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INHIBITION BY MORPHINE OF PROSTAGLANDIN E_1-STIMULATED SECRETION AND CYCLIC ADENOSINE 3',5'-MONOPHOSPHATE FORMATION IN THE RAT JEJUNUM *in vivo*

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1 The effects were studied of prostaglandin E_1 (PGE_1), theophylline and morphine on net water flux and mucosal cyclic adenosine 3',5'-monophosphate (cyclic AMP) levels in the jejunum of anaesthetized rats *in vivo*.

2 Infusion of PGE_1 (3.2 μg/min, i.a.) caused a reversal from net water absorption to net secretion and enhanced the mucosal cyclic AMP content by 54%.

3 Theophylline (5 mg/ml, intraluminal) similarly produced a reversal from net water absorption to net secretion and increased mucosal cyclic AMP content by 54%. Additional intra-arterial infusion of PGE_1 resulted in a massive increase in net water secretion and an increase in mucosal cyclic AMP content by about 200%.

4 Pretreatment with morphine (10 mg/kg, s.c.) reduced the effect of PGE_1 on net water flux and completely inhibited its effect on the mucosal cyclic AMP content. Naloxone (10 mg/kg, s.c.) abolished both effects of morphine.

5 A good correlation ($r = 0.99$) was demonstrated between mucosal cyclic AMP levels and net water flux.

6 The present results demonstrate that PGE_1 stimulates intestinal fluid secretion by increasing mucosal cyclic AMP levels. The antidiarrhoeal effect of morphine can be explained by its inhibition of the PGE-mediated increase in cyclic AMP levels, which, in turn, leads to a reduction in intestinal secretion.

Introduction

The antidiarrhoeal activity of opiates is explained at present by increased contractile activity of the circular muscle and inhibition of the propulsive activity in the intestinal tract (Vaughan Williams & Van Streeten, 1950; Niemegeers, Laenaerts & Janssen, 1974; Burks, 1976; Dajani, Bianchi, East, Bloss, Adelstein & Yen, 1977). Recently it has been demonstated that morphine and morphine-like compounds also inhibit intestinal fluid secretion induced by crude cholera enterotoxin, prostaglandin E_1 (PGE_1) and vasoactive intestinal polypeptide (VIP) (Valiulis & Long, 1973; Karim & Adaikan, 1977; Coupar, 1978; Beubler & Lembeck, 1979).

All these agents have been shown to stimulate intestinal mucosal adenylate cyclase *in vitro* (Kimberg, Field, Johnson, Henderson & Gershon, 1971; 1974; Schwartz, Kimberg, Sheering, Field & Said, 1974; Gaginella, Phillips, Dozois & Go, 1978). Cyclic adenosine 3',5'-monophosphate (cyclic AMP) has been implicated as mediator of cholera enterotoxin-, PGE- and VIP-induced small intestinal secretion (for references see Kimberg, 1974), although mucosal cyclic AMP levels have not been determined in *in vivo* experiments.

Inhibition of PGE-stimulated adenylate cyclase by morphine has been demonstrated in rat brain homogenate (Collier & Roy, 1974a), in neuroblastoma × glioma hybrid cells (Sharma, Nirenberg & Klee, 1975; Traber, Fischer, Latzin & Hamprecht, 1975) and in slices of rat striata (Havemann & Kuschinsky, 1978).

The aim of the present study was to investigate (1) whether PGE_1-induced intestinal secretion is accompanied by an increase in mucosal cyclic AMP levels and (2) whether morphine inhibits both intestinal secretion and cyclic AMP formation in the rat jejunum *in vivo*.

Methods

Female Sprague Dawley rats (Mus Rattus Co., Brunnthal, Fed. Rep. Ger., 200 ± 10 g) were used; they were deprived of food 24 h before the experiment, but had free access to water.

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Preparation

The rats were anaesthetized with pentobarbitone sodium (65 mg/kg, s.c.) 1 h before the start of the experiments. The abdomen was opened and a jejunum segment about 20 cm in length was tied off at the distal end, fitted with a polyethylene tube at the proximal end and replaced in the abdominal cavity. The trachea was cannulated and another cannula was introduced into the left carotid artery and pushed down until its tip lay near the origin of the artery from the aorta. Infusion of either 0.9% w/v NaCl solution (saline) or PGE_1 (100 μg/ml) was started with an infusion pump (Braun/Melsungen, Fed. Rep. Ger., 0.032 ml/min) after a waiting period of 30 min. A gravimetric method was used to determine net water transport. Five min after the start of the infusion, the jejunum was filled with 2 ml Tyrode solution and tied off; 20 min later, the loop was removed, weighed (weight A), opened along the mesenteric border and blotted with tissue paper. The mucosa was immediately scraped off and frozen in liquid nitrogen for determination of cyclic AMP. The duration of the procedure from removal of the loop to freezing of the mucosa was exactly 2 min. The frozen mucosa plus the remaining tissue were also weighed (weight B). Net water flux was obtained from the difference between weight A and weight B after subtraction of the 2.0 g Tyrode solution with which the jejunum was filled. A negative value denotes net absorption and a positive value net secretion. Net water flux was expressed as μl per g wet weight of jejunum during 20 min perfusion (Coupar, 1978).

Indomethacin (10 mg/kg, s.c.) was injected together with pentobarbitone sodium to avoid PGE-like release as a result of mechanical stimulation (Coupar, 1978; Beubler & Juan, 1978a). Naloxone (10 mg/kg, s.c.) was injected 60 min, and morphine (10 mg/kg, s.c.) 40 min before the start of the saline or PGE_1 infusion. Theophylline was added to the Tyrode solution and instilled intraluminally.

Cyclic AMP assay

The frozen mucosa was crushed for 30 s in a Teflon vial by a tungsten carbide ball on a microdismembrator (Braun/Melsungen, Fed. Rep. Ger.) at maximum force. Homogenization in the frozen state was continued after addition of 2 ml of trichloracetic acid (5% w/w) until thawing was complete. After centrifugation (2000 *g*, 20 min), the supernatant was removed, 0.1 volumes of 1 N HCl added and trichloracetic acid extracted 5 times with 3 volumes of diethyl ether. The aqueous residue was used for determination of cyclic AMP without further purification (Kukovetz, Pöch, Holzmann, Wurm & Rinner, 1978). Cyclic AMP was determined according to Tovey, Oldham & Whelan (1979) with the cyclic AMP assay kit (Radiochemical Centre, Amersham).

The protein content of the remaining pellet was determined by the method of Lowry, Rosebrough, Farr & Randall (1951). The cyclic AMP content of the mucosa was expressed in picomoles (pmol) per mg of protein.

Statistics

Experiments were performed in balanced blocks. Statistical significance of the differences of the means was evaluated by the two sample Student's *t* test, and all values are given as the mean ± s.e. mean.

Drugs

The following drugs were used: PGE_1 (Upjohn Comp., Kalamazoo, Mich.), indomethacin (Merck, Sharpe & Dohme, Rahway, N.J.), morphine hydrochloride (Heilmittelwerke, Vienna), naloxone hydrochloride (Endo Laboratories, Richmond Hill, N.Y.), pentobarbitone sodium (Abbott Laboratories, Ill.) and theophylline (Calbiochem., Los Angeles).

Results

Effects of prostaglandin E_1 and theophylline

Net water absorption in the tied off jejunum loop of control rats given intra-arterial saline infusion, was -220 ± 40 μl/g wet weight in 20 min ($n = 20$). The mean cyclic AMP levels in the jejunal mucosa of these control animals was 6.45 ± 0.36 pmol per mg of protein ($n = 20$, Figure 1). This mean cyclic AMP level in controls agrees closely with the results of Donowitz & Binder (1975), who found 6.53 ± 0.83 pmol per mg protein.

Infusion of PGE_1 (3.2 μg/min) into the carotid artery caused a reversal of the net water absorption to a significant net secretion of $+50 \pm 50$ μl/g in 20 min ($n = 14$, $P < 0.01$) and significantly enhanced mucosal cyclic AMP levels by 54% (9.99 ± 0.83 pmol per mg of protein, $n = 14$, $P < 0.01$) (Figure 1).

Intraluminal administration of the phosphodiesterase inhibitor, theophylline (5 mg/ml) similarly converted the net water absorption into a net secretion of $+70 \pm 80$ μl/g in 20 min ($n = 8$, $P < 0.01$). Theophylline increased mucosal cyclic AMP levels by 54% (9.96 ± 0.57 pmol per mg protein, $n = 8$, $P < 0.01$). Additional intra-arterial infusion of PGE_1 (3.2 μg/min) resulted in a massive net water secretion of $+400 \pm 50$ μl/g in 20 min ($n = 8$, $P < 0.01$) and in a 195% increase in mucosal cyclic AMP content (18.99 ± 1.97 pmol per mg protein, $n = 8$, $P < 0.01$) (Figure 1).

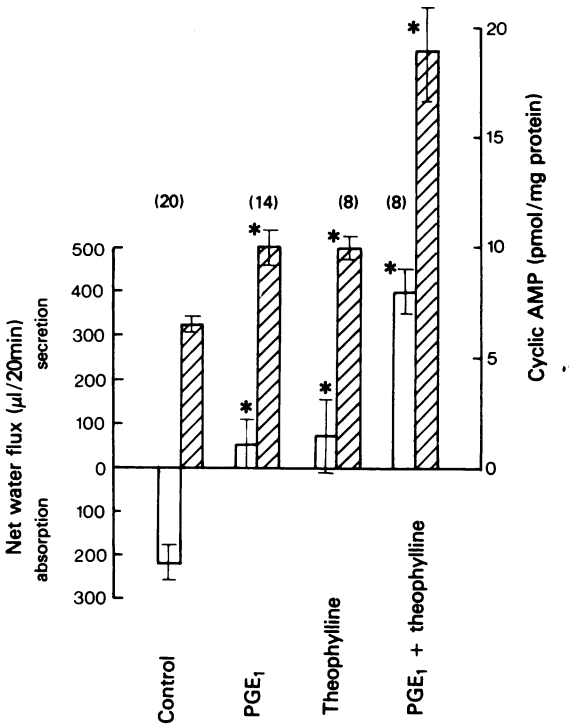

Figure 1 Net water flux and mucosal cyclic AMP levels in the rat jejunum *in vivo*. Infusion of prostaglandin E_1 (PGE_1, 3.2 µg/min i.a.) and administration of theophylline (5 mg/ml intraluminal) stimulate net water flux from blood to lumen (secretion) and elevate the mucosal cyclic AMP levels. Infusion of PGE_1 plus intraluminal administration of theophylline augmented both effects. The open columns indicate the net water flux (left ordinate scale) and the hatched columns the mucosal cyclic AMP levels (right ordinate scale). Mean values are shown; vertical lines indicate s.e. mean. The numbers in parentheses indicate the number of experiments. $^*P < 0.01$ relative to controls.

Effects of morphine and naloxone

Pretreatment of control rats with morphine (10 mg/kg, s.c.), 40 min before the start of the saline infusion influenced neither net water absorption (-189 ± 37 µl/g in 20 min) nor the cyclic AMP content in the mucosa (6.25 ± 0.33 pmol per mg protein, $n = 7$) (Figure 2).

Pretreatment of control rats with the morphine antagonist, naloxone (10 mg/kg, s.c.) 60 min before the start of the saline infusion caused a slight, insignificant reduction in net water absorption (-120 ± 30 µl/g in 20 min) and did not change the mucosal cyclic AMP content (6.35 ± 0.71 pmol per mg protein, $n = 10$) (Figure 2).

Influence of morphine and naloxone on the effect of prostaglandin E_1

Morphine significantly reduced the effect of PGE_1 on net water flux (-142 ± 50 µl/g in 20 min, $P < 0.01$) and completely abolished the PGE_1-induced increase in mucosal cyclic AMP content (6.65 ± 0.56 pmol per mg protein, $n = 8$, $P < 0.01$) (Figure 2). The inhibitory effect of morphine both on PGE_1-stimulated fluid secretion and mucosal cyclic AMP increase was significantly blocked by pretreatment of the rats with naloxone (net water flux: $+50 \pm 30$ µl/g in 20 min, $P < 0.05$; cyclic AMP content: 9.42 ± 0.64 pmol per mg protein, $P < 0.01$, $n = 10$) (Figure 2). Naloxone itself moderately enhanced the effect of PGE_1 on net water flux ($+118 \pm 20$ µl/g in 20 min, $P > 0.05$) and on cyclic AMP formation (11.22 ± 0.71 pmol per mg protein, $n = 8$, $P > 0.05$).

Discussion

Over the past decade a number of investigations have revealed that prostaglandins appear to act on the intestine both in a physiological and pathological capacity, as well as following iatrogenic intervention. Prostaglandins cause a net secretion of water and electrolytes into the gut and, therefore, increase the intestinal fluid volume (Pierce, Carpenter, Elliot & Greenough, 1971; Robert, Nezamis, Lancaster, Hanchar & Klepper, 1976; Beubler, Lembeck & Schweditsch, 1978); prostaglandins cause diarrhoea when they are released pathologically or are used for termination of pregnancy (Sandler, Karim & Williams, 1968; Karim & Amy, 1975). Diarrhoea associated with radiotherapy is also thought to be caused by PGE release (Mennie, Dalley, Dinneen & Collier, 1975). Prostaglandins appear to play a physiological role in the regulation of intestinal blood flow and transmucosal water movement (Beubler & Juan, 1977). They are released into the gut after mechanical stimulation of the mucosa (Beubler & Juan, 1978a) and are, furthermore, considered to be implicated in the mechanism of action of non-osmotic laxatives (Beubler & Juan, 1978b, c; 1979).

Prostaglandins are reported to stimulate adenylate cyclase in several tissues (Collier & Roy, 1974a, b; Sharma *et al.*, 1975). Prostaglandins, like vasoactive intestinal polypeptide and cholera enterotoxin, stimulate intestinal mucosal adenylate cyclase; from these *in vitro* experiments it has been suggested that cyclic AMP might be the final common mediator of electrolyte and fluid secretion in the intestine (Kimberg *et al.*, 1971; 1974; Kimberg, 1974; Powell, Farris & Carbonetto, 1974; Gaginella *et al.*, 1978). The aim of the present experiments was to confirm this hypothesis *in vivo*, with concomitant determination of net water flux and mucosal cyclic AMP content in the intestine.

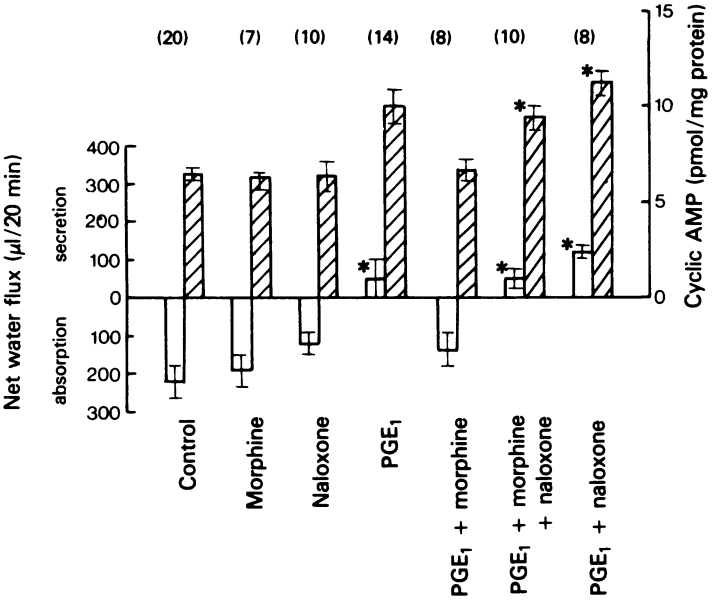

Figure 2 Net water flux and mucosal cyclic AMP levels in the rat jejunum *in vivo*. Morphine (10 mg/kg, s.c.) and naloxone (10 mg/kg, s.c.) are without significant effect on net water flux and cyclic AMP levels. Prostaglandin E_1 (PGE_1) stimulates net water flux from blood to lumen (secretion) and elevates the mucosal cyclic AMP levels. Both effects of PGE_1 are inhibited by morphine. The inhibitory effect of morphine is blocked by naloxone. The open columns indicate the net water flux (left ordinate scale) and the hatched columns the mucosal cyclic AMP levels (right ordinate scale). Mean values are shown; vertical lines indicate s.e. mean. The numbers in parentheses indicate the number of experiments. $*P < 0.01$ relative to controls.

In order to prove the validity of the experimental model, the effect of theophylline was tested on net water flux and cyclic AMP levels because theophylline is known to elevate cyclic AMP levels and to stimulate intestinal secretion (Pierce *et al.*, 1971). This secretory effect of theophylline is due to its ability to increase cyclic AMP levels (Powell *et al.*, 1974) by inhibiting the phosphodiesterase which converts cyclic AMP to AMP (Butcher & Sutherland, 1962). Both theophylline and PGE_1 increase intestinal blood flow (Beubler & Lembeck, 1976; Beubler & Juan, 1977), probably also as a result of the ability of both agents to increase cyclic AMP levels.

In the present experiments, theophylline (5 mg/ml intraluminal) increased both net water flux and cyclic AMP levels. The effects of PGE_1 at the given dosage (3.2 µg/min) on net water flux and cyclic AMP levels were of approximately the same magnitude as elicited by theophylline. The infusion rate of PGE_1 used in these experiments is regarded as maximally effective, because no further secretion was reported to occur on infusion of higher amounts of PGE_1 (Coupar, 1978).

As to net water flux and cyclic AMP formation, theophylline augmented the effect of PGE_1 in a more than additive manner (Figure 1). This result is in agreement with the assumption that PGE_1 and theophylline elevate cyclic AMP levels in different ways, namely PGE_1 via stimulation of adenylate cyclase (Gaginella *et al.*, 1978) and theophylline via inhibition of phosphodiesterase (Butcher & Sutherland, 1962). Accordingly, the final common pathway of fluid secretion stimulated by PGE_1 and theophylline appears to be the increase in mucosal cyclic AMP (see also Figure 3).

Morphine and morphine-like antidiarrhoeal compounds such as diphenoxylate and loperamide, inhibit prostaglandin-induced diarrhoea in man and animals (Karim & Adaikan, 1977; Lange, Secher & Amery, 1977; Dajani *et al.*, 1977; Chapaux, Chapaux & Royer, 1978). Recent studies (Coupar, 1978; Beubler & Lembeck, 1979) suggest that the antidiarrhoeal activity of opiates stems from their ability to inhibit intestinal fluid secretion.

The present results show that morphine antagonizes the PGE_1-induced increase in intestinal secretion, as well as PGE_1-induced cyclic AMP formation. The morphine antagonist, naloxone, blocked both effects of morphine, thus revealing the involvement of opiate receptors in the inhibition by morphine of PGE_1-induced stimulation of the intestine. Since PGE-induced a morphine-reversible intestinal fluid secretion in the pithed rat (Beubler *et al.*, 1978,

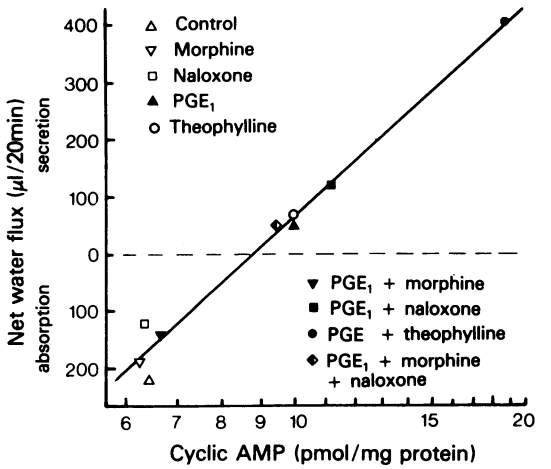

Figure 3 Correlation between net water flux (ordinate, scale, linear) and mucosal cyclic AMP levels (abscissa scale, log) in the rat jejunal loop *in vivo*. The correlation coefficient $r = 0.99$.

Beubler & Lembeck, 1979), the site of interaction between PGE and morphine is probably located in the intestine.

Inhibition of PGE-stimulated adenylate cyclase by opiates has already been demonstrated in neuroblastoma-glioma hybrid cells (Traber, Fischer, Latzin & Hamprecht, 1974; Sharma *et al.*, 1975; Traber *et al.*, 1975). The present results confirm preliminary *in vitro* results of Collier & Roy (1974a), who showed that morphine inhibited adenylate cyclase stimulation by PGE$_1$ in a rat intestinal homogenate and already suggested that this blockade of PGE effects in intestinal tissue might suppress diarrhoea. The hypothesis that morphine inhibits cyclic AMP-mediated secretion is supported by the findings that the secretory effect of other agents such as vasoactive intestinal polypeptide and cholera enterotoxin, which also stimulate mucosal adenylate cyclase, are blocked by morphine as well (Valiulis & Long, 1973; Beubler & Lembeck, 1979).

The morphine antagonist, naloxone, slightly reduced the net water absorption, but did not change the mucosal cyclic AMP content in controls; furthermore, naloxone slightly, but not significantly, enhanced the effect of PGE$_1$ on net water secretion and cyclic AMP levels. Recent experiments (Beubler & Lembeck, 1979) have shown that naloxone significantly enhanced the PGE- and vasoactive intestinal polypeptide-induced increase in intestinal fluid volume. A physiological amount of enkephalin in the intestine may have a protective function (Schaumann, 1954) against stimulation of fluid secretion. Naloxone counters this regulatory effect of enkephalins on fluid secretion.

A good correlation ($r = 0.99$) is demonstrated if log cyclic AMP content is plotted against the change in net water flux in controls and in experiments with PGE$_1$, morphine, naloxone and theophylline and the various combinations (Figure 3). The correlation strongly indicates that changes in net water flux are caused by changes in cyclic AMP levels.

In summary, the present results suggest that PGE$_1$ stimulates intestinal fluid secretion by increasing mucosal cyclic AMP levels through activation of the mucosal adenylate cyclase. It is concluded that the antidiarrhoeal effect of morphine can be explained by its inhibition of this PGE-mediated effect.

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