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COMMUNICATIONS

Studies to determine whether there is tolerance or cross-tolerance to the antisecretory effect of morphine and clonidine in the rat intestine

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Abstract—Intra-arterial infusions (4 μg min^{-1}) of prostaglandin E_2 (PGE_2) were used to stimulate intestinal fluid secretion in anaesthetized rats. Morphine (0·625–40 mg kg^{-1}) produced a dose-dependent restoration of fluid transport from secretion to the normal rate of absorption. Pretreatment with up to eight doses of morphine (20 mg kg^{-1}) did not induce tolerance, rather it enhanced the antisecretory effect of a subsequent acute dose of morphine. It seems probable that this was caused by the accumulation of morphine in the intestine. Clonidine (4–1000 μg kg^{-1}, like morphine, produced a dose-dependent reversal of stimulated fluid secretion. Pretreatment with clonidine (4 × 0·25 mg kg^{-1}) caused a shift of the clonidine antisecretory dose-response curve to the right, demonstrating tolerance. Pretreatment with clonidine also caused cross-tolerance to the antisecretory effect of morphine. The results suggest that there is a close relationship between opioid- and α_2-adrenoceptors in controlling inhibition of intestinal fluid secretion.

Morphine produces constipation by delaying intestinal transit and enhancing intestinal fluid absorption. In 1926, Miller & Plant stated that the intestine does not become tolerant to morphine. This was based on their finding that the morphine-induced rise in intraluminal pressure recorded from the ileum of conscious dogs remained undiminished throughout chronic morphine treatment. This, together with clinical observations has maintained the belief that morphine does not induce tolerance to its constipating action (Jaffe 1985). However, there are reports showing that the intestine of conscious rats does become tolerant to the antitransit effect of morphine (Burks et al 1976; Weisbrodt et al 1977; Brown et al 1988). Additionally there is indirect evidence, based on myoelectric recordings, indicating that morphine induces tolerance in the rat, as well as the dog intestine (Weisbrodt et al 1980; Kuperman et al 1987).

The potential of morphine to induce tolerance to its pro-absorptive effect has also been investigated. Warhurst et al (1983) showed that when an acute dose of morphine (10 mg kg^{-1}, s.c.) was administered to rats there was an approximately 65% increase in the basal rate of water absorption from the small intestine. However, an acute dose of morphine (10 mg kg^{-1}, s.c.) given to rats made tolerant by treatment for 48 h with a slow-release preparation containing 75 mg kg^{-1} morphine, resulted in only a 20–25% increase in basal water absorption (Warhurst et al 1984).

The aim of this study was to determine if tolerance occurs to the intestinal antisecretory action of morphine. Prostaglandin E_2 (PGE_2) was selected as the secretogogue, as it is the major prostaglandin of the small intestine and is the cause of intestinal fluid loss in many diarrhoeal states (Rask-Madsen & Bukhave 1979).

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The possibility that cross-tolerance occurs between morphine and clonidine was investigated since it has been shown that clonidine pretreatment causes tolerance to the antinociceptive action of morphine (Bentley et al 1983; Yamazaki & Kaneto 1985).

Materials and methods

Net water transport. Fluid absorption from, and secretion into, the lumen of the rat small intestine was measured by the method described by Coupar (1985). In brief, rats were anaesthetized with pentobarbitone (60 mg kg^{-1} i.p.) and a cannula was introduced retrogradely into the left common carotid artery for constant intra-arterial (i.a.) infusions of 0·9% NaCl (saline) or PGE_2 in saline at a rate of 40 μL min^{-1}. The jugular vein was cannulated for acute administration of test drugs. A recirculation technique was used to measure the net amount of fluid transported by the jejunum. The lumen of the jejunum was perfused with 8 mL of an isosmotic solution, containing (mM) NaCl 148, KCl 5, dextrose 5·5. Phenol red 0·05 mM was also added to the solution to act as a non-absorbable volume marker. A loop of intestine, 20 to 30 cm in length starting distal from the ligament of Trietz was continuously recirculated with this solution. The solution was contained in a reservoir maintained at 37°C and was driven by a gas lift of moistened 5% CO_2 in O_2.

At the end of the 20 min perfusion the fluid from the loop and reservoir was recovered for analysis of phenol red, morphine and morphine 3-glucuronide. For phenol red analysis, samples were diluted with buffer and peak absorbance was measured with a spectrophotometer at 560 nm. Absorbance at 520 and 600 nm was also measured to correct for non-specific interferences as described by Miller & Schedl (1972). Results are expressed as the net amount of water absorbed (+) or secreted (−) per gram wet weight of jejunum during the 20 min perfusion.

Morphine and morphine-3-glucuronide analysis. The HPLC system consisted of a Waters Millipore Model 510 solvent delivery pump, a Rheodyne 1725 injection valve and a reversed phase Phenomenex Ultracarb ODS column (Spherisorb 5 μm particles, 250 × 4·6 mm) mounted on a Quik-Loc column station. UV absorption was measured using a Waters 490E programmable multiwavelength detector and recorded on a BBC Goerz Metrawatt SE-120 chart recorder.

The mobile phase consisted of 0·02 M potassium dihydrogen orthophosphate buffer (pH 2·1) and acetonitrile (81:19, v/v) containing 25 mg L^{-1} octane sulphonic acid. Flow rate was 1·0 mL min^{-1} with the column at ambient temperature. UV absorbance was simultaneously monitored at 210 and 230 nm at 0·01 aufs.

C-18 Sep Paks (Waters-Millipore) were activated by washing with 5 mL methanol followed by 10 mL of water. A 1 mL sample of the luminal perfusate was pretreated as described by Milne et

Table 1. Pretreatments.

Pretreatment	Unit dose (mg kg^{-1} s.c.)	Number of injections	Total dose (mg kg^{-1})	Number of days of treatment	Time after last injection (h)
Morphine M1	10	2	20	1	12
Morphine M2	10	4	40	2	12
Morphine M3	20	8	160	4	48
Clonidine	0·25	4	1	2	12

al (1991) in their second method modification for determining morphine in plasma. Extraction efficiency was greater than 90%.

Treatment protocols. For acute treatment, groups of 5 to 7 rats were given a single i.v. dose of either morphine (0·625, 2·5, 10 and 40 mg kg^{-1}) or clonidine (4, 16, 64, 250 and 1000 μg kg^{-1}). Five min later, the infusion of PGE_2 (4 μg min^{-1}) was commenced and after a further 5 min, the luminal perfusion was also commenced. For all experiments both with acute and chronic morphine treatments, the perfusion was terminated after 20 min. The fluid in the lumen was collected for assay of phenol red, and for morphine and morphine-3-glucuronide where appropriate.

Chronic pretreatments were performed as shown in Table 1. Three regimens were used. These were 2 or 4 doses of morphine, 10 mg kg^{-1}, given at intervals of 12 h (M1 and M2, respectively), or eight doses of 20 mg kg^{-1} morphine given over 4 days (M3). Clonidine was given at 0·25 mg kg^{-1} in four doses, also at the same time intervals of 12 h. The effects of these treatments were tested 12 h after the final dose except for the highest morphine regimen (M3) which was tested after 48 h.

Statistical analysis. Pairs of means were compared by multiple comparison analysis (MLTCOMP). Dunnett's *t*-test was used to compare responses with their control values, and regression analysis was used to estimate ED50 values and to compare dose-response curves. Each mean is given with its associated s.e.m. which is represented by bars in Figs 1 and 2. Effects were judged statistically significant when the value of P was equal to or smaller than 0·05.

Drugs. Prostaglandin E_2 (PGE_2) (Upjohn) was diluted in saline and infused at 40 μL min^{-1} to given the required intra-arterial infusion rate of 4 μg min^{-1}. All other drugs were diluted in saline and administered intravenously in volumes of 0·1 mL per 100 g body weight, the doses being expressed as the free base. Morphine was obtained from Macfarlane Smith as the hydrochloride, clonidine from Boehringer Ingelheim as the hydrochloride and naloxone from Endo as the hydrochloride.

Results

Morphine. (i) Functional effects. The rate of fluid absorption from the jejunum was reversed to net secretion by infusion of PGE_2 at 4 μg min^{-1} (from $+308 \pm 36$ (n = 5) to -256 ± 40 (n = 7) μL g^{-1} in 20 min, $P < 0{\cdot}05$). This secretion was inhibited by morphine in a dose-dependent manner. The ED50 for this effect was 2·5 mg kg^{-1} (95% CI 1 – 3·9, n = 18, Fig. 1). Animals that had been pretreated with morphine (M1, M2, M3, Table 1) showed some differences from non-pretreated rats. Although the basal absorption rates were not changed, PGE_2 was less effective at stimulating secretion in these animals. In addition it was surprising to find that the standard acute dose of morphine appeared to cause a greater reduction in secretion compared with the non-pretreated group. When the reduced response to

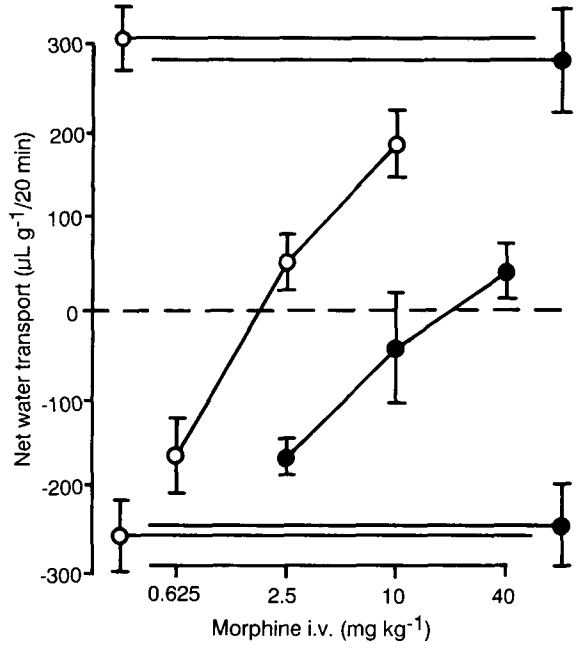

FIG. 1. Antisecretory effect of morphine before (○) and after (●) pretreatment with clonidine. The upper horizontal lines represent the mean rates of basal water absorption and the lower lines the mean rates of PGE_2-induced intestinal secretion. Increasing acute doses of morphine restored fluid transport from secretion to absorption. Sensitivity to the acute doses was decreased significantly by clonidine pretreatment ($P < 0{\cdot}05$).

PGE_2 was taken into consideration it was found that the actual effect of the standard acute dose was not significantly different in any of the pretreatment groups compared with the non-pretreated group (Table 2). Thus none of the pretreatments had caused any tolerance to the standard acute dose of morphine.

(ii) Amount in intestinal loops. The recovered samples of luminal perfusate from morphine-pretreated animals and those administered the standard acute dose were all found to contain significant amounts of both morphine itself and morphine-3-glucuronide. The amounts of morphine were similar regardless of the treatment but the level of morphine-3-glucuronide was significantly greater after the acute dose than after the pretreatment (Table 3).

Clonidine. Clonidine produced a dose-related inhibition of PGE_2-induced fluid secretion with an ED50 of 18·5 μg kg^{-1} (95% CI 17·2–19·8, n = 20, Fig. 2). Unlike morphine, pretreatment with clonidine (1 mg kg^{-1} total, Table 1) did not affect either the rate of PGE_2-induced secretion (-243 ± 39 (n = 5) vs -206 ± 40 (n = 7) μL g^{-1} in 20 min, $P > 0{\cdot}05$) or the rate of absorption ($+281 \pm 40$ (n = 5) vs $+308 \pm 36$ (n = 5) μL g^{-1} in 20 min, $P > 0{\cdot}05$). However, clonidine pretreatment produced a

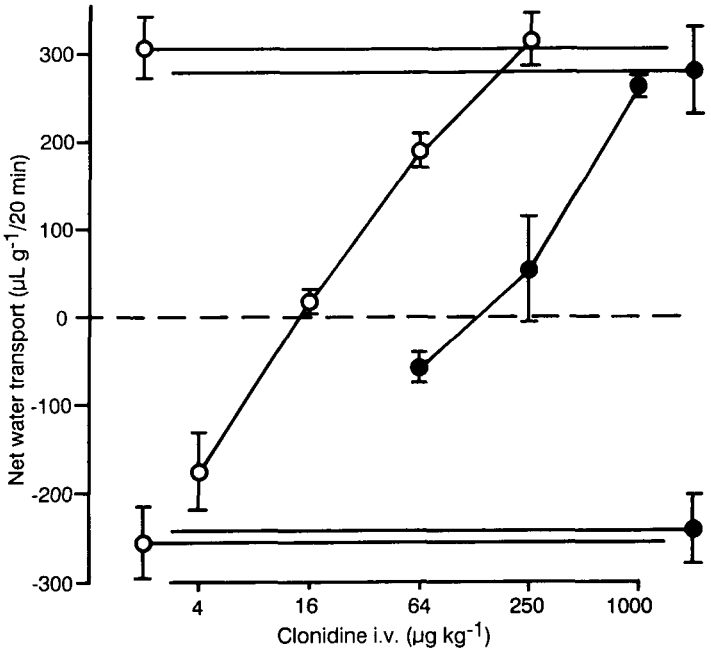

FIG. 2. Antisecretory effect of clonidine before (○) and after (●) clonidine pretreatment. The upper, horizontal lines represent the mean rates of basal water absorption and the lower lines the mean rates of PGE_2-induced intestinal secretion. Increasing acute doses of clonidine restored fluid transport from secretion to absorption. The two dose-response curves are significantly different ($P<0.05$).

statistically significant 7·6-fold reduction in the sensitivity to the acute antisecretory doses of clonidine ($P<0.05$, Fig. 2). Similarly clonidine pretreatment caused a statistically significant 11-fold decrease in the acute antisecretory effect of morphine ($P<0.05$, Fig. 1). When the pretreatment time with clonidine was reduced to two doses of 0·25 mg kg^{-1} at 12 h intervals there was no reduction in the effect of the standard acute dose of morphine.

Discussion

The major findings of this study were that clonidine, an α_2-adrenoceptor agonist, induced tolerance to its own antisecretory effect in the small intestine, as well as inducing cross-tolerance to morphine. However, no tolerance to morphine could be demonstrated by various schedules of pretreatment with morphine.

Previous studies have established that tolerance does occur to some effects of morphine in the intestine. For example, Weisbrodt et al (1977) demonstrated that tolerance occurs to the anti-transit effect in the rat small intestine, and Warhurst et al (1983, 1984) showed that tolerance also occurs to the pro-absorptive effect.

Table 3. Amounts of morphine and morphine-3-glucuronide entering intestinal loops (ng g^{-1} in 20 min) after different morphine treatments.

Morphine treatment/ pretreatment	Morphine	Morphine-3-glucuronide
Standard acute dose	906±220	3433±573*
M1	678±83	740±99
M2	711±220	686±104
M3	487±54	664±27

All animals were infused with PGE_2 (4 μg min^{-1}). * The amount of morphine-3-glucuronide in animals treated with the standard acute dose (2·5 mg kg^{-1}, i.v.) was significantly higher than in the pretreated groups (MLTCOMP, $P<0.05$). All other pair-wise comparisons are not significant (MLTCOMP, $P>0.05$). n=5 all groups.

In this study the antisecretory effect of morphine was assessed in 3 groups of animals each given different pretreatments with morphine. One and two days of pretreatment followed by the standard dose of morphine 12 h later revealed, surprisingly, that the antisecretory effect was enhanced significantly. Therefore, a

Table 2. Effect of morphine pretreatment on fluid transport rates (μL g^{-1} in 20 min).

	Pretreatment			
Treatment	None	M1	M2	M3
1 Absorption (saline i.a., i.v.)	+308±36	+261±34·5	+174±30	+296±64
2 Secretion (PGE_2 i.a., saline i.v.)	−256±40	+3±30*	+192±22*	−91±22*
3 Acute morphine response (PGE_1 i.a., 2·5 mg kg^{-1} morphine i.v.)	+54±32	+304·5±40*	+485±40*	+183·5±58
4 Extent of PGE_2 reversal by acute morphine (3–2)	+310	+301·5	+293	+274·5

* Significantly different from non-pretreated control group (Dunnett's t-test, $P<0.05$).

The pretreatments did not change the (4) difference between PGE_2-induced water transport rates with (3) and without (2) the standard acute dose ($P>0.05$, Student's unpaired t-test using summed variance of effect mean (3) and baseline mean (2)). n=5–6 all groups.

further group of animals was pretreated for a longer period (4 days) with higher doses (4×40 mg kg^{-1}) of morphine but with a longer wait (48 h) between the last dose and the test. However, even under these conditions, there was still no sign of tolerance, although the antisecretory response to the standard acute dose was not as great as with the other pretreatments. It was shown that the morphine pretreatments did not increase the rate of water transport as such but rather they reduced PGE_2-stimulated fluid secretion. When these elevated baselines are taken into consideration it is apparent that the pretreatments do not change the effect of the standard acute dose. Hence morphine does not induce tolerance to its own antisecretory effect over the dose and time ranges used in these experiments.

The reduced effect of PGE_2 after morphine pretreatment is a functional indication that morphine levels accumulate and remain high for a relatively long period of time. It is well established that morphine undergoes enterohepatic circulation (Jaffe & Martin 1985), and morphine has previously been shown to achieve a higher concentration in the small intestine than in the plasma or brain following intravenous administration. This finding has been used to explain how morphine exerts a persistent antitransit effect (Bianchi et al 1983).

Our results revealed that morphine was present in the intestinal lumen 12 and even 48 h after the last pretreatment dose. Morphine accumulated rapidly, so that the amount present within the lumen 30 min after a single ED50 injection was similar to the amounts present 12 and 48 h following the pretreatments. The amount that accumulates in the 20 min experimental period cannot originate from the bile duct since the upper duodenum is not perfused in our experiments, and in addition our method involves washing bile and other contents from the jejunum before perfusion. The effect of the standard acute dose and the pretreatments on PGE_2-induced secretion correlate relatively well with the actual accumulation rates of morphine within the intestinal lumen (Tables 2, 3). In addition morphine-3-glucuronide was also detected in the luminal perfusates following the 3 pretreatments. The amount of this metabolite was significantly greater after the standard acute dose than the chronic doses. This result should be expected in view of the much shorter time allowed for renal excretion to take place.

α-Adrenoceptor agonists and morphine have similar effects in the rat small intestine. For example, clonidine delays transit in the rat small intestine (Ruwart et al 1979), and both clonidine and oxymetazoline produce an antisecretory effect (Nakaki et al 1981). In addition, the results of this study show that clonidine induces tolerance to its own antisecretory effect as well as a cross-tolerance to morphine. There is a striking similarity in this profile of actions and interactions on the intestine compared with the CNS. For example, clonidine is a potent analgesic, and tolerance develops to its effect (Yamazaki & Kaneto 1985). Also, clonidine induces cross-tolerance to morphine. In the abdominal constriction test as modified by Bentley et al (1983), even a single low dose of clonidine induces tolerance to the antinociceptive effect of morphine.

The fact that clonidine is able to produce a similar antisecretory response to morphine, and that tolerance occurs to its action as well as cross-tolerance to morphine shows that there is a close relationship between the opioid and α_2-adrenoceptors that control the antisecretory response of the intestine. However, it is not clear why tolerance does not develop to morphine especially as the drug accumulates and persists in the intestine.

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