Evidence for naloxone and opiates as GABA antagonists

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In several species the behavioural effects of opiates change from inhibition to excitation and finally convulsions, as the dose is increased. We report here several lines of evidence that the convulsant properties of drugs with chemical structures related to morphine, and in particular naloxone, may be the result of GABA receptor blockade. For each experiment male T.O. Strain mice 25–30 g were randomly divided into five groups of eight, and used only once. Bicuculline, strychnine HCl and naloxone HCl were administered intraperitoneally, and the presence of convulsions (end point: bilateral clonic or tonic extensions of hindlimbs) was noted over the following 30 minutes. The data were analysed by the method of Litchfield & Wilcoxon (1949). Naloxone caused convulsions in mice, with an ED_{50} of 190 mg/kg (as the base). Pretreatment of mice with a subconvulsant dose of naloxone (90 mg/kg i.p. 5 min before challenge) significantly ($P < 0.05$) reduced the ED_{50} for bicuculline convulsions (potency ratio = 2.2), while not affecting the dose-response for strychnine (potency ratio = 1.1). On the other hand, diazepam pretreatment (5 mg/kg i.p. 30 min before challenge) significantly increased the ED_{50} of both bicuculline and naloxone (in both cases potency ratio = 0.5) while not affecting strychnine-induced convulsions (potency ratio = 1.2).

In other experiments, bicuculline, naloxone, morphine, levorphanol and dextrorphan were found to displace [^{3}H]-GABA from GABA receptor sites in homogenates of human cerebellum, using the GABA binding assay of Enna & Snyder (1975). Each drug was tested with 4–6 concentrations, each in duplicate. The IC_{50}s (in μM) for the above drugs were 8, 308, 400, 250 and 300, respectively.

Finally, naloxone was tested for its ability to block the inhibition evoked by iontophoretically applied GABA on single neurones in the rat nucleus accumbens and olfactory tubercle. Multibarrelled pipettes (tips 4–6 μ) were filled with 4 M NaCl (recording and current balancing barrels), 1 M GABA pH 4.8 and 50 mM naloxone in 100 mM NaCl, pH 5.0. When tested in this way, naloxone applied by microiontophoresis completely and reversibly antagonized GABA-evoked inhibition in 6 of 10 neurones.

It is postulated that the behavioural excitation seen after administering large amounts of some opiates or opiate antagonists may reflect functional blockade of GABA-inhibitory systems.

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Actions of enkephalin and morphine on spinal cord and brain stem neurones

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Enkephalin occurs in brain tissue as two pentapeptides, methionine (ME) and leucine enkephalin (LE) (Hughes, Smith, Kosterlitz, Fothergill, Morgan & Morris, 1975) and has been suggested to be the endogenous ligand for the opiate receptor in the CNS (Kosterlitz & Hughes, 1975). While this compound mimics the actions of morphine at peripheral sites (Hughes, 1975; Hughes *et al.*, 1975) and possesses analgesic activity when administered intracerebroventricularly (Belluzzi, Grant, Garsky, Sarantakis, Wise & Stein, 1976) its effects on individual central neurones are unknown.

Conventional electrophysiological and micro-electrophoretic techniques have been used to investigate the actions of synthetic methionine and leucine enkephalin on single neurones in the spinal cord of the pentobarbitone anaesthetized cat and in the pons-medulla of the urethane anaesthetized rat. The same multi-barrelled glass micropipettes were used in experiments on each species.

In the spinal cord, morphine consistently excited Renshaw cells but not other non-cholinoceptive interneurones (Duggan, Davies & Hall, 1976). This property was shared by enkephalin, ME being more consistent in this respect than LE. Both morphine and enkephalin-induced excitation was reversibly reduced by the narcotic antagonist naloxone. Neither

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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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