British Journal of Pharmacology (1997) 120, 892–898

Role of α_2-adrenoceptors in the regulation of intestinal water transport

Lu Liu & [1]Ian M. Coupar

Department of Pharmaceutical Biology and Pharmacology, Victorian College of Pharmacy, Monash University, Parkville, Vic, 3052, Australia

1 The influence of the sympathetic nervous system on intestinal fluid transport by the jejunum and ileum of the anaesthetized rat was investigated under basal conditions and during active secretion induced by intra-arterial infusion of vasoactive intestinal peptide (VIP).

2 Intra-arterial infusion of noradrenaline (3, 10, 30 nmol min^{-1}, i.a.) and i.v. injection of the selective α_2-adrenoceptor agonist UK 14,304 (1 μmol kg^{-1}, i.v.) increased the rate of basal fluid absorption. The effect of UK 14,304 was blocked by yohimbine (10 μmol kg^{-1}, i.v). However, the selective α_1-adrenoceptor agonist phenylephrine (5 μmol kg^{-1}, i.v.) did not alter either the jejunal or ileal absorption rate.

3 The α_2-adrenoceptor antagonists yohimbine (0.3, 1.0, 3 and 10 μmol kg^{-1}, i.v.) and rauwolscine (10 μmol kg^{-1}, i.v.) decreased the basal absorption rate, while the α_1-adrenoceptor antagonist prazosin (3 μmol kg^{-1}, i.v.) was without effect. Intracerebroventricular injection of yohimbine (3 μmol kg^{-1}) caused a significant antiabsorptive effect in the jejunum but not ileum.

4 Peripheral chemical sympathectomy induced by pretreating animals with 6-hydroxydopamine (150 mg kg^{-1}, i.p., total dose) induced a trend towards impaired absorption in the jejunum and ileum.

5 The findings provide evidence that the sympathetic nervous system exerts tonic control on intestinal fluid transport and that the effect is mainly through peripheral α_2-adrenoceptors.

6 The subtype determination of α_2-adrenoceptors in modulating intestinal fluid transport was assessed by determining the effects of α_2-adrenoceptor agents on intestinal fluid secretion induced by i.a. infusion of VIP (0.8 μg min^{-1}).

7 Intravenous administration of UK 14,304 caused a dose-dependent reversal of the secretory phase of the VIP-induced response, but failed to restore fluid transport to the control level of net absorption. EC_{50} values were 0.17 μmol kg^{-1} in the jejunum and 0.22 μmol kg^{-1} in the ileum.

8 The effect of UK 14,304 was blocked by the selective $\alpha_{2A/D}$ antagonist BRL 44408 and the non-selective α_2 antagonist yohimbine (each 10 μmol kg^{-1}). The selective $\alpha_{2B/C}$ antagonist ARC 239 (10 μmol kg^{-1}) did not affect the antisecretory action of UK 14,304. It is suggested that the α_2-adrenoceptors in the rat intestinal epithelium are the α_{2D} or α_{2A}-like subtype.

Keywords: Rat jejunum and ileum; absorption; secretion; fluid transport; α_2-adrenoceptors; α_2-adrenoceptor subtype; sympathetic nervous system

Introduction

Studies in experimental animals and normal volunteers have shown that α_2-adrenoceptor agonists inhibit intestinal motility and promote the absorption of fluid from the lumen of the intestine (Chang *et al*., 1982; Schiller *et al*., 1985; Fondacaro *et al*., 1988; Hildebrand & Brown, 1992). It has also been shown that α_2-adrenoceptor agonists inhibit intestinal fluid secretion induced by prostaglandin E_1, (PGE_1), vasoactive intestinal peptide (VIP), dibutyryl adenosine 3′:5′-cyclic monophosphate (cyclic AMP) and cholera toxin in rat jejunum (Nakaki *et al*., 1982). The enterocytes of the small intestinal epithelium are innervated by adrenergic neurones (Furness & Costa, 1980; Thomas & Templeton, 1982) and radioligand binding studies have identified α_2-adrenoceptors on their membranes (Chang *et al*., 1983; Nakaki *et al*., 1983).

It is generally accepted that stimulation of the sympathetic nervous system leads to inhibition of gastrointestinal motility and promotion of intestinal water and electrolyte absorption. However, the question concerning the existence of a tonic sympathetic control on gut motility and fluid transport under basal conditions is still unresolved. Therefore, the present investigations were carried out to assess whether the sympathetic nervous system has a role in controlling the net amount of fluid absorbed by the intestine. For this purpose, experiments were designed to evaluate whether α_2-adrenoceptor agonist and antagonists have the ability to alter basal water absorption in the rat intestine *in vivo*.

It has become apparent that α_2-adrenoceptors represent a heterogeneous population. Four distinct subtypes have been identified based on functional, radioligand binding and molecular biology studies and termed α_{2A}, α_{2B}, α_{2C} and α_{2D}-adrenoceptors. The rat and mouse α_{2D}-adrenoceptor appears to be a species homologue of the human α_{2A} subtype (Ruffolo *et al*., 1993; Bylund *et al*., 1995).

Identification of receptor subtypes provides an opportunity to develop therapeutic drugs with selective action. However, no functional study has been made so far to characterize the α_2-adrenoceptor subtype involved in the regulation of fluid and electrolyte transport in the small intestine. In the present study, the α_2-adrenoceptor agonist UK 14,304 and several antagonists were used in an attempt to characterize functionally the α_2-adrenoceptors involved in attenuating VIP-induced fluid secretion from the rat jejunum and ileum.

Methods

The method of measuring fluid transport by the rat jejunum and ileum follows that described in detail by Coupar (1985). A brief outline is given below.

[1] Author for correspondence.

Surgical and analytical procedures

Hooded Wistar rats of either sex (220–300 g weight) were deprived of food over night but had free access to water. Rats were anaesthetized with pentobarbitone sodium (60 mg kg^{-1}, s.c., supplemented when necessary) and placed on an electrically heated pad at approximately 35°C. The trachea was cannulated and a second cannula was introduced into the left jugular vein for administration of the adrenoceptor agonist and/or antagonist, or in the case of controls, saline or vehicle at volumes of 0.1 ml 100 g^{-1}. Another cannula was introduced into the left common carotid artery for constant infusions of saline as control, noradrenaline or VIP (0.8 μg min^{-1}) in saline into the aortic arch at a rate of 40 μl min^{-1} by means of a microinjection pump (CMA/microdialysis, Stockholm). Mean systemic blood pressure (MAP) was recorded to monitor the condition of the animals from a side-arm off the carotid cannula by means of a Gould pressure transducer (Statham, U.S.A.) connected to a Neotrace (Neomedix Systems, Sydney) recorder.

A recirculation technique was used to measure the net fluid transport rates of the jejunum and ileum. Twenty to 30 cm lengths of the proximal jejunum (beginning distal from the ligament of Trietz) and distal ileum (ending 2 cm from the ileocecal junction) were isolated, cannulated at each end and rinsed free of luminal debris. Each loop was continuously perfused with 8 ml of an isosmotic solution containing (mM): NaCl 148, KCl 5, glucose, 5.5 and phenol red 0.05, as a non-absorbable water marker. The solutions were contained in reservoirs maintained at 37°C and recirculated through each lumen by gas lift with moistened CO_2 (5%) in O_2 for a period of 20 min. The pressure in the loops was 10 cmH_2O and the flow rates were approximately 60 ml min^{-1}.

The fluid from each loop was recovered at the end of the 20 min perfusion period, centrifuged and then diluted (2:25) with $NaHCO_3/Na_2CO_3$ buffer (pH 10.5). Peak absorbances were measured at 560 nm as well as 520 and 600 nm to correct for non-specific interferences as described by Miller and Schedl (1972). The results are expressed as the amount absorbed (+) or secreted (−) in μl g^{-1} wet weight tissue during the 20 min perfusion.

Administration of drugs

Systemic drug administration In studies to determine the effect of drug treatments on basal absorption, the administration sequence commenced with the i.v. injection of UK 14,304, vehicle or saline as the control. Five min later, the intra-arterial infusion of saline was commenced with associated measurement of MAP. The loops of jejunum and ileum were washed before intestinal perfusion was started at 10 min. At 30 min the loops were removed and the perfusion fluid was collected for analysis. The segments were weighed after the removal of fluid content. The testing of the antagonists followed a similar protocol to above, except that the antagonists were injected i.v. 5 min before the injection of UK 14,304 or saline. In some experiments, noradrenaline was infused i.a. at rates of 3, 10 and 30 nmol min^{-1} and continued for the length of the 20 min luminal perfusion which commenced 5 min later.

VIP was infused i.a. to induce intestinal fluid secretion in the studies investigating the antisecretory activity of UK 14,304. The administration sequence and experimental protocol in these experiments followed the steps described above.

Pretreatment An initial dose of 6-hydroxydopamine (6-OHDA; 50 mg kg^{-1}) was administered i.p. (day one), followed by 100 mg kg^{-1} in two doses separated by 9 h on the third day. Rats were used 4 days after the initial injection. This method of chemical sympathectomy was similar to that described by Coupar and Taylor (1987), who showed that the treatment reduced the amount of noradrenaline in the rat small intestine by 93%.

Intracerebroventricular injection Immediately after isolation of intestinal loops, rats were positioned stereotaxically and a small hole was drilled through the skull. A 30-G needle attached to PE-20 polyethylene tubing was positioned such that the needle tip was placed in the lateral ventricle (coordinates relative to bregma: AP = 0.8 mm, L = 1.4 mm and V = 3.5 mm). Yohimbine was administered to give a dose of 3 μmol kg^{-1} in a total volume 0.01 ml 100 g^{-1}. The vehicle, which consisted of 5% glucose, was given in control experiments. The volumes of yohimbine and vehicle were injected over a 3 min period by a microinjection pump (CMA/microdialysis, Stockholm) and the needle was left in place for a further 1 min to minimize leakage of the solution. The needle was then removed, the hole covered with self-cure acrylic repair material (AD International, London), and the intestinal perfusion was started 15 min after completion of the i.c.v. injection. The i.c.v. site of injection was verified in a separate group of animals which were injected with a dye.

Drugs

Atropine sulphate, 6-hydroxydopamine hydrobromide, noradrenaline bitartrate (Sigma, St. Louis, U.S.A.) ARC-239 hydrochloride (2-(2,4-(O-methoxyphenyl)-piperazine-1-yl)-ethyl-4,4-dimethyl-1,3-(2H,4H)-isoquinolinedine; Boehringer Ingelheim, Artarmon, Australia), BRL 44408 (2-(2H-(1-methyl-1,3-dihydroisoindole) methyl)-4,5-dihydro-imidazoline; SmithKline Beecham, Welwyn, U.K.), rauwolscine hydrochloride, U.K. 14,304 (5-bromo-6-(2-imidazolin-2-yl-amino)-quinoxaline; Research Biochemicals International, Natick, U.S.A.), prazosin hydrochloride (Pfizer, Sydney, Australia), yohimbine hydrochloride (ICN Biochemicals, Aurora, U.S.A.).

BRL 44408 was dissolved in 0.01 M HCl solution and adjusted close to pH 6 with 0.1 M NaOH and then diluted with saline to give the required dose; 6-OHDA and noradrenaline were dissolved in deoxygenated saline containing 1 mg ml^{-1} of ascorbic acid; UK 14,304 was dissolved in dimethyl sulphoxide (DMSO) and further diluted with saline. The dilution of DMSO (8%) did not influence basal values of absorption in this study or VIP-induced secretion in the study of Hancock and Coupar (1997). Yohimbine was dissolved in 5% isotonic glucose solution for the i.c.v. experiments. All other drugs, including yohimbine for i.v. administration, were dissolved in 0.9% saline and injected in volumes of 0.1 ml 100 g^{-1}.

Statistical analysis

Results are presented as arithmetic means ± s.e.mean. The potency of UK 14,304 for inhibiting secretion induced by VIP was expressed as an EC_{50} value with a 95% confidence limit obtained by a semilogarithmic nonlinear regression analysis programme (Graph Pad Prism, Graph Pad Software Inc, San Diego, U.S.A.). This value was estimated with the response to 1 μmol kg^{-1} as the upper range of the dose-response curve. Student's unpaired t test was used to compare individual means for differences, one-way ANOVA followed by Dunnett's t test to compare treatment means with a common control and Bonferroni's test for multiple comparisons. (Graph Pad Prism, Graph Pad Software Inc, San Diego, U.S.A.). The criterion for statistical significance was set at $P < 0.05$.

Results

Absorption

Control values Basal net fluid transport was an absorptive state. The values from the jejunum and ileum of rats infused i.a. and injected i.v. with saline as a control were 161 ± 20 ($n = 12$) and 328 ± 25 ($n = 12$) μl g^{-1} wet weight in 20 min respectively. Mean arterial blood pressure (MAP) was 125 ± 3 mmHg ($n = 12$) (Table 1).

Effects of adrenoceptor agonists Infusion of noradrenaline into the aortic arch via the left common carotid artery resulted in an infusion-dependent increase in the rate of net fluid absorption from both jejunum and ileum (Figure 1). Noradrenaline at 3, 10 and 30 nmol min^{-1} (1.1, 3.6 and 10.9 μg min^{-1}) infusions enhanced the fluid transport rate by $11\pm17\%$ ($n=6$), $126\pm27\%$ ($n=6$) and $170\pm26\%$ ($n=5$), respectively, in the jejunum, and $23\pm17\%$ ($n=6$), $53\pm4\%$ ($n=6$) and $97\pm6\%$ ($n=5$), respectively, in the ileum. Noradrenaline also increased MAP by $21\pm4\%$ ($n=6$), $32\pm5\%$ ($n=6$) and $57\pm3\%$ ($n=5$) at the infusions of 3, 10 and 30 nmol min^{-1}, respectively.

The selective α_2-adrenoceptor agonist UK 14,304 caused significant enhancements of fluid absorption from both jejunum and ileum at a dose of 1 μmol kg^{-1}, i.v. (0.3 mg kg^{-1}). UK 14,304 also produced a biphasic effect on MAP consisting of a transient hypertension followed by prolonged hypotension. The depressor effect was $31\pm1\%$ of the control MAP and remained stable during the 20 min of intestinal perfusion (Table 1).

The selective α_1-adrenoceptor agonist phenylephrine at 5 μmol kg^{-1}, i.v. (1 mg kg^{-1}) did not alter the basal rates of absorption from the jejunum or ileum, but induced a significant increase in MAP (Table 1).

Effects of adrenoceptor antagonists Intravenous injection of the α_2-adrenoceptor antagonist yohimbine caused a dose-dependent decrease in the rate of fluid absorbed from both the jejunum and ileum. Yohimbine at 0.3, 1.0, 3.0 and 10 μmol kg^{-1} (0.12, 0.39, 1.2 and 3.9 mg kg^{-1}) reduced absorption by $33\pm10\%$ ($n=6$), $67\pm17\%$ ($n=7$), $80\pm15\%$ ($n=6$) and $89\pm25\%$ ($n=6$), respectively, in the jejunum, and $-7\pm14\%$, $16\pm10\%$, $55\pm11\%$ and $47\pm10\%$, respectively, in the ileum. The two lower doses of yohimbine did not alter MAP. However, a significant decrease was induced at the two higher doses amounting to $29\pm5\%$ and $40\pm4\%$ reductions (Table 1, Figure 2).

Rauwolscine, at 10 μmol kg^{-1} (3.9 mg kg^{-1}), caused a greater effect on fluid transport than the same dose of yohimbine, whereby the absorption rate was reduced in the ileum and a net secretion occurred in the jejunum. Like yohimbine, rauwolscine also reduced MAP (Table 1). Prazosin at the dose of 3 μmol kg^{-1} (1.3 mg kg^{-1}), which has been shown to block α_1-adrenoceptors effectively and selectively (Drew & Whiting, 1979), was shown to decrease MAP to the same extent as yohimbine (10 μmol kg^{-1}), but it did not induce significant changes in fluid transport rates from either the jejunum or ileum (Table 1).

The effect of the α_2-adrenoceptor agonist UK 14,304 on the response to yohimbine The selective α_2-adrenoceptor agonist UK 14,304 was used to determine whether yohimbine decreased basal absorption by blocking α_2-adrenoceptors. Pretreatment with yohimbine at a dose of 10 μmol kg^{-1}, i.v., attenuated the proabsorbtive effect of UK 14,304 1 μmol kg^{-1}, i.v. (Table 1).

Table 1 Effects of drug treatments on mean arterial blood pressure (MAP) and the rate of fluid transport from the small intestine

Drug	*MAP* (mmHg)		*Fluid transport rate* (μl g^{-1} in 20 min) *Jejunum*		*Ileum*	
Control (i.a. and i.v.-saline)	125 ± 3	(12)	161 ± 20	(12)	328 ± 25	(12)
Yohimbine (10 μmol kg^{-1})	75 ± 6***	(10)	17 ± 41**	(8)	173 ± 31*	(8)
Rauwolscine (10 μmol kg^{-1})	86 ± 8***	(4)	-45 ± 34***	(5)	63 ± 45***	(5)
6-OHDA	107 ± 3***	(6)	56 ± 36	(6)	207 ± 50	(6)
Phenylephrine (5 μmol kg^{-1})	137 ± 6*	(5)	206 ± 66	(5)	297 ± 77	(5)
Prazosin (3 μmol kg^{-1})	77 ± 5***	(5)	113 ± 55	(5)	303 ± 54	(5)
Control (Vehicle)	123 ± 7	(5)	171 ± 22	(5)	274 ± 42	(5)
UK 14,304 (1 μmol kg^{-1})	89 ± 2***	(5)	389 ± 43**	(5)	456 ± 63*	(5)
Yohimbine (10 μmol kg^{-1}) + UK 14,304 (1 μmol kg^{-1})	75 ± 3***$^{++}$	(5)	$224\pm48^{+}$	(5)	$284\pm32^{+}$	(5)

Values are expressed as means $\pm$ s.e.mean. The number of experiments is shown in parentheses. *$P<0.05$, **$P<0.01$; ***$P<0.001$ vs the control (Dunnett's t test); $^{+}P<0.05$; $^{++}P<0.01$ vs the UK 14,304-treated group alone (Bonferroni's multiple comparison test). MAP are values of mean arterial blood pressure.

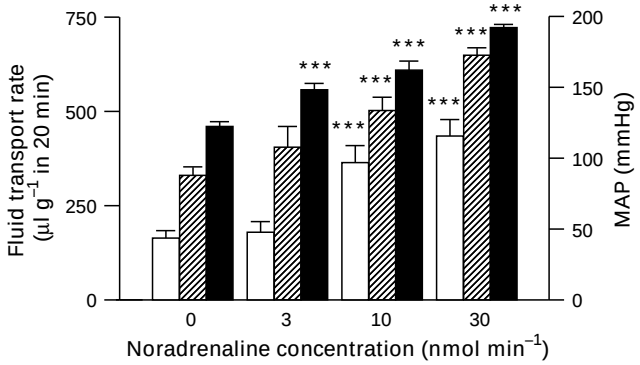

Figure 1 Effects of i.a. infusion of noradrenaline on the rate of net absorption from the small intestine and mean arterial blood pressure (MAP). The open columns indicate the rate of fluid absorption in the jejunum, the hatched columns the ileum (left ordinate scale), and the solid columns the MAP (right ordinate scale). Values are means $\pm$ s.e.mean of 5–12 rats. ***$P<0.001$ vs the individual control (Dunnett's t test).

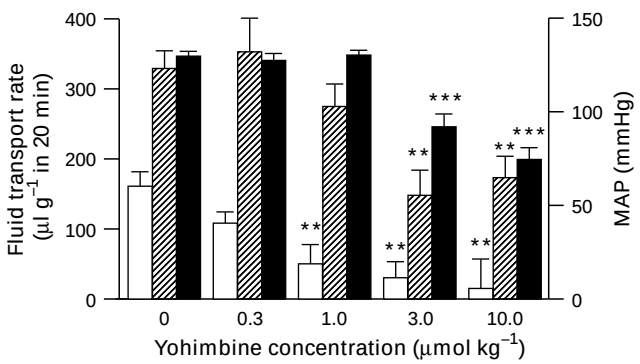

Figure 2 Effects of i.v. injection of yohimbine on net fluid transport from the small intestine and mean arterial blood pressure (MAP). The open columns indicate the rate of fluid absorption in the jejunum, the hatched columns the ileum (left ordinate scale), and the solid columns the MAP (right ordinate scale). Values are means $\pm$ s.e.mean of 5–12 rats. **$P<0.01$; ***$P<0.001$ vs the individual control (Dunnett's t test).

Chemical sympathectomy Pretreatment of the animals with 6-OHDA resulted in a $65 \pm 22\%$ reduction in the rate of fluid absorption from the jejunum and a $37 \pm 15\%$ reduction from the ileum. However, the actual rates of fluid transport were not significantly reduced by 6-OHDA compared to control by use of Dunnett's t test ($P > 0.05$). The MAP was significantly decreased in 6-OHDA-treated rats compared to untreated controls (Table 1).

Central administration of yohimbine The basal net fluid absorption rates from the jejunum and ileum of animals injected i.c.v. with yohimbine vehicle were 194 ± 35 and 291 ± 20 μl g^{-1} in 20 min ($n = 5$), respectively. Yohimbine (3 μmol kg^{-1}) significantly decreased the rate of absorption from the jejunum to 97 ± 25 μl g^{-1} in 20 min ($n = 8$, $P = 0.04$). There was also a trend towards decreased absorption in the ileum where the absorption value was 187 ± 42 μl g^{-1} in 20 min ($n = 8$, $P = 0.09$).

Secretion induced by VIP

Control value 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 from net absorption to secretion. The values were 267 ± 20 μl g^{-1} in 20 min secreted into the lumen of the jejunum and 355 ± 41 μl g^{-1} in 20 min secreted by the ileum ($n = 8$ for both regions).

Antisecretory effect of UK 14,304 UK 14,304 caused a dose-related inhibition of the VIP-induced fluid secretion. The maximal effect of UK 14,304 extended to complete block of secretion, but it failed to restore net absorption to the control value in either jejunum or ileum (Figure 3). The EC_{50} values for UK 14,304 at inhibiting VIP-induced secretion were 0.17 (95% CI, 0.11 – 0.24, $n = 5$) μmol kg^{-1} in the jejunum and 0.22 (95% CI, 0.11 – 0.43, $n = 5$) μmol kg^{-1} in the ileum. These values were estimated from the response at the maximally effective dose of UK 14,304 (1 μmol kg^{-1}).

Comparison of the antisecretory with the proabsorptive effect of UK 14,304 UK 14,304 (1 μmol kg^{-1}, i.v.) enhanced basal absorption by 218 ± 43 μl g^{-1} in 20 min in the jejunum and by 182 ± 63 μl g^{-1} in 20 min in the ileum ($n = 5$, see Table 1 for actual values). In animals infused with VIP, the same dose of UK 14,304 reversed secretion by 312 ± 21 μl g^{-1} in 20 min in the jejunum and by 311 ± 24 ($n = 8$) in the ileum. The antisecretory effect of UK 14,304 was significantly larger than the proabsorptive effect in the jejunum and ileum ($P < 0.05$, Student's unpaired t test).

Characterization of α_2-adrenoceptors involved in the antisecretory effect Yohimbine (non selective), BRL 44408 (28 to 110 fold $\alpha_{2A/D}$ selective relative to $\alpha_{2B/C}$; Young *et al*., 1989; Smith & Docherty, 1992) and ARC 239 (68 to 100 fold $\alpha_{2B/C}$ selective relative to $\alpha_{2A/D}$; Bylund *et al*., 1988) were chosen to differentiate the subtype of α_2-adrenoceptor involved in attenuating the antisecretory activity of UK 14,304 in the rat small intestine. The antagonists did not affect the values of VIP-induced secretion in either jejunum or ileum, when tested separately at doses of 10 μmol kg^{-1} ($P > 0.05$, $n = 3$). The results of agonist-antagonist interaction experiments are shown in Figure 4, where, yohimbine and BRL 44408 significantly antagonized the inhibitory effect exerted by UK 14,304 (1 μmol kg^{-1}) on VIP-induced secretion in the jejunum ($P < 0.001$, $n = 5$) and ileum ($P < 0.001$, $n = 5$). However, ARC 239 did not alter the antisecretory response to UK 14,304 (1 μmol kg^{-1}) in either region ($P > 0.05$, $n = 5$).

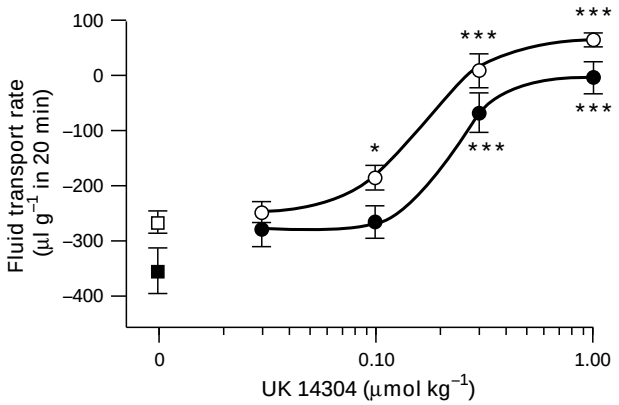

Figure 3 Inhibition by UK 14,304 of vasoactive intestinal peptide (VIP)-induced secretion in the rat jejunum (○) and ileum (●). The points on the left are the control values of fluid secretion in response to intra-arterial infusion of VIP 0.8 μg min^{-1} in the jejunum (□) and ileum (■). Values are means of 5–8 rats; vertical lines show s.e.mean. *$P < 0.05$; ***$P < 0.001$ vs the individual control (Dunnett's t test).

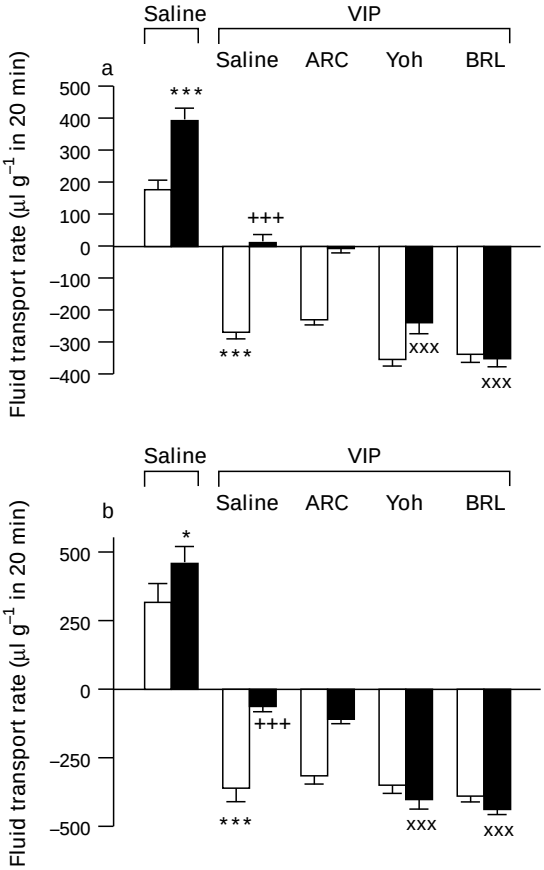

Figure 4 Effects of α_2-adrenoceptor antagonists ARC 239, yohimbine and BRL 44408 (10 μmol kg^{-1}, i.v.) on the antisecretory action of UK 14,304 (1 μmol kg^{-1}, i.v.) in (a) jejunum and (b) ileum. Solid columns indicate groups of animals treated with UK 14,304 and open columns without UK 14,304. Secretion was induced by i.a. infusion of VIP (0.8 μg min^{-1}). Values are mean ± s.e.mean of 3 – 11 rats. UK 14,304 caused a significant enhancement of absorption in the jejunum (***$P < 0.001$) and ileum (*$P < 0.05$) of rats infused i.a. with saline. VIP reversed fluid transport from net absorption to secretion in both jejunum and ileum (***$P < 0.001$). UK 14,304 reversed the fluid secretion induced by VIP in the jejunum ($^{+++}P < 0.001$) and inhibited VIP-induced secretion in the ileum ($^{+++}P < 0.001$). Yohimbine and BRL 44408 both blocked ($^{xxx}P < 0.001$) the antisecretory effect of UK 14,304 in the jejunum and ileum. However, ARC 239 did not affect the antisecretory effect of UK 14,304 ($P > 0.05$, Bonferroni's test for all comparisons).

Discussion

Effects of sympathomimetic drugs on basal absorption

The results obtained by use of drugs with well defined actions on sympathetic nerves and associated adrenoceptors confirm and extend those of previous studies which have established that sympathetic nerve activation enhances fluid absorption from the small intestine (Brunsson *et al*., 1979; Hemlin *et al*., 1989).

In the present series of experiments noradrenaline was shown to cause a large proabsorptive effect in the small intestine of the rat. The effect was more pronounced in the jejunum than ileum, with absorption rates increased by 170% and 97%, respectively, in response to noradrenaline infused at 30 nmol min^{-1} (10.9 μg min^{-1}). This result is consistent with that obtained by Levens *et al*. (1979), who showed that infusion of noradrenaline into the femoral artery of anaesthetized rats increased the fluid absorption rate from the jejunum.

Noradrenaline has been shown to stimulate fluid absorption by activating α_1-adrenoceptors in the rat jejunum and both α_1- and α_2-adrenoceptors in the ileum (Parsons *et al*., 1983). The receptors are located in the intestine, since the everted sac method was used. The presently described study could find no evidence to support the existence of functional α_1-adrenoceptors. However, this conclusion was based solely on the negative results that the selective α_1-adrenoceptor agonist phenylephrine (5 μmol kg^{-1}) and the α_1-antagonist prazosin (3 μmol kg^{-1}, i.v.) did not influence jejunal and ileal transport rates. The differences in results between the two studies probably lies in the different experimental approaches used. For instance, it is possible that the *in vitro* conditions used by Parsons *et al*. (1983) allow the expression of α_1-adrenoceptor activity which is normally suppressed by reflexes *in vivo*. The presently described *in vivo* study does reveal the existence of functional α_2-adrenoceptor populations in both jejunum and ileum. This is based partly on the finding that the selective α_2-adrenoceptor agonist, UK 14,304 (1 μmol kg^{-1}), caused a significant increase in the basal rate of fluid absorption, the effect like that of noradrenaline, being more extensive in the jejunum than ileum. The results are an indication that the rate of fluid transport is under the influence of the adrenergic system via α_2-adrenoceptors.

Thus the present results with the α-adrenoceptor agonists supplement those of previous studies which have identified functional α_2-adrenoceptors in the rat small intestine (Chang *et al*., 1983; Nakaki *et al*., 1983) and the presence of α_2-adrenoceptor binding sites on the basolateral membranes of enterocytes (Cotterell *et al*., 1982). Together the results are consistent with the view that the adrenergic system alters water and electrolyte transport through a direct action on enterocytes. However, an action on α_2-adrenoceptors on neurones within the submucosal plexus is also a possibility. In support of this, it has been shown that spontaneous inhibitory postsynaptic potentials (i.p.s.ps) occur in the submucosal plexus which can be inhibited by α_2-adrenoceptor antagonists such as yohimbine, or after neuronal release of noradrenaline is blocked by guanethidine (Brown & Miller, 1995). Thus the sympathetic nerves may alter ion and water transport by inhibiting tonic activity in submucosal neurones. An equivalent control operates to inhibit peristalsis in the rat ileum where UK 14,304 and other α_2-adrenoceptor agonists act by suppressing the activity of cholinergic neurones (Liu & Coupar, 1996).

The responses of intestinal mucosa to the α_2-adrenoceptor antagonists yohimbine and rauwolscine were unexpectedly large. The effect of yohimbine was dose-related; it caused 89% reduction in fluid absorption from the jejunum at 10 μmol kg^{-1}, i.v. An equivalent dose of rauwolscine produced an even greater effect on the net secretion. Both antagonists affected the ileum, although not to the same extent as the jejunum. These results provide the first evidence that the small intestinal mucosa is under tonic proabsorptive influence from sympathetic nerves. It is possible that these nerves tonically oppose a secretomotor pathway (cholinergic and/or noncholinergic), at least under the conditions of the presently described *in vivo* experiments. However, adrenoceptor antagonists alone generally have no effect on basal or stimulated ion movement *in vitro* (Chang *et al*., 1982), which implies that noradrenaline is not released continuously to alter mucosal transport when noradrenergic terminals are separated from their cell bodies.

The lack of effect of prazosin on basal absorption precludes the involvement of α_1-adrenoceptors in the antiabsorptive effect of adrenoceptor antagonists. Prazosin was administered at a dose sufficiently high to block the pressor responses to selective α_1-adrenoceptor agonists (Drew & Whiting, 1979). This finding is in line with previous observations that α_1-adrenoceptor stimulation did not affect electrolyte transport in the small intestine of rabbits and rats (Durbin *et al*., 1982; Fondacaro *et al*., 1988). Yohimbine on the other hand, is a fairly selective α_2 antagonist (Goldberg & Robertsson, 1983) and at a dose of 10 μmol kg^{-1} it decreased the basal absorption and antagonized the enhanced absorption induced by the selective α_2-adrenoceptor agonist UK 14,304 in both jejunum and ileum. Again these results are consistent with the view that the control of fluid absorption in the intestinal tract is influenced by the adrenergic system mainly through α_2-adrenoceptors.

The above conclusion is supported by the results derived from animals that were chemically sympathectomised with 6-OHDA, although with reservation. This is because there was only a trend towards a decrease in jejunal and ileal fluid absorption following sympathectomy. The method of chemical sympathectomy used in this study has been shown previously to deplete 93% of noradrenaline stores from the rat intestine (Coupar & Taylor, 1987). This extent of sympathetic impairment, therefore would be expected to produce a greater effect than was observed. It is possible that increased synthesis and turnover of adrenal catecholamines might compensate for the destroyed sympathetic nerve terminals, since it is known that treatment with 6-OHDA leaves the adrenal medulla unaffected (Thoenen & Tranzer, 1973). Alternatively, the central nervous system might compensate for dysfunctional sympathetic nerves, since 6-OHDA does not cross the blood brain barrier (Porter *et al*., 1963; Laverty *et al*., 1965). The unexpectedly large antiabsorptive effects of the α_2-adrenoceptor antagonists, but relatively small effect produced by peripheral chemical sympathectomy implied that centrally located α_2-adrenoceptors might be partially involved in the response to the α_2-adrenoceptor antagonists. Therefore, yohimbine was selected for further study where it was injected into the lateral ventricle at a dose (3 μmol kg^{-1}) equivalent to that shown to have an antiabsorptive effect when administered intravenously. The fact that the reduction of basal fluid absorption produced by i.c.v. yohimbine was small, and statistically significant in the jejunum only, trends to indicate that it acts on tonically activated α_2-adrenoceptors, which are located predominantly in the intestine itself.

The general finding that the responses of the jejunum to α_2-adrenoceptor agonists and antagonists were larger than the corresponding responses in the ileum indicates that the sympathetic nervous system plays a dominant role in regulating fluid transport in the upper small intestine. This observation is different from that of Chang *et al*. (1985); they showed that the sympathetic nervous system plays a more important role in regulating fluid and electrolyte transport in the ileum and colon than the jejunum. This conclusion was based on results with 6-OHDA, while the conclusions of the present study draw on results obtained with adrenoceptor agonists and antagonists in addition to 6-OHDA.

It might be inferred from the present results that the effects of noradrenaline, and α_2-adrenoceptor antagonists on fluid absorption might be directly linked to the changes in arterial blood pressure produced by them. The current evidence did not support this conclusion. First, UK 14,304 was as effective

as noradrenaline in increasing intestinal absorption. Although UK 14,304 caused a transient pressure response, the depressor phase of the response was already well established and maintained by the time intestinal perfusion was started. Also, prazosin caused vasodilatation to the same extent as yohimbine, yet was ineffective in altering intestinal transport. Finally, Brunsson *et al.* (1979) have demonstrated that the net water absorption rate is largely unaffected by vasodilatation induced by close arterial infusion of isoprenaline. These findings suggest that alterations in blood pressure alone cannot account for changes in intestinal fluid absorption.

It is of interest to note that i.v. administration of α_2-adrenoceptor antagonists, such as yohimbine, have also been found to lower mean arterial pressure in the rat (Jonson & Fandriks, 1988). It is now generally accepted that certain vascular beds in both experimental animals and man contain postjunctional α_2-adrenoceptors which, when activated, induce vasoconstriction (Timmermans & van Zwieten, 1981). It has been suggested that the postjunctional vascular α_2-adrenoceptors may play an even more important role in the hypertensive state, especially under conditions where circulating catecholamines are elevated (Medgett *et al.*, 1984). Thus, it could be presumed that yohimbine and rauwolscine decrease blood pressure under the presently described experimental situation by acting on postjunctional α_2-adrenoceptors. However, we cannot exclude the possibility that the depressor response of yohimbine and rauwolscine may also consist of α_1-adrenoceptor blockade.

Subtype of α_2-adrenoceptors mediating antisecretory activity

The present study has shown that the antisecretory effect of UK 14,304 was significantly larger than the proabsorptive effect in the jejunum and ileum. This might imply that stimulation of absorption and inhibition of secretion are mediated at different levels along the crypt-villus axis. Absorption and secretion are thought to be independent processes and it has been suggested that the crypt cells regulate secretion while the villus cells are responsible for absorption (Welsh *et al.*, 1982). It is possible that there is a greater density of α_2-adrenoceptors in the crypt than villus enterocytes, as has been established in the rabbit colon (Senard *et al.*, 1990). However, the method used in the present study does not allow detailed identification of the sites of drug action.

α_2-Adrenoceptors have been subdivided into different subtypes on the basis of their differential affinity for several ligands with varying selectivities (Ruffolo *et al.*, 1993; Bylund *et al.*, 1995). However, very few studies have been undertaken to characterize α_2-adrenoceptor subtypes with *in vivo* models. In the present study, we attempted to characterize the α_2-adrenoceptor subtype involved in the modulation of intestinal secretion at the peripheral level. The antisecretory rather than the proabsorptive response to UK 14,304 was chosen because it was larger and hence gave better discrimination in pharmacological analysis. For this purpose, intestinal fluid secretion was elicited by intra-arterial infusion of the directly acting secretagogue VIP. In this respect, our data, showing that the VIP-induced secretion was markedly reduced by UK 14,304, confirm the results of a previous study, which showed that clonidine inhibited prostaglandin and VIP-induced fluid secretion in the rat jejunum (Nakaki *et al.*, 1982). However, since it was shown that UK 14,304 pretreatment increased the control level of fluid absorption in both jejunum and ileum, our results indicate that UK 14,304 has a proabsorptive component to its antisecretory effect.

This study provides functional evidence that the adrenoceptors in the rat intestine responsible for attenuating intestinal fluid secretion resemble α_{2A} or α_{2D} more than α_{2B} or α_{2C}-adrenoceptors. This conclusion was reached on the basis that the preferential $\alpha_{2A/D}$ adrenoceptor antagonist, BRL 44408 (Uhlen *et al.*, 1994), blocked the antisecretory activity of UK 14,304, whereas, the preferential $\alpha_{2B/C}$ adrenoceptor antagonist, ARC 239 (Uhlen *et al*, 1994), was ineffective. In addition a previous study established that the selective $\alpha_{2A/D}$ adrenoceptor agonist oxymetazoline potently inhibited prostaglandin E_1-induced secretion (Nakaki *et al.*, 1982). Moreover, radioligand binding studies have shown that α_{2D}- binding sites are located on enterocytes of the rat jejunum (Paris *et al.*, 1990). It is interesting to note that the α_2-adrenoceptor subclass involved in modulating intestinal fluid transport is the same (or similar to) the subtype we have previously characterized on enteric neurones involved in the control of peristalsis in rat ileum (Liu & Coupar, 1996).

Only three subtypes of α_2-adrenoceptor have been cloned from human and rat tissues and across the species, the three genes form three groups of homologue (Bylund, 1995). It is now accepted that the rat α_{2D} subtype represents a species homology of the human α_{2A} receptor subtype, and that the rat α_{2D}-adrenoceptor should be better classified as α_{2A}-like adrenoceptor (MacKinnon *et al.*, 1994). However, there is sufficient pharmacological difference between α_{2A}- and α_{2D}-adrenoceptors (Starke *et al.*, 1995), so α_{2D} is used in this paper following the original nomenclature (Simonneaux *et al.*, 1991). The α_{2D} subtype expresses a peculiar pharmacological property, which exhibits a relative low affinity for classic α_2-adrenoceptor antagonists, yohimbine and rauwolscine (MacKinnon *et al.*, 1994).

In conclusion, the present study suggests that the adrenergic system has an important role in regulating intestinal transport. Also, in this study functional evidence was obtained that an α_{2D} or α_{2A}-like adrenoceptor subtype is responsible for mediating intestinal antisecretory action. Although α_2-adrenoceptor agonists are clearly therapeutically useful antisecretory agents, their clinical usefulness is limited by side effects, such as sedation and hypotension. The recognition that the α_{2D}-adrenoceptor subtype is involved in the control of intestinal transport and that its location is mainly peripheral would help in the development of new antidiarrheal drugs.

We wish to thank the Pharmaceutical Companies listed for their generous gifts of research compounds.

References

BROWN, D.R. & MILLER, R.J. (1995). Neurohormonal control of fluid and electrolyte transport in intestinal mucosa. In *Handbook of Physiology – The Gastrointestinal System*. ed. Schultz, S.G., Field, M. & Frizzel, R.A. Vol IV. pp. 527 – 589, Bethesda, New York: Md publishers.

BRUNSSON, I., EKLUND, S., JODAL, M., LUNDGREN, O. & SJOVALL, H. (1979). The effect of vasodilatation and sympathetic nerve activation on net water absorption in the cat's small intestine. *Acta Physiol. Scand.*, **106,** 61 – 68.

BYLUND, D.B. (1988). Subtypes of α_2-adrenoceptor: Pharmacological and molecular biological evidence converge. *Trends Pharmacol. Sci.*, **9,** 351 – 356.

BYLUND, D.B., HASS, N.A., CERUTIS, D.R. & BLAXALL, H.S. (1995). Characterization of α_2-adrenergic receptor subtypes. In *α_2-Adrenoceptors: Structure, Function and Pharmacology*. ed. Ruffolo, R.R. Jr. pp. 87 – 90. Harwood Academic Publishers.

CHANG, E.B., BERGENSTAL, R.M. & FIELD, M. (1985). Diarrhea in streptozocin-treated rats: loss of adrenergic regulation of intestinal fluid and electrolyte transport. *J. Clin. Invest.*, **75,** 1666 – 1670.

CHANG, E.B., BROWN, D.R., FIELD, M. & MILLER, R.J. (1983). Enterocyte α_2-adrenergic receptors: yohimbine and p-aminoclonidine binding relative to ion transport. *Am. J. Physiol.*, **244,** G76 – 82.

CHANG, E.B., FIELD, M. & MILLER, R.J. (1982). α-Adrenergic receptor regulation of ion transport in rabbit ileum. *Am. J. Physiol.*, **242,** G237–242.

COTTERELL, D.J., MUNDAY, K.A. & POAT, J.A. (1982). The binding of [^{3}H]-prazosin and [^{3}H]-clonidine to crude basolateral membranes from rat jejunum. *Br. J. Pharmacol.*, **76,** 277P.

COUPAR, I.M. (1985). Choice of anaesthetic for intestinal absorption and secretion experiments using rats. *J. Pharmacol. Methods*, **13,** 331–338.

COUPAR, I.M & TAYLOR, D.A. (1987). Evidence for tryptaminergic and noradrenergic involvement in the antisecretory action of morphine in the rat jejunum. *J. Pharm. Pharmacol.*, **39,** 363–369.

DREW, G.M. & WHITING, S.B. (1979). Evidence for two distinct types of postsynaptic α-adrenoceptor in vascular smooth muscle *in vivo*. *Br. J. Pharmacol.*, **67,** 207–215.

DURBIN, T., ROSENTHAL, L., MCARTHUR, K., ANDERSON, D. & DHARMSATHAPHORN, K. (1982). Clonidine and lidamidine (WHR-1142) stimulate sodium and chloride absorption in the rabbit intestine. *Gastroenterology*, **82,** 1352–1358.

FONDACARO, J.D., KOLPAK, D. & MCCAFFERTY, G.P. (1988). Selective alpha-2 adrenoceptor agonists alter fluid and electrolyte transport in mammalian small intestine. *J. Pharmacol. Exp. Ther.*, **247,** 481–486.

FURNESS, J.B. & COSTA, M. (1980). Types of nerves in the enteric nervous system. *Neuroscience*, **5,** 1–20.

GOLDBERG, M.R. & ROBERTSSON, D. (1983). Yohimbine: a pharmacological probe for study of the alpha2-adrenoceptor. *Pharmacol. Rev.*, **35,** 143–180.

HANCOCK, D.L. & COUPAR, I.M. (1997). Involvement of adenosine in the antisecretory action of morphine. *Gen. Pharmacol.*, (in press).

HEMLIN, M. (1989). Interaction between sympathetic nerve fibres and epithelial transport in the rat jejunal mucosa in vivo. *Acta Physiol. Scand.*, **137,** 365–374.

HILDEBRAND, K.R. & BROWN, D.R. (1992). Norepinephrine and alpha-2 adrenoceptors modulate active ion transport in porcine small intestine. *J. Pharmacol. Exp. Ther.*, **263,** 510–519.

JONSON, C. & FANDRIKS, L. (1988). Afferent electrical stimulation of mesenteric nerves inhibits duodenal HCO_3^- secretion via a spinal reflex activation of the splanchnic nerves in the rat. *Acta Physiol. Scand.*, **133,** 545–550.

LAVERTY, R., SHARMAN, D.F. & VOLT, M. (1965). Action of 2, 4, 5-trihydroxyphenylethylamine on the storage and release of noradrenaline. *Br. J. Pharmacol.*, **24,** 549–560.

LEVENS, N.S., MUNDAY, K.A., PARSONS, B.J., POAT, J.A. & STEWART, C.P. (1979). Noradrenaline as a possible mediator of the actions of angiotensin on fluid transport by rat jejunum in vivo. *J. Physiol.*, **286,** 351–360.

LIU, L. & COUPAR, I.M. (1996). Evidence for functional α_{2D}-adrenoceptors in the rat intestine. *Br. J. Pharmacol.*, **177,** 787–792.

MACKINNON, A.C., SPEDDING, M. & BROWN, C.M. (1994). α_2-Adrenoceptors: more subtypes but fewer functional differences. *Trends Pharmacol. Sci.*, **15,** 119–123.

MEDGETT, I.C., HICKS, I.E. & LANGER, S.Z. (1984). Smooth muscle alpha-2 adrenoceptors mediate vasoconstrictor response to exogenous norepinephrine and to sympathetic stimulation to a greater extent in spontaneously hypertensive than in Wistar Kyoto rat tail arteries. *J. Pharmacol. Exp. Ther.*, **231,** 159–165.

MILLER, D.L. & SCHEDL, H.P. (1972). Non-absorbable indicators: a comparison of phenol red and inulin ^{14}C and effects of perfusion techniques. *Gastroenterology*, **62,** 45–48.

NAKAKI, T., NAKADATE, T., YAMAMOTO, S. & KATO, R. (1982). Alpha-2 adrenergic inhibition of intestinal secretion induced by prostaglandin E_1, vasoactive intestinal peptide and dibutyryl cyclic AMP in rat jejunum. *J. Pharmacol. Exp. Ther.*, **220,** 637–641.

NAKAKI, T., NAKADATE, T., YAMAMOTO, S. & KATO, R. (1983). Alpha$_2$-adrenergic receptor in intestinal epithelial cells: identification by [^{3}H]yohimbine and failure to inhibit cyclic AMP accumulation. *Mol. Pharmacol.*, **23,** 228–234.

PARIS, H., VOISIN, T., REMAURY, A., ROUYER-FESSARD, C., DAVIAUD, D., LANGIN, D. & LABURTHE, M. (1990). Alpha-2 adrenoceptor in rat jejunum epithelial cells: characterization with [^{3}H]RX 821002 and distribution along the villus-crypt axis. *J. Pharmacol. Exp. Ther.*, **254,** 888–893.

PARSONS, B.J., POAT, J.A. & ROBERTS, P.A. (1983). α-Receptors associated with fluid absorption in rat jejunum and ileum. *Br. J. Pharmacol.*, **80,** 307P.

PORTER, C.C., TOTARO, J.A. & STONE, C.A. (1963). Effect of 6-hydroxydopamine and some other compounds on the concentration of norepinephrine in the hearts of mice. *J. Pharmacol. Exp. Ther.*, **140,** 308–316.

RUFFOLO, Jr, R.R., NICHOLS, A.J., STADEL, J.M. & HIEBLE, J.P. (1993). Pharmacologic and therapeutic applications of α_2-adrenoceptor subtypes. *Ann. Rev. Pharmacol. Toxicol.*, **32,** 243–279.

SCHILLER, L.R., SANTA ANA, C.A., MORAWSKI, S.G. & FORDTRAN, J.S. (1985). Studies of the antidiarrheal action of clonidine: effects on motility and intestinal absorption. *Gastroenterology*, **89,** 982–988.

SENARD, J.M., LANGIN, D., ESTAN, L. & PARIS, H. (1990). Identification of α_2-adrenoceptors and non-adrenergic idazoxan binding sites in rabbit colon epithelial cells. *Eur. J. Pharmacol.*, **191,** 59–68.

SIMMONNEAUX, V., EBADI, M. & BYLUND, D.B. (1991). Identification and characterization of α_{2D}-adrenergic receptors in bovine pineal gland. *Mol. Pharmacol.*, **40,** 235–241.

SMITH, K. & DOCHERTY, J.R. (1992). Are the prejunctional α_2-adrenoceptors of the rat vas deferens and submandibular gland of the α_{2A}- or α_{2D}-subtype? *Eur. J. Pharmacol.*, **219,** 203–210.

STARKE, K., TRENDELENBURG, A.U. & LIMBERGER, N. (1995). Presynaptic α_2-adrenoceptors: subtype determination. In *α_2-Adrenoceptors: Structure, Function and Pharmacology*. ed. Ruffolo, Jr, R.R. pp. 99–108, Harwood Academic Publishers.

TIMMERMANS, P.B.M.W.M. & VAN ZWIETEN, P.A. (1981). Mini-review: The postsynaptic α_2-adrenoceptor. *J. Auton. Pharmacol.*, **1,** 171–183.

THOENEN, H. & TRANZER, J.P. (1973). The pharmacology of 6-hydroxydopamine. *Ann. Rev. Pharmacol.*, **13,** 169–180.

THOMAS, E.M. & TEMPLETON, D. (1982). Sites of noradrenergic innervation of the jejunal villus. *Gen. Pharmacol.*, **13,** 67–69.

UHLEN, S., PORTER, A.C. & NEUBIG, R.R. (1994). The novel alpha-adrenergic radioligand [3H]-MK912 is alpha-2C selective among human alpha-2A, alpha-2B and alpha-2C adrenoceptors. *J. Pharmacol. Exp. Ther.*, **271,** 1558–1565.

WELSH, M.J., SMITH, P.L., FROMM, M. & FRIZZELL, R.A. (1982). Crypts are the site of intestinal fluid and electrolyte secretion. *Science*, **218,** 1219–1221.

YOUNG, P., BERGE, J., CHAPMAN, H. & CAUTHORNE, M.A. (1989). Novel α_2-adrenoceptor antagonists show selectivity for α_{2A} and α_{2B}-adrenoceptor subtypes. *Eur. J. Pharmacol.*, **168,** 381–386.

(Received September 11, 1996
Revised October 31, 1996
Accepted November 7, 1996)