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DOPAMINE AND THE DEPRESSANT ACTION OF MORPHINE ON STIMULATED GUINEA-PIG ILEUM

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1 Morphine reduces the amplitude of the contractions induced by electrical stimulation, in the myenteric plexus-longitudinal muscle preparation of guinea-pig ileum. Dopamine and apomorphine have the same effect but at much higher concentrations.

2 Dopamine, at concentrations lower than those which would normally be inhibitory, partially reverses the depressant effect of morphine.

3 Pre-treatment of guinea-pigs with 6-hydroxydopamine results in a slight supersensitivity of innervated longitudinal muscle preparations to dopamine and has no effect on morphine activity.

4 Naloxone antagonizes the depressant effect of morphine but not that of dopamine or apomorphine.

5 The response of the ileum preparation to morphine is not affected by phentolamine or propranolol; the effect of dopamine, however, is abolished by α-adrenoceptor blockade.

Introduction

Morphine decreases the amplitude of the electrically-evoked contractions in the myenteric plexus-longitudinal muscle preparation of the guinea-pig ileum (Paton, 1957; Gyang & Kosterlitz, 1966). This test has been used in order to study the basic mechanism of the effects of narcotic analgesics, since it has been shown that the receptors of the myenteric plexus are like those in the central nervous system (Fennessey, Heimans & Rand, 1969; Ehrenpreis, Light & Schonbuch, 1972). A reduced release of acetylcholine is considered responsible for the effect of morphine on the ileum (Cox & Weinstock, 1966) while an efflux of noradrenaline is generally excluded (Gyang & Kosterlitz, 1966; Kosterlitz & Watt, 1968) since α- and β-adrenoceptor blocking drugs do not modify the response of the ileum to narcotic agonists. It is also possible that morphine, in addition to the prevention of the release of acetylcholine (an excitatory neuro-transmitter substance in the gut), may cause a release of an inhibitory modulator of transmission; this substance may be dopamine as suggested by Goldstein & Schulz (1973). On the other hand, there is evidence to support the possibility of a relationship between dopamine and some effects of morphine in the central nervous system (Fennessy & Lee, 1972). We have, therefore, carried out this investigation in which the effects of dopamine and of the dopaminergic drug, apomorphine were examined and compared with the effect of morphine on the myenteric plexus-longitudinal muscle preparation of ileum taken from normal guinea-pigs and guinea-pigs pre-treated with 6-hydroxydopamine; the latter drug causes selective destruction of catecholamine-containing nerve terminals (Sachs & Jonsson, 1975).

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Methods

Animal pretreatment

Guinea-pigs of either sex weighing between 300 and 500 g were given an intraperitoneal injection of 30 mg/kg 6-hydroxydopamine dissolved in 0.9% w/v NaCl solution (saline) containing ascorbic acid (1 mg/ml) 36 h before they were killed.

Control animals received only the solvent.

Ileum preparation

The myenteric plexus-longitudinal muscle preparation from the guinea-pig (Paton & Vizi, 1969) was set up in a 5 ml organ bath containing Krebs solution of the following composition (mM): NaCl 118, KCl 4.75, KH_2PO_4 1.19, $NaHCO_3$ 25, glucose 11, $MgSO_4$ 1.2, $CaCl_2$ 2.5 and choline chloride 20 μM. A platinum ring electrode (anode) was placed at the top of the bath; the cathode was a platinum hook at the bottom of the bath. Electrical rectangular pulses were used of 1 ms duration, 0.2 Hz frequency and supramaximal (80 V) voltage (1.3 times the maximal voltage). Before addition of drugs to the bath fluid, the preparations were allowed to equilibrate for at least 60 min, with renewal of the bath fluid every 10 minutes.

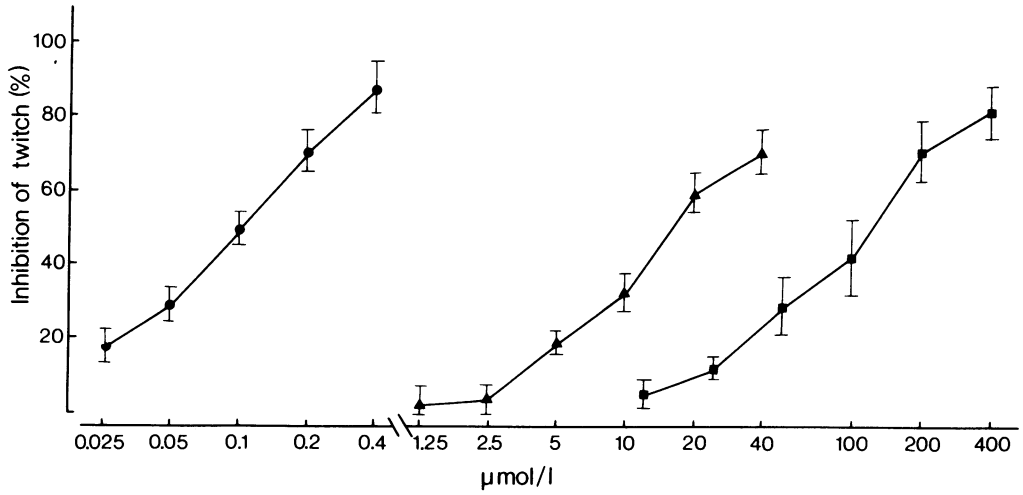

Figure 1 Inhibitory effects of morphine (●), dopamine (▲) and apomorphine (■) on the electrically induced twitches of the longitudinal muscles of guinea-pig ileum: each point represents the mean of 6 experiments. Vertical lines show s.e. mean.

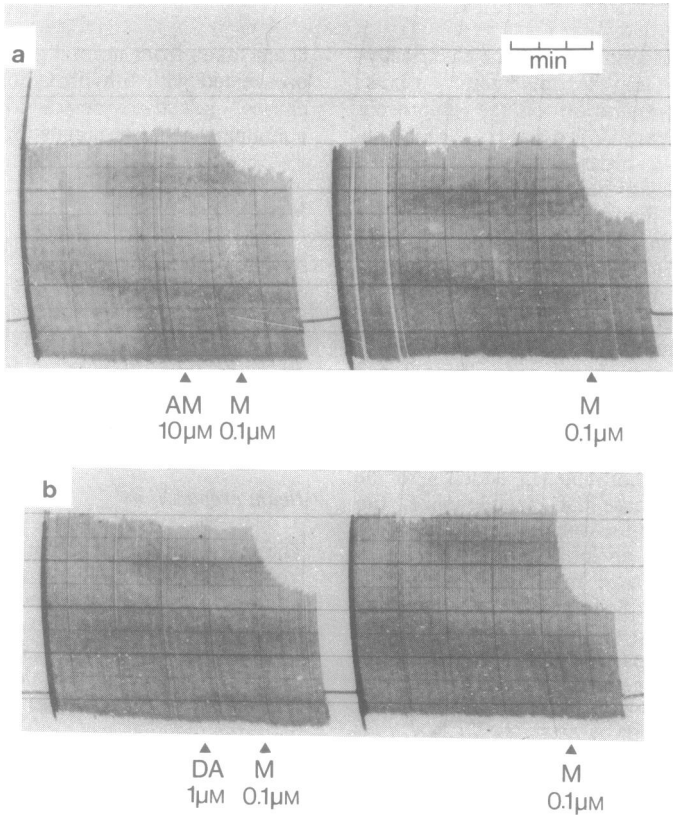

Figure 2 Antagonism by low concentrations of (a) apomorphine (AM) and (b) dopamine (DA) of the inhibitory effects of 100 nM morphine (M) on the electrically induced twitches of guinea-pig myenteric plexus-longitudinal muscle preparation.

Drugs

The drugs used were: morphine hydrochloride (C. Erba), dopamine hydrochloride (Sigma), apomorphine hydrochloride (Biosintex), naloxone hydrochloride (Endo), phentolamine hydrochloride (Ciba), propranolol hydrochloride (ICI), 6-hydroxydopamine hydrobromide (Hoffman La Roche). All drug concentrations are expressed in terms of the base.

Statistical analysis

Student's *t*-test was calculated in the usual manner (Steel & Torrie, 1960).

Results

Effect of morphine, dopamine and apomorphine on the responses of the longitudinal muscle to electrical stimulation

The amplitude of contraction (twitches) of electrically stimulated myenteric plexus-longitudinal muscle preparations was reduced by all three drugs (Figure 1); 50% depression of twitches required final bath concentrations of 100 nM morphine, 16 μM dopamine and 120 μM apomorphine. When smaller concentrations of dopamine (1 μM) or apomorphine (10 μM) were added to the bath fluid, approximately 2 min before morphine (100 nM), the inhibitory effect of the analgesic was reduced (Figure 2) but was never completely abolished; higher concentrations of dopamine and apomorphine could not be used owing to their depressant effect on the ileum.

Effect of 6-hydroxydopamine on the response of the ileum to morphine and dopamine

The depressant action of morphine on the electrically stimulated ileum was not affected by pretreatment of guinea-pigs with 6-hydroxydopamine; however, the preparation showed an increased sensitivity to dopamine. In a separate series of six experiments, it was found that the maximal (percentage) inhibition of the twitch produced to 10 μM dopamine was significantly ($P<0.05$) increased from 33.97 ± 4.50 (mean with s.e. mean) in controls to 52.61 ± 5.20 (mean with s.e. mean) in ilea from guinea-pigs pretreated with 6-hydroxydopamine.

Effects of antagonists

Naloxone abolished the depressant effect of morphine (100 nM) at a concentration of 10 nM but not that of dopamine (10 μM) or apomorphine (100 μM) even at higher concentrations (Table 1). The α-adrenoceptor antagonist, phentolamine, in concentrations of 355 nM and 1 μM, abolished the effect of dopamine but not that of morphine. The β-adrenoceptor antagonist, propranolol, did not alter the response to any of the tested drugs (Table 1).

Table 1 Effect of naloxone and of adrenoceptor blocking drugs on the action of morphine (100 nM), dopamine (10 μM) and apomorphine (100 μM) on the responses of myenteric plexus-longitudinal muscle preparation to electrical stimulation

Inhibiting drug	*Antagonist*	*No. of expts*	*Inhibition of twitch (%) Without antagonist*	*With antagonist*	P[1]
Morphine	Naloxone 10 nM	5	47.92 ± 4.22	0.46 ± 1.01	<0.01
Morphine	Phentolamine 355 nM	6	49.63 ± 4.20	48.89 ± 3.94	>0.05
Morphine	Phentolamine 1 μM	6	48.45 ± 5.10	41.88 ± 3.65	>0.05
Morphine	Propranolol 1.2 μM	6	49.93 ± 5.63	52.10 ± 6.15	>0.05
Morphine	Propranolol 4 μM	6	51.11 ± 7.38	45.29 ± 7.18	>0.05
Dopamine	Naloxone 10 nM	6	40.79 ± 3.41	41.31 ± 1.44	>0.05
Dopamine	Naloxone 100 nM	6	38.42 ± 5.21	37.41 ± 6.01	>0.05
Dopamine	Naloxone 1 μM	6	42.05 ± 6.60	41.90 ± 6.12	>0.05
Dopamine	Phentolamine 355 nM	6	37.20 ± 7.71	5.66 ± 1.69	<0.01
Dopamine	Phentolamine 1 μM	6	35.58 ± 5.72	0.88 ± 2.40	<0.01
Dopamine	Propranolol 1.2 μM	6	35.78 ± 6.19	34.95 ± 8.73	>0.05
Dopamine	Propranolol 4 μM	6	31.14 ± 5.15	29.29 ± 4.37	>0.05
Apomorphine	Naloxone 10 nM	6	32.43 ± 5.62	31.14 ± 4.23	>0.05
Apomorphine	Naloxone 1 μM	6	28.17 ± 5.43	30.24 ± 4.48	>0.05

[1] Compared with experiments without antagonist by *t*-test.

Discussion

The potency of morphine in reducing the amplitude of contractions induced by electrical stimulation in the longitudinal muscle preparation of ileum taken from guinea-pigs was not affected by pretreatment with 6-hydroxydopamine. We have confirmed the inhibitory effect of dopamine at high concentrations (Goldstein & Schulz, 1973), and shown that it is present, and even increased, in 6-hydroxydopamine-treated preparations.

These findings suggest that the action of morphine is not mediated by a release of endogenous dopamine in the intestine. In contrast, Heimans (1975) found a reduced activity of morphine in this preparation from guinea-pigs pretreated with reserpine. However, it is possible that the effect of reserpine is less specific. It is more likely that morphine and dopamine, mimicking morphine, act on separate receptors that are independent of each other. In fact, only a specific inhibitor of analgesics, naloxone, abolished the action of morphine on twitches while the blockade of the adrenoceptors was wholly ineffective; contrariwise, the inhibitory action of dopamine seems mediated by α-adrenoceptor stimulation since it was inhibited by phentolamine. These findings are in agreement with those of Kosterlitz & Watt (1968) who showed that the depressant action of morphine on the ileum is unimpaired after blockade of α- and β-adrenoceptors, and also with the data of Heimans (1975) who showed that haloperidol, an antagonist of dopamine receptors also in peripheral tissues (Yeh, McNay & Goldberg, 1969), does not alter the action of the narcotic agonist on the ileum. On the other hand, dopamine and apomorphine can mimic the effect of morphine on guinea-pig ileum preparations only at concentrations which are much higher than those of morphine.

The apparent antagonism of morphine by dopamine and apomorphine in low concentrations is not readily explained. However, in this context, some recent unpublished observations on analgesia in rats are of interest; depending on the dose, dopamine administered intra-cerebroventricularly had both morphine-like and morphine-inhibitory effects.

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