Br. J. Pharmac. (1984), **83**, 763–772

A microiontophoretic study of the actions of μ-, δ- and κ-opiate receptor agonists in the rat brain

P.B. Bradley & Anne Brookes

Department of Pharmacology, University of Birmingham, The Medical School, Birmingham B15 2TJ

1 The actions of μ-, δ- and κ-opiate receptor agonists have been compared on the activity of single neurones in the brain stem, caudate nucleus and hippocampus of the rat, using the technique of microiontophoresis.

2 In the brain stem and caudate nucleus the predominant effect of all the opiate agonists tested was depression of neuronal activity which was antagonized by naloxone. The selectivity of naloxone as an opiate receptor antagonist was indicated by its lack of effect on γ-aminobutyric acid (GABA)-induced responses.

3 In the hippocampus both μ- and δ-agonists mainly caused an increase in neuronal firing rates, though some neurones were depressed. In contrast, all the κ-agonists, including the proposed endogenous ligand for the κ-receptor, dynorphin, caused depression of neuronal activity. All of these effects were antagonized by naloxone.

4 There was a clear distinction in the areas within the hippocampus in which the μ- and δ-agonists produced different effects. Neurones in the pyramidal cell layer were always excited by these drugs, whereas neurones in the granule cell layer of the dentate gyrus were always depressed by the same drug.

Introduction

The first indication that opiate receptors in the central nervous system are not a homogenous population came from the results of experiments on the chronic spinal dog (Martin *et al.*, 1976) and was closely followed and supported by receptor binding studies (Lord *et al.*, 1976). These findings generated considerable interest and many workers in this field have since produced a considerable volume of evidence to support the concept. Both behavioural (Woods *et al.*, 1978; Frenk *et al.*, 1978; Tyers, 1980; Iwamoto, 1981) and biochemical (Lord *et al.*, 1977; Chang *et al.*, 1979; Goodman *et al.*, 1980; Kosterlitz & Paterson, 1980) approaches have been extensively used but little comparative work has been carried out at the single neurone level.

The endogenous ligands for the opiate receptor, leucine and methionine enkephalin, are thought to be neurotransmitters in the CNS (Elde *et al.*, 1976; Costa *et al.*, 1978; Hughes, 1979), and their actions on single neurones in the brain, together with those of a number of opiate drugs and other opioid peptides are now well documented (Nicoll *et al.*, 1977; Bradley *et al.*, 1978; Zieglgänsberger *et al.*, 1978). However, in none of these studies was the possibility considered that opiate agonists, known to have selective actions on μ-, δ- or κ-receptors, might have differential effects on neurones in different regions of the brain. The present investigation represents an attempt to examine this possibility. In addition to their actions being compared on the same neurone, the different classes of opioid agonists were tested on single neurones in three different regions of the rat brain. These were the brain stem, caudate nucleus and hippocampus and were chosen on the basis of knowledge of the neuroanatomy, electrophysiology (Nicoll *et al.*, 1977; Zieglgänsberger *et al.*, 1978) and the distribution of opiate receptors in these regions (Atweh & Kuhar, 1977a, b).

Preliminary reports of some of these results have been published (Bradley & Brookes, 1981, Brookes & Bradley, 1984).

Methods

Male Sprague-Dawley rats, weighing 180–330 g, were anaesthetized with urethane (1.8 g kg^{-1}) by the intraperitoneal route. For recording in the brain stem the animals were partially cerebellectomised and the floor of the fourth ventricle exposed. Electrode

penetrations were made in the brain stem between P5.7 and 8.2 (The Stereotaxic Atlas of König and Klippel (1963) is referred to throughout). For recording in the caudate nucleus, holes were drilled bilaterally in the skull, 1–4 mm rostral to bregma and 1–4 mm lateral to the midline. The electrodes were passed through the cortex to reach the caudate nucleus. Recordings were made at a depth of 3–7 mm at A8.2 to 9.9. A similar procedure was used for recording in the hippocampus. Holes were drilled bilaterally in the skull 2–5 mm caudal to bregma and 1–4 mm lateral to the midline. Recordings were made in the dorsal hippocampus at a depth of 2½–5 mm and between A2.4 and 4.6. At the end of the experiment the animals were killed and the brain removed and fixed for histological examination.

Conventional five- and seven-barrelled microelectrodes were used. The recording barrel contained 4 M saline and one of the remaining barrels always contained a solution of the dye Pontamine Sky Blue. This barrel was used for current balancing and for ejection of the dye so that the position of the neurones which had been studied could be determined histologically. The other barrels of the electrode were filled with a selection of the following drugs: *μ agonists:* morphine hydrochloride, 25 mM, pH 4.5 (MacFarlane Smith); normorphine base, 30 mM, pH 4.5 (Wellcome); FK 33,824 (D-Ala^2-MePhe^4-Met-O-ol enkephalin), 15 mM, pH 4.3 (gift from Dr Römer, Sandoz); *δ agonists:* leucine enkephalin, 15 mM, pH 4.3 (Penninsula; Sigma); BW 180C (D-Ala^2, D-Leu^5 enkephalin), 15 mM, pH 5.0 (Wellcome); *κ agonists:* ethylketocyclazocine methane sulphonate, 100 mM, pH 4.3 (Sterling Winthrop; gift from Dr McCarthy, Reckitt & Colman); MR 2034[(−)-α-(1R, 5R, 9R)-5, 9-dimethyl-2-(L-tetrahydrofurfuryl-2′ -hydro-6, 7-benzomorphan)], 15 mM, pH 4.0 (gift from Dr Merz, Boehringer-Ingelheim); bremazocine hydrochloride, 40 mM pH 5.0 (gift from Dr Römer, Sandoz); dynorphin (1–13), 875 μM, pH 4.7 (gift from Dr Goldstein).

Other drugs: monosodium glutamate, 500 mM, pH8 (Koch light); γ-aminobutyric acid (GABA), 250 mM, pH 4.7 (Koch Light); naloxone hydrochloride, 20 mM, pH 5.3 (Endo).

Where possible more than one type of opiate agonist applied to the same neurone and, on some occasions, a non-opiate agonist, eg. GABA, was applied as a 'control' agonist for comparison. The antagonism of a response by naloxone was used as the criterion for classifying the response as opiate or non-opiate (see Gayton *et al.*, 1978). All neurones studied in the brain stem and hippocampus were spontaneously active. However, in the caudate nucleus few spontaneously active neurones were encountered so a constant low level of glutamate ions was ejected (0–9 nA) to excite the cells artificially. Retaining currents of 15 to 30 nA were used between drug applications to prevent leakage which might affect the spontaneous activity of the neurone being recorded.

Results

The brain stem

The predominant effect on the firing rate of single neurones in the brain stem, following microiontophoretic application of μ-, δ- and κ-agonists, was depression (Figure 1). The μ-agonist morphine was the only drug to cause any significant proportion of excitatory responses in the brain stem. It was tested

Table 1 Responses of single neurones in the brain stem to microiontophoretic application of μ-, δ- and κ-agonists and their antagonism by naloxone

	Response			*Antagonism*	
Drug	↓	↑	0	↓	↑
μ-agonists					
Morphine	63(53)	30(25)	7(6)	100(22)	14(7)
Normorphine	88(63)	4(3)	8(6)	100(11)	0(0)
FK 33,824	93(100)	2(2)	5(5)	100 (9)	0(0)
δ-agonists					
Leucine enkephalin	88(97)	2(2)	10(11)	86(12)	—
BW 180C	91(206)	0(1)	9(20)	100(10)	—
κ-agonists					
Ethylketocyclazocine	89(80)	0(0)	11(10)	91(10)	—
Bremazocine	89(68)	0(0)	11 (8)	91(10)	—
MR 2034	85(38)	2(1)	13 (6)	94(17)	—

The figures are percentages, except those in parentheses which represent the actual numbers of neurones. ↓ = depression; ↑ = excitation; 0 – no effect.

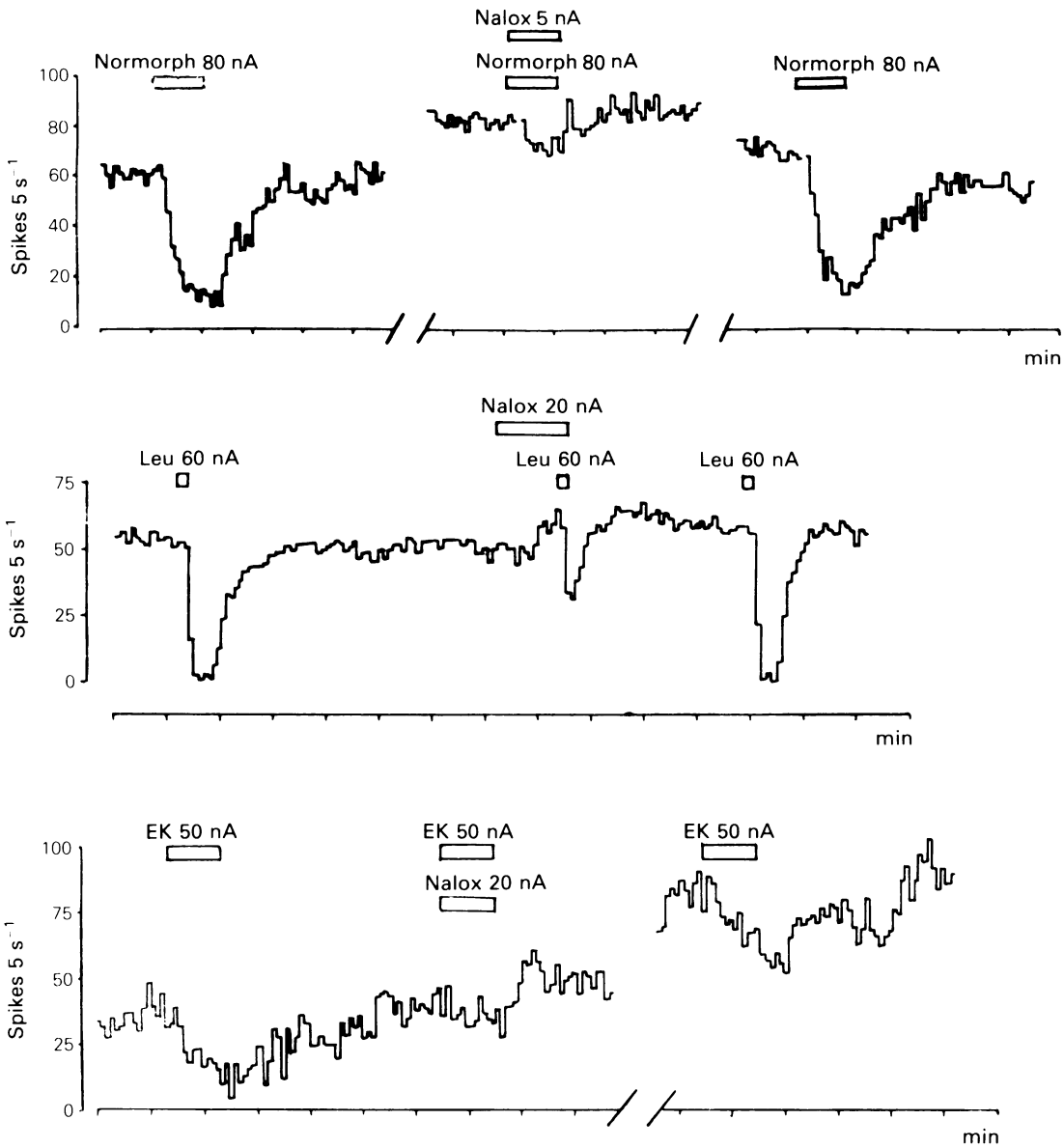

Figure 1 Examples of the effects of different classes of opioid agonist on the firing rates of single neurones in the brain stem. The firing rate is plotted against time in each case. The three traces represent three different neurones. Normorph = normorphine; Leu = leucine enkephalin; EK = ethylketocyclazocine; Nalox = naloxone.

on 84 neurones of which 53 were depressed, 25 excited and 6 unaffected. Naloxone antagonized all the depressant responses on which it was tested (22/22) but only blocked one (out of 7) excitatory response. Normorphine was tested on 72 neurones, of which it depressed 63 and excited 3, having no effect on 6. Again, naloxone antagonized all depressions tested (11/11). FK 33,824 was applied to 107 neurones, depressed 100, excited 2 and had no effect on 5. Naloxone antagonized 9/9 depressant responses.

The δ-agonist, leucine enkephalin, was applied to 110 neurones, of which 97 were depressed, 2 excited and 11 unaffected. Twelve out of 14 depressant responses to leucine enkephalin were antagonised by naloxone. However, the depression of neuronal fir-

ing produced by leucine enkephalin was of much shorter duration than the responses produced by other opioid agonists (Figure 1). BW 180C was applied to 227 neurones, depressed 206, excited one and had no effect on 20. Naloxone antagonized all the BW 180C-induced depressions on which it was tested (10/10).

The κ-agonist, ethylketocyclazocine, was applied to 90 neurones, of which 80 were depressed (Figure 1) and 10 unaffected. No excitations were observed with this drug; naloxone antagonized 10/11 depressions. Bremazocine was tested on 76 neurones, depressing 68 and leaving 8 unaffected. Again no excitations were found and naloxone antagonized 10/11 depressant responses. Finally, MR 2034 was applied to 45 neurones, depressed 38, excited one and had no effect on six. Naloxone antagonised 17/18 depressions.

The effects of the three classes of opioid agonists on single neurones in the brain stem are summarised in Table 1. With the exception of morphine, it is clear that the μ-, δ- and κ-opiate receptor agonists all produced qualitatively similar effects. Another possible exception is leucine enkephalin, which produced responses of shorter duration than those of the other agonists but this is thought to be due to the rapid enzymatic degradation of this peptide in the tissues (Hambrook *et al.*, 1976). γ-Aminobutyric acid (GABA) depressed all the neurones to which it was applied (20) but naloxone was never effective in antagonizing these responses (0/7), indicating that, in the brain stem, naloxone is a selective opiate antagonist (Figure 2). Histological examination of the dye spots in brain slices showed that the neurones studied were located mainly in three brainstem nuclei, the nucleus reticularis lateralis, the nucleus reticularis ventralis and nucleus reticularis gigantocellularis.

The caudate nucleus

As in the brain stem, all the μ-, δ- and κ-agonists tested in the caudate nucleus caused depression of the activity of single neurones (Figure 3). In this region no excitatory response to morphine was observed. Thus, morphine was applied to 46 neurones and depressed 39, having no effect on 7. Naloxone antagonized 9/9 depressant responses. Normorphine was applied to 22 neurones, depressing 20 with 2 unaffected, and again naloxone was effective in antagonizing all depressions on which it was tested (9/9).

Leucine enkephalin was applied to 51 neurones and depressed the activity of all of them, naloxone antagonising 8/8 of these responses. The responses to leucine enkephalin were of shorter duration than those to other opioid agonists, as was seen in the brain stem. BW 180C was tested on 51 neurones, of which it depressed 47 (Figure 3), leaving three unaffected; naloxone antagonized 14/14 of these depressions.

Ethylketocyclazocine was tested on 49 neurones in the caudate and depressed 47, leaving two unaffected, while naloxone antagonized 11/11 depressant

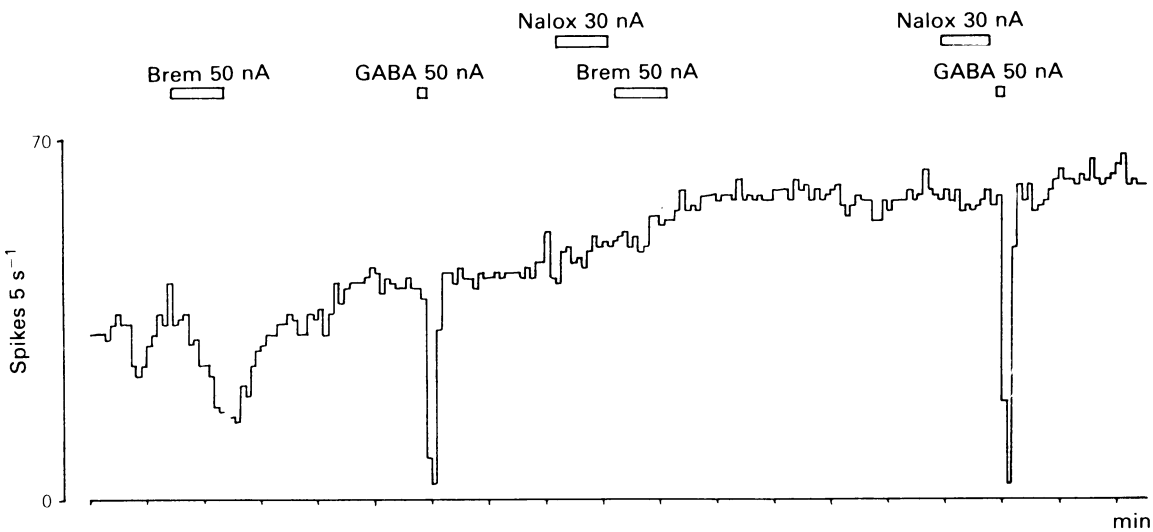

Figure 2 Depression of the firing rate of a brain stem neurone induced by bremazocine is antagonized by naloxone, whereas the depression induced by γ-aminobutyric acid (GABA) is unaffected. Brem = bremazocine. Nalox = naloxone.

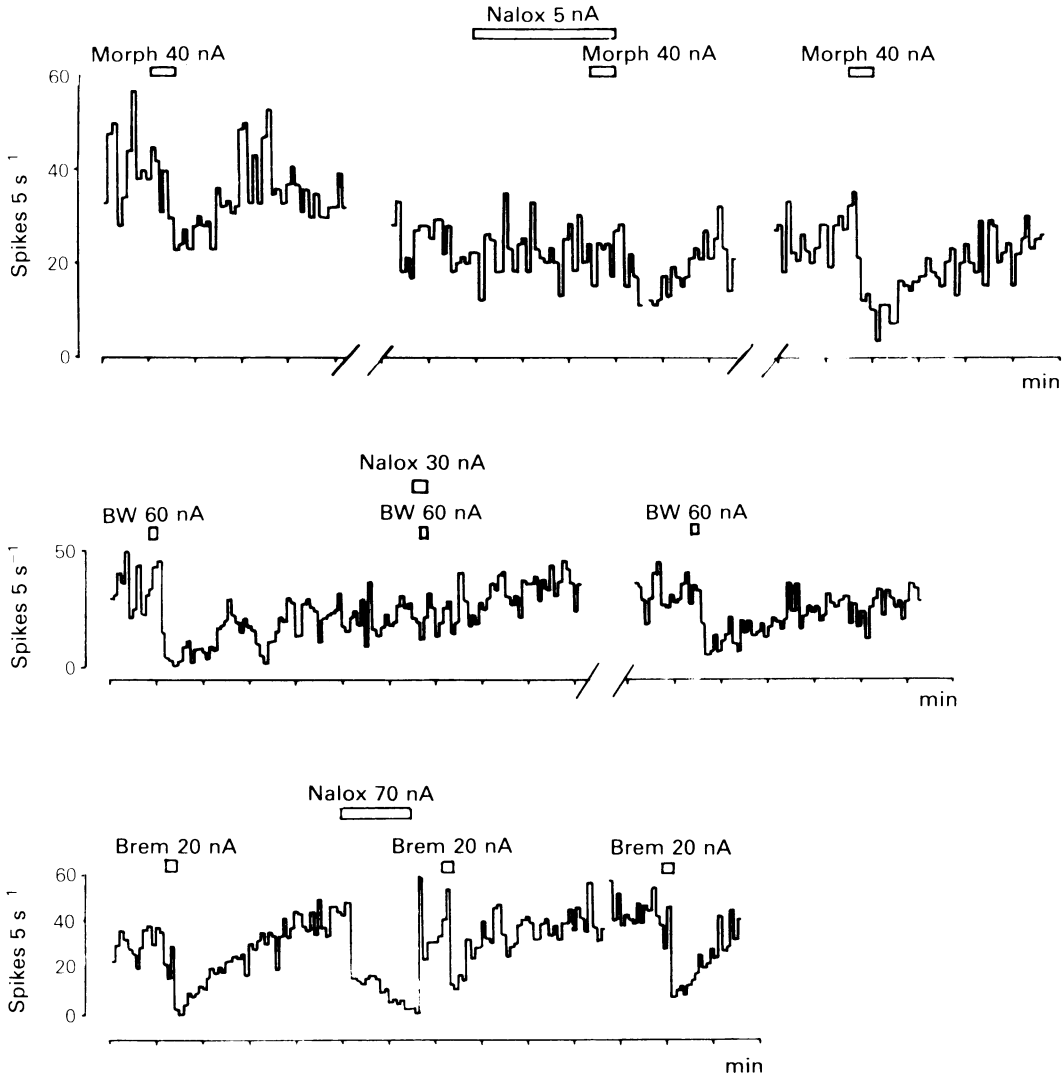

Figure 3 Examples of the effects of different classes of opioid agonists on the firing rates of single neurones in the caudate nucleus. Morph = morphine; BW = BW 180C; Brem = bremazocine; Nalox = naloxone.

responses. Bremazocine depressed the activity of all the neurones to which it was applied (37) and naloxone antagonized 17/23 responses (Figure 3). Similarly, MR 2034 depressed 49 out of 55 neurones to which it was applied, one neurone being excited and 5 unaffected. Naloxone antagonized 14 out of 21 of these depressions. Thus, in the case of bremazocine and MR 2034, a slightly smaller proportion of the responses was antagonized by naloxone than was observed with the other opioids.

Table 2 summarises the effects of the μ-, δ- and κ-opiate receptor agonists on glutamate-activated neurones in the caudate nucleus. As found with neurones in the brain stem, the predominant effect of all three classes of opiate agonists was depression of cell firing rates. γ-Aminobutyric acid was applied to 14 neurones and caused depression of activity in all cases, but naloxone failed to block any of the GABA-induced depressant responses on which it was tested (8), thus suggesting that here, as in the brain stem, it is a selective opiate antagonist. Histological examination of the brain slices showed that the electrode penetrations were all in the head of the caudate nucleus.

Table 2 Responses of single neurones in the caudate nucleus to microiontophoretic application of μ-, δ- and κ-agonists and their antagonism by naloxone

Drug	Response ↓	Response ↑	Response 0	Antagonism ↓	Antagonism ↑
μ-agonists					
Morphine	85(39)	0(0)	15(7)	100 (9)	—
Normorphine	91(20)	0(0)	9(2)	100 (9)	—
FK 33,824	100(29)	0(0)	0(0)	100(12)	—
δ-agonists					
Leucine enkephalin	100(51)	0(0)	0(0)	100 (8)	—
BW 180C	94(47)	0(0)	6(3)	100(14)	—
κ-agonists					
Ethylketocyclazocine	96(47)	0(0)	4(2)	100(11)	—
Bremazocine	100(37)	0(0)	0(0)	67(17)	—
MR 2034	89(49)	2(1)	9(5)	74(14)	—

The figures are percentages, except those in parentheses which represent the actual numbers of neurones. ↓ = depression; ↑ = excitation; 0 = no effect.

The hippocampus

In contrast to the effects observed with the opioid agonists in the brain stem and caudate nucleus, different effects were found with iontophoretic application to hippocampal neurones. Thus, the μ- and δ-agonists mainly caused excitation of neurones in the hippocampus but the κ-agonists were exclusively depressant (Figure 4).

Thus, the μ-agonist morphine, applied to 36 hippocampal neurones, excited 26, depressed 9, leaving one neurone unaffected. Naloxone antagonized 10/11 of the excitations and 1/1 depression. Normorphine, applied to 33 neurones, excited 25 and depressed 8, with none unaffected. Naloxone antagonized 8/8 excitations and 1/1 depression. FK 33,824 was tested on 104 neurones of which it excited 81 and depressed 23. Naloxone antagonized 18/18 excitatory responses and 1/1 depressions (Figure 4).

Leucine enkephalin was tested on 64 hippocampal neurones, of which 45 were excited, 15 depressed and 4 unaffected. Naloxone antagonized 6/6 of the excitations and 2/2 depressions. BW 180C, applied to 59 neurones, excited 46, depressed 11, with two

Table 3 Responses of single neurones in the hippocampus to microiontophoretic application of μ-, δ- and κ-agonists and their antagonism by naloxone

Drug	Response ↓	Response ↑	Response 0	Antagonism ↓	Antagonism ↑
μ-agonists					
Morphine	25 (9)	72(26)	3(1)	100(1)	91(10)
Normorphine	24 (8)	76(25)	0(0)	100(1)	100 (8)
FK 33,824	22(23)	78(81)	0(0)	100(1)	100(18)
δ-agonists					
Leucine enkephalin	23(15)	71(45)	6(4)	100(2)	100 (6)
BW 180C	19(11)	78(46)	3(2)	100(2)	100 (7)
κ-agonists					
Ethylketocyclazocine	90(38)	10(4)	0(0)	100(8)	——
Bremazocine	85(29)	3(1)	12(4)	100(7)	——
MR 2034	100(37)	0(0)	0(0)	100(7)	——
Dynorphin (1–13)	81(39)	8(4)	11(5)	67(4)	100 (1)

The figures are percentages, except those in parentheses which represent the actual numbers of neurones. ↓ = depression; ↑ = excitation; 0 = no effect.

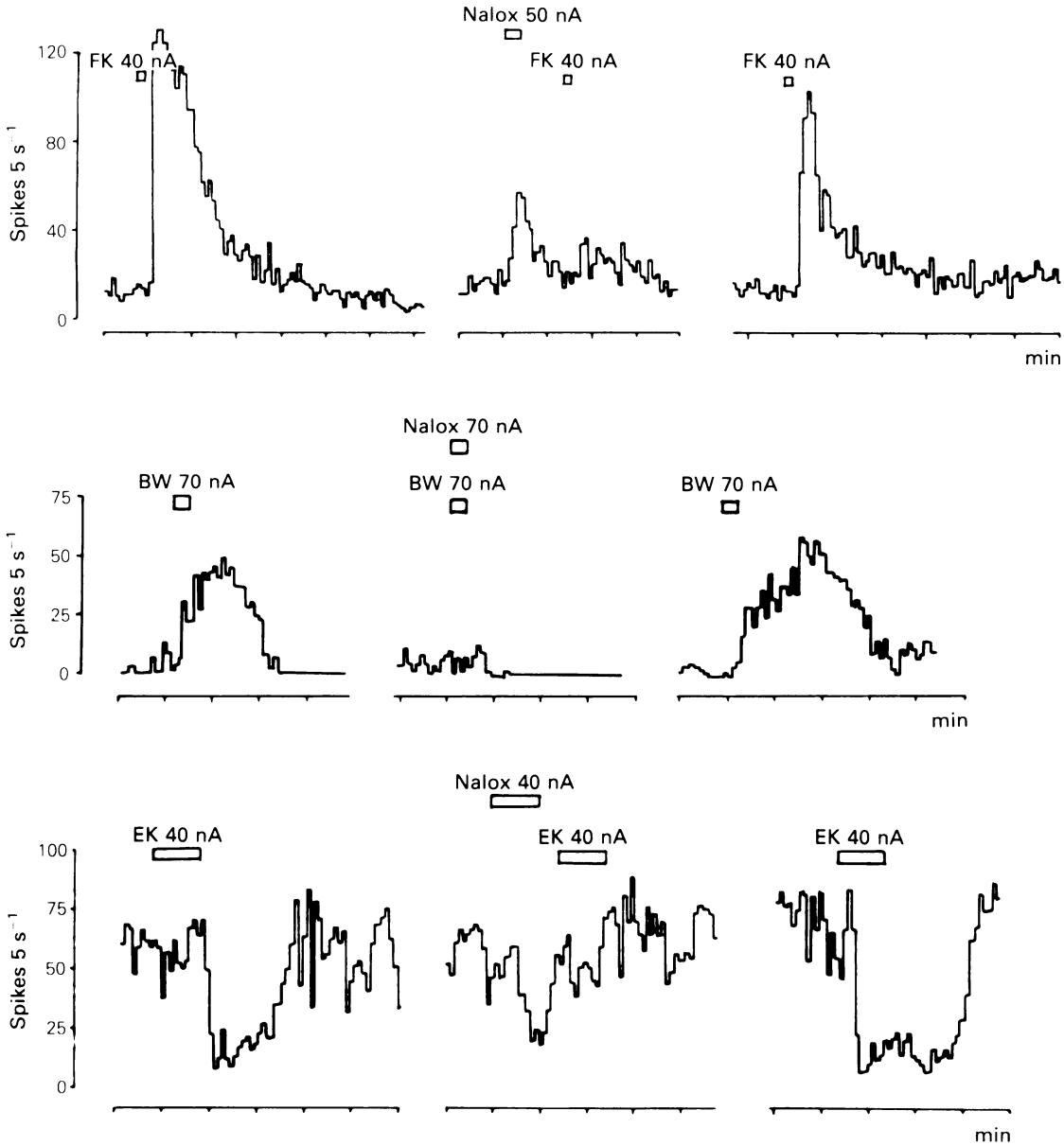

Figure 4 Examples of the effects of different classes of opioid agonists on the firing rates of single neurones in the hippocampus. FK = FK 33,824; BW = BW 180C; EK = ethylketocyclazocine; Nalox =naloxone.

unaffected; naloxone antagonized 7/7 excitations and 2/2 depressions (Figure 4).

Ethylketocyclazocine was applied to 42 hippocampal neurones. It depressed the activity of 38 and excited 4, no neurones being unaffected. Naloxone blocked all the depressant responses (8/8) on which it was tested (Figure 4). Bremazocine, tested on 34 neurones, depressed 29, excited one and had no effect on four. Naloxone antagonized the depressions (7/7). MR 2034 was applied to 37 neurones and depressed the activity of all. No neurones were excited or unaffected by this drug. Again, naloxone antagonized the depressions (7/7).

During this study, a small quantity of dynorphin

(1–13) became available and was tested on neurones in the hippocampus. Dynorphin was applied iontophoretically to 48 neurones in the hippocampus and depressed the activity of 39, it excited 4, with 5 neurones unaffected. Naloxone antagonized 4/6 of these depressions and 1/1 excitation.

The effects of the μ-, δ- and κ-opiate agonists on single neurones in the hippocampus are summarised in Table 3. The predominant effect of the μ- and δ-agonists was excitation of neuronal activity as has been previously observed, although a significant proportion of the neurones was depressed by these agonists. On the other hand, the κ-agonists exclusively depressed the firing rates of spontaneously active hippocampal neurones. Naloxone was found to antagonize both the excitatory and depressant responses. γ-Aminobutyric acid was applied to 18 hippocampal neurones, all of which were depressed, but naloxone did not affect these responses (0/4), thus indicating its selectivitity as an opiate antagonist in the hippocampus as well as in the brain stem and caudate nucleus.

Histological examination of the brain showed that the recordings in the hippocampus had been from neurones in two cell layers, the pyramidal cell layer and the granule cell layer of the dentate gyrus. Since these two regions of the hippocampus contain relatively large neurones, it is likely that, because of the type of electrode used, the recordings were from either pyramidal cells or from dentate gyrus granule cells. Furthermore, it was possible to distinguish anatomically between the excitant and depressant responses of the μ- and δ-agonists. Thus, when excitatory responses to μ- and δ-agonists were recorded, the neurones were found to be localised to the pyramidal cell layer, whereas the depressant responses were found with neurones in the granule cell layer of the denate gyrus of the hippocampus. However, depressant responses to the application of the κ-agonists occurred with neurones recorded in both the pyramidal cell layer and the granule cell layer.

Discussion

The effects of microiontophoretic application of the μ-, δ- and κ-opiate agonists on the activity of single neurones in the brain stem were similar to those described previously from this laboratory (Bradley & Dray, 1974; Bradley & Bramwell, 1977) and by others (Nicoll *et al.*, 1977). This was predominantly depression of neuronal firing which was readily reversed by microiontophoretically applied naloxone. The exception to this was morphine which, as in previous studies, produced a significant proportion of excitatory neuronal responses, but these were resistant to antagonism by naloxone and are probably not mediated by opiate receptors (Bradley & Bramwell, 1977). In fact, there is now evidence to suggest that excitatory neuronal responses produced by morphine may be mediated by a cholinergic mechanism (Hewson, 1984). The depressant responses of brain stem neurones to the different opiate agonists were all similar in form with the exception of those produced by the opioid peptide, leucine enkephalin, which were of shorter duration than those produced by opiate drugs. This may have been due to the more rapid enzymatic degradation of the endogenous peptide in the tissues (Hambrook *et al.*, 1976) as the stable peptide, BW 180C had a longer duration of action, comparable to that of morphine and other opiate drugs, the actions of which are presumably terminated by diffusion away from the receptor site. The inability of naloxone, when ejected microiontophoretically in amounts sufficient to antagonize opiate-induced depressions, to affect the responses to GABA, suggests that in the brain stem naloxone can act as a selective opiate antagonist.

Similarly, in the caudate nucleus all the opiate agonists studied here produced similar effects which were predominantly depression of neuronal activity, and the responses were similar to those reported previously for morphine (Gayton & Bradley, 1976). Once again, naloxone was an effective antagonist of opiate-induced responses but failed to antagonize the effects of GABA, although it must be noted that in this region naloxone was less effective against two of the κ-agonists (bremazocine and MR 2034).

It was in the hippocampus that striking differences in the effects of the different classes of opioid agonists on neuronal firing were observed. In contrast to their effects in the brain stem and caudate nucleus, the main effect of μ- and δ-agonists in the hippocampus was to cause an increase in neuronal firing, i.e. excitation, although a significant proportion of neurones in the hippocampus was depressed by the μ- and δ-agonists. These effects were consistent with the results of other studies with some of these agonists (Nicoll *et al.*, 1977). However, in the present experiments the κ-agonists consistently depressed neuronal firing in the hippocampus and all the substances which were tested, and which on the basis of other criteria have been classed as κ-agonists, produced this effect, including dynorphin. Both the excitation and depression of neuronal firing produced by the three classes of opiate agonists in the hippocampus were antagonized by naloxone. This is in contrast to the results obtained by Henriksen *et al.*, (1982) and by Walker *et al.*, (1982). Depression of neuronal activity by GABA was not affected, providing evidence for the selectivity of naloxone as an opiate antagonist in this region. Histological examination of the brain sections showed that the different responses to the μ- and δ-agonists could be attributed to differ-

ent anatomical locations within the hippocampus. Thus, the neurones which were excited by the μ- and δ-agonists were localised to the pyramidal cell layer, whilst those which were depressed were in the granule cell layer of the dentate gyrus. Neurones depressed by the κ-agonists, however, were found in both regions. Thus, the differential effects produced by the different classes of opiate agonists were only manifested in the pyramidal cell layer. It seems likely that both the depressant and excitatory responses may be mediated by opiate receptors as both responses were blocked by naloxone (c. the effects of morphine in the brain stem). However, it is quite possible that the different effects were brought about by different types of opiate receptors, although in this study it was not possible to differentiate between the sub-types of opiate receptor by their differing sensitivity to naloxone, since at the concentration of naloxone used, responses to μ-, δ- and κ-agonists were all readily antagonized (except perhaps for some effects of bremazocine and MR 2034 in the caudate).

The ability of dynorphin (1–13) to depress neuronal activity in the hippocampus confirms the capacity of this endogenous peptide to act like other κ-agonists and supports the proposed role for dynorphin as the endogenous ligand at the κ receptor (Chavkin *et al.*, 1982). The other κ-agonists tested in this study were all benzomorphan derivatives and, therefore, until dynorphin became available, it was not known whether the effects observed were simply peculiar to the benzomorphan structure rather than a property of the κ-agonists as a whole. The fact that dynorphin (1–13) produced the same effect as the other κ-agonists suggests that the consistent depression of neuronal activity in the pyramidal cell layer of the hippocampus is indeed common to κ-agonists generally and may be mediated by the κ-sub-type of opiate receptor, whilst excitation in the hippocampus is probably mediated by μ- and δ-receptors.

It is generally believed that the principal role of endogenous opioid peptides in the mammalian nervous system is an inhibitory one (North, 1979). Thus, inhibitory actions of opiates in various regions of the central nervous system have been regarded as their main effect and various mechanisms have been postulated to account for the excitatory actions of opioids on hippocampal neurones. Some of the mechanisms proposed are (a) disinhibition due to a primary inhibitory effect resulting in excitation (Zieglgänsberger *et al.*, 1979), (b) presynaptic facilitation (Haas & Ryall, 1980) and (c) non-specific actions (Fry *et al.*, 1979). It has also been suggested that the inhibitory effects of opioids in this region are the result of direct activation of opiate receptors (Tielen *et al.*, 1981). However, no consideration has as yet been given to the possibility that different responses might be mediated by different opiate receptors. This conclusion is the most appropriate one to explain the present findings. Thus, it seems likely that excitatory effects of opiates in the pyramidal cell layer are mediated by μ- and/or δ-receptors, whereas the depressant effects are mediated by κ-receptors. However, this does not imply that the receptors mediating the different responses are necessarily located on the same neurone. Therefore, the two responses may be produced at different sites in the pyramidal cell layer. This possibility warrants further investigation.

Given that the different types of opiate receptor agonist produce different effects on neuronal activity, the physiological consequences of such processes need to be considered. Excitatory effects on the EEG of rats after intraventricular injection of a number of opioids have been well documented (Frenk *et al.*, 1978; Henriksen *et al.*, 1978) and recently it has been shown that κ-receptor agonists are far less potent at producing seizure-like activity in the EEG than μ- and δ-agonists (Snead & Bearden, 1982).

We are grateful to Drs Goldstein, McCarthy and Römer for drugs and to the Science and Engineering Research Council and Reckitt & Colman for support.

References

ATWEH, S.F. & KUHAR, M.J. (1977a). Autoradiographic localisation of opiate receptors in rat brain. I Spinal cord and lower medulla. *Brain Res.*, **124**, 53–67.

ATWEH, S.F. & KUHAR, M.J. (1977b). Autoradiographic localisation of opiate receptors in rat brain. III The telencephalon. *Brain Res.*, **134**, 393–405.

BRADLEY, P.B. & BRAMWELL, G.J. (1977). Stereospecific actions of morphine on single neurones in the brainstem of the rat. *Neuropharmac.*, **16**, 519–526.

BRADLEY, P.B. & BROOKES, ANNE (1981). A comparative study of the actions of opioids on single neurones in different brain regions. *Br. J. Pharmac.*, **74**, 285P.

BRADLEY, P.B. & DRAY, A. (1974). Morphine and neurotransmitter substances: microiontophoretic study in the rat brainstem. *Br. J. Pharmac.*, **50**, 47–55.

BRADLEY, P.B., GAYTON, R.J. & LAMBERT, L.A (1978). Electrophysiological effects of opiates and opioid peptides. In *Centrally Acting Peptides* ed. Hughes, J. pp. 215–229. London: MacMillan.

BROOKES, A. & BRADLEY, P.B. (1984). Electrophysiological evidence for κ-agonist activity of dynorphin in rat brain. *Neuropharmac.*, **23**, 207–210..

CHANG, K.J., COOPER, B., HAZUM, E. & CUATRECASAS, P. (1979). Multiple Opiate Receptors: Different regional distribution in the brain and differential binding of opiates and opioid peptides. *Mol Pharmac.*, **16**, 91–104.

CHAVKIN, C., JAMES, I. & GOLDSTEIN, A. (1982). Dynorphin is a specific endogenous ligand of the κ-opiate receptor. *Science*, **215**, 413–415.

COSTA, E., FRATTA, W., HONG, J.S., MORONI, F. & YANG, H-Y. (1978). Interactions with enkephalinergic and other neuronal systems. *Adv. Biochem. Psychopharmac.*, **18**, 217–226.

ELDE, R., HÖKFELT, T., JOHANSSON, O. & TERENIUS, L. (1976). Immunohistochemical studies using anti-bodies to leucine-enkephalin: initial observations on the nervous system of the rat. *Neurosci.*, **1**, 349–351.

FRENK, H., McCARTY, B. & LIEBESKIND, J. (1978). Different brain areas mediate the analgesic and epileptic properties of enkephalin. *Science*, **200**, 335–337.

FRENK, H., URCA, G. & LIEBESKIND, J. (1978). Epileptic properties of leu- and met-enkephalin: comparison with morphine and reversibility by naloxone. *Brain Res.*, **147**, 327–337.

FRY, J.P., ZIEGLGÄNSBERGER, W. & HERZ, A. (1979). Specific versus non-specific actions of opioids on hippocampal neurones in the rat brain. *Brain Res.*, **163**, 295–305.

GAYTON, R.J. & BRADLEY, P.B. (1976). Comparison of the effects of morphine and related substances with those of dopamine on single neurones in the rat caudate nucleus. In *Opiates and Endogenous Opioid Peptides*. ed. Kosterlitz, H.W. pp. 213–216. Amsterdam: Elsevier/North Holland.

GAYTON, R.J., LAMBERT, L.A. & BRADLEY, P.B. (1978). Failure of (+) naloxone to antagonise responses to opioid peptides. *Neuropharmac.*, **17**, 549–551.

GOODMAN, R.R., SNYDER, S.H., KUHAR, M.J. & YOUNG, W.S. (1980). Differentiation of δ and μ opiate receptor localisations by light microscopic autoradiography. *Proc. natn. Acad. Sci. U.S.A.*, **77**, 6239–6243.

HAAS, H.L. & RYALL, R.W. (1980). Is excitation by enkephalins of hippocampal neurones in the rat due to presynaptic facilitation or to disinhibition? *J. Physiol.*, **308**, 315–330.

HAMBROOK, J., MORGAN, B., RANCE, M. & SMITH, C. (1979). Mode of deactivation of the enkephalins by rat and human plasma and rat brain homogenate. *Nature*, **262**, 782–783.

HENRIKSEN, S., BLOOM, F., McCOY, F., LING, N. & GUILLEMIN, R. (1978). β-Endorphin induces non-convulsive limbic seizures. *Proc. natn. Acad. Sci. U.S.A.*, **75**, 5221–5225.

HENRIKSEN, S., CHOUVET, G. & BLOOM, F. (1982). *In vivo* cellular responses to electrophoretically applied dynorphin in the rat hippocampus. *Life Sci.*, **31**, 1785–1788.

HEWSON, G. (1984). A microiontophoretic study of the effects of opioids on respiratory-related and non-respiratory neurones in the rat brain stem. *Ph.D. Thesis, University of Birmingham.*

HUGHES, J. (1979). Release, biosynthesis and metabolism of the enkephalins. In *Mechanisms of Pain and Analgesic Compounds*. ed. Beers, R.F. & Bassett, E.G. pp. 215–226. New York: Raven Press.

IWAMOTO, E.T. (1981). Locomotor activity and antinociception after putative μ, κ and δ opioid receptor agonists in the rat: influence of dopaminergic agonists and antagonists. *J. Pharmac. exp. Ther.*, **217**, 451–460.

KONIG, J.F.R. & KLIPPEL, R.A. (1963). *The Rat Brain. A Stereotaxic Atlas of the Forebrain and Lower Parts of the Brainstem*. Baltimore: Williams & Wilkins.

KOSTERLITZ, H.W. & PATERSON, S.J. (1980). Characterisation of opioid receptors in nervous tissue. *Proc. R. Soc. Lond. B.*, **210**, 113–122.

LORD, J., WATERFIELD, A., HUGHES, J. & KOSTERLITZ, H. (1976). Multiple opiate receptors. In *Opiates and Endogenous Opioid Peptides*. ed. Kosterlitz, H.W. pp. 275–280. Amsterdam: Elsevier/North Holland.

LORD, J., WATERFIELD, A, HUGHES, J. & KOSTERLITZ, H. (1977). Endogenous opioid peptides: multiple agonists and receptors. *Nature*, **267**, 495–499.

MARTIN, W.R., EADES, C.G., THOMPSON, J.A., HUPPLER, R.E. & GILBERT, P.E. (1976). The effects of morphine and nalorphine-like drugs in the non-dependent and morphine-dependent chronic spinal dog. *J. Pharmac. exp. Ther.*, **197**, 517–532.

NICOLL, R., SIGGINS, G., LING, N., BLOOM, F. & GUILLEMIN, R. (1977). Neuronal actions of endorphins and enkephalins among brain regions in a comparative microiontophoretic study. *Proc. natn. Acad. Sci. U.S.A.*, **74**, 2584–2588.

NORTH, R.A. (1979). Opiates, opioid peptides and single neurones. *Life Sci.*, 1527–1546.

SNEAD, O.C. & BEARDEN, L.J. (1982). Epileptogenic spectrum of opiate agonists. *Neuropharmac.*, **21**, 1137–1144.

TIELEN, A.M., LOPES DA SILVA, F.H., MOLLEVANGER, W.J. & DE JONGE, F.H. (1981). Differential effects of enkephalin within hippocampal areas. *Exp. Brain Res.*, **44**, 343–346.

TYERS, M.B. (1980). A classification of opiate receptors that mediate antinociception in animals. *Br. J. Pharmac.*, **69**, 503–512.

WALKER, J.M., MOISES, H.C., COY, D.H., BALDRIGHI, G. & AKIL, H. (1982). Nonopiate effects of dynorphin and Des-Tyr-dynorphin. *Science*, **218**, 1136–1138.

WOODS, J., FLY, C. & SWAIN, H. (1978). Behavioural actions of some N-furyl benzomorphans and ketazocines in rhesus monkeys and mice. In *Characteristics and Functions of Opioids*. ed. Van Ree, J. & Terenius, L. pp. 403–411. Amsterdam: Elsevier/North Holland.

ZIEGLGÄNSBERGER, W., FRENCH, E., SIGGINS, G. & BLOOM, F. (1979). Opioid peptides may excite hippocampal pyramidal neurones by inhibiting adjacent inhibitory interneurones. *Science*, **205**, 415–417.

ZIEGLGÄNSBERGER, W., SIGGINS, G., FRENCH, E. & BLOOM, F. (1978). Effects of opioids on single unit activity. In *Characteristics and Function of Opioids*, ed Van Rees, J.M. & Terenius, L. pp. 75–86. Amsterdam: Elsevier/North Holland.

(*Received April 4, 1984.*)