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Novel 5-HT_2-like receptor mediates neurogenic relaxation of the guinea-pig proximal colon

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Abstract

The aim of the current investigation was to characterize the 5-HT receptors that mediate neurogenic relaxation of the guinea-pig proximal colon. After blockade of 5-HT_{2A}, 5-HT_3 and 5-HT_4 receptor-mediated contractions, 5-hydroxytryptamine (5-HT) induced relaxations yielding a biphasic concentration-response curve. Other tryptamines were also agonists with the following rank order of potency: 5-HT > 5-carboxamidotryptamine = 5-methoxytryptamine ≥ α-methyl-5-HT (partial agonist) > tryptamine (partial agonist). 5-Hydroxytryptophan, 2-methyl-5-HT and *N*-methyltryptamine were virtually inactive as agonists. The curve to 5-HT was not affected by pargyline, citalopram, phentolamine, or by the 5-HT_4 receptor antagonists 2-methoxy-4-amino-5-chloro-benzoic acid 2-(diethylamino)ethyl ester (SDZ 205-557) and (1-butyl-4-piperidinylmethyl)-8-amino-7-chloro-1,4-benzodioxan-5-carboxylate (SB 204070). 8-Hydroxy-2-(di-*n*-propylamino)-tetralin (8-OH-DPAT), 5-methoxy-3[1,2,3,6-tetrahydroxypyridin-4-yl]-1*H*-indole (RU 24969), 2-(2,6-dimethoxyphenoxyethyl)aminomethyl-1,4-benzodioxane (WB 4101), 1-(3-chlorophenyl)piperazine (mCPP), 1-(*m*-trifluoromethylphenyl)-piperazine (TFMPP), flesinoxan, sumatriptan and 6-chloro-2-(piperazinyl)-pyrazine (MK212) were inactive as 5-HT receptor agonists. The first phase of the curve to 5-HT was inhibited by: metergoline ($pA_2 = 8.8 \pm 0.3$, against 5-methoxytryptamine 9.3 ± 0.3), methysergide (non-surmountable), methiothepin (non-surmountable), spiroxatrine (non-surmountable), MK212 (non-surmountable), mesulergine (7.8 ± 0.3), mCPP (7.1 ± 0.1), mianserin (7.0 ± 0.4), ritanserin (8.9 ± 0.2), rauwolscine (7.0 ± 0.2), yohimbine (6.2 ± 0.2), 1-(1-naphthyl)-piperazine (7.7 ± 0.2) and RU 24969 (6.4 ± 0.1), but not by 1-(2-methoxyphenyl)-4-[4-(2-phthalimidobtyl]-piperazine (NAN-190), spiperone, sumatriptan, 8-OH-DPAT and flesinoxan. It is suggested that the 5-HT receptor under study could be considered an unknown 5-HT_2-like receptor.

Keywords: 5-HT receptor; Colon, guinea pig; Gastrointestinal motility; Enteric nerve; Relaxation

1. Introduction

In the guinea-pig gastrointestinal tract, several receptors for serotonin (5-hydroxytryptamine; 5-HT) have been identified that are involved in motor responses: classical 5-HT_2 receptors mediating smooth muscle contraction (this receptor is currently designated 5-HT_{2A}, Humphrey et al., 1993), and 5-HT_3 and 5-HT_4 receptors on the enteric nerves, which cause an increase in acetylcholine release upon stimulation (Engel et al., 1984; Elswood et al., 1991; Briejer et al., 1993). Furthermore, acetylcholine release-inhibiting prejunctional 5-HT_{1A} receptors have been identified on the myenteric nerves (Fozard and Kilbinger, 1985; Galligan, 1992). However, several studies have appeared, presenting data on 5-HT receptors in guinea-pig gastrointestinal tissues that cannot be classified according to the 5-HT receptor classification scheme as proposed by Humphrey et al. (1993). In the stomach fundus and in the ileum, 5-HT receptors have been identified on the smooth muscle with 5-HT_1-like properties (Feniuk et al., 1983; Kalkman et al., 1986; Kojima et al., 1992). In the proximal colon, neuronal 5-HT receptors were found to mediate relaxation involving nitric oxide (Kojima, 1991; Elswood and Bunce, 1992;

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Briejer et al., 1992,1995). Classically, responses to 5-HT that are inhibited by the 5-HT_1/5-HT_2 receptor antagonists methiothepin and methysergide, but not by the 5-HT_2 receptor antagonist ketanserin and a 5-HT_3 receptor antagonist like tropisetron, would be designated as mediated by 5-HT_1 receptors (Bradley et al., 1986). Based on these criteria, Kojima (1991) and Elswood and Bunce (1992) suggested that the 5-HT receptor that mediates relaxation in the guinea-pig colon was a 5-HT_1 receptor subtype. As in the meantime many new receptor subtypes were characterized, the 5-HT receptor classification scheme was extended, and new criteria for classification were proposed (Humphrey et al., 1993). According to these novel guidelines, some of the above described 'orphan' receptors would probably not be designated 5-HT_1-like.

The aim of the current study was to try and characterize the high-affinity 5-HT receptor mediating relaxation of the guinea-pig proximal colon longitudinal muscle. We therefore tested both 5-HT receptor agonists and antagonists and compared observed activity and affinity to literature data, in the view of the novel 5-HT receptor classification scheme.

2. Materials and methods

2.1. Preparation

Dunkin-Hartley guinea-pigs of both sexes, weighing 400–600 g, were killed by stunning and decapitation. The proximal colon was removed, and the luminal contents were washed out with De Jalon solution (mM: KCl 5.6, $CaCl_2$ 0.5, $NaHCO_3$ 6.0, NaCl 155, D-(+)-glucose 2.8). The mesentery was carefully removed. Starting at the proximal end, about 1 cm distal from the caecum, the colon was divided into four segments of circa 2.5 cm. These intact segments were individually mounted vertically for isotonic measurement into a tissue bath containing 20 ml De Jalon solution. This solution was kept at 37° C and gassed with 95% O_2, 5% CO_2. The tissues were subjected to a load of 2 g and allowed to stabilize for half an hour. Ketanserin (0.3 μM) and tropisetron (3 μM) were continuously present in the De Jalon solution during all experiments to block contractile responses mediated by 5-HT_{2A}, 5-HT_3 and 5-HT_4 receptors. After stabilization, the longitudinal muscle was challenged with 0.3 μM methacholine. After washing and 10 min stabilization, the procedure was repeated in order to stabilize the response of the tissue.

2.2. Protocols

For the construction of concentration-response curves, drugs were applied directly to the tissue bath (added volume $\leq$ 1% of tissue bath volume). Methacholine (0.3 μM) was added to the bath in order to precontract the muscle.

As the relaxations to 5-HT were previously shown to be prone to desensitization (Briejer et al., 1992), non-cumulative concentration-response curves to the agonists were established, beginning 10 min after the administration of methacholine. As soon as a maximum relaxation to a concentration of agonist occurred (after 2–4 min), methacholine and the agonist were washed out by replacing the bathing fluid twice and stabilization of 10 min was allowed. This 20 min dosing cycle was repeated applying the next concentration of agonist (ascending order). The response to repetitive administration of methacholine was stable throughout the experiment. Each segment was first challenged with 10 μM 5-HT in the absence of an antagonist (ketanserin and tropisetron present). After washout, this cycle was repeated, applying again 10 μM 5-HT. The relaxation to this latter administration of 5-HT was taken as 100% relaxation. Consecutively, drugs that might interfere with the agonist under study were administered, and were left with the preparations for 15 min. Then, non-cumulative concentration-response curves were established, applying the above described 20 min dosing cycle. Drugs were re-added directly after each washout. Of 4 segments that were taken from each animal, one strip was utilized as a control (i.e. one curve per preparation).

For the construction of concentration-response curves to methacholine, a similar protocol was used, but the concentrations were added cumulatively instead of non-cumulatively. Hence, first a curve was established in the absence of the drug to be tested, and then, after washout, drug addition and 15 min stabilization the curve was repeated (2 curves per preparation). Concentration-response curves to methacholine were reproducible.

2.3. Data analysis

Means $\pm$ standard error of the mean (S.E.M.) were calculated. Differences between control and treated segments were evaluated with one-way analysis of variance (ANOVA) and subsequently Dunnett's t-test for multiple comparisons (Wallenstein et al., 1980). The level of significance was set at $P < 0.05$. EC_{50} values were determined with linear regression analysis. Only the first phase of the concentration-response curves to 5-HT (0.01–1 μM) and 5-methoxytryptamine (0.1–10 μM) were considered for the calculation of affinity parameters. The maximum effects of the first phases were estimated with a Lineweaver-Burk plot followed by linear regression analysis (Tallarida and Murray, 1981). pA_2 values and their standard errors were estimated with the Schild-Gaddum equation, or were de-

termined with a Schild plot, according to the methods described by Tallarida and Murray (1981). In some cases of non-surmountable antagonism, a pD'_2 value (−log (concentration) of antagonist that causes 50% depression) was calculated according to Van Rossum (1963).

2.4. Chemicals

The following drugs were used: ketanserin tartrate, tropisetron, (1-butyl-4-piperidinylmethyl)-8-amino-7-chloro-1,4-benzodioxan-5-carboxylate (SB 204070), 2-methoxy-4-amino-5-chloro-benzoic acid 2-(diethylamino)ethyl ester (SDZ 205-557), 1-(2-methoxyphenyl)-4-[4-(2-phthalimidobtyl]-piperazine hydrochloride (NAN-190), spiperone, 1-(3-chlorophenyl)piperazine (mCPP), 1-(*m*-trifluoromethylphenyl)piperazine cyclohexanesulfamate (TFMPP), 2-methyl-5-hydroxytryptamine, 8-hydroxy-2-(di-*n*-propylamino)-tetralin hydrobromide (8-OH-DPAT), phentolamine HCl, sumatriptan, mesulergine HCl, ritanserin, spiroxatrine (Janssen Research Foundation, Belgium), metergoline (Gruppo Montedison, Italy), methiothepin maleate (Hoffmann-La Roche, Switzerland), dopamine HCl, 5-hydroxytryptophan, *N*-methyltryptamine, tryptamine HCl, 5-methoxytryptamine HCl (Janssen Chimica, Belgium), methacholine HCl (E. Merck, Germany), 5-hydroxytryptamine creatinine sulphate (Serva, Germany), paroxetine HCl (Ferrosan, Denmark), citalopram HBr (Lundbeck, Denmark), pargyline HCl (Abbott, USA), 5-carboxamidotryptamine maleate, α-methyl-5-hydroxytryptamine maleate (Cookson Chemicals, UK), 1-(1-naphthyl)-piperazine HCl (Lilly, UK), flesinoxan HCl (Duphar, Netherlands), mianserin HCl (Organon, Netherlands), methysergide maleate (Sandoz, Switzerland), 6-chloro-2-(piperazinyl)-pyrazine hydrochloride (MK212) (MSD, USA), rauwolscine HCl (Carlrooth, UK), 5-methoxy-3-[1,2,3,6-tetrahydroxypyridin-4-yl]-1*H*-indole hemitartrate (RU 24969) (Roussel-Uclaf, France), yohimbine HCl (Sigma, Belgium), 2-(2,6-dimethoxyphenoxyethyl)-aminomethyl-1,4-benzodioxane (WB 4101) (Amersham, UK). All compounds were dissolved in distilled water, except for the bases, which were dissolved in distilled water acidified with tartaric acid in the stock solution (pH ≥ 3). The stock solution for 5-HT contained 0.25 μM ascorbic acid. This vehicle had no effect on the strips.

3. Results

3.1. 5-HT and analogues

As previously described (Briejer et al., 1992, 1995), 5-hydroxytryptamine induced relaxations from 10 nM onwards, yielding a biphasic concentration-response curve (Fig. 1). The relaxations reached a maximum effect in 1–2 min, and then decayed in the course of minutes. The first phase ranged from 10 nM to 1 μM (maximum 83%; $pEC_{50} = 7.00 \pm 0.04$; $n = 79$), and the second phase ranged from 1 μM to 100 μM. 5-Methoxytryptamine and 5-carboxamidotryptamine induced relaxations, yielding a biphasic and a monophasic concentration-response curve, respectively (pEC_{50} values 6.16 ± 0.03 and 6.10 ± 0.02 respectively; $n = 22$ and 10) (Fig. 1). α-Methyl-5-HT and tryptamine also induced relaxations, but with a lower potency and efficacy (Fig. 1). 5-Hydroxytryptophan, 2-methyl-5-HT and *N*-methyltryptamine were virtually inactive (Fig. 1).

3.2. Other putative 5-HT receptor agonists

Relaxations to 5-HT are highly sensitive to the NO synthase inhibitor N^G-nitro-L-arginine (L-NNA; 100 μM) (Briejer et al., 1992,1995) as well as to methysergide (1 μM) (see below). All compounds mentioned in Table 1 induced relaxations only at micromolar concentrations, though these relaxations were not sensitive to L-NNA and/or to methysergide. Thus, a mechanism other than 5-HT receptors was suspected to account for the relaxations to these compounds. Therefore, these compounds (except for WB4101) were tested against contractions to methacholine, and it was found that they shifted and/or depressed the concentration-response curve to methacholine (see Table 1). Sumatriptan (100 μM) did not affect the concentra-

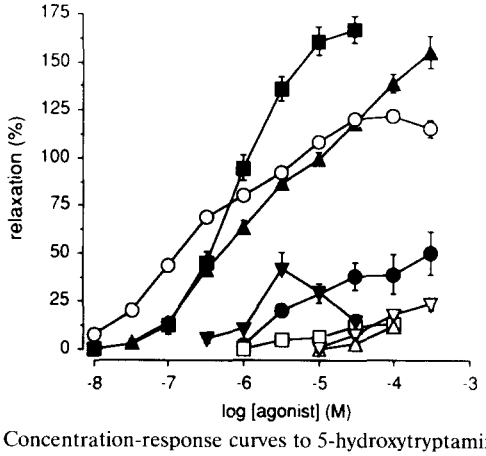

Fig. 1. Concentration-response curves to 5-hydroxytryptamine (5-HT) (○; $n = 79$) and its analogues 5-carboxamidotryptamine (■, $n = 10$), 5-methoxytryptamine (▲, $n = 22$), α-methyl-5-HT (▼, $n = 4$), tryptamine (●, $n = 8$), 2-methyl-5-HT (□, $n = 4$), *N*-methyltryptamine (△, $n = 6$) and 5-hydroxytryptophan (▽, $n = 4$). All experiments were done in the presence of ketanserin 0.3 μM and tropisetron 3 μM, and the strips were precontracted with methacholine 0.3 μM. Relaxations were expressed as a percentage of relaxations induced by 5-HT 10 μM. Points represent the mean ± S.E.M.

Table 1
Intrinsic effects of drugs and effects against 5-HT- and methacholine-induced responses on the guinea-pig colon

Drug	Induces relaxations exceeding	Inhibition of contractions [a] to methacholine	Inhibition of relaxations to 5-HT	Sensitive to	
				Methysergide	L-NNA
8-OH-DPAT	1 μM	$pA_2 = 5.2$ [b]; depression 40% (100 μM)	1 μM no effect	No	NT
Dopamine	100 μM	NT	NT	NT	No
Flesinoxan	100 μM	Depression 60%; no shift (100 μM)	1 μM no effect	No	NT
mCPP	30 μM	$pA_2 \sim 4.5$; 30% depression (100 μM)	NT	No	NT
TFMPP	10 μM	$pA_2 \sim 5$; 20% depression (30 μM)	NT	No	NT
MK212	3 μM	$pA_2 \sim 5.5$	$pD'_2 = 5.6$	No	NT
WB4101	10 μM	NT	NT	No	No
Sumatriptan	30 μM	100 μM no effect	1 μM no effect	NT	No [c]
RU 24969	30 μM	$pA_2 \sim 4.5$; 20% depression (100 μM)	$pA_2 = 6.4 \pm 0.1$	No	No

All experiments were performed in the presence of ketanserin (0.3 μM) and tropisetron (3 μM). [a] A pA_2 value was estimated with the Schild equation, if possible. [b] pA_2 value of 8-OH-DPAT 10 μM and 30 μM respectively 5.20 ± 0.01 and 5.20 ± 0.09. [c] Relaxations to 5-HT, but not to sumatriptan, were abolished by tetrodotoxin 0.3 μM. NT = not tested.

tion-response curve to methacholine, suggesting even a different mode of action ($n = 4$; not shown).

3.3. Various drugs

Relaxations to 5-HT were not affected by the α-adrenoceptor antagonist phentolamine (1 μM; $n = 4$) (not shown). The monoamine oxidase inhibitor pargyline (1–10 μM) depressed the concentration-response curves to 5-HT, 5-carboxamidotryptamine and 5-methoxytryptamine slightly, but no shift to the left (potentiation) was observed ($n = 4$; not shown). Incubation with the 5-HT uptake blocker citalopram (1 μM) had no effect ($n = 4$; not shown) on 5-HT-induced

Table 2
Estimated pEC_{50} and pA_2 values of 5-HT receptor ligands against the high-affinity phase of the concentration-relaxation curve to 5-HT, as compared to literature pEC_{50} values and pA_2 values/pK_i binding affinities

Agonist	$pEC_{50} \pm$ S.E.M.	n	5-HT_{1A}	5-HT_{1B}	5-HT_{1D}	5-HT_{1E}	5-HT_{1F}	5-HT_{2A}	5-HT_{2B}	5-HT_{2C}
5-HT	7.00 ± 0.04	79	7.6	7.8	7.6	7.3	8.1	7.6	8.0–8.4	6.5
5-Carboxamidotryptamine	6.10 ± 0.02	10	8.6	7.9	8.1	4.7	6.0	3.5	6.9–7.6	5.7
5-Methoxytryptamine	6.16 ± 0.03	22	8.0 [a]	6.4 [a]	8.4 [a]	5.3	5.9 [a]	5.5 [a]	7.8	7.6 [a]
α-Methyl-5-HT	~ 5.7	4	–	–	–	6.9 [a]	6.7 [a]	7.3	8.0	7.3
Tryptamine	~ 5.5	6	6.8 [a]	5.0 [a]	7.1 [a]	5.6–6.5 [a]	5.6 [a]	6.0 [a]	6.7–7.2	7.3
2-Methyl-5-HT	i	6	5.8 [a]	6.1 [a]	5.8 [a]	6.1 [a]	6.4 [a]	< 5.0 [a]	6.2–7.0	6.3 [a]
Antagonist	**$pA_2 \pm$ S.E.M.**	**n**	**5-HT_{1A}**	**5-HT_{1B}**	**5-HT_{1D}**	**5-HT_{1E}**	**5-HT_{1F}**	**5-HT_{2A}**	**5-HT_{2B}**	**5-HT_{2C}**
Ritanserin	8.9 ± 0.2	6	5.2–6.1	4.0–5.8	5.8–6.4	–	–	8.5–9.3	8.3	8.6–9.3
Metergoline	8.8 ± 0.3 [b]	6	8.1	7.4	9.1	6.0	6.5	8.5 [d]	8.5–9.0 [d]	10.6 [d]
Mesulergine	7.8 ± 0.3 [g]	6	6.2–6.8	4.9–5.9	5.2–5.4	–	< 5.0	9.1 [d]	7.4	9.1 [d]
1-(1-Naphthyl)-piperazine	7.7 ± 0.2	6	7.2	6.6	–	6.7	7.3	7.2	8.9–9.0 [d]	8.2
mCPP	7.1 ± 0.1	6	6.5	6.6	5.8	5.5	–	6.7	7.9 [d]	7.7
Mianserin	7.0 ± 0.4	6	6.0	6.0 [d]	6.5 [d]	7.0	–	10.1 [d]	6.7–7.3	8.0 [d]
Rauwolscine	7.0 ± 0.2	8	6.9	5.3	7.7	5.4	6.0	6.1	8.5 [d]	5.8
RU 24969	6.4 ± 0.1	5	8.1	8.2–8.4	7.3–7.4	7.2	–	5.8–6.0	7.5 [d]	6.5–7.3
Yohimbine	6.2 ± 0.2	8	6.9	5.5	7.1	5.9–6.4	7.0	6.0	7.9–8.2 [d]	4.4
NAN-190	0.3 μM i	6	8.9	6.2	6.7	–	6.7	6.7	–	6.2
Sumatriptan	1.0 μM i	4	6.1–6.6	6.4–6.8	7.2–7.5	5.6–5.7	7.6	3.7	< 5.0	4.1–5.1
8-OH-DPAT	1.0 μM i	4	8.6–8.7	4.2–5.8	5.9–6.0	5.5–6.1	5.8	< 5.0–5.0	5.1–5.4	5.1–5.2
Flesinoxan	1.0 μM i	3	8.8	6.1	6.8	–	–	5.4	–	< 5.0
Spiperone	1.0 μM i [c]	6	7.2 [d]	4.4 [d]	4.8 [d]	5.0–5.3	< 5.0	8.8–9.4	< 4.0–5.5	5.9
Ketanserin	3.0 μM i	4	< 5.0–5.9	< 5.0–5.7	5.7–6.0	4.1	< 5.0	9.3 [d]	5.4	6.6 [d]
Spiroxatrine	nd	6	8.1–8.4	3.6–3.9	5.1	–	–	6.2–6.9	< 5.0 [d]	5.1
MK212	5.6 [e]	3	5.3	5.0	–	4.3	–	4.8	< 4–6.4	6.2
Methysergide	9.0 [e]	4	7.6	5.8	8.4	6.5–7.2	7.5	8.6	7.1–8.2 [f]	8.6
Methiothepin	7.8 [e]	6	7.1	7.3	6.3	6.7–6.9	6.2	8.8	–	7.6

i = inactive, nd = not determined; [a] pK_i binding affinity; [b] pA_2 against 5-MeOT 9.3 ± 0.3 ($n = 6$), estimated with metergoline 3 nM; [c] depression of second phase of concentration-relaxation curve to 5-HT; [d] pA_2 value instead of pK_i binding affinity; [e] pD'_2 values; [f] $pIC_{50} = 8.7$; [g] pA_2 estimated with 30 nM mesulergine, estimation with 100 nM mesulergine yields a pA_2 of 7.9 ± 0.1 ($n = 3$). Literature affinity data inferred from: Clineschmidt et al., 1985; Nelson and Taylor, 1986; Cohen and Fludzinski, 1987; Hoyer, 1988; Leonhardt et al., 1989; Van Wijngaarden et al., 1990; Hoyer and Schoeffter, 1991; Kalkman and Fozard, 1991; Foguet et al., 1992; Kursar et al., 1992; Zgombick et al., 1992; Adham et al., 1993; Beer et al., 1993; Gudermann et al., 1993; Wainscott et al., 1993.

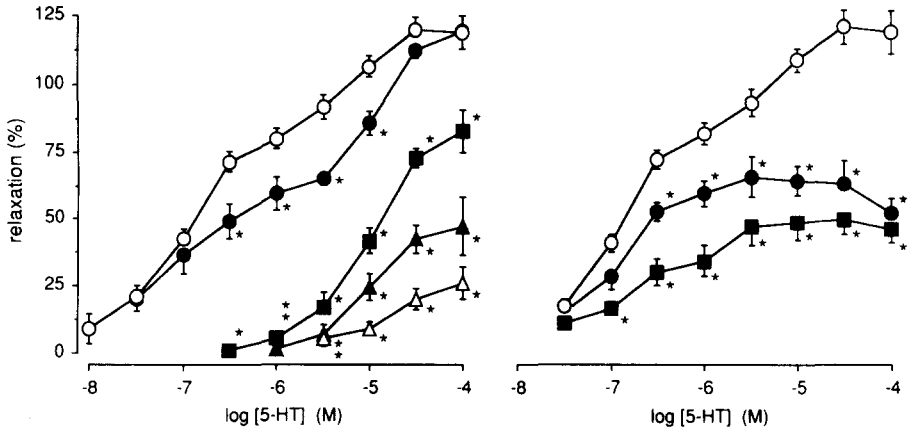

Fig. 2. Concentration-response curves to 5-HT in the absence (○; $n = 6$–10) and presence of: (left panel) methysergide 0.3 nM (●, $n = 4$), 3 nM (■, $n = 4$), 30 nM (▲, $n = 6$) and 300 nM (△, $n = 6$); (right panel) methiothepin 3 nM (●, $n = 6$) and 30 nM (■, $n = 6$). All experiments were done in the presence of ketanserin 0.3 μM and tropisetron 3 μM, and the strips were precontracted with methacholine 0.3 μM. Relaxations were expressed as a percentage of relaxations induced by 5-HT 10 μM. Points represent the mean ± S.E.M. Mean values that are significantly different from control ($P < 0.05$) are marked with an asterisk.

relaxations. Hence, in further experiments no α-adrenoceptor antagonist, monoamine oxidase inhibitor or uptake blocker was present.

3.4. 5-HT receptor antagonists

Increasing the concentration of ketanserin (a selective 5-HT_{2A} receptor antagonist, Table 2) 10-fold to 3 μM, had no significant effect on the concentration-response curve to 5-HT, as compared to the 'standard' concentration of 0.3 μM ($n = 8$; not shown). The 5-HT_4 receptor antagonists SDZ 205-557 (1 μM) or SB 204070 (10 nM) had also no effect ($n = 6$–7, not shown).

The 5-HT_1/5-HT_2 receptor antagonist methysergide (Table 2) inhibited both the first and second phase of the concentration-response curve to 5-HT, though to a different extent (Fig. 2). At 0.3 nM, the first phase was depressed relatively more than the second phase ($n = 4$), while at 3 nM ($n = 4$), the first phase was almost blocked. Increasing the concentration methysergide to 30 nM and 300 nM ($n = 6$) also depressed the second phase to an increasing extent (Fig. 2). For inhibition of the first phase, a pD'_2 value of 9.0 was estimated. The 5-HT_1/5-HT_2 receptor antagonist methiothepin (Table 2) (3 and 30 nM; $n = 6$) preferentially depressed the second phase of the concentration-response curve to 5-HT, though the first phase was also depressed (Fig. 2). The 5-HT_{1A}/5-HT_{2A} receptor antagonist spiperone (Table 2) (1 μM) only depressed the second phase of the curve to 5-HT ($n = 6$), leaving the first phase unaffected (Fig. 3). The 5-HT_{1A} receptor antagonist spiroxatrine (Table 2) (0.3 μM) virtually blocked the first phase of the concentration-response curve to 5-HT ($n = 6$), but at a concentration of 3 nM, it had no effect. Only in the concentration range coinciding with the second phase, relaxations were not fully blocked by spiroxatrine (0.3 μM) (Fig. 3). Neither the selective 5-HT_{1A} receptor antagonists NAN-190 (0.3 μM), flesinoxan (1 μM) and the 5-HT_{1A} receptor agonist 8-OH-DPAT (1 μM), nor the moderately selective 5-HT_{1D} receptor agonist sumatriptan (1 μM) (see for selectivities Table 2) did affect the relaxations to 5-HT ($n = 3$–6). Metergoline (5-HT_1/5-HT_2 receptor antagonist, Table 2) (3, 10 and 30 nM, $n = 6$) inhibited the relaxations to 5-HT and 5-methoxytryptamine in a surmountable fashion. As can be seen from Fig. 4, metergoline preferentially shifted the first phase of the concentration-response curves to 5-HT. Schild analysis of the inhibition against 5-HT yielded a slope of 1.01 ± 0.21 (Fig. 4) and a pA_2 of

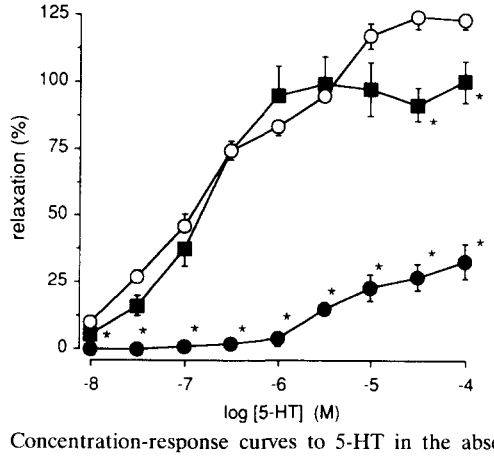

Fig. 3. Concentration-response curves to 5-HT in the absence (○; $n = 6$) and presence of: spiroxatrine 0.3 μM (●, $n = 6$) and spiperone 1 μM (■, $n = 6$). All experiments were done in the presence of ketanserin 0.3 μM and tropisetron 3 μM, and the strips were precontracted with methacholine 0.3 μM. Relaxations were expressed as a percentage of relaxations induced by 5-HT 10 μM. Points represent the mean ± S.E.M. Mean values that are significantly different from control ($P < 0.05$) are marked with an asterisk.

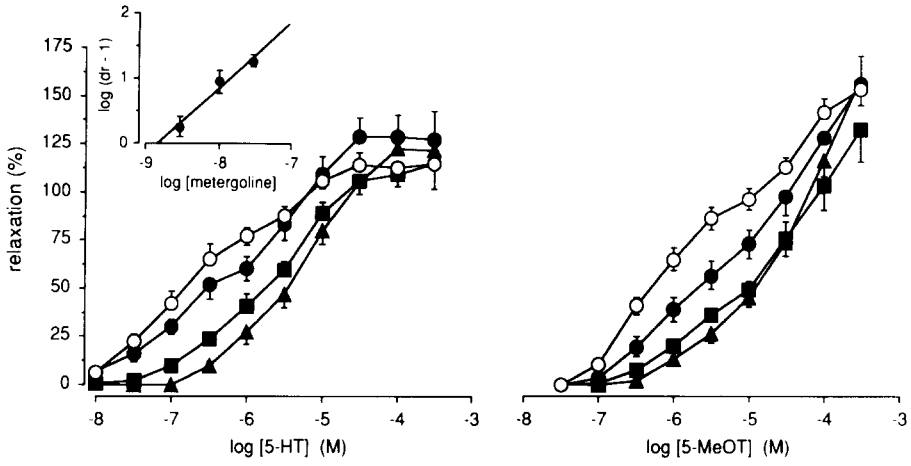

Fig. 4. Concentration-response curves to 5-HT (left panel) and 5-MeOT (right panel) in the absence (○; $n = 6$) and presence of metergoline 3 nM (●, $n = 6$), 10 nM (■, $n = 6$) and 30 nM (▲, $n = 6$). All experiments were done in the presence of ketanserin 0.3 μM and tropisetron 3 μM, and the strips were precontracted with methacholine 0.3 μM. Relaxations were expressed as a percentage of relaxations induced by 5-HT 10 μM. Points represent the mean ± S.E.M. The inserted figure on the left represents a Schild plot (dr = dose ratio). The line was fitted with the least-squares method, and did not significantly differ from unity. All concentrations caused a significant shift of the EC_{50} of the high-affinity phase of the concentration-response curve to 5-HT and 5-methoxytryptamine ($P < 0.05$), except for 3 nM against 5-HT ($P < 0.10$).

8.8 ± 0.3 (constrained at unity: $pK_b = 8.9 \pm 0.1$). Schild analysis of the antagonism against 5-methoxytryptamine, however, yielded a slope (0.58 ± 0.26) that differed from unity. The pA_2 was estimated by using only the shift caused by 3 nM metergoline, and was calculated to be 9.3 ± 0.3. Yohimbine (1 μM), rauwolscine (1 μM), 1-(1-naphthyl)-piperazine (0.3 μM), mianserin (0.3 μM), ritanserin (0.1 μM), RU 24969 (1 μM), mesulergine (0.03 and 0.1 μM) and mCPP (1 μM) (compounds that all interfere with 5-HT_1 and/or 5-HT_2

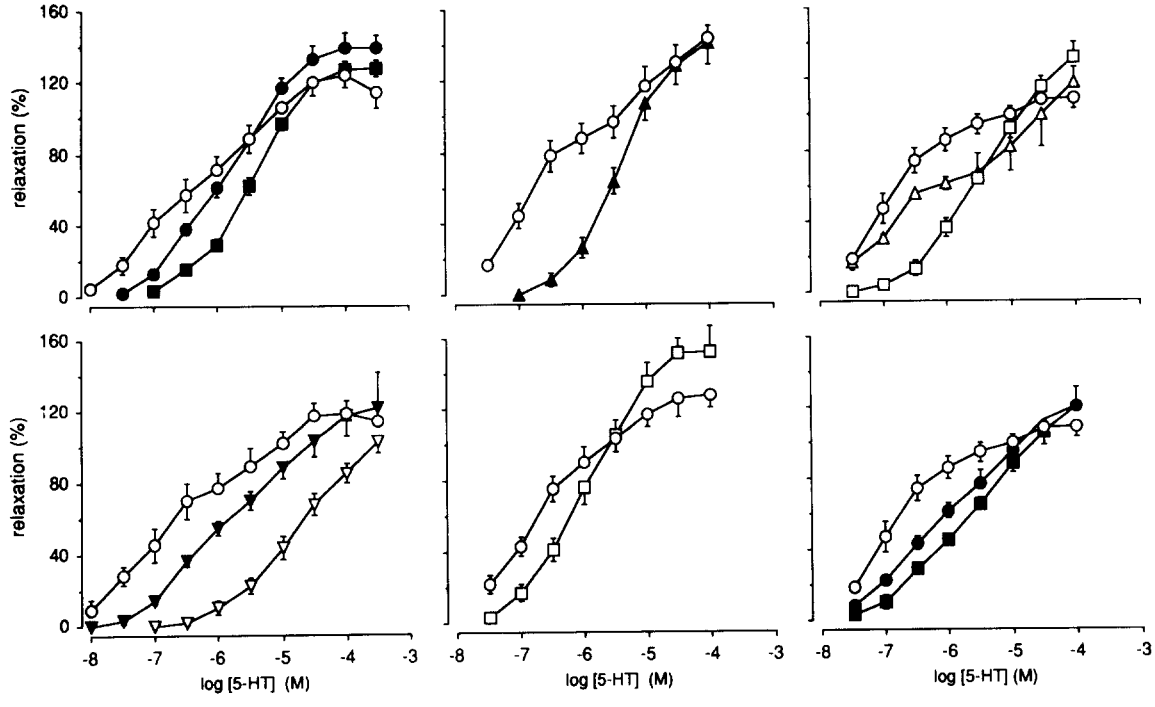

Fig. 5. Concentration-response curves to 5-HT in the absence (○; $n = 5$–8) and presence of: (upper left panel) yohimbine 1 μM (●, $n = 8$), rauwolscine 1 μM (■, $n = 8$); (upper middle panel) 1-(1-naphthyl)-piperazine 0.3 μM (▲, $n = 6$); (upper right panel) MK212 1 μM (△, $n = 3$), mCPP 1 μM (□, $n = 6$); (lower left panel) mianserin 0.3 μM (▼, $n = 6$), ritanserin 0.1 μM (▽, $n = 6$); (lower middle panel) RU 24969 1 μM (□, $n = 5$); (lower right panel) mesulergine 0.03 μM (●, $n = 6$) and 0.1 μM (■, $n = 3$). All experiments were done in the presence of ketanserin 0.3 μM and tropisetron 3 μM, and the strips were precontracted with methacholine 0.3 μM. Relaxations were expressed as a percentage of relaxations induced by 5-HT 10 μM. Points represent the mean ± S.E.M. All antagonists caused a significant shift of the EC_{50} of the high affinity phase of the concentration-response curve to 5-HT ($P < 0.05$).

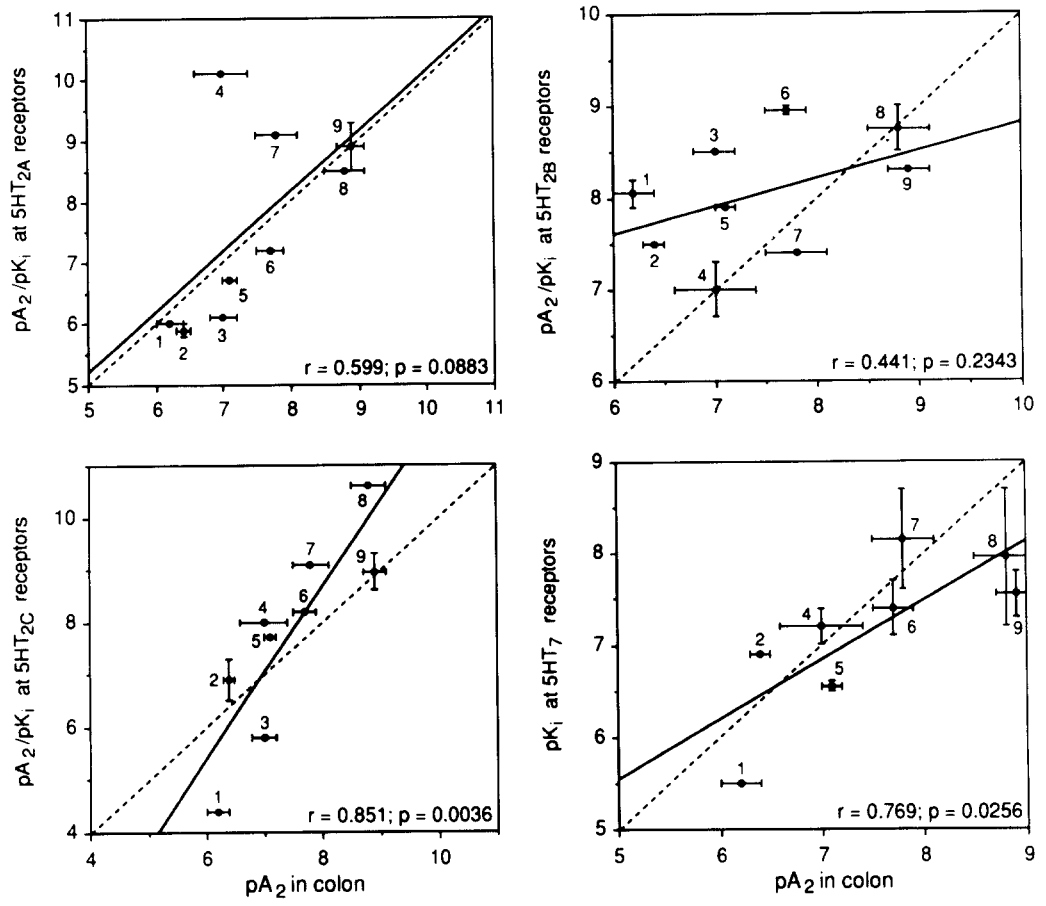

Fig. 6. Correlations of affinity values of antagonists for 5-HT_{2A}, 5-HT_{2B}, 5-HT_{2C} and 5-ht_7 receptors on one hand, and for the relaxation-mediating 5-HT receptor in the guinea-pig colon on the other hand. pA_2 values (±S.E.M.) in the colon of 8–9 compounds (x-axis) are plotted against pA_2 values or, if not available, pK_i binding values inferred from the literature (see also Table 2) on the y-axis. In case more than 2 values were found in literature, the value inbetween the extremes was used (the error bar represents the extreme values found in literature). The lines were fitted with the least-squares method (r represents the correlation coefficient) and analysed statistically with ANOVA followed by the F-test for significance of correlation, denoted with p. Data were obtained from the following compounds: yohimbine (1), RU 24969 (2), rauwolscine (3), mianserin (4), mCPP (5), 1-(1-naphthyl)-piperazine (6), mesulergine (7), metergoline (8) and ritanserin (9). The upper left panel shows the correlation with 5-HT_{2A} receptor affinity values, the upper right panel with 5-HT_{2B} receptors, lower left panel with 5-HT_{2C} receptors and the lower right panel with 5-ht_7 receptors.

receptors with variable selectivities: see Table 2 for affinities) ($n = 5–8$) inhibited the relaxations to 5-HT surmountably, causing a shift of the first phase of the concentration-response curve to 5-HT (Fig. 5; for estimated pA_2 values see Table 2). MK212 (1 μM) caused a depression (rather than a shift to the right) of the first but not the second phase of the concentration-response curve to 5-HT ($n = 3$; Fig. 5).

Of the antagonists tested, 9 allowed estimation of a pA_2 value. These values correlated near-significantly with literature affinity values for 5-HT_{2A} receptors ($r = 0.599$; $P = 0.0883$; slope = 0.983; $n = 9$) and significantly for 5-HT_{2C} receptors ($r = 0.851$; $P = 0.0036$; slope = 1.648; $n = 9$), as shown in Fig. 6. No significant correlation was found with affinity values for 5-HT_{1A}, 5-HT_{1B}, 5-HT_{1D}, 5-HT_{1E}, 5-HT_{1F}, 5-HT_{2B} receptors (see Fig. 6). Comparison of the affinities of antagonists in the colon with those for the recently cloned 5-ht_{5A}, 5-ht_{5B}, 5-ht_6 and 5-ht_7 receptors yielded only a significant correlation with affinity values for 5-ht_7 receptors ($r = 0.769$; $P = 0.0256$; slope = 0.647; $n = 8$) (Fig. 6).

4. Discussion

The concentration-response curve to 5-HT in the guinea-pig colon preparation was biphasic, suggesting that two receptors are involved. Though the author did not make special reference to it, the concentration-relaxation curve to 5-HT in the identical preparation as used by Kojima (1991) was also biphasic, with an inflection at a similar concentration of 5-HT. As compared to our experimental circumstances (intact segments, 5-HT_2, 5-HT_3 and 5-HT_4 receptors blocked), Kojima used strips of which the mucosa was removed, atropine was present in the bath solution to prevent cholinergic

contractions via 5-HT_3 and 5-HT_4 receptors and ketanserin (10 μM) to block 5-HT_2 receptors, and the strips were not precontracted (Kojima, 1991). The observed differential inhibition of these two phases, notably by methysergide, methiothepin, spiroxatrine, spiperone, but also by other compounds, also suggests a two-receptor system. As the low-affinity receptor, which mediates the second phase, is much more difficult to quantatively analyze, the receptor characterization as described below focussed only on the 5-HT receptor that accounts for the first, high-affinity phase of the concentration-response curve to 5-HT.

In the guinea-pig gut, 5-HT_{1A} receptors are known to be present on the enteric neurons (Fozard and Kilbinger, 1985; Galligan, 1992). For 5-HT_{1A} receptors, some selective compounds are available (see Table 2), e.g. 8-OH-DPAT and flesinoxan, which are agonists (Fozard and Kilbinger, 1985; Schipper et al., 1990), and NAN-190, spiperone and spiroxatrine, which are antagonists (though in some systems they behave as partial agonists) (Nelson and Taylor, 1986; Pauwels et al., 1993). At relevant concentrations (see Table 2), however, these compounds showed no consistent effects, and spiperone could only inhibit the second phase of the concentration-response curve to 5-HT. These results suggest that 5-HT_{1A} receptors do not play a role in the 5-HT-induced relaxations. 5-HT_{1B} and 5-HT_{1D} receptors have not (yet) been shown to be present in the gastrointestinal tract. The current results do also not provide evidence for their presence. Compounds that are known to interact with 5-HT_{1B} and/or 5-HT_{1D} receptor subtypes, such as RU 24969, yohimbine, rauwolscine, sumatriptan, metergoline, methysergide and methiothepin (Schoeffter and Hoyer, 1989; Hoyer and Schoeffter, 1991; Miller et al., 1992), had no effects that were consistent with the affinities reported in literature (see Table 2). Functional correlates of the more recently discovered 5-HT_{1E} and 5-HT_{1F} receptors have not been encountered yet, and no selective antagonists at 5-HT_{1E} and 5-HT_{1F} receptors (Leonhardt et al., 1989; Zgombick et al., 1992; Adham et al., 1993; Gudermann et al., 1993) are available. The affinities as available from the literature are too scarce to provide decisive evidence as to whether either 5-HT_1 receptor subtype is involved. However, 5-carboxamidotryptamine is more than 100 times less potent than 5-HT at 5-HT_{1E} or 5-HT_{1F} receptors, which was not the case in our model, rendering involvement of these 5-HT_1 receptor subtypes rather unlikely (see also Table 2). At classical 5-HT_1 receptors, 5-carboxamidotryptamine is more potent than 5-HT, which was one of the criteria for designating a 5-HT receptor 5-HT_1 (Bradley et al., 1986; Table 2). In the colon, however, 5-carboxamidotryptamine was less potent than 5-HT. Hence, taken together, the above described results do not provide evidence that the relaxations to 5-HT in the guinea-pig colon are mediated by a known 5-HT_1 receptor subtype.

Ritanserin and metergoline were potent antagonists of the 5-HT-induced relaxations, and mesulergine, yohimbine, rauwolscine, mCPP and mianserin were moderately potent antagonists. Comparing these data with literature receptor affinity values suggests that a 5-HT_2 receptor subtype could be involved (see Table 2). The affinities of 9 antagonists that were tested showed a near-significant correlation with literature affinities for 5-HT_{2A} receptors, suggesting some resemblance with this receptor subtype. The potent 5-HT_{2A} receptor antagonists ketanserin and spiperone did, however, not affect the concentration-response curve to 5-HT. Hence the receptor under study is not identical to the 5-HT_{2A} receptor subtype. The 5-HT_{2B} and 5-HT_{2C} receptors are rather insensitive to inhibition by spiperone and ketanserin (Cohen and Wittenauer, 1985; Hoyer and Schoeffter, 1991; Foguet et al., 1992; Growcott et al., 1993; Wainscott et al., 1993) as are the relaxations described here in the guinea-pig colon (see Table 2; Kojima, 1991; Elswood and Bunce, 1992). In the rat fundus, the 5-HT_{2B} receptor is located on the smooth muscle and mediates contraction. In the guinea-pig colon, on the other hand, 5-HT acts on the nerves, as the relaxations are readily blocked by the neurotoxin tetrodotoxin (Briejer et al., 1992). This is not an argument to exclude that the receptors are similar, as, for example, smooth muscle 5-HT_4 receptors in the rat gastrointestinal tract cause relaxation, while in the guinea-pig they are neurogenic and mediate contraction (Elswood et al., 1991; Reeves et al., 1991; Baxter et al., 1991; Briejer et al., 1993). Though there are some antagonists that have similar affinities both for the colon receptor and the 5-HT_{2B} receptor (see Table 2: ritanserin, metergoline, mesulergine), there are, however, also great differences (see Table 2: 1-(1-naphthyl)-piperazine, rauwolscine, yohimbine). Spiroxatrine has been reported to have no effect on rat fundus contractions to 5-HT up to a concentration of respectively 1 μM (Cohen and Fludzinski, 1987), while in our preparation it strongly inhibited the 5-HT-induced relaxations at 0.3 μM. These authors also found that mianserin up to 0.3 μM did not affect contractions in the rat fundus to 5-HT (Cohen and Fludzinski, 1987), though it was active in our preparation, with a pA_2 value estimated to be 7.0, which is in agreement with receptor binding data (see Table 2). α-Methyl-5-HT was a poor agonist in the colon, while in the rat fundus it is a full and potent agonist (Kalkman and Fozard, 1991; Growcott et al., 1993). Methysergide seemed to have a higher affinity for the colon receptor than for the 5-HT_{2B} receptor (Table 2), but in the colon it behaved as a non-competitive antagonist, which makes it difficult to calculate a reliable affinity parameter. Comparison of antagonist affinities at the rat

fundus receptor (5-HT_{2B}) and the guinea-pig colon receptor showed no significant correlation. There was, however, a significant correlation with 5-HT_{2C} receptor affinities, but also here some differences with the colon receptor were apparent. The rank order of potency at 5-HT_{2C} receptors is α-methyl-5-HT > 5-HT > 5-carboxamidotryptamine (Hoyer and Schoeffter, 1991), while in the colon the potency order was 5-HT > 5-carboxamidotryptamine = α-methyl-5-HT, demonstrating especially the difference with respect to α-methyl-5-HT. Yohimbine and rauwolscine have only low affinity for 5-HT_{2C} receptors, but in our model, however, yohimbine and rauwolscine showed moderate affinity. The affinities of metergoline and mesulergine do also not quite correspond for the colon receptor and for 5-HT_{2C} receptors. These differences are reflected in the steep slope of the fitted line in Fig. 6. Hence, the 5-HT receptor under study resembles most the 5-HT_{2C} receptor, but its characteristics are not identical.

RU 24969, 8-OH-DPAT, sumatriptan and MK212 (and others: Table 1) induced methysergide- and/or L-NNA-insensitive relaxations in our preparation, while those to 5-HT are blocked by methysergide or L-NNA (this paper; Briejer et al., 1992). Relaxations to RU 24969, 8-OH-DPAT and MK212 were shown to be caused by antagonism of the methacholine-induced precontraction. Galligan (1992) also showed that in the guinea-pig ileum, 8-OH-DPAT blocks histamine and muscarinic ($pA_2 = 5.5$; in our preparation $pA_2 = 5.2$) receptors. Though the compounds might also induce part of the relaxation via the 5-HT receptor under study, the non-specific effects would mask them. Affinity estimates could, however, be assessed by using them as an antagonist at a concentration that would induce no non-specific effects (see Tables 1 and 2).

Recently, a number of novel 5-HT receptors have been cloned, provisionally designated 5-ht_{5A} (REC17, 5-$ht_{5\alpha}$), 5-ht_{5B} (MR22, 5-$ht_{5\beta}$), 5-ht_6 and 5-ht_7 (PCT65, REC20, 5-ht_x) (Plassat et al., 1992, 1993; Matthes et al., 1993; Erlander et al., 1993; Monsma et al., 1993; Ruat et al., 1993a,b; Shen et al., 1993; Lovenberg et al., 1993; Bard et al., 1993). Comparison of receptor binding affinities and pEC_{50} values in cells in which such receptors were expressed showed a significant correlation with the 5-ht_7 receptor. Northern blot analysis revealed the presence of 5-ht_7 receptor gene transcripts in mouse and human, but not rat, gut (Ruat et al., 1993a,b; Plassat et al., 1993; Shen et al., 1993; Lovenberg et al., 1993; Bard et al., 1993). No functional correlates have yet been found of the putative 5-ht_7 receptor and relevant pharmacological data are too scarce to allow any thorough comparison of the putative 5-ht_7 receptor and the receptor currently under study.

In the guinea-pig ileum, a smooth muscle receptor mediates relaxations to 5-HT. These responses could be inhibited by metergoline, spiperone and methysergide, but with quite different pA_2 values as compared to the current results (Feniuk et al., 1983,1984; Kalkman et al., 1986), which suggests that the receptor involved is of another subtype. Also in the guinea-pig stomach fundus circular muscle, 5-HT induces relaxations via a smooth muscle receptor (Kojima et al., 1992). Here, the rank order of potency was 5-carboxamidotryptamine $\gg$ 5-HT = 5-methoxytryptamine > 5-methyl-5-HT; TFMPP and 8-OH-DPAT were partial agonists, and α-methyl-5-HT, 2-methyl-5-HT and sumatriptan were virtually inactive. The relaxations to 5-HT in this preparation were inhibited by methiothepin and mianserin, but also by ketanserin and spiperone. These data indicate that the 5-HT receptor in the guinea-pig stomach fundus is also different from the 5-HT receptor in the colon.

It is concluded that the neuronal 5-HT receptor that mediates the first phase of the concentration-response curve to 5-HT (NO-mediated part) is not a known 5-HT receptor, and could be considered a 5-HT_2-like receptor.

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References

Adham, N., H.-T. Kao, L.E. Schlechter. J. Bard, M. Olsen, D. Urquhart, M. Durkin, P.R. Hartig, R.L. Weinshank and T.A. Branchek, 1993, Cloning of another human serotonin receptor (5-HT_{1F}): a fifth 5-HT_1 receptor subtype coupled to the inhibition of adenylate cyclase, Proc. Natl. Acad. Sci. USA 90, 408.

Bard, J.A., J. Zgombick, N. Adham, P. Vaysse, T.A. Branchek and R.L. Weinshank, 1993, Cloning of a novel human serotonin receptor (5-HT_7) positively linked to adenylate cyclase, J. Biol. Chem. 268, 23422.

Baxter, G.S., D.A. Craig and D.E. Clarke, 1991, 5-Hydroxytryptamine$_4$ receptors mediate relaxation of the rat oesophageal tunica muscularis mucosae, Naunyn-Schmied. Arch. Pharmacol. 343, 439.

Beer, M.S., D.N. Middlemiss and G. McAllister, 1993, 5-HT_1-like receptors: six down and still counting, Trends Pharmacol. Sci. 14, 228.

Bradley, P.B., G. Engel, W. Feniuk, J.R. Fozard, P.P.A. Humphrey, D.N. Middlemiss, E.J. Mylecharane, B.P. Richardson and P.R. Saxena, 1986, Proposals for the classification and nomenclature of functional receptors for 5-hydroxytryptamine, Neuropharmacology 25, 563.

Briejer, M.R., L.M.A. Akkermans, A.L. Meulemans, R.A. Lefebvre and J.A.J. Schuurkes, 1992, Nitric oxide is involved in 5-HT-induced relaxations of the guinea-pig colon ascendens in vitro, Br. J. Pharmacol. 107, 756.

Briejer, M.R., L.M.A. Akkermans, A.L. Meulemans, R.A. Lefebvre and J.A.J. Schuurkes, 1993, Pharmacological characterization of 5-hydroxytryptamine receptor types in the guinea-pig proximal colon, J. Gastroint. Motil. 5, 239.

Briejer, M.R., L.M.A. Akkermans, A.L. Meulemans, R.A. Lefebvre and J.A.J. Schuurkes, 1995, 5-HT-induced neurogenic relaxations of the guinea-pig proximal colon: investigation into the role of ATP and VIP in addition to nitric oxide, Naunyn-Schmied. Arch. Pharmacol. 351, 126.

Clineschmidt, B.V., D.R. Reiss, D.J. Pettibone and J.L. Robinson, 1985, Characterization of 5-hydroxytryptamine receptors in rat stomach fundus, J. Pharmacol. Exp. Ther. 235, 696.

Cohen, M.L. and L.A. Fludzinski, 1987, Contractile serotonergic receptor in rat stomach fundus, J. Pharmacol. Exp. Ther. 243, 264.

Cohen, M.L. and L.A. Wittenauer, 1985, Relationship between serotonin and tryptamine receptors in the rat stomach fundus, J. Pharmacol. Exp. Ther. 233, 75.

Elswood, C.J. and K.T. Bunce, 1992, Investigation of the 5-HT receptor mediating relaxation in guinea-pig proximal colon, J. Pharm. Pharmacol. 44, 264.

Elswood, C.J., K.T. Bunce and P.P.A. Humphrey, 1991, Identification of putative 5-HT_4 receptors in guinea-pig ascending colon, Eur. J. Pharmacol. 196, 149.

Engel, G., D. Hoyer, H.O. Kalkman and M.B. Wick, 1984, Identification of 5-HT_2 receptors on longitudinal muscle of the guinea pig ileum, J. Recept. Res. 4, 113.

Erlander, M.G., T.W. Lovenberg, B.M. Baron, L. De Lecea, P.E. Danielson, M. Racke, A.L. Slone, B.W. Siegel, P.E. Foye, K. Cannon, J.E. Burns and J.G. Sutcliffe, 1993, Two members of a distinct subfamily of 5-hydroxytryptamine receptors differentially expressed in rat brain, Proc. Natl. Acad. Sci. USA 90, 3452.

Feniuk, W., P.P.A. Humphrey and A.D. Watts, 1983, 5-Hydroxytryptamine-induced relaxation of isolated mammalian smooth muscle, Eur. J. Pharmacol. 96, 71.

Feniuk, W., P.P.A. Humphrey and A.D. Watts, 1984, 5-Carboxamido-tryptamine – a potent agonist at 5-hydroxytryptamine receptors mediating relaxation, Br. J. Pharmacol. 82, 209P.

Foguet, M., D. Hoyer, L.A. Pardo, A. Parekh, F.W. Kluxen, H.O. Kalkman, W. Stühmer amd H. Lübbert, 1992, Cloning and functional characterization of the rat stomach fundus serotonin receptor, EMBO J. 11, 3481.

Fozard, J.J. and H. Kilbinger, 1985, 8-OH-DPAT inhibits transmitter release from guinea-pig enteric cholinergic neurones by activating 5-HT_{1A} receptors, Br. J. Pharmacol. 86, 601P.

Galligan, J.J., 1992, Differential inhibition of cholinergic and non-cholinergic neurogenic contractions by 5-hydroxytryptamine$_{1A}$ receptor agonists in guinea pig ileum, J. Pharmacol. Exp. Ther. 260, 306.

Growcott, J.W., B. Cox and T.P. Blackburn, 1993, A pharmacological comparison of the receptors mediating contractile responses to 5-hydroxytryptamine in the rat isolated caudal artery and fundic strip, J. Pharm. Pharmacol. 45, 876.

Gudermann, T., F.O. Levy, M. Birnbaumer, L. Birnbaumer and A.J. Kaumann, 1993, Human S31 serotonin receptor clone encodes a 5-hydroxytryptamine$_{1E}$-like serotonin receptor, Mol. Pharmacol. 43, 412.

Hoyer, D., 1988, Functional correlates of serotonin 5-HT_1 recognition sites, J. Recept. Res. 8, 59.

Hoyer, D. and P. Schoeffter, 1991, 5-HT receptors: subtypes and second messengers, J. Recept. Res. 11, 197.

Humphrey, P.P.A., P. Hartig and D. Hoyer, 1993, A proposed new nomenclature for 5-HT receptors, Trends Pharmacol. Sci. 14, 233.

Kalkman, H.O. and J.R. Fozard, 1991, Further definition of the 5-HT receptor mediating contraction of rat stomach fundus: relation to 5-HT_{1D} recognition sites, in: Serotonin: Molecular Biology, Receptors and Functional Effects, eds. J.R. Fozard and P.R. Saxena (Birkhäuser Verlag, Basel, Switzerland) p. 153.

Kalkman, H.O., G. Engel and D. Hoyer, 1986, Inhibition of 5-carboxamidotryptamine-induced relaxation of guinea-pig ileum correlates with [^{125}I]LSD binding, Eur. J. Pharmacol. 129, 139.

Kojima, S., 1991, Characterization of 5-hydroxytryptamine-induced relaxations of guinea-pig proximal colon, Arch. Int. Pharmacodyn. 313, 23.

Kojima, S., R. Ishizaki and Y. Shimo, 1992, Investigation into the 5-hydroxytryptamine-induced relaxation of the circular smooth muscle of guinea-pig stomach fundus, Eur. J. Pharmacol. 224, 45.

Kursar, J.D., D.L. Nelson, D.B. Wainscott, M.L. Cohen and M. Baez, 1992, Molecular cloning, functional expression, and pharmacological characterization of a novel receptor (5-hydroxytryptamine$_{2F}$) from rat stomach fundus, Mol. Pharmacol. 42, 549.

Leonhardt, S., K. Herrick-Davis and M. Titeler, 1989, Detection of a novel serotonin receptor subtype (5-HT_{1E}) in human brain: interaction with a GTP-binding protein, J. Neurochem. 53, 465.

Lovenberg, T.W., B.M. Baron, L. De Lecea, J.D. Miller, R.A. Prosser, M.A. Rea, P.E. Foye, M. Racke, A.L. Slone, B.W. Siegel, P.E. Danielson, J.G. Sutcliffe and M.G. Erlander, 1993, A novel adenylyl cyclase-activating serotonin receptor (5-HT_7) implicated in the regulation of mammalian circadian rhythms, Neuron 11, 449.

Matthes, H., U. Boschert, N. Amlaiky, J.-L. Plassat, F. Muscatelli, M.-G. Mattei and R. Hen, 1993, Mouse 5-hydroxytryptamine$_{5A}$ and 5-hydroxytryptamine$_{5B}$ receptors define a new family of serotonin receptors: cloning, functional expression, and chromosomal localization, Mol. Pharmacol. 43, 313.

Miller, K.J., A. King, L. Demchyshyn, H. Niznik and M. Teitler, 1992, Agonist activity of sumatriptan and metergoline at the human 5-HT_{1Db} receptor: further evidence for a role of the 5-HT_{1D} receptor in the action of sumatriptan, Eur. J. Pharmacol. Mol. Pharmacol. 227, 99.

Monsma, F.J., Y. Shen, R.P. Ward, M.W. Hamblin and D.R. Sibley, 1993, Cloning and functional expression of a novel serotonin receptor with high affinity for tricyclic psychotropic drugs, Mol. Pharmacol. 43, 320.

Nelson, D.L. and E.W. Taylor, 1986, Spiroxatrine: a selective serotonin$_{1A}$ receptor antagonist, Eur. J. Pharmacol. 124, 207.

Pauwels, P.J., P. Van Gompel and J.E. Leysen, 1993, Activity of serotonin (5-HT) receptor agonists, partial agonists and antagonists at cloned human 5-HT_{1A} receptors that are negatively coupled to adenylate cyclase in permanently transfected HELA cells, Biochem. Pharmacol. 45, 375.

Plassat, J.-L., U. Boschert, N. Amkaily and R. Hen, 1992, The mouse 5-HT_5 receptor reveals a remarkable heterogeneity within the 5-HT_{1D} receptor family, EMBO J. 11, 4779.

Plassat, J.-L., N. Amlaiky and R. Hen, 1993, Molecular cloning of a mammalian receptor that activates adenylate cyclase, Mol. Pharmacol. 44, 229.

Reeves, J.J., K.T. Bunce and P.P.A. Humphrey, 1991, Investigation into the 5-hydroxytryptamine receptor mediating smooth muscle relaxation in the rat oesophagus, Br. J. Pharmacol. 102, 1067.

Ruat, M., E. Traiffort, R. Leurs, J. Tarvidel-Lacombe, J. Diaz, J.-M. Arrang and J.-C. Schwartz, 1993a, Molecular cloning, characterization, and localization of a high affinity serotonin receptor (5-HT_7) activating cAMP formation, Proc. Natl. Acad. Sci. USA 90, 8547.

Ruat, M., E. Traiffort, J.-M. Arrang, J. Tarvidel-Lacombe, J. Diaz, R. Leurs and J.-C. Schwartz, 1993b, A novel rat serotonin (5-HT_6) receptor: molecular cloning, localization and stimulation of cAMP accumulation, Biochem. Biophys. Res. Commun. 193, 268.

Schipper, J., M. Tulp, H. Sijbesma, F. Krijzer, J. Mos, J. v.d. Heyden and B. Olivier, 1990, Preclinical evaluation of flesinoxan as a potential anxiolytic and antidepressant drug, Eur. J. Pharmacol. 183, 2059.

Schoeffter, P. and D. Hoyer, 1989, 5-Hydroxytryptamine 5-HT_{1B} and 5-HT_{1D} receptors mediating inhibition of adenylate cyclase activ-

ity. Pharmacological comparison with special reference to the effects of yohimbine, rauwolscine and some β-adrenoceptor antagonists, Naunyn-Schmied Arch. Pharmacol. 340, 285.

Shen, Y., F.J. Monsma, M.A. Metcalf, P.A. Jose, M.W. Hamblin, D.R. Sibley, 1993, Molecular cloning and expression of a 5-hydroxytryptamine$_7$ serotonin receptor subtype, J. Biol. Chem. 268, 18200.

Tallarida, R.J. and R.B. Murray, 1981, Manual of Pharmacologic Calculations with Computer Programs (Springer-Verlag, New York, Heidelberg, Berlin).

Van Rossum, 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.

Van Wijngaarden, I., M.Th.M. Tulp and W. Soudijn, 1990, The concept of selectivity in 5-HT receptor research, Eur. J. Pharmacol. Mol. Pharmacol. 188, 301.

Wainscott, D.B., M.L. Cohen, K.W. Schenk, J.E. Audia, J.S. Nissen, M. Baez, J.D. Kursar, V.L. Lucaites and D.L. Nelson, 1993, Pharmacological characteristics of the newly cloned rat 5-hydroxytryptamine$_{2F}$ receptor, Mol. Pharmacol. 43, 419.

Wallenstein, S., C.L. Zucker and J.L. Fleiss, 1980, Some statistical methods useful in circulation research, Circ. Res. 47, 1.

Zgombick, J.M., L.E. Schechter, M. Macchi, P.R. Hartig, T.A. Branchek and R.L. Weinshank, 1992, Human gene S31 encodes the pharmacologically defined serotonin 5-hydroxytryptamine$_{1E}$ receptor, Mol. Pharmacol. 42, 180.