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Pharmacological characterization of 5-hydroxytryptamine-induced motor activity (in vitro) in the guinea pig gastric antrum and corpus

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Abstract

In order to characterize the receptor subtypes involved in 5-hydroxytryptamine (5-HT)-induced circular muscle motor responses of the guinea pig gastric antrum and corpus, we examined the effects of several antagonists in vitro. 5-HT evoked concentration-dependent contractions of the gastric antrum and relaxations of the corpus. 5-HT-induced antral contractions were abolished by pretreatment with atropine and tetrodotoxin. Methysergide, ketanserin, granisetron and [1-[2-(methylsulphonylamino)ethyl]-4-piperidinyl]methyl 1-methyl-1*H*-indole-3-carboxylate maleate salt (GR113808A), but neither 1-(2-methoxyphenyl)-4-[4-(2-phthalimido)butyl]piperazine (NAN-190) nor N^2-[(4*R*)-4-hydroxy-1-(1-methyl-1*H*-indol-3-yl)carbonyl-L-prolyl]-*N*-methyl-*N*-phenylmethyl-3-(2-naphthyl)-L-alaninamide (FK888), inhibited 5-HT (3×10^{-6} M: submaximal concentration)-induced antral contractions concentration dependently and shifted the 5-HT concentration-response curve to the right. 5-HT (3×10^{-6} M)-induced corporal relaxation was not affected by tetrodotoxin, ketanserin, granisetron or GR113808A. At 10^{-7} M, neither methysergide nor NAN-190 affected corporal relaxation, but at a high concentration (10^{-6} M) they both inhibited it and shifted the 5-HT concentration-response curve to the right. We conclude that 5-HT-induced antral contraction is mediated by cholinergic neurons via 5-HT_{2A}, 5-HT_3 and 5-HT_4 receptors, whereas corporal relaxation is mediated via 5-HT_1-like receptors on smooth muscle that are sensitive to methysergide and NAN-190.

Keywords: 5-HT (5-hydroxytryptamine, serotonin); 5-HT receptor antagonist; Stomach; Contraction; Relaxation; (Guinea pig)

1. Introduction

5-Hydroxytryptamine (5-HT) is widely distributed throughout the gastrointestinal tract and is well known to be a neurotransmitter in the enteric nervous system. There are many subtypes of 5-HT receptor and so far they have been classified into four main types: 5-HT_1, 5-HT_2, 5-HT_3 and 5-HT_4. Furthermore, 5-HT_1 and 5-HT_2 binding sites have been subdivided into 5-HT_{1A}, 5-HT_{1B}, 5-HT_{1D}, 5-HT_{1E}, 5-HT_{1F}, 5-HT_{2A}, 5-HT_{2B} and 5-HT_{2C}, and, more recently, three new subtypes termed 5-HT_5, 5-HT_6 and 5-HT_7 have been cloned from the central nervous system (Hoyer et al., 1994). The pharmacological characteristics of the 5-HT_{1E}, 5-HT_{1F}, 5-HT_5, 5-HT_6, and 5-HT_7 receptors have not yet been defined. Furthermore, intracellular recording indicated the presence of a further receptor subtype, 5-HT_{1P}, in the myenteric plexus (Gershon, 1991). 5-HT can elicit multiple responses in the gastrointestinal tract. It causes contractions in the guinea pig ileum via 5-HT_{2A} receptors on the muscle (Engel et al., 1984) and neuronal 5-HT_3 and 5-HT_4 receptors (Read and Gwee, 1994) and similar responses of the guinea pig colon have been reported (Briejer et al., 1993, 1995). 5-HT also evokes relaxations of the guinea pig ileum precontracted with histamine (Kalkman et al., 1986), rat esophageal muscularis mucosa precontracted with a muscarinic agonist (Bieger and Triggle, 1985), rat esophageal longitudinal muscle precontracted with carbachol (Reeves et al., 1991), guinea pig stomach fundus (Moen et al., 1983; Bugge et al., 1989; Yamaguchi, 1972; Kojima et al., 1992), rat duodenum (Postorino et al., 1993), rat caecum (Uguru and Bamgbose, 1986), guinea pig colon (Briejer et al., 1992, 1995) and canine ileum (Boeckxstaens et al., 1990; Bogers et al., 1991). In the stomach, Moen et al. (1983) reported that 5-HT induced contraction in the guinea pig gastric antrum and Yamaguchi (1972) reported that 5-HT induced tetrodotoxin-resistant relaxations of guinea pig stomach fundic strips. Conversely, Meulemans et al. (1993) reported a tetrodotoxin-sensitive relaxation of the guinea pig whole stomach. In addition, the gene for the 5-HT_{2B} receptor that causes contraction in the rat stomach fundus

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was cloned recently (Kursar et al., 1992; Foguet et al., 1992).

The precise nature of the effects of 5-HT on the stomach, particularly the antrum, remains to be established. The purpose of this study was to use several antagonists to examine the mechanism of action of 5-HT on circular muscle strips obtained from the guinea pig gastric antrum and corpus.

2. Materials and methods

2.1. Preparation of smooth muscle strips from the guinea pig stomach

Male guinea pigs (350–700 g, from Imai Experimental Animal, Saitama, Japan) were killed by a blow to the head and bled from the carotid arteries. The stomach was removed quickly and opened along the lesser curvature. Following removal of the mucosa, the stomach was pinned flat on a dissecting tray filled with aerated Krebs solution (mM/l: NaCl 118, KCl 4.7, $CaCl_2$ 2.5, KH_2PO_4 1.2, $NaHCO_3$ 5.0, $MgSO_4 \cdot 7H_2O$ 1.2 and glucose 11.0). Circular muscle strips approximately 10 mm long and 3 mm wide were isolated from the antrum 5–10 mm proximal to the pylorus and those of the corpus were taken 0–5 mm distal to the esophago-gastric junction. Both sides of each muscle strip were tied with silk suture.

2.2. Recording of isometric tension

The muscle strips were suspended in 15-ml organ baths containing Krebs solution aerated with 95% O_2-5% CO_2 and maintained at 37.4°C, pH 7.4. One end of each muscle strip was anchored to the bottom of the organ bath with a tissue holder and the other end was attached to an isometric transducer (FD pickup TB-612T, Nihon Kohden Co., Tokyo, Japan) by the silk suture. The isometric tensions of the muscle strips isolated from the antrum and corpus were recorded on a multichannel polygraph (AP-612G, Nihon Kohden Co.). After the muscle strips had been mounted, the optimal resting tension of 1 g was kept constant by readjustment during the equilibration period which lasted for at least 1 h. The Krebs solution was replaced every 30 min during the experiments, and the muscle strips were washed at least three times following the application of 5-HT.

2.3. Experimental protocol

2.3.1. Characterization of the responses of guinea pig gastric antral and corporal muscle strips to exogenously applied 5-HT

The addition of 5-HT (10^{-9}–10^{-4} M) to the organ baths in a cumulative manner induced relaxations of the gastric corpus muscle. In a preliminary study, we found that the 5-HT-induced responses of the gastric antrum muscle were transient and high concentrations of 5-HT in excess of 10^{-5} M resulted in desensitization, so 5-HT (10^{-9}–10^{-4} M) was added in a non-cumulative manner to investigate the contractile responses of the gastric antrum. Submaximal contractile and relaxant responses were evoked by 3×10^{-6} M 5-HT and, therefore, 3×10^{-6} M 5-HT was used in the following studies.

2.3.2. The effects of antagonists on submaximal 5-HT-induced responses

First, 3×10^{-6} M 5-HT alone was given without antagonists and thereafter washed out by replacing the bath solution at least three times. Twenty minutes after 5-HT was washed out, an antagonist was added and 10 min later 3×10^{-6} M 5-HT was added again. In preliminary studies, we found that the responses to three successive administrations of 3×10^{-6} M 5-HT followed by washing out were not significantly different. Pretreatment with each vehicle alone also had no effect on the responses of the muscles to 5-HT.

2.3.3. The effects of antagonists on the 5-HT concentration-response curves

We also investigated the effects of the antagonists which inhibited the submaximal 5-HT-induced responses on the 5-HT concentration-response curves.

For the gastric corpus, increasing concentrations of 5-HT were added to the organ bath in a cumulative manner and then washed out. Thirty minutes later, the concentration-response series was repeated in the presence of the required antagonist and, finally, 10^{-4} M of papaverine was added to the organ bath to produce a maximal relaxant response. Each strip was used to record a single set of such concentration-response curves and, therefore, served as its own control. A preliminary study showed that two successive concentration-response curves without antagonist were not different.

For the antrum, increasing concentrations of 5-HT were added in a non-cumulative manner and then washed out. 10^{-4} M acetylcholine (ACh) was applied before and after the 5-HT concentration-response series and we confirmed that the two responses to 10^{-4} M ACh were not different. As 5-HT in excess of 10^{-5} M caused desensitization, which made it impossible to obtain a set of concentration-response curves using the same muscle strip, one strip isolated at the same time was not pretreated with antagonist and served as a control and the other strips were pretreated with various antagonists before addition of 5-HT.

2.4. Analysis of data

Contractions and relaxations were measured as an increase in the maximal tension and a decrease in the minimal tension, respectively. In the experiments using antagonists to 3×10^{-6} M 5-HT-induced responses, the

results are presented as percentages of the response to the first exposure to 5-HT. In the concentration-response studies, the contractions and relaxations are expressed as percentages of the 10^{-4} M acethylcholine-induced contraction and 10^{-4} M papaverine-induced relaxation, respectively. All the data are presented as means ± S.E. obtained from 4 to 12 observations. Statistical analysis of the data was performed with a two-tailed paired *t*-test, and *P* values less than 0.05 were regarded as significant between paired data.

The concentration of antagonist required to inhibit 50% of 5-HT (3×10^{-6} M)-induced response (IC_{50}) is referred to in the text as the pIC_{50}, indicating the negative logarithm of the original values.

The pA_2 was calculated as: $pA_2 = -\log[B] + \log[[A]_2/[A]_1 - 1]$, in which $[B]$ is the antagonist concentration, $[A]_2$ is the concentration of agonist needed to produce a response equivalent to the control half-maximal response in the presence of antagonist at concentration $[B]$, and $[A]_1$ is the concentration of agonist needed to elicit the half-maximal response in the absence of antagonist (Van Rossum, 1963).

2.5. Drugs

The following drugs were purchased: 5-hydroxytryptamine (5-HT) hydrobromide, tetrodotoxin and N^G-nitro-L-arginine (L-NNA) (Sigma, St. Louis, MO, USA), 1-(2-methoxyphenyl)-4-[4-(2-phthalimido)butyl]piperazine (NAN-190) and ketanserin tartrate (RBI, Natrick, MA, USA), hexamethonium bromide (Wako, Osaka, Japan), acetylcholine chloride (ACh) (Daiich, Tokyo, Japan), atropine sulfate (Tanabe, Osaka, Japan), phentolamine mesylate (Ciba-Geigy, Takarazuka, Japan) and propranolol hydrochloride (Zeneca, Osaka, Japan). The following drugs were kindly donated: granisetron (Smith Kline Beecham, Betchworth, Surrey, UK), methysergide (Sandoz, Basel, Switzerland), [1-[2-(methylsulphonylamino)ethyl]-4-piperidinyl]methyl 1-methyl-1*H*-indole-3-carboxylate maleate salt (GR113808A) (Glaxo, Greenford, Middlesex, UK) and N^2-[(4*R*)-4-hydroxy-1-(1-methyl-1*H*-indol-3-yl)carbonyl-L-prolyl]-*N*-methyl-*N*-phenylmethyl-3-(2-naphthyl)-L-alaninamide (FK888) (Fujisawa, Osaka, Japan). All the drugs, except for methysergide and FK888, were dissolved in distilled water. Methysergide was initially dissolved in methanol to produce 10^{-2} M and FK888 was initially dissolved in dimethyl sulfoxide to produce 10^{-3} M and then diluted to the required concentrations with distilled water.

Although methysergide has been regarded as an antagonist at 5-HT_1 or 5-HT_2 receptors or both, we considered it as a non-selective 5-HT_1-like and 5-HT_2 receptor antagonist, since the 5-HT_{1C} receptor has now been reclassified as a 5-HT_{2C} receptor. Methysergide antagonizes the 5-HT_{2C} receptor with high affinity and the 5-H_{1P} receptor with low affinity (Frieling et al., 1991; Sugden, 1990; Hoyer et al., 1994) and acts as a very weak agonist at 5-HT_{1A}, 5-HT_{1B} and 5-HT_{1D} receptors (Levy et al., 1992; Hoyer et al., 1994; Pauwels et al., 1993; Hamel et al., 1993; Yamamoto et al., 1991).

3. Results

3.1. Effect of exogenous 5-HT on the gastric antrum

5-HT (10^{-8}–10^{-4} M) evoked concentration-dependent contractions of guinea-pig antral muscle strips (Figs. 1 and 2). The most marked effect of 5-HT was an increase in phasic activity whereas it increased the tonic activity slightly. At 10^{-4} M, 5-HT caused a contraction of about 40.3% of the 10^{-4} M ACh-induced contraction.

3.2. Effect of exogenous 5-HT on the gastric corpus

5-HT (10^{-7}–10^{-4} M) evoked concentration-dependent decreases in tone of the guinea pig gastric corpus (Figs. 3 and 4). At 10^{-4} M, 5-HT caused a relaxation of about 96.7% of the 10^{-4} M papaverine-induced relaxation.

3.3. Effects of antagonists on 5-HT-induced contractions of the gastric antrum

3.3.1. Effects of antagonists on submaximal concentration of 5-HT-induced contractions of the gastric antrum

The submaximal contractions elicited by 3×10^{-6} M 5-HT were inhibited almost completely by tetrodotoxin (3×10^{-7} M; $n = 6$) and atropine (10^{-6} M; $n = 7$) (Table 1). Hexamethonium (10^{-4} M; $n = 4$), FK888 (10^{-6} M; $n = 6$), phentolamine (10^{-6} M; $n = 6$) propranolol (10^{-6} M; $n = 6$) and NAN-190 (10^{-7} M; $n = 6$) had no effect on the 5-HT-induced contractions (Table 1). Granisetron (10^{-8}–10^{-7} M; $n = 6$–9) and GR113808A (10^{-9}–10^{-7} M; $n = 6$–8) inhibited the 5-HT-induced contractions in a concentration-dependent manner (Table 1). Methysergide (10^{-8}–10^{-7} M; $n = 6$–7) and ketanserin (10^{-9}–10^{-7} M; $n = 6$–7) also inhibited the 5-HT-induced contractions in a concentration-dependent manner (Fig. 5 and Table 1). The

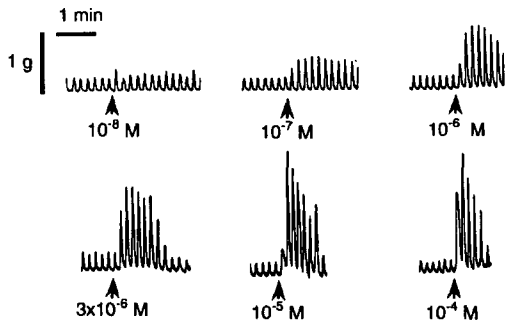

Fig. 1. Trace showing typical effect of 5-HT on circular muscle strips isolated from the guinea pig gastric antrum.

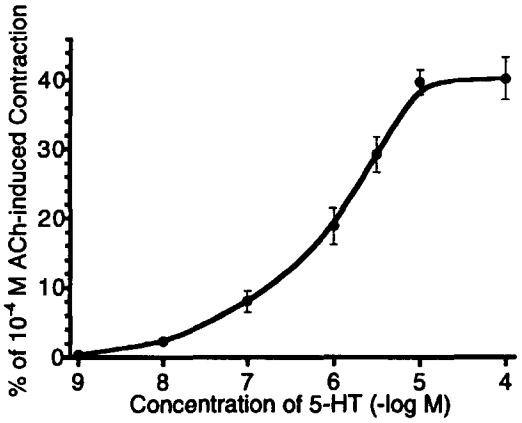

Fig. 2. A concentration-response curve for 5-HT-induced contractions of the guinea pig gastric antrum. Data are means ± S.E. ($n = 9$).

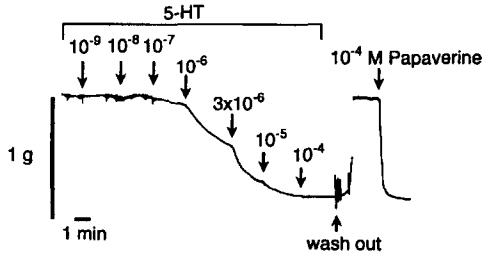

Fig. 3. Trace showing typical effect of 5-HT on circular muscle strips isolated from the guinea pig gastric corpus.

Table 1
Effects of various antagonists on 5-HT-induced contractions in the gastric antrum

Drug	Dose −log M	n	Percentage
Tetrodotoxin	6.5	6	4.5 ± 1.5 [b]
Atropine	6	7	3.4 ± 0.9 [b]
Hexamethonium	6	4	98.9 ± 3.2
Phentolamine	6	6	96.3 ± 4.1
Propranolol	6	6	101.1 ± 5.6
FK888	6	6	101.4 ± 4.6
Methysergide	9	6	99.3 ± 8.7
	8	6	49.6 ± 8.1 [b]
	7	7	36.2 ± 9.6 [b]
NAN-190	8	6	104.2 ± 5.2
	7	6	92.5 ± 4.3
Ketanserin	9	6	95.0 ± 9.2
	8	7	44.6 ± 7.0 [a]
	7	7	24.5 ± 5.5 [b]
Granisetron	9	9	96.5 ± 3.0
	8	7	71.9 ± 8.0 [b]
	7	6	69.0 ± 6.9 [b]
GR113808A	10	6	102.2 ± 4.6
	9	8	83.7 ± 5.6 [c]
	8	6	80.7 ± 5.9 [c]
	7	6	67.5 ± 5.8 [c]

Antagonists were added 10 min before the second application of 5-HT. Values represent the mean tension as a percentage of the control value (i.e. first application of 5-HT alone) ± S.E. [a,b,c] Significant difference from the control value calculated by a two-tailed paired t-test ([a] $P < 0.001$; [b] $P < 0.01$; [c] $P < 0.05$).

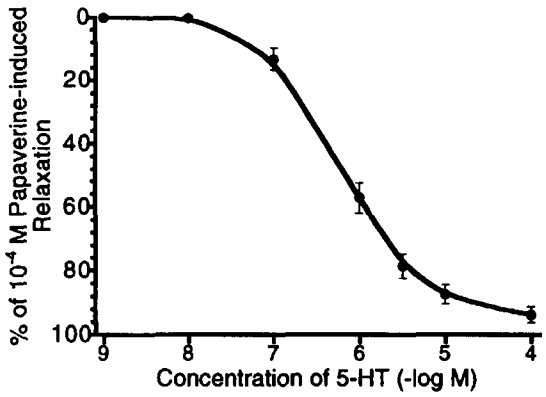

Fig. 4. A concentration-response curve for 5-HT-induced relaxations of the guinea pig gastric corpus. Data are means ± S.E. ($n = 9$).

calculated pIC_{50} value of ketanserin was 8.1.

3.3.2. *Effects of antagonists on 5-HT concentration-response curves of the gastric antrum*

Tetrodotoxin ($n = 6$) and atropine ($n = 6$) blocked the 5-HT-induced gastric antral contractions almost completely. Very small contractions were observed when high concentrations of 5-HT not less than 3×10^{-6} M were applied. At 10^{-8} M, ketanserin ($n = 6$) inhibited the 5-HT (10^{-7}–10^{-5} M)-induced contractions and made the curve biphasic. The first and second phases after pretreatment with 10^{-8} M ketanserin comprised responses to 5-HT contractions below and above 10^{-6} M, respectively. The apparent pA_2 value of ketanserin was 8.71. The effect of 10^{-8} M methysergide ($n = 7$) was similar, but it also reduced the maximal response to 5-HT. At 10^{-8} M, granisetron ($n = 7$) inhibited only the responses to 5-HT in excess of 10^{-6} M and did not inhibit those to 5-HT in concentrations not exceeding 10^{-7} M. At 10^{-8} M, GR113808A ($n = 8$) inhibited the responses to 5-HT in concentrations below 3×10^{-6} M, but not those to high

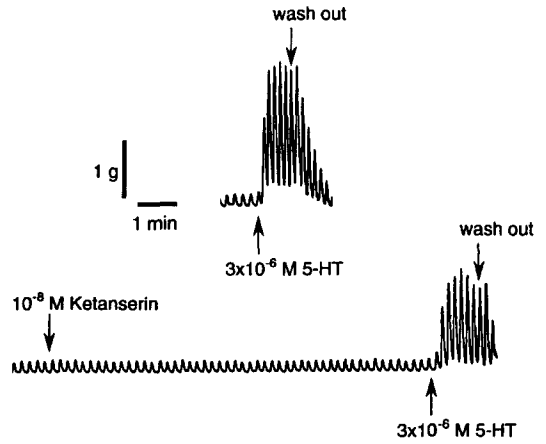

Fig. 5. Trace showing 3×10^{-6} M 5-HT-induced contractions of the guinea pig gastric antrum in the absence (upper) and presence (lower) of 10^{-8} M ketanserin. Ketanserin inhibited the 5-HT-induced antral contraction.

concentrations of 10^{-5} M and over, and made the curve steeper (Fig. 6).

3.4. Effects of antagonists on 5-HT-induced relaxation of the gastric corpus

3.4.1. Effects of antagonists on submaximal concentration of 5-HT-induced relaxation of the gastric corpus

The submaximal relaxation of the corpus induced by 3×10^{-6} M 5-HT was not inhibited by tetrodotoxin (3×10^{-7} M; $n = 8$), atropine (10^{-6} M; $n = 12$), phentolamine

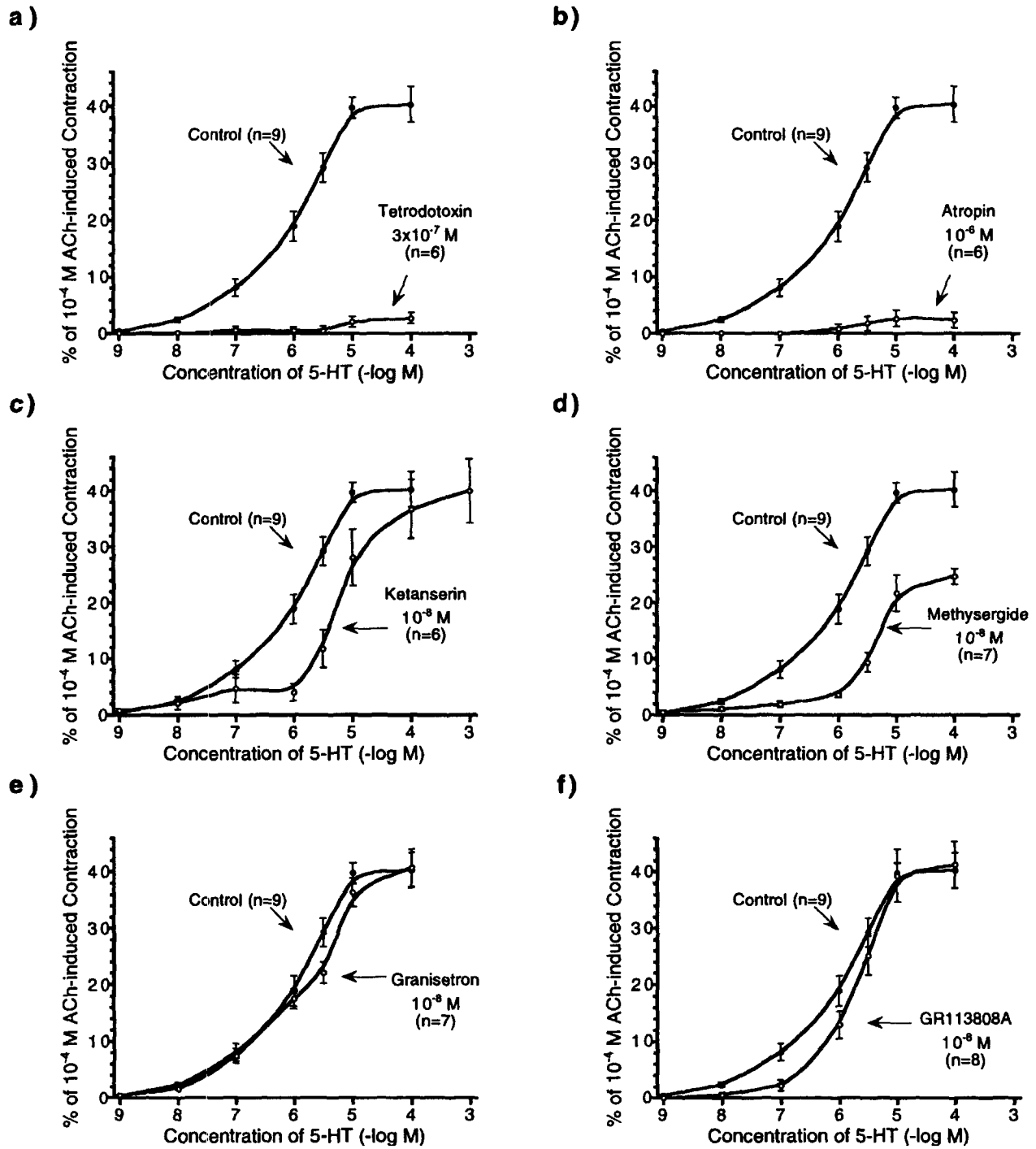

Fig. 6. Effects of various antagonists on the concentration-response curve for 5-HT-induced contractions of the guinea pig gastric antrum. (a) Tetrodotoxin and (b) atropine virtually abolished the contractions. (c) Ketanserin shifted the curve to the right. (d) Methysergide shifted the curve to the right and reduced the maximal response. (e) Granisetron inhibited the responses to 5-HT (above 10^{-6} M). (f) GR113808A inhibited the responses to 5-HT (below 3×10^{-6} M) and steepened the curve.

Table 2
Effects of various antagonists on 5-HT-induced relaxations in the gastric corpus

Drug	Dose −log M	n	Percentage
Tetrodotoxin	6.5	8	103.9±5.7
Atropine	6	12	100.5±3.8
Phentolamine	6	6	101.1±5.7
Propranolol	6	7	105.0±2.1
L-NNA	4	6	110.4±4.5
Methysergide	7	6	103.9±5.7
	6	6	49.2±4.3 [b]
NAN-190	7	6	97.5±1.0
	6	7	54.2±5.9 [b]
Ketanserin	6	7	105.5±3.0
Granisetron	6	10	97.1±2.8
GR113808A	6	8	103.0±2.9

Antagonists were added 10 min before the second application of 5-HT. Values represent the mean tension as a percentage of the control value (i.e. first application of 5-HT alone)±S.E. [a,b,c] Significant difference from the control value calculated by a two-tailed paired *t*-test ([a] $P < 0.001$; [b] $P < 0.01$).

(10^{-6} M; $n = 6$), propranolol (10^{-6} M; $n = 7$) or L-NNA (10^{-4} M; $n = 6$) (Fig. 7 and Table 2), suggesting that 5-HT acts directly on the smooth muscle to induce relaxation. L-NNA, methysergide and NAN-190 caused a slight increase in basal tone. Granisetron ($n = 10$), ketanserin ($n = 7$) and GR113808A ($n = 8$), all applied at 10^{-6} M,

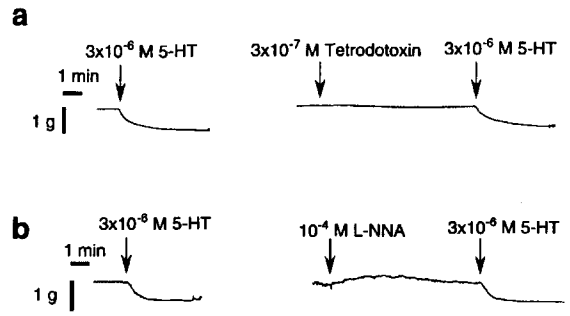

Fig. 7. Trace showing the lack of effect of (a) 3×10^{-7} M tetrodotoxin and (b) 10^{-4} M L-NNA on 3×10^{-6}M 5-HT-induced relaxation of the corpus.

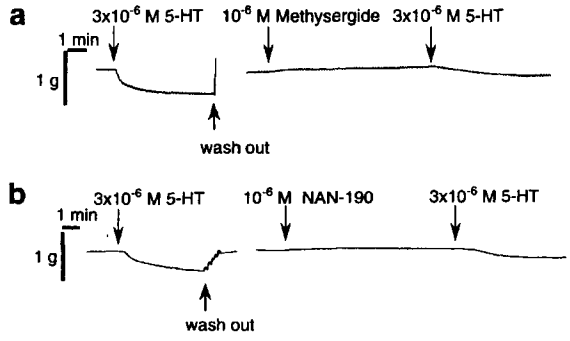

Fig. 8. Trace showing typical effects of (a) 10^{-6} M methysergide and (b) 10^{-6} M NAN-190 on 3×10^{-6} M 5-HT-induced relaxation of the corpus. Both drugs inhibited this relaxations.

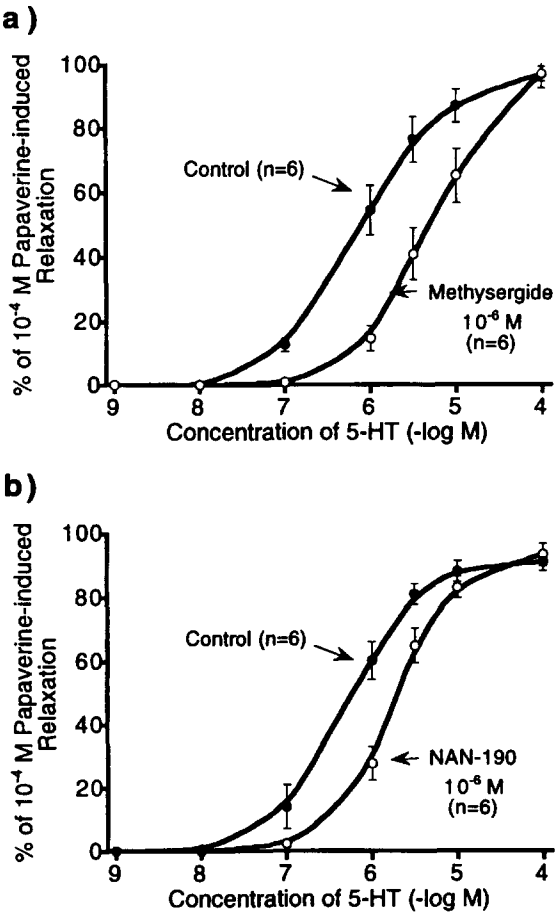

Fig. 9. Effects of the high concentration (10^{-6} M) of (a) methysergide and (b) NAN-190 on the concentration-response curve for 5-HT-induced relaxation of the guinea pig gastric corpus. Both drugs shifted the curve to the right.

had no effect on this 5-HT-induced relaxation (Table 2), whereas methysergide (10^{-6} M; $n = 6$) and NAN-190 (10^{-6} M; $n = 7$) inhibited the 5-HT-induced relaxation (Fig. 8 and Table 2).

3.4.2. Effects of antagonists on 5-HT concentration-response curves of the gastric corpus

The 5-HT concentration-response curves were shifted to the right by methysergide (10^{-6} M; $n = 6$) and NAN-190 (10^{-6} M; $n = 6$) (Fig. 9) without a decrease in the maximal effect.

Methysergide produced a dose ratio of 6.13, giving a pA_2 of 6.71, and NAN-190 produced a dose ratio of 3.98, giving a pA_2 of 6.47.

4. Discussion

4.1. 5-HT-induced contractions of the gastric antrum

4.1.1. The nature of 5-HT-induced contraction of the gastric antrum

The present study shows that exogenous 5-HT elicits different motor responses in the gastric antrum and corpus

of the guinea pig. The concentration-dependent contractions in the antrum are apparently mediated through postganglionic cholinergic neurons, since tetrodotoxin and atropine, but not hexamethonium (nicotinic receptor blocker) and FK888 (NK_1 receptor antagonist: Fujii et al., 1992), almost completely blocked 5-HT-induced contractions. These findings agree with those of Moen et al. (1983), who reported that 5-HT-induced antral contractions were blocked by tetrodotoxin, and those of Visi and Vizi (1978), who reported that 5-HT-induced ACh release from the guinea pig ileal myenteric plexus was reduced by tetrodotoxin, but not by hexamethonium.

4.1.2. Subtypes of 5-HT receptor involved in 5-HT-induced contractions of the gastric antrum

The submaximal 5-HT-induced contractions of the gastric antrum were inhibited significantly by methysergide (non-selective 5-HT_2 receptor antagonist), ketanserin (selective 5-HT_{2A} receptor antagonist), granisetron (selective 5-HT_3 receptor antagonist) and GR113808A (selective 5-HT_4 receptor antagonist), suggesting the involvement of 5-HT_{2A}, 5-HT_3 and 5-HT_4 receptors.

NAN-190 (selective 5-HT_{1A} receptor antagonist) (Glennon et al., 1988, 1989) did not affect the submaximal 5-HT-induced antral contraction. 5-HT_{1A} receptors are thought to be located on presynaptic and prejunctional nerve terminals and their activation has been reported to inhibit neurotransmitter release, reducing the amplitude of fast excitatory postsynaptic potentials in guinea pig ileal myenteric neurons (Pan and Galligan, 1994) and the gastric antrum (Tack et al., 1992). Furthermore, 5-HT_{1A} receptor activation has been shown to inhibit transmitter release from guinea pig enteric cholinergic neurons (Fozard and Kilbinger, 1985) and to inhibit cholinergic (Fozard and Kilbinger, 1985; Mir et al., 1988) and non-cholinergic (Galligan, 1992) contractions of guinea pig ileal longitudinal muscle evoked by transmural electrical stimuli. Therefore, 5-HT_{1A} receptor blockade may increase the amplitude of the contractions. However, NAN-190 had no effect on 5-HT-induced contraction of the guinea pig antrum in our study and the concentration of NAN-190 we used was considered adequate to block 5-HT_{1A} receptors, because it was of a similar order to that used by Galligan (1992). A possible reason for this lack of effect of NAN-190 is that the preganglionic neurons which possess 5-HT_{1A} receptors were not excited by exogenous 5-HT initially, as Galligan (1992) reported that 5-HT_{1A} receptors are not present on postganglionic cholinergic motor neurons. Alternatively, exogenous 5-HT did not stimulate the postganglionic cholinergic neurons via preganglionic cholinergic neuronal activation, which is supported by our finding that hexamethonium did not affect 5-HT-induced contractions.

Both tetrodotoxin and atropine abolished the contractions induced by 5-HT at concentrations not exceeding 3×10^{-6} M, suggesting these responses were mediated entirely by postganglionic cholinergic neurons. The residual small contractions seen in the presence of 5-HT at concentrations above 3×10^{-6} M may have been mediated by 5-HT_{2A} receptors on the smooth muscle, because only ketanserin (10^{-8} M) and methysergide inhibited the contraction induced by 5-HT at concentrations above 3×10^{-6} M. The inhibition of the maximal response to 5-HT by methysergide may be attributable to the lack of selectivity of this drug.

Granisetron (10^{-8} M) only inhibited contractions induced by 5-HT in excess of 10^{-6} M, suggesting that contractions elicited by relatively high concentrations of 5-HT are mediated by 5-HT_3 receptors. GR113808A (10^{-8} M) abolished the response to 10^{-8} M 5-HT and steepened the concentration-response curve, suggesting that low concentrations of 5-HT contract the gastric antral muscle mainly via 5-HT_4 receptor activation. 5-HT_3 and 5-HT_4 receptors are known to be involved in the cholinergic pathways in the myenteric plexus of the guinea pig ileum (Kilbinger and Wolf, 1992) and colon (Elswood et al., 1991), and this localization is considered to be universal in the gastrointestinal tract (Read and Gwee, 1994). Briejer et al. (Briejer et al., 1993, 1995) also reported that contractions of the guinea pig colon induced by 5-HT in concentrations below 3×10^{-6} M and above 3×10^{-6} M were mediated by 5-HT_4 and 5-HT_3 receptors, respectively. Buchheit et al. (1985) reported that 5-HT induced biphasic contractions of guinea pig ileal longitudinal muscle and that the first phase (comprising responses to lower concentrations of 5-HT) was mediated by non-5-HT_3 receptors (which may be identical to 5-HT_4 receptors) and the second phase (comprising responses to higher concentrations of 5-HT) was mediated by 5-HT_3 receptors. The results of our study on the guinea pig gastric antrum are consistent with these findings. Contradictory to our results for the antrum, Buchheit et al. (1985) proposed that the second phase, i.e. responses to higher concentrations of 5-HT, was mediated by the action of substance P on guinea pig ileal smooth muscle. However, Fox and Morton (1991) reported that selective NK_1 receptor antagonist and/or desensitization with a selective NK_3 receptor agonist had no effect on 5-HT-induced contractions of the guinea pig ileum. The 5-HT-induced antral contractions observed in our study were considered to be mediated entirely by postganglionic cholinergic neurons, because atropine, but not FK888, virtually abolished them.

Ketanserin (10^{-8} M) inhibited the contractions evoked by 5-HT at concentrations not less than 10^{-7} M and made the curve biphasic, suggesting the involvement of at least two receptors, including 5-HT_{2A} receptors. According to Fox and Morton (1990), ketanserin does not inhibit the nerve-mediated guinea pig ileal contractions induced by 5-HT at concentrations up to 10^{-6} M. Engel et al. (1984) reported that ketanserin antagonized 5-HT_{2A} receptors on the smooth muscle of the guinea pig ileum pretreated with atropine and considered that 5-HT_{2A} receptors were localized on the smooth muscle. However, in our study, the

inhibition by ketanserin was unlikely to be attributable to antagonism of receptors on smooth muscle, as the antral contractions evoked by 5-HT at concentrations below 10^{-5} M were blocked completely by tetrodotoxin. At the concentration which inhibited antral contractions, ketanserin is generally regarded as a 5-HT_{2A} receptor antagonist in the guinea pig ileum (Engel et al., 1984). Moreover, the apparent pA_2 value of ketanserin was calculated to be 8.71 and the pIC_{50} was 8.1, which are very close to its pA_2 value of 8.5–9.4 for 5-HT_{2A} receptors reported by Bradley et al. (1986) and its pIC_{50} value of 8.16 for 5-HT-induced contractions of the guinea pig ileal longitudinal muscle in the presence of atropine (10^{-6} M) reported by Engel et al. (1984). These results suggest that 5-HT_{2A} receptors are localized on the gastric antral nerves and mediate 5-HT-induced neurogenic contractions through postganglionic cholinergic nerve activation.

Electrophysiological experiments with intracellular microelectrodes revealed the existence of orphan 5-HT receptors, called 5-HT_{1P} receptors, which mediate the slow excitatory postsynaptic potentials evoked by 5-HT, in the guinea pig ileal and gastric antral myenteric plexus as well as 5-HT_{1A} and 5-HT_3 receptors (Mawe et al., 1986; Gershon, 1991; Tack et al., 1992). Ketanserin has been reported to have no effect on 5-HT-induced slow excitatory postsynaptic potentials (Tack et al., 1992), suggesting that it has no effect on 5-HT_{1P} receptors. Therefore, the results of our study suggest that functional 5-HT_{2A} receptors exist on the nerves and mediate 5-HT-induced neurogenic contraction of the guinea pig antrum. Fiorica-Howells et al. (1993) reported that the 5-HT-induced increase in cAMP levels in ganglia isolated from the myenteric plexus of the guinea pig small intestine was inhibited by ketanserin.

Our conclusions, summarized in Fig. 10, are as follows. 5-HT-induced contraction of the gastric antrum is mediated mainly via postganglionic cholinergic neurons. At low and high concentrations, 5-HT induces contraction through 5-HT_4 and 5-HT_3 receptors, respectively, on the nerves. 5-HT_{2A} receptors are located on both nerves and muscle and are involved in 5-HT-induced contraction. 5-HT only acts on the 5-HT_{2A} receptors on the muscle at very high concentrations (over 3×10^{-6} M).

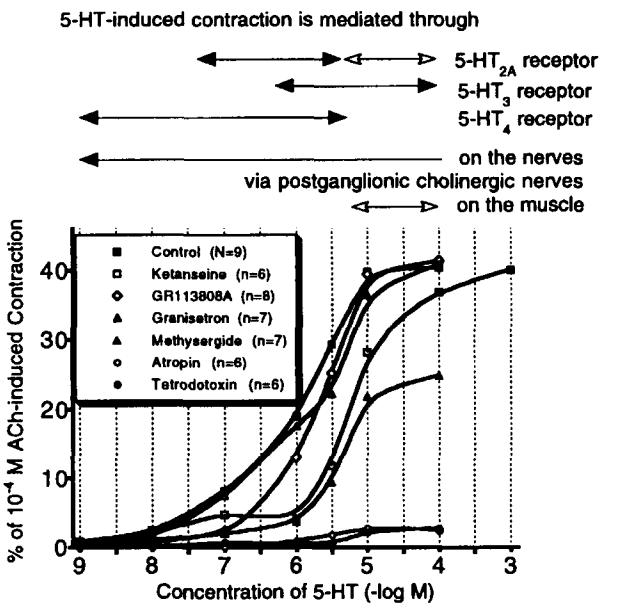

Fig. 10. Our proposed mechanisms of 5-HT-induced contraction of the guinea pig gastric antrum. 5-HT induces contraction mainly through postganglionic cholinergic neurons. At low concentrations, 5-HT induces contraction via 5-HT_4 receptors on the nerve, and at high concentrations, it does so via 5-HT_3 receptors on the nerves. Only a part of the contractile response to very high concentrations of 5-HT is mediated via 5-HT_{2A} receptors on the muscle. 5-HT_{2A} receptors also exist on the nerves and mediate 5-HT-induced contraction.

4.2. 5-HT-induced relaxation of the gastric corpus

4.2.1. The nature of 5-HT-induced relaxation of the gastric corpus

5-HT evoked concentration-dependent relaxations of the corpus, which were resistant to tetrodotoxin, atropine, phentolamine and propranolol. This suggests a direct action of 5-HT on the smooth muscle. As Moen et al. (1983), Yamaguchi (1972) and Kojima et al. (1992) have reported a similar relaxation by 5-HT in the gastric fundus of the guinea pig, we therefore presume that the mechanism of this relaxation is similar in the corpus and the fundus. It is unlikely that nitric oxide (NO) is involved in the relaxation in the corpus, since this study has shown that L-NNA, a selective inhibitor of NO-synthase (Mülsch and Busse, 1990), had no effect on the 5-HT-induced relaxation of the corpus. Therefore, 5-HT may be one of the final mediators involved in non-adrenergic non-cholinergic (NANC) relaxation. This finding disagrees with those of Meulemans et al. (1993), who demonstrated that the 5-HT-induced relaxation of guinea pig whole stomach was inhibited by both tetrodotoxin and L-NNA. This discrepancy could be explained by the difference between the total pressure changes in the whole stomach and the local responses limited regionally and directionally of circular strips of muscle. Another possible explanation is that the discrepancy results from the presence of atropine in their experiments. However, we consider the former explanation more likely, because Kojima et al. (1992) reported that 5-HT-induced relaxations of circular muscle strips of the guinea pig gastric fundus were not affected by tetrodotoxin in the presence of atropine and the 5-HT-induced relaxations observed in our present study were affected neither by 10^{-6} M atropine nor 3×10^{-6} M tetrodotoxin.

4.2.2. Subtypes of 5-HT receptor involved in 5-HT-induced relaxation of the gastric corpus

5-HT-induced relaxation of the gastric corpus was not antagonized by granisetron, GR113808A or ketanserin, suggesting that neither 5-HT_{2A}, 5-HT_3 nor 5-HT_4 receptors are involved in this relaxation, which is consistent with the results of Kojima et al. (1992).

Furthermore, NAN-190, which has been reported to act as a 5-HT_{1A} receptor antagonist, at concentrations up to 10^{-7} M had no effect on such relaxations. At 10^{-6} M, NAN-190 reduced the relaxant response and its pA_2 value was calculated to be 6.47, indicating that 5-HT_{1A} receptors are unlikely to mediate this response, as this value differs from that of 8.9 reported for 5-HT_{1A} receptors (Hoyer et al., 1994). This agrees with the conclusion of Kojima et al. (1992) that 5-HT_{1A} receptors are not involved in the 5-HT-induced relaxation in guinea pig gastric fundus, because 8-hydroxy-2-(di-*n*-propylamino)tetralin (8-OH-DPAT, a selective 5-HT_{1A} receptor agonist) failed to induce the full relaxation.

Methysergide up to 10^{-7} M also had no influence on 5-HT-induced corporal relaxation. At 10^{-6} M, methysergide reduced 5-HT-induced relaxation and its pA_2 value was calculated to be 6.71, which differs considerably from the reported value of 8.9 for 5-HT_{2C} receptors. Furthermore, methysergide, which has also been reported to act as an agonist at 5-HT_{1A}, 5-HT_{1B} and 5-HT_{1D} receptors, itself did not cause relaxation. Moreover, neither propranolol, which has been reported to have affinity for 5-HT_{1B} binding sites, nor ketanserin, which has low affinity for 5-HT_{2B} and 5-HT_{2C} receptors ($pK_i = 7.0$) (Baxter et al., 1995), at concentrations of up to 10^{-6} M, inhibited corporal relaxation. Therefore, we consider that 5-HT_{1A}, 5-HT_{1B}, 5-HT_{1D}, 5-HT_{2B} and 5-HT_{2C} receptors are also unlikely to mediate this response.

Relaxation of the corpus may be mediated by non-5-HT_{1A}, non-5-HT_{1B}, non-5-HT_{1D}, '5-HT_1-like receptors' which are antagonized by both methysergide and NAN-190. Methysergide has been suggested to be able to bind to 5-HT_5, 5-HT_6 and 5-HT_7 receptors and to antagonize 5-HT_7 (Hoyer et al., 1994) and some '5-HT_1-like' (Bradley et al., 1986; Dhasmana et al., 1993; Hoyer et al., 1994) receptors and NAN-190 has been suggested to be able to bind to 5-HT_7, but not to 5-HT_6, receptors (Hoyer et al., 1994). Therefore, the receptors which mediate 5-HT-induced relaxation of the guinea pig gastric corpus may be 5-HT_7 and/or 5-HT_5 receptors. Carter et al. (1995) reported that the postjunctional 5-HT receptors mediating guinea pig isolated ileal relaxation exhibited an operational profile similar to that of guinea pig cloned 5-HT_7 receptors.

The slight and transient increase in basal tone induced by L-NNA may have been the result of blockade of the basal tonic excitation of the inhibitory nerves, consistent with the finding that NO itself is involved in NANC relaxation of the proximal stomach (Kamata et al., 1993), although it was not involved in the 5-HT-induced relaxations seen in our study. Methysergide and NAN-190 also caused slight and transient increases in basal tone, which may also have been due to blockade of the basal tonic excitation of the inhibitory nerves, because these antagonists did inhibit 5-HT-induced relaxation in our study.

In conclusion, we have demonstrated and characterized the different motor activity responses of the guinea pig gastric antrum and corpus to 5-HT in vitro. 5-HT-induced antral contraction is mediated mainly by postganglionic cholinergic neurons via multiple 5-HT receptors, i.e., 5-HT_{2A}, 5-HT_3 and 5-HT_4 receptors. 5-HT-induced relaxation of the corpus is mediated by 5-HT_1-like receptors, which can be antagonized by methysergide and NAN-190 and may be 5-HT_7 and/or 5-HT_5 receptors.

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