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Evidence for tryptaminergic and noradrenergic involvement in the antisecretory action of morphine in the rat jejunum

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Experiments have been performed to determine whether the antisecretory (antidiarrhoeal) effect of morphine in the intestine is mediated by a direct action of morphine on enteric nerves. Rats were pretreated with 6-hydroxydopamine (6-OHDA) or *p*-chlorophenylalanine (PCPA) to deplete intestinal stores of noradrenaline and 5-hydroxytryptamine (5-HT). Intraperitoneal injection of 6-OHDA (3 doses at 50 mg kg^{-1}) caused a selective reduction in the level of noradrenaline in the jejunum to 7·3% of control. Intraperitoneal injection of PCPA (200 mg kg^{-1}) selectively reduced the jejunal level of 5-HT to 30·5% of control. Groups of rats that had been treated as described above were anaesthetized and then injected intravenously with saline or with blocking doses of either atropine (0·25 mg kg^{-1}), hexamethonium (20 mg kg^{-1}), ketanserin (30 μg kg^{-1}), methysergide (30 μg kg^{-1}), phentolamine (2 mg kg^{-1}) or propranolol (1 mg kg^{-1}). Following perfusion of the lumen of the jejunum, the rate of glucose absorption was measured to assess the integrity of the mucosa. Glucose absorption was unaltered in animals pretreated with hexamethonium and propranolol but there was a small enhancement in animals pretreated with atropine, PCPA, methysergide, 6-OHDA and phentolamine. The rate of net water absorption from the lumen of the jejunum and the rate of fluid secretion into the lumen following intra-arterial infusion of vasoactive intestinal peptide (VIP, 0·8 μg min^{-1}) were unaltered by any of the drug treatments. Intravenous injection of morphine (10 mg kg^{-1}) did not alter the levels of noradrenaline or 5-HT in the whole jejunum. However, this dose of morphine did cause a 63·5% decrease in the VIP-induced change in water transport. This antisecretory effect of morphine was unaltered in animals pretreated with atropine, hexamethonium and propranolol. In contrast, methysergide, ketanserin and 6-OHDA abolished the antisecretory effect of morphine. PCPA and phentolamine produced a partial inhibition of morphine's antisecretory effect. It is concluded that morphine produces its antisecretory effect in the jejunum by activation of noradrenergic and tryptaminergic systems.

The major cause of constipation following acute administration of morphine is due to inhibition of gastrointestinal propulsion. This action is commonly attributed to the ability of morphine to decrease acetylcholine release. Whereas this is true for the guinea-pig ileum (Paton 1957), morphine does not produce this effect in the rat intestine (Gaginella & Wu 1983) and even appears to release acetylcholine in the human intestine (Daniel et al 1959). Morphine does, however, release 5-hydroxytryptamine (5-HT) in the rat intestine (Burks 1976). These effects in human and rat intestine help to explain how morphine increases muscle tone, especially that of the circular layer. The consequence of this action is an overall decrease in the efficiency of co-ordinated propulsive movements (Daniel et al 1959; Weinstock 1971).

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It is now recognized that the antidiarrhoeal as opposed to the constipating action of opiates and related compounds is due to their ability to inhibit intestinal fluid secretion stimulated by a variety of ingested and endogenous diarrhoea-producing substances (see Awouters et al 1983 for further information). As with the constipating action of morphine, the antisecretory action is caused by morphine acting on the intestine itself. This is evident because the effect of morphine remains undiminished after destruction of the central nervous system (Lembeck & Beubler 1979; Lee & Coupar 1982). Additionally, opiate binding sites have been identified within the villi of the rat jejunum (Dashwood et al 1985) which could correspond to the receptors mediating the pharmacological response which have been classified as μ-opioid receptors (Coupar 1983). However, opioid binding sites are not present on the secretory epithelium of the intestine (Gaginella et al 1983), nor

does morphine directly reduce the biochemical marker of enterocyte secretory activity, cyclic AMP (Hardcastle et al 1982). These observations suggest the presence of intrinsic neuronal transmission linking opioid binding at μ-opioid receptors to the intestinal epithelium. This is further supported by the finding that the proabsorptive effect of methionine enkephalin is inhibited by tetrodotoxin in rabbit ileal mucosa (Binder et al 1984). It is possible that the nerves involved terminate as noradrenergic endings since the crypt and villus epithelium does receive a noradrenergic innervation (Thomas & Templeton 1982) and α_2-agonists have a remarkably similar spectrum of antisecretory effects to morphine (Nakaki et al 1982).

We have therefore undertaken pharmacological analysis to determine whether noradrenergic as well as other enteric nerves are activated by morphine to inhibit intestinal fluid secretion. The secretagogue selected for the following experiments was vasoactive intestinal peptide (VIP) since it is a causative agent in some pathogenic diarrhoeal states (Gaginella & O'Dorisio 1979) and acts directly on the epithelial cells of the small intestine (Laburthe et al 1979; Prieto et al 1981; Sarrieau et al 1983).

METHODS

The method of measuring fluid absorption from, and VIP-stimulated secretion into, the lumen of the rat jejunum, has recently been described (Coupar 1985). In brief, male and female Hooded Wistar rats (230–290 g) are anaesthetized with pentobarbitone sodium (60 mg kg^{-1}). A cannula is introduced into the left jugular vein for drug administration and into the left common carotid artery for constant intra-arterial (i.a.) infusions of saline or VIP in saline at a rate of 40 μL min^{-1}. Mean systemic blood pressure is recorded from a side-arm off the carotid cannula by means of a Statham pressure transducer connected to a Grass polygraph (Model 79C). A recirculation technique is used to measure the net amount of fluid transported by the jejunum. The perfusing solution (8 mL of an isosmotic solution containing NaCl 8·57, KCl 0·37, dextrose 1·0 and phenol red 0·02 g L^{-1} as a non-absorbable marker) is recirculated through the intestinal loop (20 to 30 cm in length starting distal from the Ligament of Trietz) from a reservoir maintained at 37 °C by gas-lift consisting of moistened 5% CO_2 in O_2.

At the end of the 20 min perfusion the fluid from the loop is recovered and peak absorbance in samples diluted with buffer is measured at 560 nM as well as 520 and 600 nM 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.

Glucose absorption is calculated from the fluid volume and the glucose concentration before and after perfusion by the commercially available hexokinase method (Glucoquant, Boehringer-Mannheim).

Pretreatment of animals with 6-hydroxydopamine (6-OHDA) to produce chemical sympathectomy (Thoenen & Tranzer 1973), and *p*-chlorophenylalanine (PCPA) to deplete 5-HT (Koe & Weissman 1966) were carried out as follows: 6-OHDA was dissolved in deoxygenated saline containing 1 mg mL^{-1} of ascorbic acid. One dose was administered intraperitoneally (i.p.) in volumes of 1 mL kg^{-1} followed by two doses three days later. Animals were used for experiments 4–5 days after the initial injection. PCPA was dissolved in 0·9% NaCl (saline) and injected in a volume of 2 mL kg^{-1} two days before experiments. On the day of the experiment following the onset of anaesthesia, the intravenously (i.v.) administered drugs used for the pharmacological analysis were dissolved in saline and injected (time zero) in volumes of 1 mL kg^{-1}. Control animals received injections of saline. Five min later, morphine (10 mg kg^{-1}) or saline was injected. After a further 5 min, intra-arterial (i.a.) infusion of VIP or saline as control was commenced and continued for the length of the 20 min luminal perfusion which commenced at 15 min (i.e. a further 5 min later).

The selectivities of the pretreatments in depleting noradrenaline and 5-HT were determined by measuring the intestinal levels of the amines. Pilot experiments established that the i.v. doses of antagonists used caused effective receptor blockade by the following criteria. Atropine (0·25 mg kg^{-1}) and hexamethonium (20 mg kg^{-1}) were both shown to block bradycardia induced by electrical stimulation of the vagus throughout the duration of the experiment. Methysergide (30 μg kg^{-1}) and ketanserin (30 μg kg^{-1}) each inhibited 5-HT-induced pressor responses in pithed rats. Propranolol (1 mg kg^{-1}) blocked isoprenaline-induced depressor responses and phentolamine (2 mg kg^{-1}) inhibited noradrenaline-induced pressor responses in anaesthetized rats.

Amine determinations

Rats pretreated with saline, 6-OHDA or PCPA were killed by desanguination. Approximately 20 cm of jejunum, similar in position and length to the

intestinal loop used in recirculation experiments, was removed, washed with cold saline, blotted dry, frozen in liquid nitrogen and stored at −20 °C. In another group of rats lengths of jejunum were removed 30 min after i.v. administration of morphine (10 mg kg^{-1}) and treated and stored as above. For amine determinations the frozen tissues were weighed and after thawing in a solution of 0·4 M perchloric acid containing 0·2% sodium edetate were chopped and then homogenized using an Ultra-Turrax homogenizer. Noradrenaline and 5-HT were extracted from the homogenate using Bio-Rex 70 (Bio-Rad Laboratories) according to the column procedure of Barchas et al (1972) and assayed spectrofluorometrically as previously described (Fennessy & Taylor 1977). The concentrations of amines are expressed in terms of the free base or acid.

Statistics

The results were subjected to appropriate statistical evaluations. The values for glucose absorption were analysed by one-way analysis of co-variance to correct for the negative regression of intestinal weight on glucose absorption. Pairs of resultant adjusted means were compared for statistical differences by the conventional Student's *t*-test (BMDP Statistical Software 1981). Means of water absorption and secretion were subjected to one-way analysis of variance and individual means compared using the Student's unpaired *t*-test. The statistical significance of differences between means of amine levels were determined by use of Student's unpaired *t*-test. Statistical significance was assumed if $P < 0·05$.

Drugs

Atropine sulphate (Sigma), hexamethonium bromide (Koch-Light Laboratories), 6-hydroxydopamine hydrobromide (6-OHDA, Sigma), ketanserin (Janssen), methysergide hydrogen maleate (Sandoz), *p*-chlorophenylalanine methylester hydrochloride (PCPA, Sigma), pentobarbitone sodium (Nembutal, Abbott), phentolamine mesylate (Regitine, Sigma), propranolol hydrochloride (ICI) and vasoactive intestinal peptide (VIP, Karolinska Institute).

RESULTS

Amine levels

The effect of the drug treatments on the levels of noradrenaline and 5-HT are given in Table 1. Three injections of 50 mg kg^{-1} 6-OHDA over a 3 day period as described above resulted in a 93% reduction in the level of noradrenaline but the level of 5-HT was unaltered compared to the level determined in vehicle-treated animals. PCPA (200 mg kg^{-1}) resulted in a reduction in the level of 5-HT to 30·5% of control without altering the level of noradrenaline. These dosing schedules of the depleting agents were chosen on the basis of their causing statistically significant and selective depletion of noradrenaline or 5-HT from the jejunum. In saline-pretreated rats, morphine (10 mg kg^{-1}) did not alter the amine levels in the jejunum during the 30 min following injection, which is equivalent to the duration of recirculation experiments (Table 1).

Table 1. The effect of pretreatment with 6-hydroxydopamine (6-OHDA), *p*-chlorophenylalanine (PCPA) or morphine on the levels of noradrenaline and 5-hydroxytryptamine (5-HT) in the rat jejunum.

	Noradrenaline	5-HT
Control (μg g^{-1})	0·33 ± 0·03	1·86 ± 0·20
6-OHDA pretreated (% control)		
Dose: i.p.		
25 mg kg^{-1}	14·1 ± 8·8*	96·9 ± 16·7
50 mg kg^{-1}	7·3 ± 9·2*	127·7 ± 26·5
100 mg kg^{-1}	7·8 ± 8·8*	112·3 ± 23·5
PCPA pretreated (% control)		
Dose: i.p.		
100 mg kg^{-1}	86·9 ± 9·7	36·9 ± 11·2*
200 mg kg^{-1}	88·1 ± 7·6	30·5 ± 11·1*
400 mg kg^{-1}	67·4 ± 7·6*	26·5 ± 11·1*
Morphine pretreated (% control)		
Dose: i.v.		
10 mg kg^{-1}	101·5 ± 9·7	109·7 ± 22·1

The levels of controls are expressed as μg g^{-1} wet weight ± s.e.m. The levels of each treatment group are expressed as a percentage of control ± relative s.e. The values are the means of the levels determined in at least 4 tissues. *$P <$ 0·05 compared with control.

Absorption

The rate of glucose absorption in control animals was 4·19 ± 0·16 mg g^{-1} in 20 min (Fig. 1). Some of the treatments exerted a small, though statistically significant, effect on the rate of glucose absorption from the lumen of the jejunum. In animals treated with hexamethonium and propranolol, the rate of glucose absorption was unaltered but was significantly higher in those animals pretreated with atropine, PCPA, methysergide, 6-OHDA and phentolamine (P = 0·003, analysis of co-variance, Fig. 1A).

The values of net water absorption from the jejunum were more variable, both within and between groups, than values for glucose absorption. The control rate of water absorption was 216 ± 27 μL g^{-1} in 20 min and there was no significant difference between the different treatment groups (P

> 0·1, analysis of variance, Fig. 1B). There was no correlation between the rates of glucose and water absorption for the different treatments (r = 0·26).

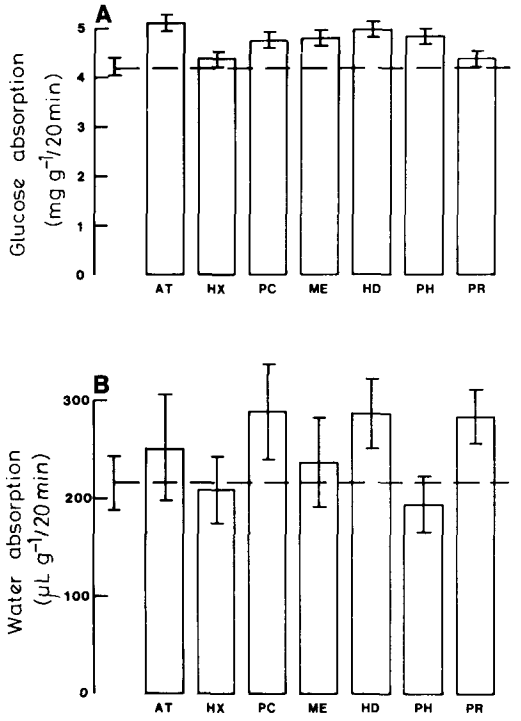

FIG. 1. A. Effect of drug treatments on glucose absorption from the lumen of the jejunum. The broken line represents the level of glucose absorption occurring in non-drug treated control animals. The treatments exerted a statistically significant effect on glucose absorption (P = 0·003, analysis of co-variance). The individual means significantly greater than control are atropine (AT, P = 0·009), *p*-chlorophenylalanine (PC, P = 0·025), methysergide (ME, P = 0·01), 6-hydroxydopamine (HD, P = 0·0015) and phentolamine (PE, P = 0·008). The means for hexamethonium (HX) and propranolol (PR) were not significantly different from control (P > 0·05, Student's unpaired *t*-test). B. Values of net water absorption from the lumen of the jejunum following drug treatments. The broken line is the rate of water absorption in control animals. The means are not significantly different (analysis of variance, P > 0·1). Bars in this and Fig. 2 are standard errors of the mean, n = 5 for all groups.

Secretion and the antisecretory effect of morphine

Infusion of VIP (0·8 μg min^{-1} i.a.) starting 5 min before and continuing during the 20 min perfusion totally reversed water transport to net secretion similar in magnitude to the maximum secretory effect of VIP in pentobarbitone-anaesthetized rats (Coupar 1985). The drug treatments did not significantly effect the magnitude of the VIP-induced secretion (P > 0·1, analysis of variance). Intravenous injection of morphine (10 mg kg^{-1}), 10 min before commencing perfusion of the jejunum, blocked the VIP-induced secretion to the extent that no significant net fluid transport occurred across the mucosa of the jejunum. This antisecretory effect of morphine was equivalent to a 64% reversal of the VIP-induced change in fluid transport, and was unmodified in animals pretreated with atropine, hexamethonium or propranolol.

In contrast phentolamine, 6-OHDA, PCPA, methysergide and ketanserin all inhibited the antisecretory effect of morphine (Table 2, Fig. 2).

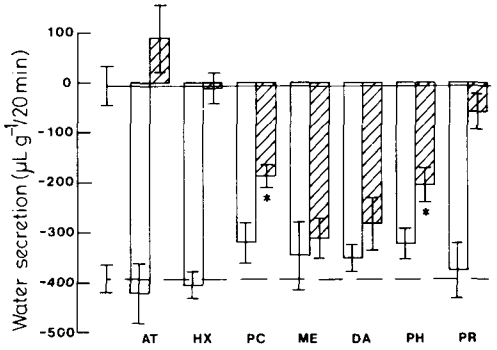

FIG. 2. The lower broken line is the control level of water secretion in response to intra-arterial infusion of VIP (0·8 μg kg^{-1}), while the open bars are the responses to VIP in drug-treated animals. There is no significant alteration in the rate of water secretion between the different treatments (analysis of variance, P > 0·1). The upper continuous line shows the water transport rate occurring in animals injected intravenously with morphine (10 mg kg^{-1}) and infused with VIP. The alteration of this antisecretory effect of morphine by the drug treatments is shown as hatched bars. The probability values for the differences between VIP and VIP/morphine-treated groups with the same pretreatments are atropine (AT, P = 0·005), hexamethonium (HX, P = 0·001), *p*-chlorophenylalanine (PC, P = 0·02), methysergide (ME, P = 0·66), 6-hydroxydopamine (HD, P = 0·28), phentolamine (PH, P = 0·03) and propranolol (PR, P = 0·01) (Student's unpaired *t*-test).

DISCUSSION

The central findings of this study suggest that morphine transmits its antisecretory effect in the small intestine through neuronal pathways. Since pharmacological manipulations were used it was necessary to demonstrate that the various drug treatments did not adversely decrease the normal functional activity of the intestine. For this reason the primary physiological functions of glucose and water absorption were measured. Surprisingly, several of the drug treatments were shown to enhance active glucose absorption from the lumen of the jejunum. These were atropine, 6-OHDA, phentolamine, PCPA and methysergide. There is a

Table 2. Effects of drug treatments on water absorption, secretion rates in response to VIP, and the antisecretory activity of morphine.

Treatment	Water transport rates μL g^{-1} in 20 min			% Reversal of VIP by morphine
	Saline	VIP	Morphine + VIP	
Saline	+216 ± 27	−392 ± 26	−6 ± 38	64
Atropine	+250 ± 53	−421 ± 59	+89 ± 67	76
Hexamethonium	+209 ± 34	−405 ± 26	−11 ± 31	64
Propranolol	+282 ± 28	−375 ± 55	−58 ± 34	48
Phentolamine	+193 ± 28	−322 ± 31	−204 ± 34*	23
6-OHDA	+286 ± 35	−350 ± 26	−281 ± 53†	11
PCPA	+288 ± 48	−320 ± 40	−187 ± 22*	22
Methysergide	+236 ± 46	−346 ± 69	−310 ± 39†	6
Ketanserin	+367 ± 26	−314 ± 62	−185 ± 35†	19

* Drug treatments producing a significant decrease in the antisecretory effect of morphine compared to the morphine antisecretory control value ($P < 0.05$, Student's t-test).

† Drug treatments blocking the antisecretory effect of morphine as shown by no significant difference between the VIP-induced secretion rates in animals injected with and without (saline) morphine ($P > 0.05$, Student's t-test). $n = 5$ for all groups.

paucity of information in the literature regarding the effect of drugs on nutrient absorption. What is available indicates that some of the above treatments could enhance absorption by virtue of relaxing the villi and hence increasing absorptive area, since it is known that both acetylcholine and noradrenaline both contract the villi (Hooper & Schneider 1970). This contrasts with a study which shows that noradrenaline increased glucose absorption in rat isolated small intestine (Aulsebrook 1965). Nevertheless, regardless of the mechanism, it should be stressed that the enhancement of glucose absorption noted was small and the biological significance of such enhancement is unclear. The selective depletion of noradrenaline and 5-HT by 6-OHDA and PCPA, respectively, also demonstrates the selective nature of the treatments used.

Of prime importance to this study is the observation that the treatments did not significantly alter the magnitude of either water absorption or fluid secretion in response to infusion of VIP. However, treatments interfering with the noradrenergic and tryptaminergic systems of the jejunum by depletion and receptor blockade, clearly inhibited the antisecretory effect of morphine. Blockade of the cholinergic system, however, at muscarinic or nicotinic receptors, did not modify the effect of morphine.

It is probable that morphine exerts its antisecretory action within the intestinal tissue, since it retains antisecretory activity after destruction of the central nervous system (Lembeck & Beubler 1979; Lee & Coupar 1982). Recent autoradiographic studies of rat jejunum using [^{3}H]naloxone and [^{3}H]dihydromorphine have identified opiate binding sites concentrated almost exclusively near the crypts and the villus cores (Dashwood et al 1985). If morphine acts on these sites then noradrenaline satisfies many of the requirements for being the final mediator of morphine's antisecretory action for the following reasons. Fine noradrenergic nerve fibres are present in the mucosa of the rat small intestine innervating the crypts and epithelial cells of the villi (Schultzberg et al 1980; Thomas & Templeton 1982). α-Adrenoceptor binding sites are present on the enterocyte basolateral membrane (Cotterell et al 1982; Nakaki et al 1983). These binding sites may represent the functional receptors which, when activated, enhance fluid absorption and physiologically antagonize the secretory effect of secretagogues such as VIP (Cotterell et al 1983; Nakaki et al 1983; Parsons et al 1984). In addition, the receptors for VIP are also located on the membrane of the enterocytes which are in turn innervated by VIPergic fibres (Schultzberg et al 1980; Prieto et al 1981; Sarrieau et al 1983). Our results are therefore consistent with morphine releasing noradrenaline onto the mucosal epithelium. 6-OHDA, which caused large and selective depletion of intestinal noradrenaline, completely blocked the effect of morphine. The α-adrenoceptor antagonist phentolamine was not as effective as 6-OHDA which is to be expected if it is assumed that the mucosal noradrenergic nerve terminals possess functional α_2-adrenoceptors. The observation that morphine did not cause a detectable increase in noradrenaline levels is not surprising and should not be considered evidence against involvement of noradrenaline since the proportion of noradrenergic nerves innervating the secretory cells

is only small compared with the total noradrenergic nerves of the whole jejunum (Thomas & Templeton 1982). Future studies involving the measurement of noradrenaline turnover rate in the jejunal mucosa may be more applicable.

Our results also show clearly that inhibition of the tryptaminergic system leads to a block of the antisecretory action of morphine. This may appear surprising for two considerations. Firstly, it is well established that 5-HT is not an inhibitor but actually a stimulant of intestinal fluid secretion (Donowitz et al 1979: Hardcastle et al 1981). Therefore, depletion of 5-HT or inhibition of its effect would be expected to enhance the resting rate of water absorption and perhaps lead indirectly to an enhancement of the antisecretory effect of morphine. Secondly, release of 5-HT from the intestine has been implicated as a factor that initiates intestinal fluid secretion characteristic of the morphine-withdrawal syndrome (Beubler et al 1984). On the other hand, acutely administered morphine in the rat also results in 5-HT release which has been suggested as being associated with antimotility and hence the constipating action of morphine (Burks 1976). Clearly 5-HT affects many functions of the intestine depending on the location of its release. Our results indicate the involvement of 5-HT in the acute antisecretory action of morphine, since the effect of morphine was blocked by low doses of both ketanserin and methysergide. The assumption has been made that the doses of ketanserin and methysergide are selective from studies of these drugs on the cardiovascular system. It may also be assumed that the receptor activated by 5-HT in rat blood vessels and intestine is similar since the putative 5-HT_2 receptor antagonist ketanserin at the low dose of 30 $\mu g\,kg^{-1}$ i.v. used in this study does cause a large inhibition of 5-HT-induced pressor responses in pithed rats (Conolan et al 1985). A higher dose of 100 $\mu g\,kg^{-1}$ has been shown to inhibit intestinal fluid secretion on opiate withdrawal in rats (Beubler et al 1984), however, doses above this level have the potential to block α-adrenoceptors (Fozard 1982; Conolan et al 1986). The reason why the effect of morphine was not totally blocked in PCP A-treated animals is explained by the observation that the selective dose of PCPA used did not cause complete depletion of intestinal 5-HT.

As with noradrenaline, no change in the whole intestinal level of 5-HT was detected after i.v. injection of morphine. This may indicate that the pool of 5-HT involved with the antisecretory effect of morphine is small, being masked by the large pool of enterochromaffin 5-HT which perhaps is involved in the withdrawal-secretory response (Beubler et al 1984) and myenteric plexus 5-HT which is probably responsible for causing the antimotility effects of morphine (Burks 1973).

It is only possible to hypothesize as to the location and interconnections of noradrenergic and tryptaminergic nerves that are involved with mediating the antisecretory effect of morphine. Although most information has been derived from the guinea-pig, it is known that tryptaminergic cell bodies occur in the myenteric plexus of the rat small intestine, which send single, fine processes ending as varicosities in the ganglia of the myenteric and submucous plexus (Costa et al 1982; Furness & Costa 1982). In the guinea-pig, the noradrenergic nerves innervating the intestinal mucosa are extrinsic and therefore do not have cell bodies within the intestinal wall (Keast et al 1984). It is possible therefore that the action of morphine on opiate-sensitive neurons in the intestine activates tryptaminergic nerve-cell bodies. We speculatively suggest that the resultant release of 5-HT excites noradrenergic nerve endings and the released noradrenaline physiologically antagonizes the secretory effect of VIP.

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