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Rapid communication

COMPETITIVE ANTAGONISM OF MORPHINE ACTION IN VITRO BY CALCIUM

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Recent observations suggest an important role for calcium in the interaction of opiates with neuronal tissue. The intracerebroventricular injection of calcium antagonized the antinociceptive effect of morphine. Morphine administration reduced the concentration of brain calcium and the effect seemed to be predominantly located in fractions containing nerve endings (for references see Yamamoto et al., 1978). It has been shown that the opiate action on the guinea-pig ileum preparation in vitro is similar in many respects to the opiate interaction with the brain. It was thus of interest to study the significance of calcium for the inhibitory effect of morphine on the electrically induced contractions of the guinea-pig ileum preparation in vitro.

Myenteric plexus—longitudinal muscle strips were prepared from guinea-pig ileums and mounted in tissue chambers containing Krebs-bicarbonate solution (37°C). Experimental procedure and equipment have been described previously (Opmeer and van Ree, 1978). Two sets of experiments were performed. First, the inhibitory action of a fixed dose of morphine was determined in the presence of graded concentrations of Ca^{2+}. Between the test doses of morphine the preparation was extensively washed by renewing the bath fluid. The basal response to stimulation was not affected by modulating the Ca^{2+} concentration except that a concentration of 0.63 mM slightly reduced twitch tension. Morphine (400 nM) added to the Krebs solution with a normal Ca^{2+} concentration (2.54 mM) produced about 30% inhibition of twitch amplitude, which could be antagonized by naloxone (50 nM) added before or after morphine. The sensitivity of the strips to the inhibitory action of morphine appeared to be dose-dependently related to the external Ca^{2+} concentration. Doubling this concentration in the Krebs solution (to 5.08 mM) diminished the morphine response to 85.7 ± 4.0% of the response observed under standard conditions ($n = 14$, $P < 0.02$, Student's paired t-test) (see also Heimans, 1975). On the other hand lowering the Ca^{2+} concentration (to 1.27 or 0.63 mM) increased the morphine response to 118 ± 9% ($n = 14$) resp. 140 ± 8% ($n = 14$) of the response under standard conditions ($P < 0.05$ and $P < 0.05$). To investigate the influence of calcium on an already established response to morphine, a second set of experiments was performed in which the Ca^{2+} concentration of the Krebs solution was increased after the addition of morphine, without washing the preparation. Various doses of morphine (100—800 mM) were added to a Krebs solution containing 0.63 mM Ca^{2+}. After the response to a particular dose of morphine appeared to be constant, a small quantity of a concentrated $CaCl_2$ solution was added to the bath fluid to a final concentration of 2.54 mM Ca^{2+}. Subsequently, using the same procedure,

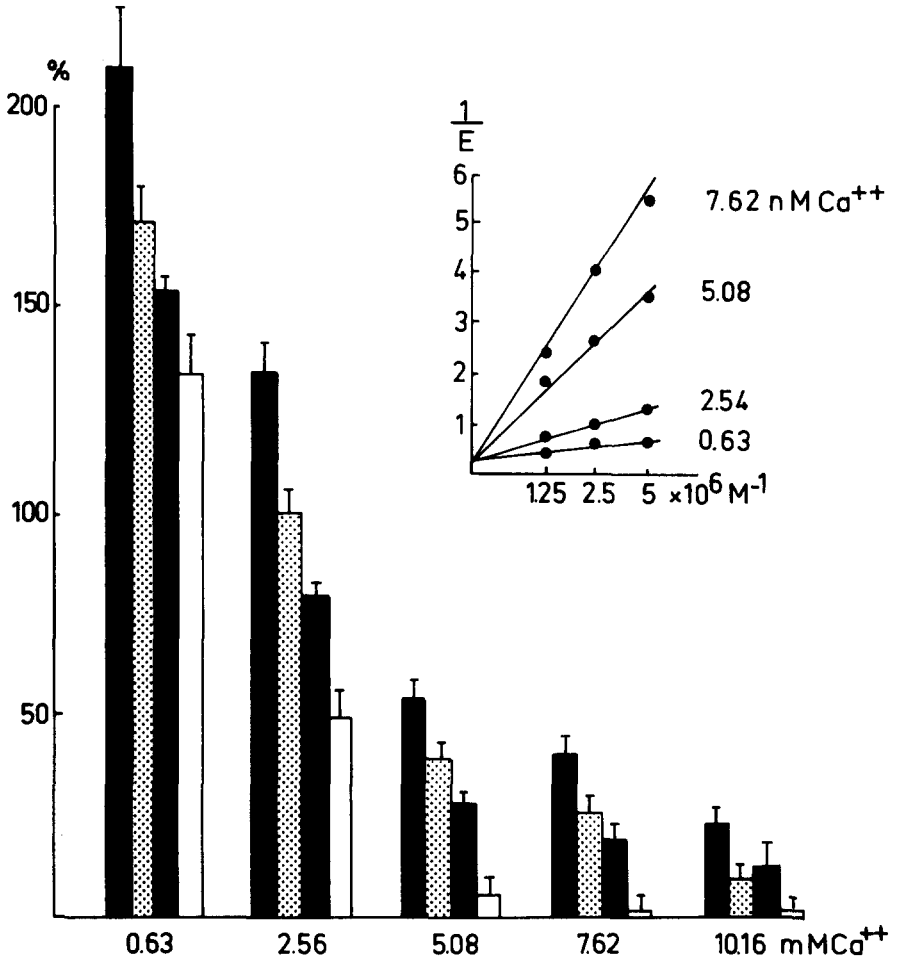

Fig.1. The influence of increasing the Ca^{2+} concentration in Krebs solution (starting at 0.63 mM) on the inhibitory response to graded doses of morphine (▪ 800 nM; ⊡ 400 nM; ■ 200 nM; □ 100 nM) by an electrically stimulated longitudinal muscle—myenteric plexus preparation of the guinea-pig ileum. Each histogram shows the mean inhibitory response to morphine (n = 4—7) expressed as percentage of the mean response obtained at 400 nM morphine with normal Krebs solution (2.54 mM Ca^{2+}). Vertical bars represent the S.E.M. Insert: Double reciprocal analysis (Lineweaver—Burk plot) of the interaction of graded concentrations of Ca^{2+} with the response to morphine. The morphine concentration (M) was plotted versus the response divided by the value obtained at 400 nM morphine with normal Krebs solution (E).

higher concentrations of Ca^{2+} in the medium up to 10.16 mM were studied. It was observed that Ca^{2+} dose-dependently diminished the response to morphine (see fig. 1). The highest Ca^{2+} concentration completely reversed the action of morphine. Analysing the data according to a Lineweaver—Burk transformation, it appeared that the interaction of Ca^{2+} and morphine resembled competitive antagonism (see fig. 1; insert).

The mechanism underlying this competitive antagonism is not yet clear. Not only an effect of Ca^{2+} on the interaction of morphine with its receptors, but all inhibitory actions of Ca^{2+} on any process between receptor activation and the measured response which lead to an apparent 'affinity' reduction are classed as competitive. The morphine—receptor interaction may elicit efflux or reduced uptake of synaptosomal Ca^{2+}, resulting in a decreased Ca^{2+} content (Yamamoto et al., 1978). Increasing the extracellular Ca^{2+} concentration may restore the disturbed balance between intra- and extracellular

Ca^{2+} and consequently diminish the effect of morphine. Since the response to morphine is modulated by changing the extracellular Ca^{2+} concentration, it is reasonable to consider that Ca^{2+} disposition plays a critical role in the response to morphine. Normal synaptic transmission in both the central and peripheral nervous system requires a precisely controlled release of neurotransmitter, which depends on the influx of Ca^{2+} into nerve terminals (Rubin, 1974). Thus, it may be postulated that morphine alters Ca^{2+} disposition in neurones affected by this drug, resulting in a reduced neurotransmitter release i.e. diminished acetylcholine release in the guinea-pig ileum (Paton, 1957).

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