morphine nor enkephalin depressed the firing rate of any of the spinal neurones studied, however, enkephalin selectively reduced acetylcholine-induced but not morphine-induced excitation of Renshaw cells. By contrast, morphine enhanced the actions of acetylcholine and amino acid excitants on these neurones and also antagonized the depressant action of glycine on spinal neurones whereas enkephalin did not modify this action of glycine.

The effects of morphine and enkephalin on brain stem neurones were qualitatively similar to those observed on spinal cord neurones. Hence, excitation was the predominant effect of enkephalin and morphine on brain stem neurones. These neurones could also be excited by acetylcholine. Occasionally, enkephalin and morphine caused a reduction in cell firing rate. As in the spinal cord ME was a more effective excitant than LE. Morphine enhanced the excitatory effect of acetylcholine and reduced glycine-induced depression whereas enkephalin reduced the response to acetylcholine and did not affect glycine depression.

These observations provide direct evidence that enkephalin acts on central opiate receptors and are consistent with the hypothesis that enkephalin is the endogenous ligand for the stereospecific opiate receptor (Kosterlitz & Hughes, 1975). However, it is not clear whether the additional properties of enkephalin, not shared by morphine, are of physiological importance.

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References

BELLUZZI, J.D., GRANT, N., GARSKY, V., SARANTAKIS, D., WISE, C.D. & STEIN, L. (1976). Analgesia induced *in vivo* by central administration of enkephalin in rat. *Nature (Lond.)*, **260**, 625–626.

DUGGAN, A.W., DAVIES, J. & HALL, J.G. (1976). Effects of opiate agonists and antagonists on central neurones of the cat. *J. Pharmac. exp. Ther.*, **196**, 107–120.

HUGHES, J. (1975). Isolation of an endogenous compound from the brain with pharmacological properties similar to morphine. *Brain Res.*, **88**, 295–308.

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 (Lond.)*, **258**, 577–579.

KOSTERLITZ, H.W. & HUGHES, J. (1975). Some thoughts on the significance of enkephalin, the endogenous ligand. *Life Sci.*, **17**, 91–96.

The effects of morphine and met-enkephalin on nociceptive neurones in the rat thalamus

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Morphine applied iontophoretically has been found to both excite and depress the activity of single central neurones (Bradley & Dray, 1974; Satoh, Zieglgänsberger & Herz, 1975; Duggan, Davies & Hall, 1976). In contrast, we have found that iontophoretically applied met-enkephalin (Hughes, Smith, Kosterlitz, Fothergill, Morgan & Morris, 1975) is predominantly depressant (Hill, Pepper & Mitchell, 1976). We have therefore investigated the actions of morphine and met-enkephalin on the same population of neurones and, to increase the relevance of the study to analgesic mechanisms, we have confined our study to nociceptive neurones located in the rat thalamus.

Adult male rats were anaesthetized with 1% halothane in oxygen. Single unit recordings were made from the central barrel of a 5-barrelled micropipette stereotaxically placed in the thalamus. Recording and current balancing barrels were filled with 4 M NaCl. Other barrels contained combinations of L-glutamate (0.5 M, pH 8.5), GABA (0.5 M, pH 3.0) morphine hydrochloride (50 mM, no adjustment of pH) and synthetic met-enkephalin (8 mM, no adjustment of pH) for iontophoretic application. Techniques for extracellular recording of action potentials and iontophoresis of drugs were conventional. Nociceptive neurones were identified by their consistent increases in firing rate following noxious stimulation of the tail (Hellon & Mitchell, 1975). Stimuli used were immersion of the tail in hot (50–55°C) water for 30–45s or a strong pinch with a thermometer clamp. Twenty-two nociceptive neurones were identified in 16 rats.

Intravenous morphine (hydrochloride; 0.6 to 5.0 mg/kg) prevented or greatly reduced the increase in firing rate produced by noxious stimulation. This effect was typically rapid in onset (<2 min) and of long duration (up to 70 min). Intravenous naloxone (hydrochloride; 0.15 to 0.6 mg/kg) reversed this action of morphine. Similar results have been obtained by others in studies of nociceptive neurones in the dorsal horn of the cat spinal cord (Le Bars, Menetrey, Conseiller & Besson, 1975).

The change in the firing rate of the thalamic neurones in our study may have been secondary to a change in the presynaptic volley exciting them, since

the discharge in ventrolateral tract axons is much reduced by intravenous morphine (Jurna & Grossman, 1976). However, direct iontophoretic application of either morphine (10–40 nA) or met-enkephalin (0–100 nA) to nociceptive neurones was found to depress both spontaneous and glutamate evoked activity and to prevent the excitant effects of noxious stimulation. No evidence of excitation of these neurones by morphine was seen.

Thus, on this restricted population of neurones at least, the actions of morphine and enkephalin are similar. This investigation would therefore lend support to the suggestion (Kosterlitz & Hughes, 1975) that enkephalin may function in the brain as an endogenous morphine-like factor controlling the appreciation of noxious stimuli.

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References

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

DUGGAN, A.W., DAVIES, J. & HALL, J.G. (1976). Effects of opiate agonists and antagonists on central neurones of the cat. *J. Pharmac. exp. Ther.*, **196**, 107–120.

HELLON, R.F. & MITCHELL, D. (1975). Characteristics of neurones in the ventrobasal thalamus of the rat which respond to noxious stimulation of the tail. *J. Physiol. (Lond.)*, **250**, 29–30P.

HILL, R.G., PEPPER, C.M. & MITCHELL, J.F. (1976). The depression of nociceptive and other neurones in the rat brain by iontophoretically applied met-enkephalin. *Nature (Lond.)* (in press).

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 (Lond.)*, **258**, 577–579.

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.

KOSTERLITZ, H.W. & HUGHES, J. (1975). Some thoughts on the significance of enkephalin, the endogenous ligand. *Life Sci.*, **17**, 91–96.

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 Res.*, **98**, 261–277.

SATOH, M., ZIEGLGÄNSBERGER, W. & HERZ, A. (1975). Interaction between morphine and putative excitatory neurotransmitters in cortical neurones in naive and tolerant rats. *Life Sci.*, **17**, 75–80.

Interactions of peptides derived from the C-fragment of β-lipotropin with brain opiate receptors

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The pentapeptide sequence of methionine–enkephalin (met–E, Tyr-Gly-Gly-Phe-Met) is also the N-terminal sequence of the C-Fragment of β-lipotropin, a 31 residue peptide which is found in considerable quantity in the pituitary (Bradbury, Smyth & Snell, 1975). This peptide has a high affinity for brain opiate receptors, as measured by competition with [^{3}H]-naloxone and [^{3}H]-dihydromorphine for binding to a washed membrane preparation. The potency of C-Fragment is equally great against the two tritiated opiates, a characteristic of morphinoid antagonists, but not of agonists (Pert & Snyder, 1974) or methionine–enkephalin, which display greater potency against dihydromorphine than against naloxone (Bradbury, Smyth, Snell, Birdsall & Hulme, 1976).

The removal of residues 30 and 31 (Gly-Gln) from C-Fragment has little effect on the binding properties, but further removal of residues 28 and 29 (Lys-Lys) reduces the affinity by a factor of 20 (Bradbury *et al.*, 1976). This suggests that interaction of C-terminal residues, including lysines 28 and 29, with the receptor may make an important contribution to the binding of C-Fragment, both augmenting the affinity and modifying the binding properties of the N-terminal pentapeptide sequence. Direct evidence for the existence of a binding site for the C-terminal comes from the observation that the tridecapeptide, composed of residues 19–31, inhibits [^{3}H]-naloxone and [^{3}H]-dihydromorphine binding, with an IC_{50} of 3×10^{-6}M, despite the absence of the met–E sequence.

The binding site for the N-terminal region of C-Fragment has been probed by investigating the binding properties of a series of analogues of met–E. N-methylation of met–E, or conversion of the carboxyl terminal to an amide group produces a 2–4 fold increase in the affinity without affecting the morphine-like binding properties of met–E. However, N-carbamylation, benzylation of the tyrosine hydroxyl group, or substitution of the 2-glycine residue by 1-proline, or 1-alanine all drastically reduce binding affinity. Substitution of sarcosine in the 2-