM-1 μM) was described. The effect of morphine was less in high calcium solutions.

b. SINGLE NEURONES. Sato et al. (428, 429) first showed inhibition by opiates of spontaneous firing of neurones in the myenteric plexus of the guinea-pig ileum—a tissue known for several years to contain morphine-sensitive neurones (285). Subsequent studies (131–133, 162, 375) showed that this inhibition by opiates was concentration-dependent. Some cells were inhibited by concentrations of normorphine as low as 1 nM, and 100 nM inhibited cell firing by at least 50%. Morphine, normorphine, ketocyclazocine, cyclazocine, buprenorphine, and levorphanol (but not dextrorphan) all had similar inhibitory action and the effective concentrations were similar to those required to inhibit the acetylcholine output from the guinea-pig ileum excited by electrical field stimulation. The effects of these agonists were reversed or prevented by naloxone [but not (+)-naloxone] and by other opiate antagonists (267).

In experiments of this type, the spontaneous firing of myenteric neurones is probably induced by the contact of the recording suction electrode with the excitable axon hillock region of the neurone. Opiates also inhibited the excitation of myenteric neurones by 5-HT (428, 429, 131–133), sodium picrate, caerulein, pentagastrin, and acetylcholine (429). The inhibitory action of morphine was not affected by the absence of calcium from the perfusing solution, indicating both that it is a direct action on the cell from which activity is being recorded, and that the action itself does not require extracellular calcium ions (132, 375). Evidence has been presented (266) that this acute inhibitory effect of morphine on myenteric neurone firing probably does not result from inhibition of adenylate cyclase and a reduction in intracellular cyclic AMP levels. The inhibitory action of morphine was unaffected in four conditions in which intracellular cyclic AMP levels might be expected to be elevated; these were prior and concomitant exposure to cyclic AMP, dibutyryl cyclic AMP, prostaglandin E_2 and the phosphodiesterase inhibitor, 3-isobutyl-1-methylxanthine.

[Met]enkephalin, [Leu]enkephalin, and β-endorphin also inhibited cell firing, and the potency of these substances was similar to or slightly greater than that of normorphine (374, 499). In the circumstances of these experiments enzymatic breakdown of enkephalin was likely to be minimal—the very small amount of tissue present (a few milligrams) was constantly superfused by a peptide-containing solution.

The same neurones that were inhibited by dihydromorphine were also inhibited by [D-Ala^2,D-Leu^5]enkephalin (160). In some neurones, the effective concentrations (1 to 10 nM) of these agonists were so low that it might reasonably be expected that they were acting on the μ- and δ-binding sites known to occur in the myenteric plexus (313). Indeed, naloxone (1 nM) was more effective in antagonizing dihydromorphine inhibitions than [D-Ala^2,D-Leu^5]enkephalin inhibitions. This constitutes evidence that both μ and δ receptors may sometimes occur on the same cell; the immediate consequences of each receptor occupation may differ but both lead to inhibition of cell firing.

Tachyphylaxis to the inhibitory action of normorphine on cell firing occurred when low concentrations (50 nM) were used, and when the inhibition of firing was slight (267). During application of higher concentrations of normorphine (100 nM to 1 μM), the inhibition of cell firing persisted throughout the period of application (up to 1 hr). However, after such a prolonged application, the neurone was less sensitive to a subsequent test dose with a lower concentration (267). The firing of neurones that had been incubated for 24 hr in opiate-containing solutions was no longer inhibited by even higher concentrations of morphine than those previously effective. Control incubations in morphine-free solutions did not change the sensitivity of the neurones to normorphine. Levorphanol incubation had similar effects, but neurones incubated with dextrorphan retained their usual sensitivity to opiates (369). Activity has also been recorded from neurones in tissue removed from animals treated during 3 days with increasing dose of morphine. The tissue was placed in a solution in vitro that contained normorphine or morphine so as to simulate the in vivo environment. Such neurones were similar in all respects to those incubated for 24 hr in the presence of morphine; tolerance to the usual inhibitory effects of opiate agonists was apparent (267).

Neurones that had been exposed to morphine either in vivo (by pretreatment of the guinea-pig) or in vitro (by 24 hr of incubation) were excited by naloxone (369, 381). This occurred within seconds of naloxone reaching the neurones and cells fired at rates that were significantly higher than those observed in any other experimental circumstances. This excitation appeared to be the result of the displacement of the agonist from the opiate receptor for it was caused only by the (−)-isomer but not the (+)-isomer of naloxone and another opiate antagonist (267). Incubation of tissue in solutions that contained levorphanol also sensitized the neurones to the excitatory effects of naloxone, but naloxone did not cause excitations of neurones that had been incubated with an equal concentration of dextrorphan.

The naloxone excitation described above presumably underlies the naloxone contracture of the ileum that has been removed from a morphine-treated guinea-pig (161, 441). That is, the contracture, which is largely blocked by atropine (179, 441), is due to a massive release of acetylcholine by action potentials in the myenteric neurones. An important point is that the release of acetylcholine is not the *cause* of the naloxone excitation—because the excitation persists in the presence of atropine and hexamethonium. This implies that the release of

acetylcholine is the *result* of the naloxone excitation, which in turn implies that the primary action of naloxone on tissue chronically exposed to morphine is not an increase in the amount of transmitter released per action potential, but an increased number of action potentials resulting from the hyperexcitability of the neurone. In addition, the excitation of single cells by naloxone is probably much potentiated by the release of noncholinergic synaptic transmitters (such as substance P) onto each other. This could provide a strong positive feedback and is presumed to underlie the particularly large excitation of single cells observed electrophysiologically and the very substantial release of acetylcholine.

2. Intracellular Recording. a. MYENTERIC PLEXUS NEURONES. Morphine, normorphine, [Met]enkephalin, [Leu]enkephalin, and levorphanol (but not dextrorphan) each hyperpolarized a proportion of myenteric neurones in the guinea-pig ileum (366, 370, 371). Figure 10 illustrates this action of morphine and [Met]enkephalin. The effective concentrations (1 nM to 1 μM) were similar to

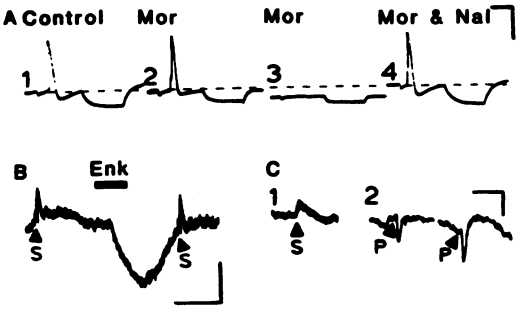

FIG. 10. Effect of normorphine and enkephalin on three different myenteric neurones. Intracellular recordings of membrane potential. A, a single focal stimulus was applied to the surface of the myenteric ganglion (note stimulus artifact). This excited a cell process of the impaled neurone; the action potential propagated to the soma where it was recorded after a delay of a few milliseconds. After the action potential a current (500 pA) pulse was passed through the recording electrode; the resulting hyperpolarization is a measure of neuronal input resistance. 1, control; 2, 2 min after changing to a solution containing normorphine (1 μM). The hyperpolarization that occurred accentuated the inflexion on the rising phase of the action potential; this is due to a delay in invasion of the soma from the proximal process. 3, 3.5 min after changing to the normorphine solution. The membrane is hyperpolarized and input resistance is much reduced. The action potential no longer invades the soma. 4, 6 min after changing to a solution that contained both normorphine (1 μM) and naloxone (100 nM). Calibrations: horizontal 10 ms, vertical 50 mV. B, during the period indicated by ▬, the perfusing solution was changed to one that contained [Met]enkephalin (100 nM). This caused a marked hyperpolarization (the apparent delay in the onset of the hyperpolarization is accounted for by the time required for a new solution to arrive at the tissue bath). At the times indicated by ▲ (S), [Met]enkephalin was ejected by electrophoresis (80 nA, 10 s) from the tip of a micropipette positioned within 5 μm of the soma membrane. The depolarizing response was not blocked by naloxone; the hyperpolarizing response to perfusion was blocked by naloxone. C, ▲, times of electrophoretic application of [Met]enkephalin. S, application (60 nA, 20 s) to the soma membrane. P, application (60 nA, 10 s and 60 nA, 20 s) to the cell process 10 μm from the soma. Only application to the cell process caused a hyperpolarization. [Reproduced, with permission, from North and Tonini (371) and North, et al. (370)].

those required to inhibit cell firing recorded with an extracellular suction electrode (see above) and similar to those required to inhibit the acetylcholine output from the myenteric plexus following electrical field stimulation. A similar hyperpolarization by morphine was observed in cat myenteric neurones (1 to 5 μM) (506).

This membrane hyperpolarizing effect of the opiates persisted in calcium-free solutions (370). In fact, the hyperpolarization was larger and more readily observed in solutions that contained a reduced calcium ion and disappeared in solutions that contained an elevated calcium ion concentration (346). The hyperpolarization was associated with an increase in the membrane conductance to potassium ions, probably as a result of a morphine-induced increase in free intracellular calcium (346). Concentrations of morphine and enkephalin (1 to 10 nM), which were often too low to hyperpolarize the resting membrane of most neurones, prolonged the afterhyperpolarization that followed one (82) or a train (82, 474) of action potentials. This afterhyperpolarization is caused by calcium entering the neurone and activating the membrane potassium conductance. It was proposed that a primary action of morphine may be to inhibit calcium binding to an intracellular site or extrusion from the cell, thus prolonging the afterhyperpolarization. A similar opiate action in the absence of action potentials might also cause a rise in free calcium concentration leading to a membrane hyperpolarization although there is no direct evidence for this (474). The prolongation of the afterhyperpolarization may be similar to that which was recorded extracellularly in the frog sciatic (290) and cat sural nerves (258).

North et al. (370) suggested that the hyperpolarizing effect of morphine and enkephalin on myenteric neurones does not take place exclusively on the soma membrane and presented evidence that the potential change observed with an intracellular electrode in the soma may be the electrotonically spreading remnant of a larger hyperpolarization occurring on one or more cellular processes. The most likely site of the generation of the hyperpolarization is the proximal part of the cell process (347) because the propagation of action potentials initiated at a distance from the soma is blocked by enkephalin, morphine, and levorphanol but not affected by dextrorphan (347).

The opiate-induced hyperpolarization and conductance increase of myenteric neurones markedly reduces their excitability. This may contribute to the inhibition of acetylcholine release by morphine that is observed when the ileum (or stripped muscle/plexus preparation) is stimulated by an electric field. The morphine hyperpolarization could prevent the excitation of the myenteric neurones, or prevent propagation of the action potential along cell processes into the varicosities presumed to be sites of transmitter release. Such possibilities are fully discussed by North and Tonini (371), North (368), and Szerb (468). They offer an explanation for the

findings 1) that the inhibitory action of morphine on acetylcholine release can be overcome by an increase in the strength or duration of the electric field application even though the stimulus was supramaximal before morphine application (e.g. 161), 2) that the sensitivity to morphine is higher when submaximal electrical stimulation is used (95), and 3) that morphine reduces the size of the pool from which acetylcholine is released in the myenteric plexus rather than reducing the rate of release from a pool of fixed size (139).

Neurones that had been incubated in the presence of morphine (1 μM) for 24 hr had the same membrane potential as those incubated in control solutions suggesting that tolerance to the hyperpolarizing action of morphine had developed. Naloxone had no effect on the membrane potential of control incubated cells, but naloxone depolarized neurones incubated with morphine (253). This action of naloxone was stereospecific, and is presumed to underlie the excitations observed with extracellular recordings. The acute effect of morphine on myenteric neurones is a membrane hyperpolarization; after 24 hr this passes off and the depolarizing action of naloxone becomes apparent. An excitation by naloxone of neurones in morphine-dependent animals has been observed in several sites in the central nervous system (1, 163, 181, 184). This may imply that the acute action of opiates on those central neurones is also a membrane hyperpolarization. Such a hyperpolarization may not be detectable by either an intracellular or extracellular electrode located near the soma, if it is generated at a nonsomatic site, but a depolarizing effect of naloxone on the processes of a morphine-sensitive cell could initiate action potentials that invade the soma and would be readily detectable.

b. PRIMARY AFFERENT NEURONES. Opiates may act to inhibit the release of transmitter from the central terminals of primary afferent neurones including those which convey nociceptive information. In this case, one might expect to observe opiate effects on the membrane of the cell somata in the sensory ganglia. Such effects have been observed in immature sensory ganglia, and will be discussed below. However, in adult ganglia the results have been rather disappointing. Normorphine (1 μM to 1 nM) had no effect on the resting membrane potential of A or C primary afferent neurones of the rat (501). [Met]enkephalin and [D-Ala^2,D-Leu^5]enkephalin were also ineffective. None of these opioids affected input resistance or the action potential waveform, either in control conditions or after the spike was widened with 4-aminopyridine or barium. Similarly negative results were obtained in the study by Shefner et al. (449) on the rabbit isolated nodose ganglion. In identified C cells, neither morphine (1 μM to 100 μM) nor [D-Ala^2,D-Leu^5] enkephalin caused changes in action potential configuration, input resistance, or resting potential. However, lower concentrations of morphine (1 nM to 100 nM) do have effects on rabbit nodose ganglion C cells. Higashi et al. (223) reported that morphine increased the conductance of some C cells, and this was sometimes accompanied by a hyperpolarization. They also reported that the action potential waveform was affected by morphine, but these results were variable from cell to cell and frequently transient. There seems little doubt that opioids do have effects on primary afferent ganglion cell somata, but more detailed studies are required to characterize them fully. The conclusion of Higashi et al. (223) was that opiates may cause transient increases in the intracellular calcium levels. In appropriate circumstances, this could result in both an inhibition of calcium entry and a membrane hyperpolarization; both responses seem to be transient, particularly with higher concentrations. Both rabbit nodose ganglia (449) and rat DRG (172) have opiate binding sites.

B. Effects on Junctional Transmission

1.Transmission between neurones. a. INFERIOR MESENTERIC GANGLION. Konishi et al. (278) found that [Met]enkephalin, [Leu]enkephalin (1 to 20 μM), and [D-Ala^2,Met^5]enkephalinamide (100 nM to 1 μM) reduced the amplitude of the cholinergic EPSP recorded intracellularly from neurones in the guinea-pig isolated inferior mesenteric ganglion. These inhibitory effects were completely prevented by naloxone (1.5 μM); like the hyperpolarization of myenteric neurones mentioned above, the effects were more marked in solutions containing a reduced calcium ion concentration. The membrane potential and input resistance of the ganglion cells was not changed by the opioid peptides, and nor was the nicotinic depolarization evoked by microelectrophoretic applications of acetylcholine; this argues in favour of a presynaptic action to depress acetylcholine release. The EPSPs resulted from the excitation of several presynaptic nerve fibres and it is not possible to determine whether enkephalin reduced the release of transmitter per action potential, or reduced the number of action potentials invading the presynapic terminals.

Morphine has been reported to have a similar presynaptic effect on this preparation except that very high concentrations (20 to 800 μM) were required (50). Even at 20 μM morphine the depression of the EPSP was slight and took 10 to 15 min to occur. Naloxone (5.5 μM) only partially prevented and did not reverse this action of morphine, casting some doubt on the interpretation that this is mediated by opiate receptors. In a later paper, Konishi (279) proposed that the presynaptic inhibition caused by enkephalin might occur under physiological circumstances. High frequency (50 Hz, 8 s) stimulation of one set of nerve fibres entering the ganglion was followed by a period of depression of the cholinergic EPSP evoked by another input. This depression was blocked by naloxone (3 μM), indicating that it may result from release of an endogenous opioid. A noncholinergic slow EPSP in the same ganglion is also depressed in a naloxone-sensitive manner by a brief period of high

frequency stimulation of nerves entering the ganglion (251). These results suggest that an opioid peptide can be released, and its effects on synaptic transmission detected. Ideally, stereoisomers of naloxone and other antagonists should be examined. The physiological circumstances in which such a system operates are unknown; in guinea-pig coeliac ganglion, enkephalin is located within nerve terminals making only axo-somatic and axo-dendritic contacts with principal ganglion cells (277). Thus, one has the physiological evidence of presynaptic inhibition without the anatomical substrate, a situation previously noted for the dorsal horn.

b. MYENTERIC PLEXUS. Both morphine and [Met]enkephalin depressed synaptic potentials in the guinea-pig myenteric plexus (Cherubini, Morita, and North, unpublished observations) The fast EPSP, which is caused by acetylcholine, was reduced in amplitude by concentrations of [Met]enkephalin ranging from 100 pM to 10 μM. Similar findings have been made in the cat ciliary ganglion (268). Depression of the slow EPSP, which is noncholinergic and may be mediated by substance P (348), occurred at slightly higher concentrations. Both effects were blocked by naloxone (10 to 100 nM).

2. Autonomic Neuroeffector Transmission. The only junction that has received systematic electrophysiological investigation is that between the postganglionic sympathetic fibres and the smooth muscle cells of the mouse vas deferens. In these experiments, the amplitude of the excitatory junction potential (EJP) was used as a measure of the amount of transmitter released after a single stimulus to the intramural nerves. This technique offers significant advantages over experiments in which the release of transmitter is chemically assayed and which require repetitive stimulation in order to obtain detectable release. It also offers advantages over measurement of the contractile response of the longitudinal muscle of the vas deferens—for it avoids complications arising from direct actions of drugs on the muscle fibres.

Henderson and North (217) showed that normorphine caused a dose-dependent reduction in the amplitude of the EJP recorded from cells of the mouse vas deferens. Spontaneous EJPs were unaffected (216). The observation was confirmed by Illes et al. (245), Bennett and Lavidis (39), and Ito and Tajima (247). This action was reversed or prevented by naloxone, and it was also observed with levorphanol, but not by dextrorphan. The EC50 for normorphine was about 500 nM, which agrees well with the EC50 in experiments in which the reduction in the overflow of labelled NA or the contractile response of the muscle was measured. [D-Ala^2,Met^5]enkephalinamide and [D-Ala^2,D-Leu^5]enkephalin also reduced the amplitude of the EJP with ED50s of 49 nM and 510 pM, respectively (372).

This technique, which measures directly a presynaptic action of opiates, has been pursued in three directions—the role of cyclic nucleotides, the role of calcium ions, and the development of tolerance and dependence. North and Vitek (372) found that the ED50s for morphine and for [D-Ala^2,D-Leu^5]enkephalin were not changed when these agents were applied in the presence of cyclic AMP, dibutyryl cyclic AMP, or dibutyryl cyclic AMP together with a phosphodiesterase inhibitor [either 1-methyl-3-isobutylxanthine (IBMX), or 1-ethyl-4-hydrazino-IH-pyrazolo (3,4-b) pyridine-5 carboxylic acid, ethyl ester, hydrochloride (SQ 20,006)]. These findings were construed as evidence against the hypothesis that a reduction in cyclic AMP levels in the nerve terminal is an essential step in the inhibition by opiates and opioid peptides of transmitter release.

There have been several reports that an elevation of calcium ion concentration overcomes the usual inhibitory effect of morphine (39, 243, 245, 340). However, the changes in calcium ion concentration have had marked effects both on neuronal excitability and on transmitter release even in the absence of morphine. In the experiments of Illes et al. (245), the stimulus strength required to evoke an EJP of given amplitude in the presence of a reduced calcium concentration was so much higher than in the control situation that it is unlikely that the same population of intramural nerves was being excited in the two circumstances. Bennett and Lavidis (39) argued that there is a direct competition between morphine and calcium ion at a hypothetical membrane calcium binding site. This conclusion is based on a double reciprocal plot with a single concentration of normorphine (as inhibitor), and relies on the probably unfounded assumption that the amplitude of the EJP is simply related to the proportion of calcium binding sites that are occupied (the occupancy assumption). Inspection of their results (fig. 1 of Ref. 39) indicates that the percentage of depression of the EJP amplitude by normorphine is the same at various calcium concentrations. Both studies are in accordance with the hypothesis that an important action of opiates is to inhibit the voltage-dependent calcium current that occurs at the nerve terminal membrane, but neither offer direct evidence that morphine and calcium ions compete for a common site. At frequencies greater than 1 Hz, the EJP in the mouse vas deferens underwent facilitation during the first 10 pulses, an effect attributed by Bennett and Florin (38) to residual calcium in the nerve terminal. The effect of morphine was most marked on the first EJP of the train, which is again compatible with a blockade of calcium entry during the presynaptic action potential. Further evidence that a block of inward calcium currents may be involved in the opiate action is the observation that normorphine and enkephalin do not inhibit the spontaneous EJPs in the mouse vas deferens (39, 216, 247); spontaneous EJPs probably do not require transmembrane movements of calcium through a voltage-dependent calcium channel (37).

A more recent study (340) compared the action of normorphine with that of magnesium, which was assumed to inhibit NA release by blocking calcium entry

into the nerve terminals. The report drew attention to the difficulties in demonstrating "competitive" interaction even between calcium and magnesium because of the limits to the range of concentrations that could be used. Normorphine acted in a way that was clearly "non-competitive" with calcium, but the experiments failed to distinguish among the actions of morphine that could block action potential propagation, inhibit entry of calcium into depolarized terminals, or reduce the ability of intracellular calcium to evoke release. Similar negative results were obtained by nonelectrophysiological methods (243).

When mice are treated with increasing doses of morphine during 2 days they become highly tolerant and dependent. The vasa deferentia removed from such mice showed a 12-fold reduction in sensitivity to the depression by normorphine of the EJP. The sensitivity to depression of the EJP by adenosine was not changed (373). In another study, mice were implanted with morphine pellets and the vasa deferentia removed after 3 days (244). Tolerance was also apparent in the morphine-treated mice, but its degree was dependent upon the excitability of the noradrenergic fibres. Evidence was presented that the more excitable fibres displayed more tolerance to the action of opiates than the less excitable fibres, implying that an important primary action of opiates may be to alter fibre excitability. When naloxone was applied to the vas deferens of mice chronically pretreated with morphine, there was an increase in size of the EJP to levels above those that would be expected from simple reversal of acute morphine action (373).

A depression by morphine of the cholinergic EJP in both the longitudinal and circular muscle of the guinea-pig ileum has been observed (247). The noncholinergic nonadrenergic inhibitory junction potential (IJP) was unaffected. The depression of the EJP became less as the extracellular calcium concentration was increased.

3. Skeletal Neuromuscular Junction. Pinsky and Frederickson (399) showed that both morphine (10 μM) and nalorphine (20 μM) reduced the amplitude of the end-plate potential recorded intracellularly from frog sartorius fibres, but insufficient experiments were reported to make useful conclusions. Experiments in which the contractile response of skeletal muscle was measured after nerve stimulation (34, 457) indicated that the depressant actions of opiates occur only at high concentrations, the rank order of potency of various opiates did not correlate with analgesic potencies, and that the effect was not stereospecific and not antagonized by naloxone. Morphine and meperidine reduced the amplitude of the end-plate potential when applied in very high concentrations (100 μM to 1 mM); the effects were not reversed by naloxone (272, 464).

A more recent report confirmed and extended the observation of Pinsky and Frederickson to enkephalin. [Met]enkephalin (10 to 30 μM) depressed the evoked end-plate potential at the frog neuromuscular junction (48). Enkephalin did not block action-potential propagation into the terminal because it also inhibited acetylcholine release evoked by focal depolarization of the terminal in the presence of TTX. Spontaneous miniature end-plate potentials were unaffected. Naloxone (10 μM) blocked the effects; it is unknown whether lower concentrations were effective.

These results are of particular interest in view of Nicoll's report (362) that at the "other end" of the same neurones—motoneurones in the spinal cord—are hyperpolarized by enkephalin in the frog, whereas morphine was relatively ineffective. The results suggest that frog motoneurones have an opioid receptor with rather low affinity for naloxone, and which is much more readily activated by enkephalin than by morphine. The consequence of this activation in the somata is a hyperpolarization; the consequence at the terminal is diminished acetylcholine release. In their report, Bixby and Spitzer (48) drew attention to the possibility that the presynaptic inhibition could result from a depression of inward calcium current or an enhancement of outward potassium current in the nerve terminal. The latter would correlate with the hyperpolarization of cell bodies. The parallels with the situation described above for the nucleus locus coeruleus (section II B 7) are obvious.

IV. Invertebrate Nervous System

Simon and Rosenberg (452) and Frazier et al. (177, 178) studied the effects of morphine on the internally perfused voltage clamped squid giant axon. At a very high concentration (1 mM), morphine had a weak local anaesthetic action whether applied to the inside or outside of the membrane. Although there was no change in resting membrane potential, the voltage-dependent potassium and sodium channels were blocked by morphine. There was no effect of morphine at lower concentrations, and the effects of high concentrations were mimicked but not blocked by naloxone. Levorphanol and dextrorphan were equally effective. The authors therefore concluded that the effects were unlikely to be of relevance to actions of morphine on the mammalian nervous system. Similar conclusions might be drawn as to the relevance of studies that showed that morphine blocks cholinergic synaptic transmission in *Aplysia* (486) and the effect of 5-HT in mollusks (94). Both these actions were postsynaptic, in that responses to nerve-released transmitters and microelectrophoretically applied transmitters were reduced in parallel. Minimal effective concentrations of morphine were 10 to 50 μM; other agonists were not tested and naloxone reversal was not examined. A presynaptic action of morphine (10 to 100 μM) in reducing acetylcholine release in *Aplysia* was not blocked by naloxone (478). The actions of opiates on invertebrate preparations have not aroused much interest on account of the large concentrations that are necessary to obtain any effects, the substantial difficulties in measuring behavioural or other pharmacological responses with which

to correlate the effects observed, and because of the initial failure to detect opiate binding sites in invertebrate preparations (392).

More recent studies (286, 458) indicate that binding sites for opioids do exist in some invertebrate species and this may prompt further electrophysiological studies with opioid peptides. Already, one report indicates that [Met]enkephalin prevents the bursting activity of a spontaneously active neurone in *Helix pomatia* (459).

V. Immature Neurones in Culture

Electrophysiological studies of opiate action on cultured neurones were undertaken when it became known that certain neuroblastoma cell lines were rich in opiate binding sites (274). The first study (356) showed little effect of morphine on the resting potential or input resistance of the somatic cell hybrid NG108-15. Barker (personal communication) also found no effect of morphine or enkephalin on the active or passive properties of NG108-15 cells. The finding by Myers et al. (356) that morphine blocked the depolarizing response to microelectrophoretic application of dopamine is of interest because opiates inhibit adenylate cyclase in these neurones, both basal enzyme activity and that stimulated by prostaglandin E_2 (274). It is not known, however, whether dopamine exerts its depolarizing action on these cells by activating adenylate cyclase as it appears to do in some other neuronal systems. It should be stressed that the effective concentrations of morphine in the experiments of Myers et al. (356) greatly exceed those required to inhibit adenylate cyclase.

Neurones cultured from the spinal cord of foetal mice respond to microelectrophoretic application of opioid peptides in four different ways, but only a small proportion of neurones (< 10%) showed any or all of the effects (23–25). The first effect observed was a depolarization associated with a conductance increase that desensitized rapidly and had a reversal potential near +20 mV. The second effect was an inhibitory response that reversed in polarity close to the chloride equilibrium potential. These responses were only partially antagonized even by very high concentrations (100 μM) of naloxone. A third effect of enkephalin was a direct reduction of the current flow induced by microelectrophoretic application of glutamate (probably carried principally by a movement of Na^+ ions). This effect therefore appears not to be ion-specific. The effect was partly reversed by an unstated concentration of naloxone in some cells only. A fourth effect of enkephalin was an elevation of the threshold for spike generation. These experiments on spinal neurones are difficult to interpret for several reasons. First, the effects were highly variable from cell to cell. It is not known which, if any, of the effects have pharmacological importance, for the reversals by naloxone occurred only in some cells, and were generally partial even at very high antagonist concentrations. No experiments were reported with opiates, and it remains extremely important to test stereoisomers of opiate agonists. Second, the actions of the opioid peptides were shared by several other peptides and it is therefore difficult to assign any specific roles for these substances. Third, synaptic connections between neurones were pharmacologically blocked, so that extrapolations of the findings to a functioning neuronal network cannot be easily made.

In another study of cultured spinal cord cells of the mouse (325), synaptic connections were allowed to develop between these cells and cultured DRG cells. Simultaneous intracellular recordings from a DRG cell and an interconnected explant of spinal cord were made. An action potential induced in the former was followed after a short delay by an EPSP in the latter. Microelectrophoretic application of etorphine to the DRG cell did not affect the resting membrane potential, input resistance, or action potential. By contrast, etorphine applied with low microelectrophoretic currents to the spinal cord cell reversibly abolished the EPSP without changing postsynaptic membrane properties. This effect was blocked by concomitant electrophoretic application of naloxone. It was concluded that etorphine inhibited the release of transmitter from DRG cells onto spinal neurones either by preventing action potential propagation into the terminals or by a direct interference with transmitter release. The transmitter that was responsible for the EPSPs was not known, but it is unlikely to be substance P. The DRG cells that were impaled were large, whereas under these conditions, the substance P occurs primarily within smaller cells (Macdonald, personal communication).

Mudge et al. (352) studied the action of [Met]enkephalin and [D-Ala^2,Met^5]enkephalinamide on the properties of chicken DRG cells in culture. No effect was observed on resting membrane potential or input resistance. Both opioid peptides (100 nM–10 μM) produced a small shortening of the action potential duration in about two thirds of cells. This effect was reversed in each of an unstated number of experiments by naloxone (1 μM). When the plateau of the DRG action potential was greatly prolonged by the addition of barium ions (5 mM), the effect of enkephalin was more marked. Furthermore, the action potential that persisted in the presence of TTX (which is carried by calcium ions) rose more slowly in the presence of enkephalin. These experiments suggest that enkephalin may block a voltage-dependent inward calcium current although a facilitation of an outward voltage-dependent current is also possible. As enkephalin also inhibited the release of substance P evoked by high potassium solutions in the cultures (352), it was concluded that the primary effect of enkephalin in reducing transmitter release may be due to a reduction in the voltage-dependent calcium current.

DRG cells of the mouse in culture are similarly affected by opioids (488). The calcium action potential (prolonged in duration by tetraethylammonium) was shortened by [Leu]enkephalin in the concentration range of 20 nM to

5 μM. In a later study (489), the authors found that this action of [Leu]enkephalin was shared by morphiceptin, a selective μ-receptor ligand in appropriate concentrations. It was concluded that both μ and δ ligands had the same effect, a reduction in the calcium action potential, but that some cells were affected only by μ agonsits and others only by δ agonists. It cannot be considered that the ligands used at the concentrations applied are selective for one or other receptor type. Differential blockade by naloxone and other antagonists will be necessary to substantiate the receptor selectivity.

Rohon-Beard neurones are cells in the dorsal part of the spinal cord of the developing amphibian that are analogous to primary afferent neurones of mammals. Early in development these cells have long duration calcium action potentials, which disappear at a later stage. Bixby and Spitzer (47) made intracellular recordings in vivo. The found that the Rohon-Beard action potential was shortened by [Met]enkephalin (20 μM). The effect was abolished by naloxone (1 to 10 μM). Enkephalin had no effect on cells at a later stage of development when the action potential was carried predominantly by sodium ions.

In summary, one may conclude that opioids shorten the duration of a calcium action potential in cultured DRG cells of the mouse and chick and in Rohon-Beard cells. It is not certain which receptor type mediates these effects, nor whether they result from a primary reduction in calcium entry or an increase in potassium conductance.

It is generally considered that these changes occurring in the cell somata will reflect changes at transmitter release sites in the spinal cord. This may not be entirely true. First, the insensitivity of the DRG soma action potential to TTX is not shared by the processes—at least not sufficiently for calcium to support propagated activity (127). Second, opiate binding sites in explants of foetal mouse cord are much more dense on the neurites of DRG cells than on the cell bodies themselves (231). Extrapolation of the studies on cultured DRG cells of the mouse and chick to mammalian tissue may be hazardous; mouse DRG cells in culture show predominantly short action potentials (inter alia 414), and in only a small proportion of adult frog DRG cells is a significant calcium component to the action potential evident (246, 275). This is a particular concern since many of the investigations just cited were carried out in high (5 to 10 mM) calcium concentrations. Perhaps more significant is the question of whether drug and transmitter sensitivities of the immature animals persist into adulthood. For some transmitters [e.g. $GABA_B$ effects (156)] this is clearly not true. Studies on adult mouse DRG cells would be helpful.

The calcium action potential in cultured hippocampal pyramidal cells was unaffected by opioids, even though these cells do not show the same disinhibitory actions of opioids described in the adult hippocampus (205).

Crain and collaborators (100) used a cultured explant of foetal mouse spinal cord with attached DRG to study opiate effects. Extracellular electrodes were used to record depolarization of the dorsal horn neurones after a single stimulus to the DRG. The multiunit depolarizing synaptic response was almost completely eliminated by the presence in the culture medium of opiates or opioid peptides (fig. 11). The effect of opiates occurred within 3 to 10 min and reversed on washing with a drug-free solution. The effective concentrations of morphine were low (10 nM to 1 μM), the action was reversed within a few minutes by naloxone (10 nM to 1 μM), and levorphanol but not dextrorphan mimicked the effect of morphine. Naloxone itself slightly enhanced the sensory-evoked dorsal horn response suggesting that the stimulus to the DRG leads also to the release of opioid peptides from dorsal horn elements which normally reduces the

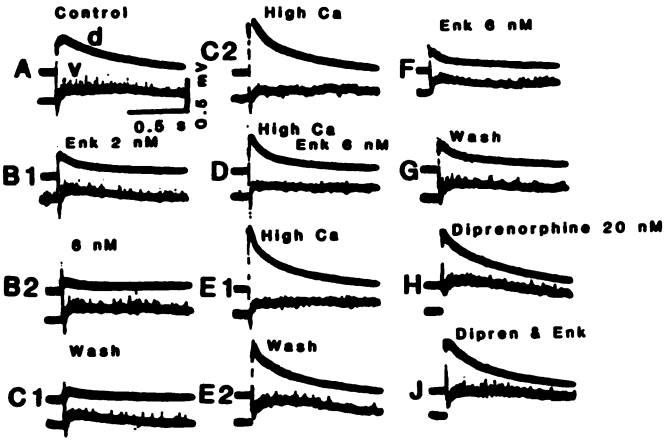

FIG. 11. Selective depression of sensory-evoked dorsal horn network responses in foetal mouse spinal cord-dorsal root ganglion (DRG) explant to low concentrations of an opioid pentapeptide [D-Ala^2, $MePhe^4$, $Met(O)^5$]enkephalin-ol. A, characteristic short latency negative slow wave evoked in dorsal cord (d) by DRG stimulus; response of ventral cord (v) consists of positive-negative slow wave sequence and spike barrage. B_1, several minutes after introduction of a low concentration of the enkephalin analogue (2 nM), the dorsal cord response was markedly attenuated whereas the ventral cord discharge showed no significant change. B_2, after increasing the enkephalin level to 6 nM, dorsal cord slow wave response was almost abolished. C_1, 5 min after return to balanced salt solution (BSS), dorsal cord response remained small. C_2, much larger dorsal cord response was evoked (about 1 hr later) after exposure to BSS containing higher Ca^{++} concentration (3 mM vs. usual 1 mM). D, after introduction of the enkephalin analogue (6 nM) in BSS with 3 mM Ca^{++} dorsal cord response was again depressed, but not as completely as in regular BSS (cf B_2). E_1, after return to BSS within 3 mM Ca^{++} the dorsal cord response was fully restored (by 30 min). E_2, dorsal cord response remained large even after 20 min in regular BSS (cf A). F, within 9 min after introduction of the enkephalin analogue (6 nM) dorsal cord response was again sharply reduced, and in this case the large ventral cord response (E_2) was also partly attenuated (cf B_2). G, slow recovery after 9 min in BSS was accelerated by introduction of diprenorphine (20 nM). By 20 min, dorsal cord potential was fully restored (H) and ventral cord discharge also became larger (cf G). (In most cultures, diprenorphine enhanced only dorsal cord responses.) J, presence of diprenorphine (20 nM) prevents depressant effects of enkephalin (6 nM). [Reproduced, with permission, from Crain et al. (99).]

amplitude of the recorded potential. The potencies of a series of opiates and opioid peptides were remarkably proportionate to their relative analgesic potency in the intact animal (99). The opiate actions were much inhibited by procedures that increased transmitter release such as 4-aminopyridine or high calcium solutions (98).

The acute depressant effect of morphine on the dorsal horn response was not observed in cultures that had been previously exposed to opiates for 2 to 3 days, even with acute increases in concentration of up to 100-fold (96). Similar tolerance occurred as a result of incubation with [D-Ala^2,MePhe^4,Met(O)^5]enkephalin-ol (10 nM). The development of tolerance could be prevented by including naloxone (100 nM) in the incubating solution, or by reducing the temperature to 20°C. A similar tolerance was induced by chronic exposure to 5-HT, and this was associated with cross-tolerance to opioids (97). The introduction of naloxone into cultures that had been incubated with morphine usually caused an increase in the size of the dorsal horn evoked response; this was considerably larger than the small increase observed when naloxone was applied to cultures that had not been chronically exposed to morphine.

It has not been determined whether the depression of the dorsal horn response represents an action to reduce transmitter release from primary afferent fibres, an interference with the postsynaptic actions of the transmitter, or simply an ongoing hyperpolarization of dorsal horn neurones. Whatever the underlying mechanism, the technique offers a useful approach to the study of the cellular mechanisms that underlie tolerance in controlled conditions.

VI. Conclusions

A. General Comments

The three major difficulties in the interpretation of electrophysiological studies of opioid action are concerned with drug concentrations, chemical specificity, and biological specificity. It is desirable to know the concentration of drug close to the neurone from which activity is being recorded. In the in vivo experiments this is never directly measured. Most workers have relied on the systemic administration of doses that produce analgesia or other effects in the unanaesthetized animal. However, the analgesic test used may be quite irrelevant to the particular action of opiates in question. For example, the dose of morphine that prolongs the tail flick latency in the rat (a spinal reflex) need not bear any relation to the dose required to inhibit the firing of a neurone in the striatum. Furthermore, many electrophysiological experiments have been carried out in the cat, a species not normally used in studying behavioural effects of opiates. When the concentration of agonist cannot be directly measured, the most useful guide has been the dose of naloxone that is required to prevent or reverse the observed effects. Assuming that the same or similar opiate receptors underlie mediation of the manifold opiate effects, then a similar antagonist concentration should prevent all the effects. In many studies, the dose of naloxone required to antagonize inhibition of cell firing is the same as that required to block morphine action in behavioural test; but in others it is significantly higher (364). While puzzling at the time, it now seems that these higher naloxone doses may be required because the agonists are acting on receptor subtypes with relatively low naloxone affinities.

Microelectrophoretic application of opioids effectively precludes any knowledge of concentration. The dose of systemically administered naloxone required to prevent or reverse effects of microelectrophoretically applied opioids should therefore be determined (see section I,B).

Only with in vitro studies has it been possible to measure concentrations accurately. In some such studies (e.g. myenteric plexus and cultured dorsal root ganglia/spinal cord), there was reasonable agreement between the effective concentrations of agonists in the electrophysiological experiments and the concentrations required to occupy most of the saturable high affinity binding sites. Spare receptors (460) probably exist under many experimental circumstances (380) and in any event agonist affinities determined in binding experiments need not correlate with effective doses. The critical experiment is the demonstration that effective concentrations of naloxone or other antagonists are similar to those required to displace radioactive ligands. This is true, for example, in the cultured DRG, myenteric plexus, and locus coeruleus.

The most convenient tool at the disposal of the electrophysiologist is the availability of many opiates, including enantiomers, that can be rank ordered both in their affinity for binding sites and in their potency in behavioural tests. In most of the electrophysiological studies in which isomers have been tested, there has been an apparent difference in potency between levorphanol and dextrorphan of at least 10 and up to 100 times. The uses of isomers is especially valuable with microelectrophoretic administration, where it can generally be assumed that the two enantiomers do not differ in their solubility, transport number, and diffusion coefficient. Only a few studies have sought to rank order agonists by their potency (e.g. Ref. 99). The majority of electrophysiological studies cited in this review have failed to demonstrate stereospecificity or present any other evidence that small changes in agonist structure can lead to large changes in the effects observed.

By biological specificity is meant the demonstration that the opiate effects are restricted to anatomically or functionally defined neurones. For example, firing of neurones of the nucleus locus coeruleus is inhibited by opiates, whereas neurones in the immediately adjacent brain structures are unaffected (45, 280). Such anatomical delineation contrasts with a lack of functional identity. For example, little significance can be attached to

the finding that locus coeruleus neurones increase their firing rate in response to a noxious stimuli; so do neurones in many parts of the brain, and, perhaps with better homology, in sympathetic ganglia. It is not known whether the responses of locus coeruleus cells are concerned with the perception of pain, and opiate actions on these cells may not be directly relevant to analgesia. On the other hand, neurones in the dorsal horn of the spinal cord can be functionally identified on the basis of their responses to noxious and non-noxious stimulation, and in a few studies also on the basis of their ascending projection. Most workers agree that opiates depress the response of these cells to excitation by noxious stimuli. By contrast, the anatomical location of the dorsal horn neurones is often not of much help.

Both functional and anatomical identification of the affected neurone is required before full biological significance can be attached to an effect of opioids. Unfortunately, a great many investigations have studied opioid effects on neurones unidentified by either of these means (e.g. in the reticular formation, or striatum); such indiscriminate testing of neuronal effects of opioids is of limited value. In the case of the opioid peptides, the only estimates of relevant concentrations can come for radioligand binding assays. Such estimates will at best be rough, as the conditions of isolation and homogenization may be expected to alter binding substantially from the in vivo situation. There is no knowledge of the concentrations of endogenously released enkephalin, and blood levels of β-endorphin appear at first sight to be too low to have any significant effects on peripheral neuronal function. Chemical specificity of enkephalin effects can and should be examined by applying a peptide analogue that has little activity in the binding assays (e.g. 387). Biological specificity can be approached by comparing the effects of application of endogenous enkephalin with the effects of stimulating identified pathways; this has so far not been very successful (see below).

B. Effects of Opiates and Opioid Peptides on the Properties of Single Neurones

Opiates and opioid peptides have inhibited the firing of neurones in all but a few regions of the central nervous system tested. The inhibition of cell firing could result from a direct action on the neurone whose activity is recorded (often, and perhaps misleadingly, said to be a "postsynaptic" action) or from inhibition of other neurones that excite the cell whose activity is being recorded (when such an effect occurs at transmitter release sites this is usually referred to as presynaptic inhibition; when it occurs elsewhere on the excitatory neurone it is disfacilitation). Studies with extracellular recording suggest that at least a component of this inhibition of firing may take place directly on the cell whose activity is recorded—the major evidence in support of this being the observation that neuronal excitation caused by L-glutamate in many areas is reduced by opiates and opioid peptides. Studies with intracellular recording from the central nervous system have provided strong support for such a direct form of inhibition. Neurones in slices of guinea-pig (389) and rat (498) locus coeruleus, and rat substantia gelatinosa (521) are hyperpolarized by opioids. This hyperpolarization results from a potassium-conductance increase. The potassium-conductance increase leads also to a shunting of the calcium action potential; this could inhibit transmitter release. In the locus coeruleus, a μ-type receptor has been shown to be responsible for this action. Other intracellular studies have shown no effects of opioids on membrane properties, but these have been from neurones (spinal motoneurones and hippocampal pyramidal cells) that appear to bear few opioid binding sites. Intracellular recording from other opioid-sensitive neurones will help to indicate how widespread in the nervous system is this hyperpolarizing effect of opioids.

Although systemic opiates excite neurones in many areas of the central nervous system little evidence has been produced to show that this results from a direct action on the cells excited. In the hippocampus, excitation is apparently the result of an action on inhibitory interneurones (disinhibition). With spinal Renshaw cells, high concentrations of opiates may excite by an action at nicotinic receptors but this probably has no relevance to the pharmacological effects of analgesic doses. The mechanism of excitation of some neurones of the hypothalamus, nucleus of Edinger-Wesphal, and brain stem raphe is unknown.

In the peripheral nervous system there is strong evidence on the mechanisms whereby opiates inhibit cell firing. Firstly, the release of excitatory transmitters is reduced. This has been proposed to be due to a hyperpolarization of nerves leading to propagation block, to a block of voltage-dependent calcium currents, or to an interference with the ability of intracellular calcium currents to promote release. In the second place, cell membranes are hyperpolarized. This is possibly due to an increase in the free intracellular calcium concentration. The hyperpolarization that follows a period of repetitive firing is particularly susceptible to enhancement by opiates.

C. Effects of Opiates on the Function of Neuronal Assemblies

The probable actions of opiates mentioned above, membrane hyperpolarization and inhibition of transmitter release from nerve varicosities, can explain in general terms many of the effects of opiates on the nervous system. The most studied by far has been the reduction in nociceptive input to dorsal horn neurones. With systemic administration of opiates, this action appears to occur primarily at the spinal level, and this has been confirmed with microelectrophoretic application. Because nociceptive inputs to a given cell are reduced selectively, it is often thought that opiates must inhibit

transmitter release selectively from small diameter primary afferent fibres conveying only pain modality. But it is not inconceivable that a similar selectivity could be conveyed by remote inhibition, mediated by hyperpolarization or conductance increase, on certain parts of the distal dendritic tree of dorsal horn neurones that preferentially receive nociceptive inputs. Or it may simply be that nociceptive information relays in the substantia gelatinosa through those cells that are hyperpolarized by opioids. Whichever mechanism operates, the most important effect of opiates is to block the transmission of nociceptive information in the ascending projections.

Present techniques cannot assess the contribution of the spinal actions of opiates to analgesia in humans but an important role remains an attractive hypothesis. It is sometimes argued that a spinal action of opiates cannot explain the well known opiate action on the affective component of pain. However, some dorsal horn cells project predominantly to the medial thalamic nuclei (11) and selective inhibition of activity in such a projection could produce a dulling of the affective component of pain, whereas it would not affect the ability to sense and localize the noxious stimulus. In addition,the affective component will of course be dulled by direct actions of opiates at relays along the palaeospinothalamic pathway into the intralaminar thalamus, and at the cortical level itself.

Inhibition of cell firing has also been described to underlie the well known effects of opiates on respiration (124), and the same primary effect of opiates on neurones in the different regions of the hypothalamus appears to underlie the action of opiates on satiety (385) and perhaps posterior pituitary hormone secretion (88).

D. Electrophysiological Evidence for a Physiological Role for Opioid Peptides

Evidence has been sought that endogenous opioids play a role in the normal function of the nervous system by two kinds of experiment. One has been the demonstration of a close similarity between the effects of opioids and those of stimulating identified pathways; the other has been the finding of a parallel action of naloxone on the effect of exogenous opioids and on the spontaneous or evoked activity of the same neurones. Evidence based on similarity of effect is insufficient. Even in situations in which the site and mechanism of action on single neurones are well known, there is no convincing evidence that stimulation of opioid fibres can mimic these effects. For this kind of evidence to be useful, it will be necessary to show mimicry in detail, including identity of ionic mechanisms, and to show that enkephalin-containing neurones are being stimulated selectively, preferably by a natural stimulus. The electrophysiological evidence to date for a functional role for enkephalin therefore resides on the use of naloxone. Naloxone alone has no significant effects on the properties of the cultured or peripheral neurones, and the majority of studies on single central neurones incidate that microelectrophoretic administration of naloxone is without effect. There are, however, some studies that have shown effects of naloxone which are not related to occupation of an opiate receptor (134, 141).

If it is accepted that naloxone can reveal activity of an endogenous opioid when given parenterally in a certain dose range, reading of this review tells one that the effects of the compound when studying single neurones in multineuronal systems have been far from uniform. For example, naloxone has been shown to enhance C-fibre reflexes in the decerebrate cat (32), though not in unanaesthetized man (497). Some investigators (417) describe that naloxone increases the response of dorsal horn neurones to C-fibre stimulation and reduces the inhibition of this response that follows stimulation in the nucleus raphe magnus; other investigators found no effect of naloxone on the responses of the same neurones to noxious heat (150) or on descending inhibition (143). The disparity in findings may reflect the fact that we know little about the precise conditions of the nervous system that are most favourable for opioid peptide release.

At a simpler level, opioid peptides appear to cause presynaptic inhibition in a paravertebral ganglia (279). They also play a role in the function of the enteric nervous system, because naloxone stereospecifically strengthens the peristaltic reflex (294); but the basis of this action at the level of communication between identified neurones remains unknown.

Stimulation of the arcuate region of the hypothalamus caused an inhibition of firing of neurones in the nucleus locus coeruleus in the rat (462). This was partly prevented by naloxone. In view of the evidence that β-endorphin-containing neurones form a projection from the arcuate nucleus that reaches as far as the locus coeruleus, it is tempting to conclude that the stimulation produced inhibition of firing is mediated by β-endorphin. On the other hand, the reversal by naloxone was only partial even with rather high concentration (5 mg/kg). Both lesion studies and local microelectrophoretic application of naloxone [and (+)-naloxone] will be required to substantiate the conclusion that the inhibition is mediated by the descending β-endorphin pathway. Electrophysiological evidence for a role for endogenous opioids was also presented by Pomeranz and Cheng (402). They found that naloxone prevented the inhibition by electroacupuncture of firing of dorsal horn neurones in response to noxious stimuli, but the precise nature and origin of the opioid-mediated inhibition remains unclear. Indeed, the entire question of the influence of circulating endogenous opioids on electrophysiological activity has not been addressed. The conditions of in vivo experimentation might be expected to be associated with significant opioid peptide release from pituitary and adrenal glands.

From the foregoing remarks it appears that the like-

lihood of observing effects of naloxone increases substantially with the level of complexity of the organization of the neuronal assembly required. Animals with an intact nervous system show several effects of naloxone in various biochemical and behavioural tests. The functional role of opioid peptides should be demonstrable by changes in the electrical properties of the nerve cells. The present challenge to the electrophysiologist is to devise the appropriate experiments which, in a relatively simplified system, may indicate the details and significance of such a role.

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