J. Pharm. Pharmacol. 1983, 35: 253–255
Communicated May 9, 1982

0022–3573/83/040253–03 $02.50/0

Inhibition by morphine of dibutyryl cyclic AMP-induced fluid secretion from the rat jejunum

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It has been suggested that the antidiarrhoeal action of morphine is better explained by its ability to decrease secretion rather than to inhibit the propulsive ability of the gut (Beubler & Lembeck 1979). However, it is still unclear how morphine acts to inhibit secretion. It is generally accepted that 3',5'-cyclic adenosine monophosphate (cAMP) is the intracellular mediator of intestinal fluid secretion, since cAMP itself causes secretion and endogenous secretagogues such as prostaglandins and vasoactive intestinal peptide (VIP) to elevate muscosal levels of cAMP in-vitro (Field 1971; Kimberg 1974; Powell et al 1974; Schwartz et al 1974; Klaeveman et al 1975; Simon & Kather 1978; Laburthe et al 1979; Simon et al 1980). Prostaglandin E_1 also elevates cAMP levels in-vivo, the effect being blocked by morphine (Beubler & Lembeck 1980).

However, it is apparent that the antisecretory mechanism of morphine cannot simply be explained on the basis of inhibition of adenylate cyclase because morphine inhibits VIP-induced secretion (Lee & Coupar 1980a) without reducing cAMP levels in-vivo (Lee & Coupar 1980b) and in-vitro (Beubler 1980). Further, the opioid loperamide inhibits cholera enterotoxin-induced secretion without preventing the associated rise in cAMP levels (Farack et al 1981). In spite of these differences, it is possible that the antisecretory action of morphine may be simply explained on the basis of inhibition at a final secretory site, distal to the generation of cAMP. Therefore, the following experiments were aimed at determining whether cAMP stimulates fluid secretion in-vivo and if so, whether its effects are inhibited by morphine. Dibutyryl cyclic AMP (dbcAMP) was used rather than cAMP because of its greater lipid solubility and resistance to phosphodiesterase (Cehovic et al 1972).

Materials and methods

Male and female hooded Wistar rats (190–290 g) were starved overnight but were given free access to drinking water. The animals were anaesthetized with a subcutaneous (s.c.) injection of pentobarbitone (60 mg kg^{-1}) and cannulae (PE 10) were introduced into the left common carotid artery for measurement of mean systemic blood pressure, and into the left external jugular vein for administration of dbcAMP.

A re-circulation technique was used to measure the net amount of fluid transported by the jejunum (approximately 20 cm) over a 20 min period, as described by Coupar (1978). Briefly, this involves using an isomotic solution containing (g $litre^{-1}$) NaCl 8·57, KCl 0·37, dextrose 1·0 and phenolsulphonphthalein 0·02 as a non-absorbable marker of water transport. This solution, at 37 °C, is re-circulated through the lumen of the jejunum by gas lift, using moistened carbon dioxide (5%) in oxygen. Results are expressed as the net amount of water absorbed (+) or secreted (−) per g wet weight of intestinal tissue during the 20 min perfusion. Morphine HCl, naloxone HCl (Endo) and the sodium salt of N^6,$O^{2'}$-dibutyryl-adenosine 3':5'-cyclic monophosphate (dbcAMP, Sigma) were dissolved in 0·9% NaCl (saline) and injected in volumes of 1 ml kg^{-1}.

Values in the text are of means ± standard error means. Individual pairs of means were compared by Student's unpaired *t*-test. Multiple comparisons between means were computed by the method of Scheffé (1959). Differences were considered significant when the value of P was smaller than 0·05.

Results

Rats injected intravenously (i.v.) with saline as control, absorbed 249 ± 65 (n = 10) µl of fluid g^{-1} of jejunum in the 20 min perfusion period. Intravenous injection of dbcAMP (20 and 40 mg kg^{-1}), 5 min before re-circulating the luminal perfusion solution, caused inhibition of net fluid absorption. The highest dose investigated (80 mg kg^{-1} dbcAMP) stimulated a net secretion of 68 ± 44 (n = 8) µl g^{-1} in 20 min. This value of secretion was significantly different from the control absorption value ($P<0·005$). The full dose-response curve for dbcAMP is shown in Fig. 1A.

Morphine (injected s.c. 35 min before re-circulation) inhibited fluid secretion induced by dbcAMP (80 mg kg^{-1} i.v.) in a dose-dependent manner, such that 10 mg kg^{-1} of morphine maintained a net absorption value of 171 ± 50 (n = 5) µl g^{-1} in 20 min. This value was significantly different from the value of secretion caused by dbcAMP alone ($P<0·001$) and not significantly different from the control value of absorption ($P=0·45$; Fig. 1B). The antisecretory effect of morphine (10 mg kg^{-1} s.c.) against dbcAMP (80 mg kg^{-1} i.v.) was blocked by naloxone (2 mg kg^{-1} with morphine s.c.). The value of secretion in this group of animals was

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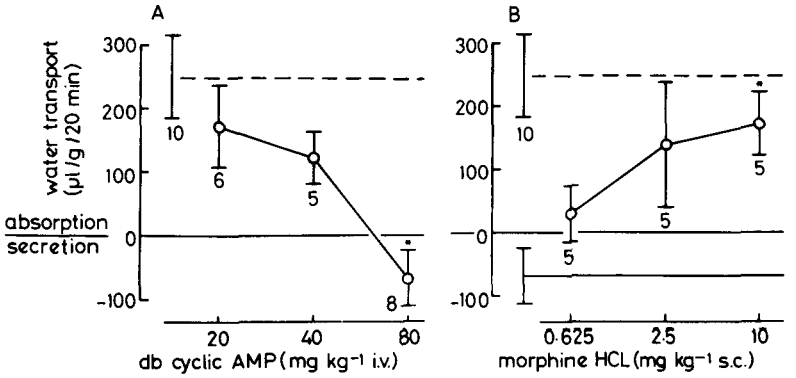

FIG. 1. Effect of (A) dbcAMP on net water transport and (B) morphine on dbcAMP-induced fluid secretion. The top broken line in each is the control value of normal fluid absorption, while the continuous line in (B) is the value of secretion in response to 80 mg kg^{-1} i.v. dbcAMP. Re-circulation of the jejunal perfusing solution was started 5 min after injecting dbcAMP and continued for 20 min. Morphine was injected s.c. 30 min before dbcAMP. * in A indicates that the mean is significantly different from the control value of absorption, while * in B indicates a significant difference from the value of secretion in response to dbcAMP. Each point is the mean ± s.e.mean. Numbers under the bars indicate the number of animals.

62 ± 18 (n = 5) µl g^{-1} in 20 min and was significantly different from the value of absorption in the dbcAMP plus morphine group ($P < 0{\cdot}005$) and not significantly different from the dbcAMP alone group ($P = 0{\cdot}92$). The dose of naloxone employed does not itself alter the value of normal water absorption (Lee & Coupar 1980a).

Dibutyryl cyclic AMP induced a dose-related decrease in mean systemic arterial blood pressure which lasted the duration of the jejunal perfusion. Morphine, and morphine plus naloxone treatments did not significantly alter the depressor effect of dbcAMP. Results at 5 and 25 min after dbcAMP were: saline 132 ± 8, 142 ± 6 n = 10; dbcAMP 61 ± 4, 69 ± 4 n = 8; dbcAMP + morphine 59 ± 4, 51 ± 4 n = 5; dbcAMP + morphine + naloxone, 45 ± 4, 56 ± 3 n = 5 mmHg.

Discussion

The results show that dbcAMP causes a dose-related reversal of water transport in the rat jejunum from net absorption to secretion, and that morphine blocks this secretory effect. Secretion occurred after a relatively large dose of dbcAMP and was associated with a large and sustained fall in mean arterial blood pressure. The possibility that the resultant changes in haemodynamics significantly influence fluid transport is unlikely for the following reasons: Although the absorption rate of water is reduced at low flow rates so also is secretion (Winne 1972a, b). Similarly, net absorption is not altered as a result of intestinal vasodilatation in-vivo (Brunsson et al 1979). Although fluid secretion occurs in pithed rats, which have a low blood pressure, it is caused by the release of prostaglandin E_2-like material rather than the lowered blood pressure (Lee & Coupar 1982). As regards the antisecretory effect of morphine, secretion is reversed to net absorption without any reversal of mean arterial blood pressure.

The finding that dbcAMP causes secretion is in agreement with the secretory effect of cAMP itself in-vitro. Nakaki et al (1982) have shown independently that dbcAMP (approximately 50 mg kg^{-1} as an intra-arterial infusion) induces fluid secretion from the rat jejunum. Their results further support the suggestion that cAMP is the intracellular mediator of intestinal fluid secretion. The involvement of cAMP in intestinal secretion is relevant to the mechanism of action of the antidiarrhoeal drugs, especially the opioids. Results from initial experiments, where PGE_1 was used as the secretagogue, indicated that morphine inhibited secretion by blocking the associated rise in the mucosal level of cAMP (Beubler & Lembeck 1980). However, it is becoming apparent that block of adenylate cyclase by opioids is not the general mechanism of their antisecretory action. For example, the inhibition by morphine of VIP-stimulated secretion is not associated with block of adenylate cyclase either in-vivo (Lee & Coupar 1980b) or in-vitro (Beubler 1980). Also loperamide inhibits fluid secretion induced by cholera enterotoxin but again not the rise in mucosal cAMP(Farack et al 1981). Since the pool of cAMP involved in secretion is probably small compared to the total cAMP of the intestine, it is possible that the block of PGE_1 stimulated adenylate cyclase by morphine is not that associated with secretion. In view of these considerations, a simple interpretation of the present results is that morphine blocks the secretory mechanism at a point after the generation of cAMP. Opioids are not the only drugs proposed to act in this manner. During the preparation of this paper, Nakaki et al (1982) published results showing that α_2-adrenoceptor agonists selectively inhibit the secretory responses to PGE_1 and VIP as well as secretion induced by dbcAMP. They suggested that α_2-agonists act at a site distal to cAMP generation.

The present results show that the inhibitory action of morphine against dbcAMP is mediated by opiate receptors since the effect is blocked by naloxone. It is

possible that the receptors are the same as are involved in inhibiting PGE_1- and VIP-induced secretion, since the antisecretory effect of morphine against these secretagogues, measured by the same experimental method, is also blocked by naloxone. Additionally, the range of morphine doses that causes inhibition of PGE_1- and VIP-induced secretion is the same (Coupar 1978: Lee & Coupar 1980a).

This project was supported financially by the National Health and Medical Research Council of Australia and the Pharmaceutical Society of Victoria.

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J. Pharm. Pharmacol. 1983, 35: 255–257
Communicated September 7, 1982

0022–3573/83/040255–03 $02.50/0

Influence of classical and atypical neuroleptics on apomorphine-induced behavioural changes and on extinction of a conditioned avoidance response

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Acquisition and extinction of conditioned avoidance behaviour are used as animal models to determine the activity of potential antipsychotic drugs. Thus, neuroleptics inhibit acquisition and facilitate extinction of conditioned avoidance behaviour. These drugs are also potent antagonists of dopamine (DA) action. Particularly, the antagonism of behavioural responses induced by apomorphine, a DA receptor stimulant, is considered as an important pharmacological test for showing blockade of DA action. Apomorphine causes a biphasic effect in rate: at low doses it decreases motor activity and induces sedation, while at high doses it increases motor activity and elicits stereotypy (see Di Chiara & Gessa 1978).

The present studies were carried out to determine a possible relation between the ability of haloperidol (a classical neuroleptic drug) and of sulpiride and clozapine (atypical neuroleptics) to antagonize the behavioural responses to apomorphine and their capacity to facilitate extinction of pole-jumping avoidance behaviour in rats.

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Materials and methods

Male Wistar rats of an inbred strain (CPB-TNO, Zeist, The Netherlands), 130–140 g, were housed 5–6 per cage, kept on a standard illumination schedule (light on between 5.00 a.m. and 17.00 p.m.) and had free access to food and water. Experimentation was carried out between 9.00 a.m. and 2.00 p.m. in a sound-proof room. Drugs were administered subcutaneously in the neck. Each rat was used once.

The behavioural effects elicited by apomorphine were observed as described before (Van Ree & Wolterink 1981; Van Ree et al 1982). Briefly, locomotor activity and rearing were measured for 3 min, starting 5 min after apomorphine injection, in a rectangular perspex observation cage. Subsequently, locomotor activity, rearing and stereotypy (duration of sniffing the cage floor) were measured for 4 min, starting 20 min after apomorphine treatment in a small open field.