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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## 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 $3 \times 10^{-6}$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-

position reduces the potency against [$^3$H]-naloxone thirty-fold, but produces a much larger effect on the potency against [$^3$H]-dihydromorphine.

Although the binding properties of C-Fragment resemble those of the alkaloid antagonists, it is a potent central analgesic (Feldberg & Smyth, 1976), and acts as a full agonist on the guinea pig ileum (Hughes & Kosterlitz, personal communication). The presence of two binding sites for C-Fragment may account for this unexpected combination of properties.

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