



An enhancing effect of 5-hydroxytryptamine on electrically evoked atropine-resistant contraction of guinea-pig proximal colon

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1 In the presence of atropine (0.2 μM) and indomethacin (2 μM), the effects of 5-hydroxytryptamine (5-HT) have been studied on electrically-evoked, neurogenic contractions of the guinea-pig proximal colon *in vitro*.

2 5-HT, at higher concentrations than 1 nM, caused an increase in electrically (1 Hz, 0.3 ms, 160 mA)-evoked, atropine-resistant contractions in a concentration-dependent manner and at 30 nM produced a maximal effect (pEC₅₀ value of 8.20 ± 0.11 , $n = 6$). The enhancing effects of 5-HT on the electrically evoked contractions were mimicked by α -methyl-5-HT (pEC₅₀ value of 6.59 ± 0.05 , $n = 6$).

3 Both hexamethonium (100 μM) and spantide (10 μM), selective antagonists for nicotinic and tachykinin receptors respectively, significantly reduced the enhancement of the electrically evoked contractions by 5-HT (30 nM).

4 DAU 6285 (3 μM), a 5-HT₄ receptor antagonist, abolished the enhancing action of 5-HT (30 nM), but metitepine (0.03 μM), a 5-HT₁/5-HT₂ receptor antagonist, ketanserin (0.01 μM), a 5-HT₂ receptor antagonist, and ondansetron (1 μM), a 5-HT₃ receptor antagonist, had no effect on the enhancement. The enhancing effects of α -methyl-5-HT (1 μM) were also abolished by DAU 6285 (3 μM).

5 Both 5-HT (30 nM) and α -methyl-5-HT (1 μM) had no effect on contractions to exogenous substance P (0.15–0.3 nM).

6 These results indicate that in the guinea-pig proximal colon, 5-HT produced an enhancement of atropine-resistant neurogenic contraction induced by electrical field stimulation through pre-junctional mechanisms and that the enhancement is mediated by the stimulation of 5-HT₄ receptors located on intramural preganglionic cholinergic neurones and tachykininergic neurones.

Keywords: 5-Hydroxytryptamine (5-HT); 5-HT₄ receptors; atropine-resistant contraction; proximal colon (guinea-pig)

Introduction

5-Hydroxytryptamine (5-HT) can exert multiple actions on the mammalian digestive tract, including contraction or relaxation of the smooth muscle and stimulation of the intramural nerve plexus (Furness & Costa, 1982). Nevertheless, the precise role of 5-HT in the control of gastrointestinal motility is not clear.

In isolated preparations of the guinea-pig proximal colon, 5-HT produces both relaxations and contractions (Costa & Furness, 1979). Our previous study demonstrated that the 5-HT-evoked relaxations are mediated by the stimulation of 5-HT₁-like receptors located on non-adrenergic, non-cholinergic inhibitory neurones (Kojima, 1991). The 5-HT-evoked contractions appear to be mediated by stimulation of cholinergic and non-cholinergic excitatory neurones (Costa & Furness, 1979). Atropine-sensitive contractions induced by 5-HT are mediated by the stimulation of 5-HT₄ receptors located on cholinergic neurones (Elswood *et al.*, 1991), while the receptor subtypes responsible for atropine-resistant neurogenic contractions remain to be determined. The aim of the present study was to provide evidence for understanding the mechanism of the excitatory action of 5-HT on non-cholinergic neurones by elucidating the effects of 5-HT on electrically-evoked, atropine-resistant contractions of guinea-pig proximal colon.

Methods

Experimental set-up

Male Dunkin Hartley guinea-pigs, weighing 250–650 g, were stunned and bled. A segment of the proximal colon, 2–9.5

cm away from the caecum was removed, and the luminal contents were washed out with a modified Tyrode solution (composition mM: NaCl 136.8, KCl 2.7, CaCl₂ 1.8, MgCl₂ 1.05, NaH₂PO₄ 0.42, NaHCO₃ 11.9, glucose 5.56, ascorbic acid 0.12). The colon was divided into three segments of circa 2.5 cm and opened longitudinally. After careful dissection of the underlying mucosa, muscle strips were suspended in longitudinal direction under a 0.5 g load in 15 ml tissue baths filled with modified Tyrode solution at 37°C and bubbled with 5% CO₂:95% O₂. The modified Tyrode solution always contained atropine (0.2 μM) and indomethacin (2 μM), to minimize cholinergic activity and endogenous prostanoid biosynthesis in response to electrical field stimulation (EFS). Tissues were allowed to equilibrate for at least 60 min before the start of experiments. During the equilibration period, the bathing medium was replaced every 15 min. Changes in mechanical activity of the tissue were recorded isotonicly (isotonic transducer, Nihon Kohden, TD-112S; Nihon Kohden recticoder, RJG-3006). To obtain nerve-mediated contractions of guinea-pig colon, the tissue was placed between bipolar platinum ring electrodes (5 mm internal diameter, 6 mm apart) and connected to a Nihon Kohden stimulator (SEN-3201). Optimal stimulus parameters (rectangular pulse with 1 Hz, 0.3 ms duration, 160 mA, 37 V, for 10 s every 10 min) to obtain tetrodotoxin-sensitive, submaximal contractions (approximately 30% of histamine (10 μM)-induced maximal contraction) with stable amplitude were determined in a preliminary study. When control responses to EFS were stable for at least 30 min, the colonic strips were incubated with a single concentration (1–3000 nM) of 5-HT or α -methyl-5-HT for 5 min and further 3–5 stimulations performed. In each experiment, histamine 10 μM was added at the first stage of the experimental protocol. When the effects of 5-HT receptor antagonists, hexamethonium or

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spantide were evaluated, the colonic strips were incubated for 30–60 min with the antagonists prior to addition of agonists. In most cases, one of three preparations served as control and the two others were used for the study of 5-HT in the presence of a set concentration of antagonist. The effects of 5-HT (30 nM) or α -methyl-5-HT (1 μ M) on exogenous substance P (0.15–0.3 nM)-induced contractions were evaluated by comparing the response before and after the addition of agonists in the same preparation at 40 min intervals.

Analysis of data

The effects of agonists and antagonists on the EFS-induced contractions were expressed as a percentage of the mean of the 3 consecutive control responses prior to drug addition. The percentage effects of the agonists were plotted as mean values to obtain log concentration-response curves. pEC_{50} ($-\log$ molar concentration of the agonist that enhances the response to EFS by 50% of the maximal effect that can be reached with that agonist) of 5-HT and α -methyl-5-HT was determined from each curve according to the method of Van Rossum (1963). Means \pm s.e. mean of n experiments are given throughout the paper. Antagonist effects were compared with control experiments and significance assessed by Student's t tests. In some cases Dunnett's test were used. Results were considered significant if $P < 0.05$.

Drugs

The following drugs were used: atropine sulphate, hexamethonium chloride dihydrate, histamine dihydrochloride (Wako, Osaka, Japan); tetrodotoxin (Sankyo, Tokyo, Japan); substance P, spantide (Peptide Institute, Osaka, Japan); 5-HT creatine sulphate (Merck, Darmstadt, Germany); indomethacin (Sigma, St. Louis, MO, U.S.A.); α -methyl-5-HT maleate, ketaserin tartrate, metitepine maleate (Research Biochemicals Inc, Natick, U.S.A.); ondansetron (gift from Glaxo Research Group, Ware, U.K.); DAU 6285 hydrochloride (endo-6-methoxy-8-methyl-8-azabicyclo [3.2.1] oct 3-yl-2, 3-dihydro-2-oxo-1H-benzimidazole-1 carboxylate hydrochloride, a gift from Dr C.A. Rizzi, Boehringer Ingelheim, Italy). All drugs were initially dissolved in saline with the following exceptions: indomethacin was dissolved in distilled water containing equimolar concentrations of Na_2CO_3 , and 5-HT or α -methyl-5-HT were dissolved in saline containing ascorbic acid (0.12 mM). All subsequent dilutions of the drugs were made with saline, except the 5-HT or α -methyl-5-HT solutions, which always contained ascorbic acid (0.12 mM). The reported concentrations are the final bath concentrations.

Results

Response to electrical field stimulation (EFS)

In the presence of atropine (0.2 μ M) and indomethacin (2 μ M), EFS (1 Hz, 0.3 ms, 160 mA, for 10 s) of longitudinal muscle of the guinea-pig proximal colon produced a phasic contraction (the amplitude of which was $29.6 \pm 0.6\%$ of the histamine (10 μ M, $n = 24$)-induced maximal contraction) within a few seconds after the 10 s stimulation period. The submaximal contractions evoked by EFS were completely abolished by tetrodotoxin (300 nM, $n = 4$, Figure 1a). Moreover, the contractions evoked by EFS were significantly reduced ($P < 0.05$) by both hexamethonium (100 μ M), a nicotinic receptor antagonist, and spantide (10 μ M), a tachykinin receptor antagonist ($76.2 \pm 3.1\%$, $n = 6$ and $72.8 \pm 5.7\%$, $n = 6$ respectively, compared to pre-drug control).

Effects of 5-HT

The administration of 5-HT (30 nM) caused a sustained increase in electrically evoked contractions ($P < 0.001$, Figure

1b). The enhancing action of 5-HT was rapid in onset and lasted for as long as the amine remained in the bath, but on washing for 40 min the effects were reversed (Figure 1b). 5-HT (1–100 nM) caused an increase in electrically evoked contractions in a concentration-dependent manner to a maximal effect of $219.9 \pm 11.7\%$ at 30 nM, resulting in a pEC_{50} value of 8.20 ± 0.11 ($n = 6$, Figure 2). 5-HT (30 nM) also evoked a transient contraction in some tissues (4 out of 6 strips), amounting to $12.7 \pm 3.6\%$ of the effect of histamine 10 μ M ($n = 6$). The enhancing effects of 5-HT were mimicked by α -methyl-5-HT (0.1–3 μ M, $n = 6$, Figure 2). In all strips the maximal effects of agonists at each concentration were reached within 5 min after drug administration. The maximal enhancement by α -methyl-5-HT was close to that produced by 5-HT ($198.0 \pm 9.9\%$ at 1 μ M), resulting in a pEC_{50} value of 6.59 ± 0.05 ($n = 6$). α -methyl-5-HT (1 μ M) also evoked a transient contraction in some tissues (3 out of 6 strips), amounting to $15.4 \pm 1.6\%$ of the effect of histamine 10 μ M ($n = 6$).

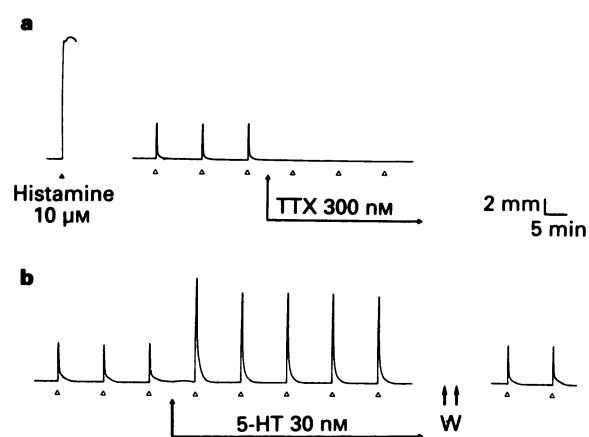


Figure 1 Typical tracing showing the effects of (a) tetrodotoxin (TTX; 300 nM) and (b) 5-hydroxytryptamine (5-HT; 30 nM) on submaximal electrically evoked (Δ ; 1 Hz, 0.3 ms, 160 mA, for 10 s) contractions of the longitudinal muscle of guinea-pig isolated proximal colon. The effects of 5-HT were removed by wash out (W = wash out for 40 min). Vertical calibration shows 2 mm shortening of the tissue; horizontal calibration shows 5 min. Atropine (0.2 μ M) and indomethacin (2 μ M) were present throughout experiments.

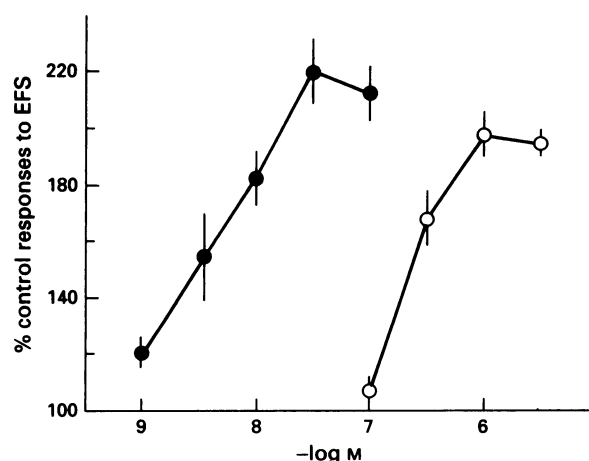


Figure 2 Concentration-response curves for the enhancing effect of 5-hydroxytryptamine (5-HT, ●) and α -methyl-5-HT (○) on electrically evoked contractions of the guinea-pig isolated proximal colon. The enhancement of neurogenic contractions are expressed as a percentage of the control response to electrical stimulation prior to the drug addition. Each point represents the mean \pm s.e. mean of 6 preparations. Atropine (0.2 μ M) and indomethacin (2 μ M) were present throughout experiments.

Effects of antagonists

To evaluate the participation of ganglionic transmission or of endogenous tachykinins in the enhancing action of 5-HT, hexamethonium and spantide were studied. When the tissue was preincubated with hexamethonium (100 μ M) for 30–40 min, the enhancement of the electrically evoked contractions induced by 5-HT (30 nM) was reduced (Figure 3). Similarly, 30–40 min preincubation of the tissue with spantide (10 μ M) reduced the 5-HT-induced enhancement of the electrically evoked contractions (Figure 3).

None of the 5-HT receptor antagonists investigated had any significant influence on the amplitude of the submaximal contractions evoked by EFS (Table 1). Moreover, as shown in Table 1, metitepine (0.03 μ M), a 5-HT₁/5-HT₂ receptor antagonist, ketanserin (0.01 μ M), a 5-HT₂ receptor antagonist and ondansetron (1 μ M), a 5-HT₃ receptor antagonist, did not affect the enhancement of electrically evoked contractions induced by 5-HT. However, DAU 6285 (0.3–3 μ M), a 5-HT₄ receptor antagonist, significantly inhibited the enhancement of EFS-induced responses by 5-HT in a concentration-dependent manner. At the higher concentration used, DAU 6285 (3 μ M) abolished the enhancing action of 5-HT. DAU 6285 (3 μ M) also abolished the enhancement of EFS-induced responses by α -methyl-5-HT (1 μ M, $n = 6$, $94.2 \pm 6.0\%$ compared to pre-drug control).

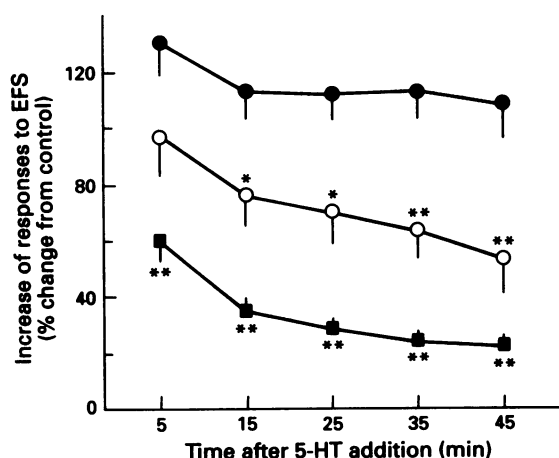


Figure 3 Time course of the enhancing effect of 5-hydroxytryptamine (5-HT, 30 nM) on electrically evoked contractions of the longitudinal muscle of guinea-pig proximal colon in the absence (●) and presence of hexamethonium (100 μ M, ○) or spantide (10 μ M, ■). All results are expressed as the % change from control responses before the addition of 5-HT. Each point represents the mean \pm s.e.mean of 6 preparations. * $P < 0.05$ and ** $P < 0.01$ as compared with drug-treatment groups versus control groups using Dunnett's multiple comparison test. Atropine (0.2 μ M) and indomethacin (2 μ M) were present throughout the experiments.

Table 1 Effect of 5-HT receptor antagonists on electrically evoked contractions and on the enhancing effect of 5-HT (30 nM) in the guinea-pig isolated proximal colon

Pretreatment	n	EFS-induced contractions (% of control)	
		Absence of 5-HT	Presence of 5-HT
None	16	100	212.6 \pm 8.3*
Metitepine (0.03 μ M)	6	97.2 \pm 1.8	205.9 \pm 13.7
Ketanserin (0.01 μ M)	6	98.0 \pm 2.1	185.6 \pm 5.9
Ondansetron (1 μ M)	6	96.1 \pm 5.1	223.8 \pm 12.2
DAU 6285 (0.3 μ M)	6	100.9 \pm 3.8	131.1 \pm 7.4*
DAU 6285 (3 μ M)	6	100.0 \pm 7.1	100.4 \pm 6.5*

Each value represents the mean \pm s.e.mean

* $P < 0.001$ in comparison with the control (None) value^a by Student's *t* test

Effects of 5-HT and α -methyl-5-HT on substance P-induced contractions

Substance P (SP, 0.15–0.3 nM) produced transient contraction of the colonic strip in a concentration-dependent manner. The SP (0.3 nM)-induced contraction was abolished by preincubation of the tissue with spantide (10 μ M, $n = 5$), but was unaffected by tetrodotoxin (0.3 μ M, $n = 5$). As shown in Table 2, both 5-HT (30 nM) and α -methyl-5-HT (1 μ M) were without effect on contractions evoked by exogenous SP (0.15 or 0.3 nM).

Discussion

In the presence of atropine and indomethacin, the EFS-induced contraction of the guinea-pig proximal colon is probably mediated via non-cholinergic excitatory neurones, because tetrodotoxin abolished the contractions. Moreover, the EFS-induced contraction was partially hexamethonium-sensitive, suggesting that the response is, at least in part, due to stimulation of intramural preganglionic cholinergic neurones. In the present study, a tachykinin receptor antagonist, spantide also significantly reduced the EFS-induced contractions. The result is consistent with previous work indicating that endogenous tachykinins are involved in the atropine-resistant and hexamethonium-sensitive excitatory responses of the guinea-pig proximal colon *in vivo* (Giuliani *et al.*, 1993). In the present study, we found that the atropine-resistant contraction of the colonic strip evoked by EFS was enhanced in the presence of 5-HT. The nicotinic receptor antagonist, hexamethonium significantly reduced the enhancement of EFS-induced responses by 5-HT, suggesting that the enhancing action of the amine is mediated in part by the facilitation of nicotinic cholinergic transmission and that 5-HT may activate preganglionic parasympathetic nerve ter-

Table 2 Effect of 5-HT (30 nM) and α -methyl-5-HT (1 μ M) on substance P (SP)-induced contractions of the guinea-pig isolated proximal colon

Compounds	Response to SP (% of 10 μ M histamine)	n
Control		
SP (0.15 nM)	23.9 \pm 2.0	13
SP (0.3 nM)	43.2 \pm 2.2	
+ 5-HT 30 nM		13
SP (0.15 nM)	25.7 \pm 2.4	8
SP (0.3 nM)	42.9 \pm 3.1	8
+ α -methyl-5-HT 1 μ M		
SP (0.15 nM)	24.6 \pm 5.0	5
SP (0.3 nM)	39.8 \pm 3.4	5

5-HT and α -methyl-5-HT were given 5–10 min prior to the addition of SP. Each value represents the mean \pm s.e.mean. 5-HT and α -methyl-5-HT did not affect SP-induced contractions ($P > 0.05$, Student's *t* test).

minals or cholinergic interneurons to release acetylcholine. Recently, Mizutani *et al.* (1992) have shown that 5-HT induced an ascending contraction through an enteric excitatory pathway formed by a series of cholinergic interneurons and final cholinergic motor neurones in canine small intestine. Spantide also inhibited the enhancement of EFS-induced responses by 5-HT, suggesting that an increase in tachykinergic neurotransmission is involved in this enhancement. 5-HT is known to potentiate the contractile responsiveness of vascular smooth muscle to noradrenaline, histamine or angiotensin II (Moreland *et al.*, 1985; Van Nueten *et al.*, 1982). However, the fact that 5-HT or α -methyl-5-HT examined in the present study did not affect the contractile response to exogenous substance P, one of the tachykinin family, suggests that the enhancement of EFS-induced contractions by 5-HT was not the result of enhanced smooth muscle responsiveness to tachykinins. This suggests that the enhancement of non-cholinergically mediated contractions by 5-HT may involve a prejunctional rather than a postjunctional mechanism, such as facilitation of tachykinin release from non-cholinergic neurones. In contrast to the present results, Galligan (1992) has previously shown that non-cholinergically mediated contractions of the guinea-pig ileum were inhibited by 5-HT in a concentration-dependent manner. Although the cause of this discrepancy is not clear, the different role of 5-HT in different regions of the intestine may be involved.

According to the current classification, four main types of 5-HT receptors, termed 5-HT₁, 5-HT₂, 5-HT₃, 5-HT₄, can be distinguished (Bradley *et al.*, 1986; Clarke *et al.*, 1989). Studies with antagonists have given some insight into the types of 5-HT receptor responsible for the enhancing effects of 5-HT on EFS-induced contractions. Metitepine, a 5-HT₁/5-HT₂ receptor antagonist, ketanserin, a 5-HT₂ receptor antagonist, and ondansetron, a 5-HT₃ receptor antagonist,

failed to block the enhancing effects of the amine, suggesting that the neuronal 5-HT receptor does not belong to either the 5-HT₁, 5-HT₂ or 5-HT₃ receptor class. On the other hand a novel 5-HT₄ receptor antagonist, DAU 6285 (Dumuis *et al.*, 1992), abolished the enhancing action of 5-HT, suggesting an involvement of the 5-HT₄ receptor subtype. α -Methyl-5-HT, a 5-HT₂ receptor agonist, produced a similar enhancement of EFS-induced contractions, but was 10 to 30 times less potent than 5-HT. This agonist appears to interact with a 5-HT₄ receptor in the guinea-pig proximal colon, since the enhancing action of α -methyl-5-HT was abolished by DAU 6285. Similar results have also been reported in the rat oesophageal tunica muscularis mucosae (Baxter *et al.*, 1991) or piglet isolated right atrium (Medhurst & Kaumann, 1993). The inhibitory effects of DAU 6285 on the enhancing action of these agonists appear not to be due to a non-specific effect, since DAU 6285 did not affect EFS-evoked contractions or contractions evoked by carbachol (data not shown). Thus, these results support the hypothesis that the activation of 5-HT₄ receptors can augment the atropine-resistant contractions evoked by EFS through the facilitation of intramural nicotinic cholinergic and tachykinergic neurotransmissions of the guinea-pig proximal colon.

In conclusion, our data indicate that in the guinea-pig proximal colon, 5-HT produced an enhancement of atropine-resistant neurogenic contractions induced by EFS through prejunctional mechanisms and that the enhancement is mediated by the stimulation of 5-HT₄ receptors located on intramural preganglionic cholinergic neurones and tachykinergic neurones.

We wish to thank Dr Rizzi (Boehringer Ingelheim, Italy) for the generous gift of DAU 6285 and Glaxo Research group (Ware, U.K.) for the generous gift of ondansetron.

References

- BAXTER, G.S., CRAIG, D.A. & CLARKE, D.E. (1991). 5-Hydroxytryptamine₄ receptors mediate relaxation of the rat oesophageal tunica muscularis mucosae. *Naunyn-Schmied. Arch. Pharmacol.*, **343**, 439–446.
- BRADLEY, P.D., ENGEL, G., FENIUK, W., FOZARD, J.R., HUMPHREY, P.P.A., MIDDLEMISS, D.N., MYLECHRANE, E.J., RICHARDSON, B.P. & SAXENA, P.R. (1986). Proposal for the classification and nomenclature of functional receptors for 5-hydroxytryptamine. *Neuropharmacology*, **25**, 563–576.
- CLARKE, D.E., CRAIG, D.A. & FOZARD, J.R. (1989). The 5-HT₄ receptor: naughty but nice. *Trends Pharmacol. Sci.*, **10**, 385–386.
- COSTA, M. & FURNESS, J.B. (1979). The sites of action of 5-hydroxytryptamine in nerve-muscle preparations from the guinea-pig small intestine and colon. *Br. J. Pharmacol.*, **65**, 237–248.
- DUMUIS, A., GOZLAN, H., SEBBAN, M., ANSANAY, H., RIZZI, C.A., TURCONI, M., MONFERINI, E., GIRALDO, E., SCHIANTARELLI, P., LADINSKY, H. & BOCKAERT, J. (1992). Characterization of a novel 5-HT₄ receptor antagonist of the azabicycloalkyl benzimidazolone class: DAU 6285. *Naunyn-Schmied. Arch. Pharmacol.*, **345**, 264–269.
- ELSWOOD, C.J., BUNCE, K.T. & HUMPHREY, P.P.A. (1991). Identification of putative 5-HT₄-receptors in guinea-pig ascending colon. *Eur. J. Pharmacol.*, **196**, 149–155.
- FURNESS, J.B. & COSTA, M. (1982). Identification of gastrointestinal transmitter. In *Mediators and Drugs in Gastrointestinal Motility*. Vol. 1, ed. Bertaccini, G. pp. 383–405. Berlin, Heidelberg, New York: Springer-Verlag.
- GALLIGAN, J.J. (1992). Differential inhibition of cholinergic and noncholinergic neurogenic contractions by 5-hydroxytryptamine_{1A} receptor agonists in guinea-pig ileum. *J. Pharmacol. Exp. Ther.*, **260**, 306–312.
- GIULIANI, S., LECCI, A., GIACHETTI, A. & MAGGI, C.A. (1993). Tachykinins and reflexly evoked atropine-resistant motility in the guinea-pig colon *in vivo*. *J. Pharmacol. Exp. Ther.*, **265**, 1224–1231.
- KOJIMA, S. (1991). Characterization of 5-hydroxytryptamine-induced relaxations of guinea-pig proximal colon. *Arch. Int. Pharmacodyn.*, **313**, 23–32.
- MEDHURST, A.D. & KAUMANN, A.J. (1993). Characterization of the 5-HT₄ receptor mediating tachycardia in piglet isolated right atrium. *Br. J. Pharmacol.*, **110**, 1023–1030.
- MIZUTANI, M., NEYA, T. & NAKAYAMA, S. (1992). Ascending contraction mediated by 5-hydroxytryptamine₃ receptors in canine small intestine. *Am. J. Physiol.*, **263**, G306–G311.
- MORELAND, R.S., VAN BREEMEN, C. & BOHR, D.F. (1985). Mechanism by which serotonin attenuates contractile response of canine mesenteric arterial smooth muscle. *J. Pharmacol. Exp. Ther.*, **232**, 322–329.
- VAN NUETEN, J.M., JANSSEN, P.A.J., DE RIDDER, W. & VAN-HOUTTE, P.M. (1982). Interaction between 5-hydroxytryptamine and other vaso-constrictor substances in the isolated femoral artery of the rabbit; effect of ketanserin (R 41 468). *Eur. J. Pharmacol.*, **77**, 281–287.
- VAN ROSSUM, J.M. (1963). Cumulative dose-response curves. II. Technique for the making of dose-response curves in isolated organs and the evaluation of drug parameters. *Arch. Int. Pharmacodyn.*, **143**, 299–330.

(Received May 3, 1994
Revised August 12, 1994
Accepted September 8, 1994)