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.

References

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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