Br. J. Pharmac. (1978), **64**, 137–143.

SELECTIVE EFFECTS OF MORPHINE ON THE NOCICEPTIVE RESPONSES OF THALAMIC NEURONES IN THE RAT

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1 The effects of intravenous morphine on the firing of single sensory neurones in the thalamus of the anaesthetized rat are described.

2 Low doses (0.38 to 2.00 mg/kg) of morphine depressed the excitation of nucleus lateralis neurones produced by natural noxious stimuli but were without effect on spontaneous activity. The excitation of some of these neurones by iontophoretically applied acetylcholine or glutamate was also depressed by morphine. These effects were reversed by intravenous (0.25 to 1.17 mg/kg) but not iontophoretically applied (50 to 200 nA) naloxone.

3 Similar or higher doses of morphine (0.58 to 5.07 mg/kg) did not prevent the excitation of ventrobasal thalamic neurones by non-noxious stimuli.

4 The possibility of a direct action of intravenous morphine in the thalamus is discussed.

Introduction

Several studies have suggested that intravenous morphine may produce analgesia by a selective depressant action on nociceptive neurones at a number of levels within the neuroaxis (e.g. Fujita, Yasuhara, Yamamoto & Ogiu, 1954; Sasa, 1969; Nakamura & Mitchell, 1971; Kitahata, Kosaka, Taub, Bonikos & Hoffert, 1974; Haigler, 1976; Shigenaga & Inoki, 1976a; Duggan, Hall & Headley, 1977). However, relatively few of these groups of investigators have directly recorded the activity of single, physiologically identified neurones. The value of some studies of supraspinal sites in particular has been limited by the use of gross evoked field potential recordings to monitor neuronal activity. High intensity electrical stimulation of peripheral nerves has been commonly used as the sole noxious stimulus and such stimuli would presumably excite non-nociceptive afferents also.

In the present study we have therefore investigated the effects of intravenous morphine on the excitation of single neurones in nucleus lateralis of the thalamus produced by natural, adequate noxious stimuli, and for comparison have also studied the action of intravenous morphine on the activation of ventrobasal thalamic neurones by low intensity, non-noxious, peripheral electrical stimuli.

A preliminary account of part of this work has been published (Hill & Pepper, 1976).

Methods

Surgical preparation

Adult male rats were anaesthetized with halothane (1 to 1.5% in humidified oxygen). The femoral artery ipsilateral to, and the external jugular vein contralateral to the recording site were cannulated to allow blood pressure to be monitored and for the intravenous administration of drugs. Body temperature was maintained at 37°C by a heating blanket. Cisternal drainage of cerebrospinal fluid was provided by removing the atlanto-occipital membrane. A discrete craniotomy (3 mm diameter) was made and the dura mater removed to allow stereotaxic positioning of the micropipette in the thalamus. The exposed cortical surface was covered with a solution of agar (3% in 0.9% w/v NaCl solution).

Micropipettes

Single or 6 barrelled micropipettes were constructed from borosilicate glass tubing with an integral fibre (Clark Electromedical GC 200F). Single barrelled micropipettes were filled with a solution of pontamine sky blue (G.T. Gurr Ltd.; 2.5% in 0.2 M sodium acetate buffer, pH 5.6). The central recording barrel of multibarrelled micropipettes was filled with 4 M NaCl. One peripheral barrel always contained 1 or 4 M NaCl and was used for automatic current neutralization so

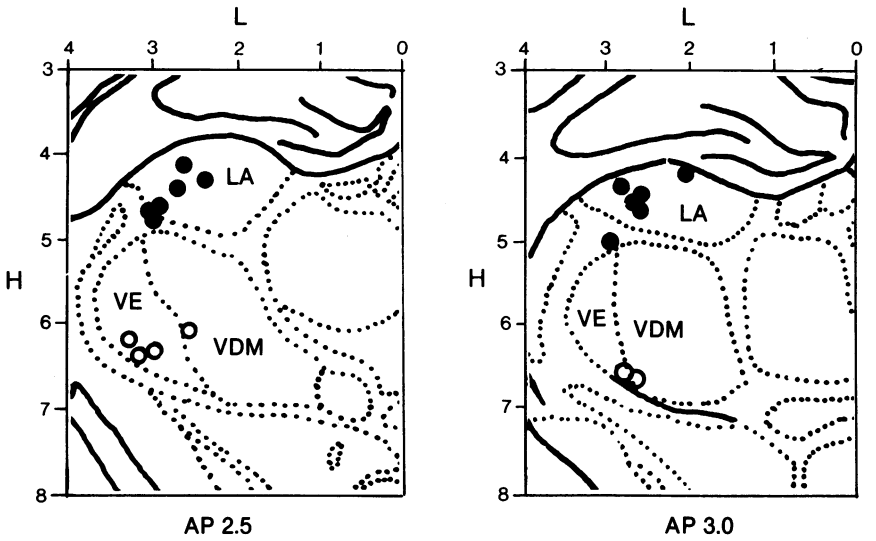

Figure 1 The location of 12 neurones that were excited by noxious stimulation of the tail (●) and 6 neurones that were excited by low intensity stimulation of the contralateral fore paw (○). Neurones are plotted on schematic drawings of coronal sections through the thalamus adapted from the atlas of Fifkova & Marsala (1967). The remaining 5 neurones could not be located histologically with sufficient accuracy and so are not included in the figure. LA = nucleus lateralis anterior; VE = nucleus ventralis thalami; VDM = nucleus ventralis pars dorsomedialis.

that direct effects of current on neuronal firing were reduced. Other barrels contained solutions of L-glutamate (Sigma; 0.5 M, pH 8) acetylcholine chloride (Sigma; 0.5 M, pH 3) and naloxone hydrochloride (Endo; 5 mM in 150 mM NaCl). Multibarrelled micropipettes were broken back immediately before use to give an overall tip diam ter of 3 to 5 μm.

Experimental procedures

Extracellular recordings were obtained from single neurones in nucleus lateralis or ventrobasal thalamus (AP 2.5 to 3.0, L 2.5 to 3.5, H 3.5 to 5.0 and 6.0 to 7.0 respectively, Fifkova & Marsala, 1967). Noxious and non-noxious stimuli were then applied to the periphery. The test noxious stimulus used was immersion of the rat's tail in water at 50.to 55°C for 10 to 30 s (Janssen, Niemegeers & Dony, 1963). Such temperatures have been reported to excite selectively polymodal nociceptors (Burgess, 1974). Receptive fields of those neurones responding to this stimulus were estimated by high intensity subcutaneous electrical stimulation (40 V, 5 ms, 1 Hz) of the limbs. The non-noxious stimulus used was low intensity subcutaneous electrical stimulation (7 to 40 V, 0.05 ms, 0.5 Hz) of the contralateral limbs. Solutions of either morphine sulphate (Macfarlan Smith) or hydrochloride (Ferris & Co. Ltd.) and naloxone hydrochloride (Endo) were made in saline and given intravenously as a bolus injection of 0.1 to 0.2 ml in volume. Doses in the text and figures are of the base. The effects of morphine were investigated on only one cell in each animal. Locations of neurones studied were determined by the examination of serial sections of the fixed brain for pontamine sky blue marks (Lodge, Caddy, Headley & Biscoe, 1974) and/or electrode tracks (Figure 1).

Results

Nucleus lateralis neurones

Only those neurones that showed reproducible excitation following repeated noxious stimulation of the tail (see Methods) were used in this study. This excitation was rapid (latency <10 s) in onset and was substantially maintained for up to 10 min. Receptive fields were large; thus high intensity electrical stimulation of the contralateral hind limb usually caused an equivalent excitation of the same neurones. In contrast, non-noxious stimuli such as hair displacement, light pressure, immersion of the tail in water at temperatures of 45°C or less, or low intensity electrical stimulation of the limbs had no effect on firing rate.

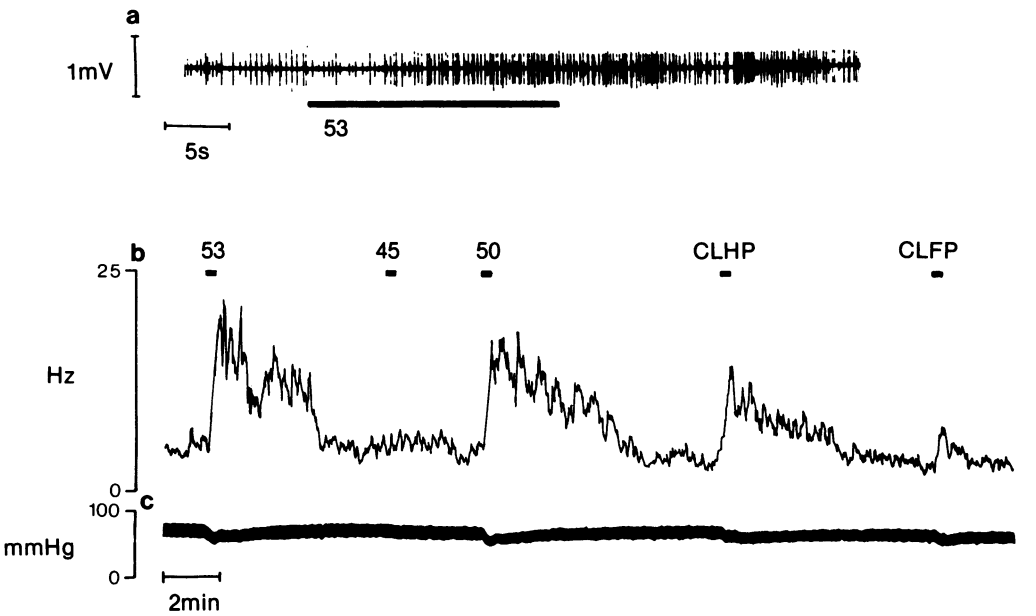

Figure 2 (a) Photographic record of the firing of a nucleus lateralis neurone which responded to immersion of the rat's tail in water at 53°C for 20 s (indicated by the bar below the record). Note that excitation followed an initial inhibition, and that there was no change in action potential amplitude. Only the larger action potentials shown in this record were counted to produce the analogue ratemeter record of this and following responses which are shown in (b). (b) This analogue ratemeter record shows that immersion of the rat's tail in water at 53°C and 50°C but not 45°C caused long lasting excitation. It also shows that high intensity electrical stimulation (40 V, 5 ms, 1 Hz for 20 s) of the contralateral hind paw (CLHP) but not the contralateral fore paw (CLFP) gave a response similar to that caused by noxious heating of the tail. (The apparent rise in firing rate during CLFP stimulation was largely caused by the stimulus artefacts being counted). (c) Simultaneous record of arterial blood pressure showing that noxious stimuli were accompanied by transient blood pressure changes. Note that although CLHP and CLFP stimulation caused similar blood pressure changes, only CLHP stimulation resulted in the characteristic neuronal excitation.

Responses of one such neurone are illustrated in Figure 2.

Figure 2 also shows that although noxious stimulation caused a transient change in blood pressure, it is unlikely that movement of the electrode relative to the neurone (which is sometimes associated with cardiovascular events) was responsible for the observed neuronal excitation since no change in action potential amplitude was observed. Neither could excitation be a result of the blood pressure change, *per se*, since the duration of the excitation greatly exceeded the duration of the blood pressure change. Furthermore blood pressure changes were not accompanied by neuronal excitation when noxious stimuli were applied outside the neurone's receptive field.

All neurones tested were also excited by the iontophoretic application of glutamate (30 to 100 nA, 8 neurones) or acetylcholine (40 to 90 nA, 4 neurones) as previously described (Hill & Pepper, 1978).

The nociceptive responses of all 16 neurones studied were greatly depressed by the intravenous administration of morphine (0.34 $\pm$ 0.09 mg/rat, mean $\pm$ s.e. mean, $n = 16$; the dose range being 0.38 to 2.00 mg/kg). Nine of these neurones no longer responded to noxious stimulation after morphine, and the responses of the remaining 7 neurones were much attenuated, both in peak excitation and response duration. The delay in the onset of morphine action could not be measured accurately because of the long interval necessary between noxious stimuli but effects were observed on the first trial, i. e. 1 to 6 min after the injection of morphine. However, the spontaneous activity of these neurones was unaffected by morphine. An inhibition of glutamate- or acetylcholine-evoked excitation was observed on 4 of 8 neurones,

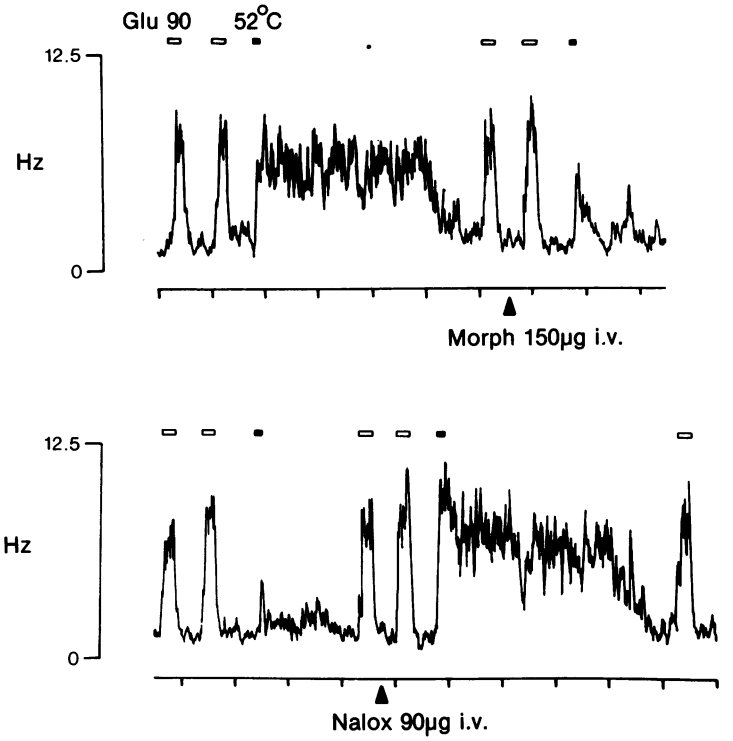

Figure 3 A continuous analogue ratemeter record of the firing of a nucleus lateralis neurone excited by immersion of the rat's tail in water at 52°C (filled bars) and by the iontophoretic application of glutamate (Glu) with a current of 90 nA (open bars). Intravenous morphine (Morph, 0.15 mg, i.e. 0.51 mg/kg) attenuated the nociceptive response but was without effect on either spontaneous activity of this neurone, or the glutamate evoked excitations. Following intravenous naloxone (Nalox, 0.09 mg, i.e. 0.30 mg/kg) the nociceptive response was restored. Each division of the time scale represents 2 min.

the responses of the remaining 4 neurones to these drugs being unchanged by morphine, even when the nociceptive response had been abolished (Figure 3).

After morphine, the nociceptive responses of 14 of these neurones remained depressed until the intravenous injection of naloxone (0.17 ± 0.03 mg/rat, $n = 14$; the dose range being 0.25 to 1.17 mg/kg) between 11 and 76 min later. Occasionally (2 neurones) the nociceptive response following naloxone was greater than pre-morphine controls. Furthermore, morphine was without effect, even at high doses (up to 2.85 mg/rat) on the nociceptive responses of 2 further cells which were located after naloxone (0.18 and 0.23 mg/rat) had been given earlier in the experiment. However, when applied directly to 3 neurones by microiontophoresis (50 to 200 nA) naloxone did not prevent or reverse the depression of the nociceptive response by intravenous morphine (0.15 mg/rat in each case).

Ventrobasal neurones

Seven neurones were studied which showed the characteristic consistent short latency (5 to 11 ms, mean 6.6 ms) excitation following low intensity electrical stimulation of a contralateral limb. Action potentials were usually superimposed upon the early negative-going wave of an evoked field potential. These cells were also excited by natural stimuli such as hair displacement or 'tapping' within their receptive fields, which were usually confined to a small area of the paw. Noxious stimulation inside or outside their receptive fields was without effect on the firing of these neurones.

In every case, intravenous morphine (0.53 ± 0.18 mg/rat, $n = 7$; the dose range being 0.58 to 5.07 mg/kg) failed to attenuate the short latency excitation of ventrobasal neurones. Indeed in one experiment (Figure 4) the number of action potentials in the

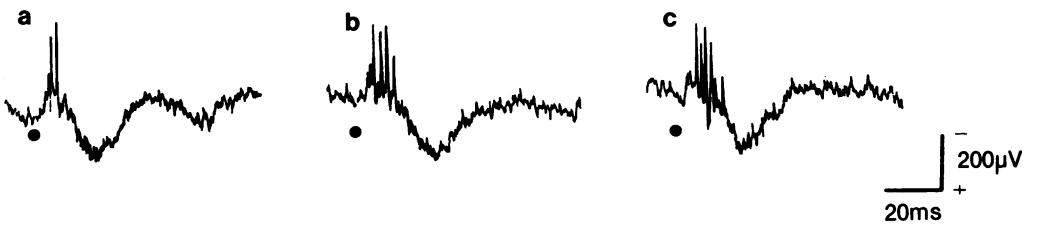

Figure 4 Photographic records showing the effect of intravenous morphine on the activation of a ventrobasal neurone by low intensity subcutaneous electrical stimulation (7 V, 0.05 ms, 0.5 Hz; i.e. 2 × threshold) of the contralateral fore paw. Each record is of a single oscilloscope sweep, the stimulus being triggered 10 ms after the start of the sweep as indicated by the dot below each record. Recordings were filtered (10 ms time constant) and negativity is shown as an upward deflection of the trace. (a) Control; (b) 13 min after morphine 0.76 mg (3.38 mg/kg); (c) 14 min after a second dose of morphine (0.76 mg), given 17.5 min after the first.

evoked burst was increased following morphine, and, in two experiments, spontaneous activity was increased. Changes in the latency of the evoked action potentials or in the amplitude of the evoked field potential were not observed (Figure 4).

Discussion

This study has shown that intravenous morphine suppressed the nociceptive response of nucleus lateralis neurones. This action was presumably due to the activation of specific opiate receptors since it was prevented or reversed by the antagonist, naloxone. The observation that post-naloxone responses to noxious stimuli were occasionally bigger than pre-morphine responses (see also Duggan *et al.*, 1977) might indicate that transmission in pathways involved in nociception is tonically controlled by endogenous, opiate-like peptides (Hughes, Smith, Kosterlitz, Fothergill, Morgan & Morris, 1975) as has been previously suggested (Frederickson & Norris, 1978).

Earlier studies of the rat thalamus have suggested that intravenous morphine has a selective depressant action on the excitation of thalamic neurones in centrum medianum (Oleson & Liebeskind, 1976) and in the posterior group of nuclei (Emmers, 1976; Shigenaga & Inoki, 1976b) produced by peripheral high intensity electrical stimuli presumed to be noxious in nature. However, in these studies rather high doses of morphine (2.5 to 20 mg/kg) were used, and at such doses, non-specific effects could occur.

In contrast, in the present study, very low doses of morphine were found to be effective and such doses are lower than those needed to attenuate the nocifensive response of conscious rats (5 mg/kg; Janssen *et al.*, 1963) to the same stimulus as was used in the present study, and which is probably selective for polymodal nociceptors (Burgess, 1974). It is therefore likely that the suppression, by morphine, of the excitation of nucleus lateralis neurones is related to its antinociceptive properties. Furthermore morphine did not prevent the excitation of ventrobasal units by low intensity stimuli. Indeed, some enhancement of unitary activity was occasionally seen. It is therefore interesting to note that morphine potentiates the Aβ component of the compound action potential recorded from primary afferents (Jurna & Grossman, 1977) and that rats treated with morphine have been found to show exaggerated behavioural responses to non-noxious stimuli (Yaksh, Yeung & Rudy, 1976).

The selective suppression of noxious evoked activity of thalamic neurones by systemically administered morphine may, of course, be secondary to a reduction in their excitatory afferent input, and indeed there is evidence for such an action. Thus, intravenous morphine has been shown to depress the noxious evoked excitation of neurones in the dorsal horn of the spinal cord (Satoh, Nakamura & Takagi, 1971; Le Bars, Menetrey, Conseiller & Besson, 1975) spinal trigeminal nucleus (Sasa, 1969) and reticular formation (Nakamura & Mitchell, 1971; Haigler, 1976; Sun & Gatipon, 1976; Mayer & Hill, 1978) and also to reduce the afferent volley in the ventrolateral tract (Jurna & Grossman, 1976). Furthermore, in the present study, intravenous morphine could suppress the nociceptive excitation of nucleus lateralis neurones without affecting their spontaneous firing.

We suggest, however, that morphine may have an additional, direct action in the thalamus. Opiate receptors are present in the rat thalamus (Pert, Kuhar & Snyder, 1975; Atweh & Kuhar, 1977) and direct, iontophoretic application of morphine to nucleus lateralis neurones was found to depress readily their nociceptive responses (Hill & Pepper, 1976, and unpublished observations). If morphine does act at sites in the thalamus, one might predict that iontophoretic naloxone would reverse the action of intravenous

morphine, as it does the action of opiates on spinal neurones (Zieglgänsberger & Bayerl, 1976). However, at thalamic sites, this reversal would, at best, only be partial because of the effects of morphine at spinal and reticular formation sites. Our failure to demonstrate even partial antagonism could well be due to the inability of the locally applied drug to occupy effectively relatively distant receptors which would be readily available to the systemic drug. Such problems have been extensively discussed in the literature (e.g. Curtis, 1976).

Our present observation that intravenous morphine could depress the nociceptive responses of nucleus lateralis neurones without affecting their spontaneous activity, or in some cases, the excitation produced by iontophoretic acetylcholine or glutamate would be consistent with a presynaptic action of morphine, preventing the release of transmitter from afferent endings. Such an action of morphine has been demonstrated in the trigeminal nucleus (Jessel & Iversen, 1977) and cerebral cortex (Taube, Starke & Borowski, 1977) *in vitro*, and would also explain the results of Duggan *et al.* (1977) who observed that morphine reduced the noxious but not the non-noxious excitation of the same neurones in the spinal cord. An entirely presynaptic action in the thalamus would not, however, account for the depression of the acetylcholine- or glutamate-evoked excitation of some nucleus lateralis neurones after morphine. A similar effect has been found to occur at spinal sites and here it was shown to be mediated by the activation of postsynaptic opiate receptors (Zieglgänsberger & Bayerl, 1976).

Thus, although the primary site of action of morphine may be at a spinal level, we suggest that post- and perhaps presynaptic effects on thalamic neurones could contribute to the production of analgesia.

We would like to thank Endo Laboratories for the gift of naloxone. C.M.P. was an MRC scholar.

References

ATWEH, S.F. & KUHAR M.J. (1977). Autoradiographic localisation of opiate receptors in rat brain. II The brain stem *Brain Research*, **129**, 1–12.

BURGESS, P.R. (1974). Patterns of discharge evoked in cutaneous nerves and their significance for sensation. *Adv. Neurol.*, **4**, 11–18.

CURTIS, D.R. (1976). The use of transmitter antagonists in micro-electrophoretic investigations of central synaptic transmission. In *Drugs and Central Synaptic Transmission*, ed. Bradley, P.B. & Dhawan, B.N. pp. 7–35. London: Macmillan.

DUGGAN, A.W., HALL, J.G. & HEADLEY, P.M. (1977). Suppression of transmission of nociceptive impulses by morphine: selective effects of morphine administered in the region of the substantia gelatinosa. *Br. J. Pharmac.*, **61**, 65–76.

EMMERS, R. (1976). Thalamic mechanisms that process a temporal pulse code for pain. *Brain Research*, **103**, 425–441.

FIFKOVA, E. & MARSALA, J. (1967). Stereotaxic atlases for the cat, rabbit and rat. In *Electrophysiological Methods in Biological Research*, ed. Bures, J., Petran, M. & Zachar, J. pp. 653–731. New York: Academic Press.

FREDERICKSON, R.C.A. & NORRIS, F.H. (1978). Enkephalins as inhibitory neurotransmitters modulating nociception. In *Iontophoresis and Transmitter Mechanisms on the Mammalian Central Nervous System*. ed. Ryall, R.W. & Kelly, J.S. pp. 320–322. Amsterdam: Elsevier.

FUJITA, S., YASUHARA, M., YAMAMOTO, S. & OGIU, K. (1954). Studies on sites of action of analgesics. 2. The effect of analgesics on afferent pathways of pain. *Jap. J. Pharmac.*, **4**, 41–51.

HAIGLER, H.J. (1976). Morphine: ability to block neuronal activity evoked by a nociceptive stimulus. *Life Sci.*, **19**, 841–858.

HILL, R. G. & PEPPER, C. M. (1976). The effects of morphine and met-enkephalin on nociceptive neurones in the rat thalamus. *Br. J. Pharmac.*, **58**, 459P.

HILL, R.G. & PEPPER, C.M. (1978). Studies on the pharmacology of nociceptive neurones in the rat thalamus. In *Iontophoresis and Transmitter Mechanisms in the Mammalian Central Nervous System*, ed. Ryall, R.W. & Kelly, J.S. pp. 329–331. Amsterdam: Elsevier.

HUGHES, J., SMITH, T.W., KOSTERLITZ, H.W., FOTHERGILL, L.A., MORGAN, B.A. & MORRIS, H.R. (1975). Identification of two related pentapeptides from the brain with potent opiate agonist activity. *Nature* **258**, 577–579.

JANSSEN, P.A.J., NIEMEGEERS, C.J.E. & DONY, J.G.H. (1963). The inhibitory effect of Fentanyl and other morphine-like analgesics on the warm water induced tail withdrawal reflex in rats. *Arzneimittel-Forsch.*, **13**, 502–507.

JESSEL, T.M. & IVERSEN, L.L. (1977). Opiate analgesics inhibit substance P release from rat trigeminal nucleus *Nature*, **268**, 549–551.

JURNA, I. & GROSSMAN, W. (1976). The effect of morphine on the activity evoked in ventrolateral tract axons of the cat spinal cord. *Exp. Brain Res.*, **24**, 473–484.

JURNA, I. & GROSSMAN, W. (1977). The effect of morphine on mammalian nerve fibres. *Eur. J. Pharmac.*, **44**, 339–348.

KITAHATA, L.M., KOSAKA, Y., TAUB, A., BONIKOS, K. & HOFFERT, M. (1974) Lamina-specific suppression of dorsal-horn unit activity by morphine sulfate. *Anesthesiology*, **41**, 39–48.

LE BARS, D., MENETREY, D., CONSEILLER, C. & BESSON, J. M. (1975) Depressive effects of morphine upon lamina V cells activities in the dorsal horn of the spinal cat. *Brain Research*, **98**, 261–277.

LODGE, D., CADDY, K. W. T., HEADLEY, P. M. & BISCOE, T. J. (1974) The location of neurones with pontamine sky blue. *Neuropharmac.* **13,** 481–485.

MAYER, M. L. & HILL, R. G. (1978) The effects of intravenous fentanyl, morphine and naloxone on nociceptive responses of neurones in the rat caudal medulla. *Neuropharmac.* (in press).

NAKAMURA, J. & MITCHELL, C. L. (1971) The effects of morphine, pentobarbitol and chlorpromazine on bioelectrical potentials evoked in the brain stem of the cat by electrical stimulation of the gingiva and toothpulp. *J. Pharmac. exp. Ther.*, **178,** 232–240.

OLESON, T. D. & LIEBESKIND, J. C. (1976) Modification of midbrain and thalamic evoked responses by analgesic brain stimulation in the rat. In *Advances in Pain Research and Therapy*, Vol. 1, ed. Bonica, J. J. & Albe-Fessard D. pp. 487–494. New York: Raven Press.

PERT, C. B., KUHAR, M. J. & SNYDER, S. H. (1975) Autoradiographic localisation of the opiate receptor in rat brain. *Life Sci.* **16,** 1849–1954.

SASA, M. (1969) Effects of morphine on unit discharges recorded from the trigeminal nuclei in cats. *Jap. J. Pharmac.*, **19,** 373–385.

SATOH, M. NAKAMURA, N. & TAKAGI, M. (1971) Effect of morphine on the bradykinin-induced unitary discharges in the spinal cord of the rabbit. *Eur. J. Pharmac.*, **16,** 245–247.

SHIGENAGA, Y. & INOKI, R. (1976a) Effects of morphine and barbiturate on the SI and SII potentials evoked by toothpulp stimulation of rats. *Eur. J. Pharmac.*, **36,** 347–353.

SHIGENAGA, Y. & INOKI, R. (1976b) Effects of morphine on single unit responses in ventrobasal complex (VB) and posterior nuclear group (PO) following toothpulp stimulation. *Brain Research*, **103,** 152–156.

SUN, C. L. & GATIPON, G. B. (1976) Effects of morphine sulfate on medial bulboreticular response to peripherally applied noxious stimuli. *Exp. Neurol.*, **52,** 1–12.

TAUBE, H. D., STARKE, K. & BOROWSKI, E. (1977) Presynaptic receptor systems on the noradrenergic neurones of rat brain. *Naunyn-Schmiedebergs Arch. Pharmac.*, **299,** 123–141.

YAKSH, T. L., YEUNG, J. C. & RUDY, T. A. (1976) Interaction of the medial thalamus and the periaqueductal gray in the modulation of the anti-nociceptive actions of morphine. In *Advances in Pain Research and Therapy*, Vol 1. ed. Bonica, J. J. & Albe-Fessard, D. pp. 621–628. New York: Raven Press.

ZIEGLGÄNSBERGER, W. & BAYERL, H. (1976) The mechanism of inhibition of neuronal activity by opiates in the spinal cord of cat. *Brain Research*, **115,** 111–128.

(Received March 16, 1978.)