British Journal of Pharmacology (1996) 117, 21-28

Multiple 5-HT receptors in the guinea-pig superior cervical ganglion

Clare J. Watkins & [1]Nigel R. Newberry

Oxford University-SmithKline Beecham Centre for Applied Neuropsychobiology, University Department of Clinical Pharmacology, Radcliffe Infirmary, Woodstock Road, Oxford OX2 6HE

1 We have studied the pharmacology of the depolarization by 5-hydroxytryptamine (5-HT) of the guinea-pig isolated superior cervical ganglion (SCG) using the grease-gap technique. We studied the effects of selective and non-selective antagonists on the responses to 5-HT and other 5-HT receptor agonists.

2 We have extended the pharmacology of the 5-HT_3 receptor in this preparation by studying the effects of granisetron, BRL 46470 and mianserin on the concentration-response curve (CRC) to 2-methyl-5-HT. As with other 5-HT_3 receptor antagonists, these compounds exhibited a lower affinity for guinea-pig 5-HT_3 receptors than for rat 5-HT_3 receptors.

3 We have confirmed that low concentrations of 5-HT ($\leqslant 1$ μM) mediate ketanserin-sensitive responses and higher concentrations of 5-HT also recruit 5-HT_3 receptors. The responses to low concentrations of 5-HT were antagonized by low concentrations of ketanserin, spiperone, mianserin, DOI and LSD indicating probably mediation by 5-HT_{2A} receptors. At high concentrations, the hallucinogen, DOI, but not LSD, evoked a ketanserin-sensitive depolarization.

4 Although mianserin could bind to the 5-HT_{2A} receptors in this preparation, we could not demonstrate a down-regulation of depolarizations evoked by these receptors after a 10 day oral treatment with mianserin (10 mg kg^{-1}, daily).

5 5-Carboxamidotryptamine (5-CT) evoked a prolonged depolarization. Although high concentrations of 5-CT ($\geqslant 1$ μM) appeared to activate 5-HT_{2A} receptors, lower concentrations of 5-CT evoked a response with a distinct pharmacology. After studying the action of 20 selective and non-selective 5-HT receptor ligands we believe that this response may be mediated by a novel receptor; but its pharmacology is closest to that of receptors in the 5-HT_2 receptor family. Like 5-CT, 5-HT (3-300 μM) could evoke an LSD-sensitive response in the presence of the 5-HT_2 receptor antagonist, ketanserin and the 5-HT_3 receptor antagonist, tropisetron (all 1 μM).

6 We conclude that 5-HT activates three pharmacologically distinct receptors to depolarize the guinea-pig SCG. Low concentrations of 5-HT appear to activate 5-HT_{2A} receptors. Higher concentrations of 5-HT also activate 5-HT_3 receptors and a possible novel 5-HT receptor. The novel receptor could be a species homologue of a 5-HT_2 receptor or an, as yet, unclassified 5-HT receptor.

Keywords: 5-Hydroxytryptamine; superior cervical ganglion, 5-HT_3 receptor; 5-HT_{2A} receptor; novel receptor; mianserin; LSD

Introduction

It is almost forty years since 5-hydroxytryptamine (5-HT) was first shown to act on mammalian sympathetic ganglion neurones (e.g. Trendelenburg, 1956). Since that time most of the work has been done on rabbit and rat superior cervical ganglia (SCG). This research has focused principally on the fast depolarizing response mediated by 5-HT_3 receptors (e.g. Wallis & North, 1978; Azami *et al.*, 1985; Ireland *et al.*, 1987; Newberry *et al.*, 1991), although the presence of other 5-HT receptors has been demonstrated (Ireland & Jordan, 1987; Gilbert & Newberry, 1987; Newberry & Gilbert, 1989). Until comparatively recently, the pharmacology of 5-HT receptors present in guinea-pig sympathetic ganglia had received less attention. In 1988, Wallis & Dun showed that guinea-pig coeliac ganglion cells responded to 5-HT with fast depolarizing responses mediated by 5-HT_3 receptors and with slow depolarizing responses mediated by methysergide-sensitive receptors.

The guinea-pig isolated superior cervical ganglion has been studied in order to demonstrate species differences among 5-HT_3 receptors (Newberry *et al.*, 1991). It seemed that considerably higher concentrations of 5-HT_3 receptor antagonists were needed to antagonize the 5-HT_3 receptor-mediated responses in guinea-pig tissues than in tissues from rats or mice. In addition to 5-HT_3 receptors, relatively low concentrations of 5-HT induced ketanserin-sensitive responses in this preparation (Newberry *et al.*, 1991). This paper describes our follow-up to those observations. We extend the pharmacology of 5-HT_3 receptors in these neurones and provide evidence that the ketanserin-sensitive depolarizing response is probably mediated by 5-HT_{2A} receptors. Although, it has been shown that rat cerebrocortical 5-HT_{2A} receptors down-regulate after chronic treatment with mianserin (Sanders-Bush, 1990), we were unable to demonstrate a down-regulation of 5-HT_{2A} receptor-mediated responses in this preparation following a 10 day oral treatment with mianserin. We also present evidence that another receptor may be activated by 5-HT, and more specifically by 5-carboxamidotryptamine (5-CT), although we were unable to define the receptor involved as one of the known 5-HT receptor subtypes.

Methods

The methods for grease-gap recording from the guinea-pig isolated superior cervical ganglion were as previously described (Newberry *et al.*, 1991). The principal difference used in this

[1] Author for correspondence.

study was that the male Dunkin-Hartley guinea-pigs (200–350 g, Harlan-Olac, Bicester, Oxon) were killed by an intraperitoneal injection of pentobarbitone (600–1000 mg kg^{-1}). We confirmed that this method of killing the animals did not affect the concentration-response curve (CRC) to 5-HT on this preparation (data not shown). After setting up the preparation and obtaining a relatively stable d.c. recording baseline, 5-HT was superfused over the ganglion for 1 min periods at 30 min intervals until the response varied by less than 10%; this usually took 3–4 applications. Unless otherwise stated, all subsequent responses were related to the amplitude of the 'last' of these responses (given the arbitrary value of 1).

Agonists were superfused over the preparation for 1 min periods allowing time for complete recovery of the response prior to subsequent application. Lengthening the application time to 3 min had no significant effect on the CRC to 5-HT. The action of 5-HT receptor antagonists and other compounds was determined with two different methods. In the most often used method, the antagonist was superfused over the ganglion for 1 h beginning 15–20 min after the 'last' application of 100 μM 5-HT. The CRC of an agonist was then determined in the presence of that antagonist. This CRC was compared with that determined on a control ganglion, usually from the same animal, which was treated identically but no antagonist was superfused over it. When necessary, the solvent used to dissolve the antagonist was superfused over the control ganglion. In another experimental protocol, the object of the experiment was to determine the effect of an antagonist on the response to a fixed concentration of agonist. The agonist was applied at $\approx$45 min intervals and the antagonist application began 15 min after one of those applications. Because of the agonist application intervals, the effect of the antagonist was determined after its application for 30, 75 or 120 min. In this way the concentration of antagonist which reduced the agonist response by 50% (its IC_{50}) could be determined. As with the previous method, drug-induced effects were compared to controls in which the agonist application was repeated in the absence of the drug.

The pooled data of a CRC are shown as the mean ($\pm$s.e.mean) response values related to the depolarization of the 'last' response to 100 μM 5-HT. These values were statistically proved to be normally distributed (cf. the rat vagus nerve, Newberry *et al.*, 1993). Individual CRCs were analysed to obtain concentration-ratios for antagonists. We used the geometric mean concentration of agonist necessary to induce a given response level in the presence or absence of the antagonist. The response level used was 50% of the largest relative response of the pooled data of the agonist in the presence of the antagonist. An apparent affinity for the antagonist was determined only if the antagonist appeared to produce a parallel rightward shift of the agonist CRC, otherwise an approximate IC_{50} was inferred from the pooled data. The apparent affinity (pK_{app}) was estimated from pK_{app} = (concentration of antagonist)/(concentration ratio -1). The EC_{50} of an agonist, the concentration evoking a half maximum response, was determined from individual ganglia and cumulated as the pEC_{50} ($-\log_{10} EC_{50}$) since these values are normally distributed. A significant action of a drug was assessed with Student's unpaired t test ($P<0.05$).

Oral pretreatment with mianserin (10 mg kg^{-1}) was carried out with 10 successive single daily doses of mianserin. The mianserin was dissolved at 5 mg ml^{-1} in water containing 0.2 g ml^{-1} sucrose and the animals, initially 250–300 g, were given the appropriate volume by mouth. Controls were provided by the same number of treatments to another group of animals with sucrose alone. Another group of animals were treated for 9 days with sucrose followed by a single dose of mianserin (1 day treatment). Twenty-four hours after the last treatment, the animals were killed and the left-hand ganglion was excised for the experiment. The CRC to 5-HT was determined in the presence of 1 μM granisetron. In these experiments the absolute depolarizing responses to 5-HT (in mV) were compared since mianserin could have altered the maximum response to 5-HT. These experiments, generally involving 4 control and 4 treated animals, were performed on several separate occasions and the data pooled. Plasma levels of mianserin determined after the animals were killed for the 10 day treatment experiment, were 6 ± 3 ng ml^{-1} (mean$\pm$ s.e.mean, $n=4$). In separate experiments, the plasma levels 2 h after a single oral treatment were 30 ± 7 ng ml^{-1} ($n=4$). The drug treatments did not significantly affect the animals' weight gain.

The drugs were obtained from the following sources: 5-HT hydrochloride, (−)-propranolol hydrochloride, atropine sulphate, dopamine hydrochloride, (−)-noradrenaline hydrochloride and pargyline hydrochloride (Sigma Chemical Co.); ketanserin tartrate and spiperone (Janssen Pharmaceutica); (endo-N-(8-methyl-8-azabicyclo[3.2.1]oct-3-yl)2,3-dihydro-3,3 dimethyl-indole-1-carboxamide, hydrochloride (BRL 46470 hydrochloride), granisetron (BRL 43694) hydrochloride, 5-carboxamidotryptamine maleate (5-CT), *N*-[2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl]-*N*-(2-pyridinyl)cyclo hexanecarboxamide trihydrochloride (WAY-100635), N-[4-methoxy-3-(4-methyl-1-piperazinyl) phenyl]-2′-methyl-4′-(5-methyl-1,2,4-oxadiazol-3-yl) [1,1-biphenyl]-4-carboxamide (GR 127935), paroxetine hydrochloride, N-(1-methyl-5-indolyl)-N′-(3-pyridyl) urea hydrochloride (SB 200646A), fluvoxamine maleate, (1-butyl-4-piperidinyl)methyl 8-amino-7-dichloro-1,4-benzodioxan-5-carboxylate hydrochloride (SB 204070), renzapride, mesulergine and (±)-mianserin hydrochloride (SmithKline Beecham Pharmaceuticals); 8-hydroxy-2-(di-n-propylamino)-tetralin (8-OH-DPAT), tropisetron (ICS 205-930), 1-(2,5-dimethoxy-iodophenyl)-2-aminopropane (DOI) and 2-methyl-5-HT maleate (Research Biochemicals Inc.); fluoxetine hydrochloride (Lilly Research Laboratories), citalopram hydrobromide (Lundbeck), methiothepin maleate (Cookson Chemicals Ltd), clozapine and methysergide maleate (Sandoz), tetrodotoxin (Calbiochem) and (+)-lysergic acid diethylamide (LSD, forensic science laboratory, Aldermaston, Berks). The drugs were dissolved at 10^{-3} to 10^{-2} M in water apart from spiperone and GR 127935 (in 0.3% glacial acetic acid) and (+)-LSD (in 100% ethanol). GR127935 and WAY-100635 were synthesized at SmithKline Beecham Pharmaceuticals, Harlow, Essex.

Results

As previously reported (Newberry *et al.*, 1991), 5-HT reproducibly depolarized the guinea-pig isolated SCG over a wide concentration range (30 nM to 1 mM). As stated in the Methods, agonist-induced responses were expressed as a ratio of the 'last' depolarization to 100 μM 5-HT at the beginning of the experiment. The absolute value of this response was 0.66 ± 0.01 mV (mean$\pm$s.e.mean, $n=366$).

We believe that the responses to both 1 and 100 μM 5-HT were evoked by a direct action on the ganglion cells since they were not significantly affected by tetrodotoxin (0.3 μM), by reducing the calcium concentration in the superfusate to 0.1 mM or by atropine (1 μM), an antagonist of the muscarinic actions of the preganglionic transmitter, acetylcholine ($n=4$, not shown). The concentration-response curve (CRC) to 5-HT ($pEC_{50}=5.03\pm0.14$, $n=19$, Figure 1a) was not significantly affected by the monoamine oxidase inhibitor, pargyline (50 μM, $n=4$) or the selective 5-HT (serotonin) reuptake inhibitors (SSRIs, Hyttel, 1994) fluoxetine, fluvoxamine or citalopram (all at 1 μM, $n=4$, not shown).

The effects of some 5-HT receptor agonists are shown (Figure 1b). We can confirm that the 5-HT_3 receptor agonist, 2-methyl 5-HT (100 μM) depolarized this preparation. This response was shown to be resistant to ketanserin and probably mediated by 5-HT_3 receptors (Newberry *et al.*, 1991). The 5-HT_{1A} and 5-HT_7 receptor agonist, 8-OH-DPAT (1 μM, Gozlan *et al.*, 1983; Middlemiss & Fozard, 1983; Tsou *et al.*, 1994) did not evoke an observable hyperpolarization; rather it produced a very small slow depolarization (relative response of 0.01–0.02, $n=4$, not shown). The hallucinogenic drug (Glennon *et*

al., 1988) and 5-HT_{2A} and 5-HT_{2C} receptor agonist, DOI (10 μM), produced a small slow depolarization which limited its usefulness as the agonist of choice (see later). In contrast to the above agonists 5-CT, an agonist of 5-HT_1, 5-ht_5 and 5-HT_7 receptors (Hoyer *et al.*, 1994), produced a pronounced and prolonged depolarizing response (see later).

Noradrenaline and dopamine also depolarized this preparation but at 100 μM the responses they produced were much smaller than those to 5-HT. Their relative responses were 0.27 ± 0.05 ($n=5$) and 0.09 ± 0.06 ($n=4$), respectively. Their response duration (not shown) was similar to that of 5-HT, rather than 5-CT.

Antagonism of responses to 2-methyl-5-HT (5-HT_3 receptor pharmacology)

It has been previously shown that tropisetron (ICS 205-930) and (+)-tubocurarine antagonize the 2-methyl-5-HT-induced depolarizing response in this preparation. We have extended the pharmacology of this response with other 5-HT_3 receptor antagonists, namely granisetron (Sanger & Nelson, 1989), BRL 46470 (Blackburn *et al.*, 1992) and mianserin (Wood *et al.*, 1993) (see Figure 2). Granisetron (0.1 μM) produced a parallel rightward shift of the CRC to 2-methyl-5-HT with an apparent affinity (pK_{app}) of 7.7. This value was similar to that obtained with granisetron against 5-HT in the presence of 1 μM ketanserin ($pK_{app}=7.5$, not shown). BRL 46470 (0.3–3.0 μM) exerted an insurmountable antagonism of 2-methyl-5-HT-induced responses with an IC_{50} of ≈ 0.3 μM. Mianserin (10 μM) produced a clear rightward shift ($pK_{app}=5.4$) of the 2-methyl-5-HT CRC. Its IC_{50} against the single responses to 100 μM 2-methyl-5-HT was ≈ 20 μM ($n=4$). LSD (1 μM), which has been shown to antagonize ligand-gated ion channel responses in molluscan neurones (Gerschenfeld & Paupardin-Tritsch, 1974) did not significantly reduce the responses to 100 and 300 μM 2-methyl-5-HT ($n=5$, not shown).

Antagonism of responses to 5-HT (multiple receptors)

We studied the effect of 5-HT_3 and 5-HT_2 receptor antagonists on the CRC to 5-HT. High concentrations of the selective 5-HT_3 receptor antagonists, tropisetron (ICS 205-930, 1 μM, Figure 3, Richardson *et al.*, 1985), granisetron (1 μM, not shown) and BRL 46470 (10 μM, not shown) reduced the maximum response to 5-HT (by $\approx 30-40\%$) but they had no effect on the responses to relatively low concentrations of 5-HT ($\leqslant 10$ μM). In contrast, high concentrations of 5-HT_2 receptor antagonists, 1 μM ketanserin (Figure 3) or 0.3 μM spiperone (not shown), antagonized the responses to low concentrations of 5-HT as well as reducing the maximum response to 5-HT. The combined actions of a 5-HT_3 and a 5-HT_2 receptor antagonist were always greater than one of these antagonists alone (see Figure 3). Similar effects were observed with the combinations of ketanserin (1 μM) with tropisetron (1 μM, Figure 3), spiperone (0.3 μM) with granisetron (1 μM) or mianserin (0.3 μM) with tropisetron (1 μM, $n=5-8$ for each combination). Note that this concentration of mianserin was too low to affect significantly the 5-HT_3 receptor-mediated response to 2-methyl-5-HT (see above).

Antagonism of 5-HT_2 receptors

Relatively low concentrations of the 5-HT_2 receptor antagonist, ketanserin (Leysen *et al.*, 1981; 10 nM, Figure 3b) and spiperone (Peroutka & Snyder, 1979; 30 nM, not shown) produced a rightward shift of the whole CRC, but increasing the concentrations of these compounds failed to produce proportionally larger rightward shifts of the CRC. A component of the CRC appeared to be resistant to them. Similar rightward

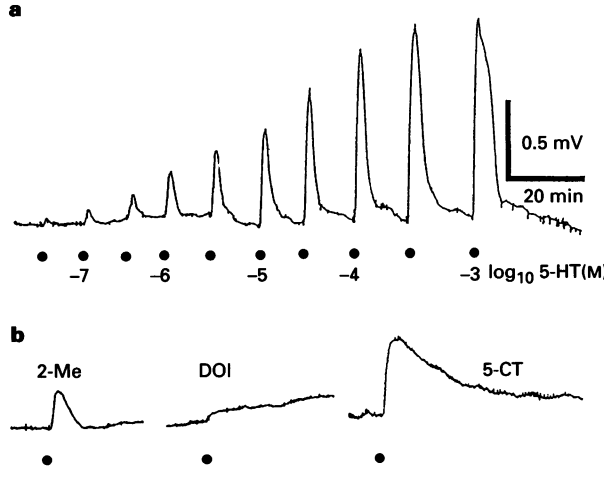

Figure 1 Chart recordings of the depolarizing responses evoked by 5-HT receptor agonists on the guinea-pig SCG. (a) Shows the concentration-dependent depolarizing responses evoked by 5-HT on the guinea-pig SCG. The superfused 5-HT was applied at concentration increasing in half $\log_{10}$ molar concentration units (0.03, 0.1, 0.3 μM etc.) and these are indicated in $\log_{10}$ units. It can be seen that the response shape varies with the concentration applied. In particular, at $\geqslant 30$ μM the depolarizing response rises more rapidly to peak and is followed by a slower response. (b) Shows depolarizing responses evoked by 2-methyl-5-HT (2-Me, 100 μM), DOI (10 μM) and 5-CT (1 μM). The calibration bar applies to all traces.

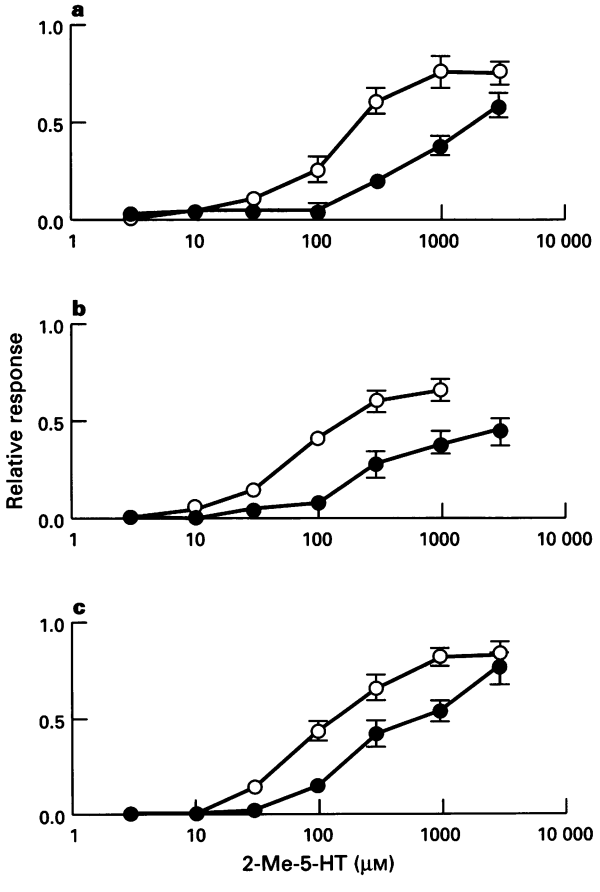

Figure 2 The effects of 5-HT_3 receptor antagonists on the concentration-response curve to 2-methyl-5-HT: (a) granisetron (0.1 μM) ($n=4$), (b) BRL 46470 (0.3 μM) ($n=4$) and (c) mianserin (10 μM) ($n=5$). Control curves ($n=4$) are shown (○) and test curves (●). The depolarizing responses are related to the response to 100 μM 5-HT before the concentration-response curve was determined (see Methods).

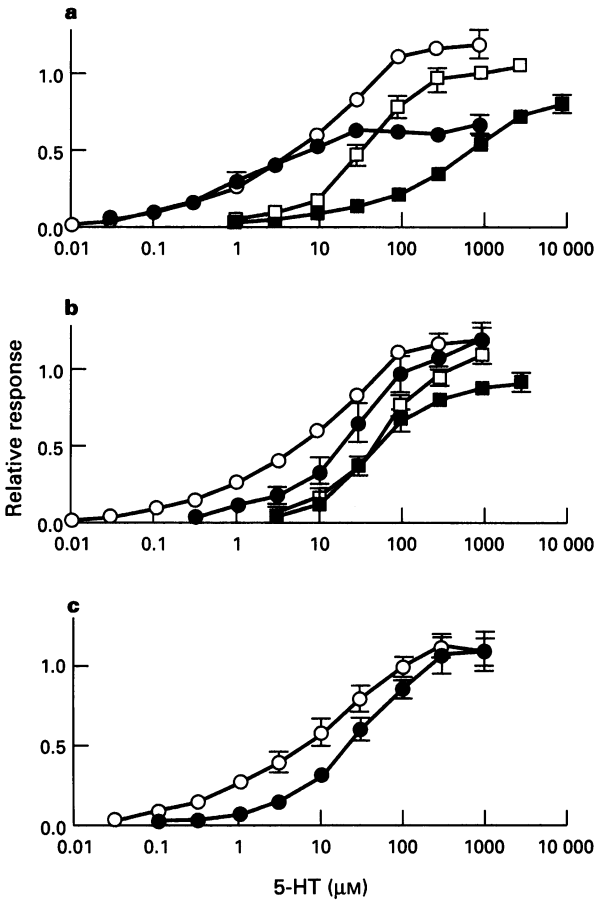

Figure 3 The effects of 5-HT_3 and 5-HT_{2A} receptor antagonists on the concentration-response curve to 5-HT: (a) shows the effect of ICS 205-930 (1 μM, ●, $n=8$), ketanserin (1 μM, □, $n=8$) and ICS 205-930 plus ketanserin (both 1 μM, ■, $n=8$). (b) Shows the effect of increasing concentrations of ketanserin: 10 nM (●, $n=4$), 100 nM (□, $n=4$) and 1 μM (■, $n=4$). (c) Shows the effect of DOI 0.1 μM (●, $n=4$). Control curves in (a), (b) and (c) are shown (○) ($n=4-9$). The depolarizing responses are related to the responses to 100 μM 5-HT before the concentration-response curve was determined (see Methods).

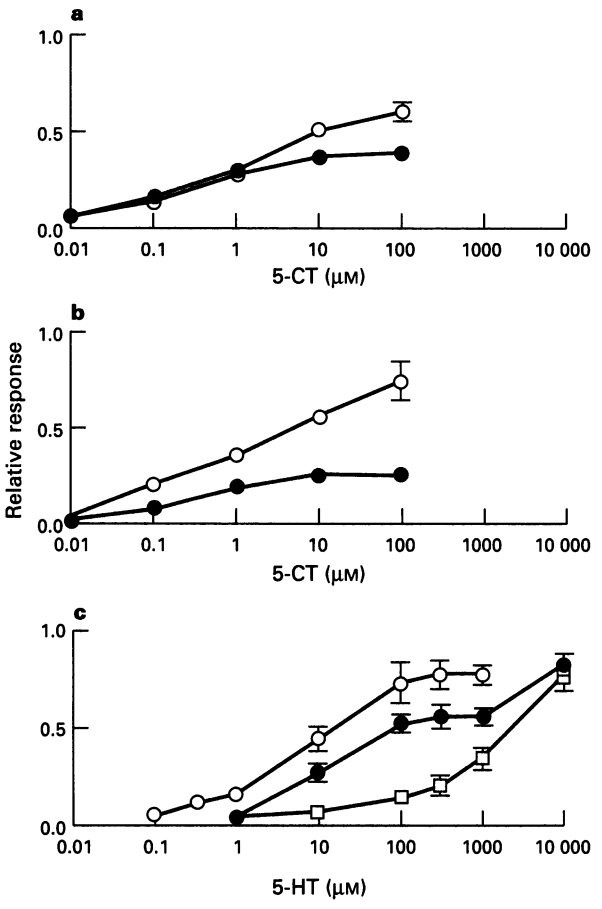

Figure 4 The effects of ketanserin and LSD on the concentration-response curve to 5-CT and 5-HT: (a) ketanserin (0.1 μM against 5-CT ($n=5$); (b) LSD (10 nM) against 5-CT ($n=5$) and (c) LSD (3 and 30 nM) against 5-HT ($n=5$ and 6). Control curves are shown (○) and test curves (●) or (□) (30 nM LSD). The depolarizing responses are related to the response to 100 μM 5-HT before the concentration-response curves were determined (see Methods). The experiment in (c) was done in the presence of granisetron (1 μM): it can be seen that the response to 10 mM 5-HT appears to be resistant to the action of LSD. This response is probably mediated by 5-HT_3 receptors.

shifts of the 5-HT CRC were observed with low concentrations of mianserin (30 nM, $n=5$) and LSD (3 nM, Figure 4c). The action of LSD was determined in detail when it was found that it antagonized the response to 5-CT (see Figure 4b). The concentration-ratios (3 to 30) produced by the above concentrations of these antagonists suggested that 5-HT_{2A} receptors were being activated by low concentrations of 5-HT (see Table 1 for affinity values). The EC_{50} for 5-HT to activate 5-HT_{2A} receptors was therefore likely to be ≈ 10 μM. This is clearly seen in experiments carried out in the presence of granisetron (1 μM), to antagonize 5-HT_3 receptors (Figure 4c).

Partial agonism by DOI

As shown in Figure 1, DOI produced a small depolarizing response at 10 μM. This response appeared to be maximal as 100 μM DOI produced no larger response ($n=2$). The response to 10 μM DOI was reduced by 1 μM ketanserin from 0.05 ± 0.04 to 0.01 ± 0.01 (mean relative ± s.e.mean, $n=4$). DOI produced a smaller response at 1 μM ($\approx 30\%$ of 10 μM) but at 0.1 μM it produced no detectable response. However, when superfused for 1 h at 0.1 μM it shifted the 5-HT CRC to the right (Figure 3c). These actions are compatible with DOI being a partial agonist of 5-HT_2 receptors.

Chronic oral treatment with mianserin

It has been shown that rat cerebrocortical 5-HT_{2A} receptors down-regulate after chronic treatment with mianserin (Sanders-Bush, 1990). We determined whether this effect occurred with guinea-pig sympathetic ganglion 5-HT_{2A} receptors. We studied the CRC to 5-HT following 1 and 10 days of single daily oral treatment with 10 mg kg^{-1} mianserin. The experiments were done in the presence of 1 μM granisetron to antagonize 5-HT_3 receptors. We found that single oral treatment ($n=8$) and chronic oral treatments ($n=13$) failed to affect the EC_{50} (≈ 10 μM) or the maximum response to 5-HT compared to controls ($n=26$) (not shown). The plasma concentrations of mianserin 2 h after a single treatment or after the 10 day treatment (100 and 20 nM, respectively, see Methods) were sufficient to bind to the 5-HT_{2A} receptors in this preparation (see above).

Antagonism of responses to 5-CT

5-CT evoked much slower depolarizations of the guinea-pig SCG than 5-HT (Figure 1). The responses to 1 μM 5-CT were resistant to tetrodotoxin (0.3 μM) and to reducing the calcium in the medium from 2.5 to 0.1 mM ($n=4$ each, not shown). In particular this response was resistant to atropine (1 μM) which is an antagonist of slow muscarinic responses in this prepara-

Table 1 Pharmacology of the depolarizing response induced by 5-CT (1 μM)

Compound	*Concentration* (nM)	(*n*)	*Receptor(s)* (Bound)	*Affinity* (nM)	*References*
Reduced (40–60%) by:					
LSD[A]	10	(5)	5-$HT_{2A,2C}$	10,4	Boess & Martin (1994); Zifa & Fillion (1992)
Mesulergine[A]	100	(4)	5-$HT_{2A,2C}$	1,1	Hoyer *et al.* (1994)
Methiothepin[A]	100	(4)	5-$HT_{2A,2C}$	1,6	Hoyer *et al.* (1994)
Mianserin[A]	300	(4)	5-$HT_{2A,2C}$	10,2	Hoyer *et al.* (1994); Boess & Martin (1994)
Methysergide	1000	(4)	5-$HT_{2A,2C}$	3,1	Zifa & Fillion (1992); Hoyer *et al.* (1994)
Ketanserin	1000	(4)	5-$HT_{2A,2C}$	0.5,320	Hoyer *et al.* (1994)
Spiperone	1000	(3)	5-$HT_{2A,2C}$	0.8, > 1000	Hoyer *et al.* (1994)
Not reduced by:					
WAY-100635	300	(4)	5-HT_{1A}	**30**[B]	Craven *et al.* (1994); (Fletcher *et al.* (1994)
8-OH-DPAT	1000	(4)	5-$HT_{1A,7}$	6,**50**	Hoyer *et al.* (1994); Tsou *et al.* (1994)
GR 127935[A]	100	(4)	5-$HT_{1B,1D\alpha,1D\beta}$	3,1,0.1	Starkey & Skingle (1994)
Sumatriptan	300	(4)	5-HT_{1D}	100	Hoyer *et al.* (1994)
Ketanserin[A]	100	(5)	5-HT_{2A}	0.5	Hoyer *et al.* (1994)
Spiperone[A]	30	(5)	5-HT_{2A}	0.8	Hoyer *et al.* (1994)
DOI	100	(3)	5-HT_{2A}	25	Hoyer *et al.* (1994)
SB 200646	1000	(3)	5-$HT_{2B,2C}$	32,130	Kennett *et al.* (1994)
Clozapine	100	(2)	5-$HT_{6,7}$	13,13	Hoyer *et al.* (1994)
Granisetron[A]	1000	(4)	5-HT_3	**20**	This paper
Tropisetron	10,000	(2)	5-$HT_{3,4}$	**63**,630	This paper, Hoyer *et al.* (1994)
SB 204070	100	(3)	5-HT_4	**0.02**	Wardle *et al.* (1994)
Renzapride	100,000	(3)	5-HT_{1P}	**100,000**[B]	Wade *et al.* (1994)
Propranolol	100	(4)	5-HT_{1A},β-Adr	250,10	Hoyer *et al.* (1994)
Atropine	1000	(2)	Muscarinic ACh	**4**	Roberts & Newberry (1990)

The table shows the concentrations of antagonists which do and do not significantly reduce the 5-CT-induced response. The receptors to which the indicated concentration of antagonist would bind appreciably are suggested from the affinity of the ligand. The 5-CT-induced response was considered to be significantly reduced by an antagonist if the pooled responses in the presence of the antagonist differed from those obtained by reapplying the 5-CT in the absence of an antagonist ($P<0.05$). The latter procedure resulted in a mean change of the response of 2% (range –23% to +10%, $n=4$). Affinity values are from the sources cited in the main reference section. The list of affinities is not intended to be comprehensive but it highlights the receptors being considered. The following unlisted receptors are unlikely to be involved: 5-ht_{1E} and 5-ht_{1F} receptors since mesulergine, ketanserin, spiperone and 5-CT have K_i values above 1 μM for them; 5-ht_{5A}, 5-ht_{5B} and 5-HT_6 receptors since mesulergine has an affinity of >1 μM for these (see Hoyer *et al.*, 1994; Boess & Martin, 1994). ([A]) indicates results obtained from a concentration-response curve; ([B]) indicates an effective concentration rather than an affinity. **Emboldened** affinity values are from guinea-pig tissues. β-Adr indicates β-adrenoceptors.

tion ($n=4$, see Roberts & Newberry, 1990). The 5-CT CRC occurred over the range of 10 nM to 100 μM but it did not clearly reach a maximum (Figure 4). Responses to high concentrations of 5-CT were reduced by ketanserin (100 nM, Figure 4a) but responses to lower concentrations of 5-CT (≤1 μM) were unaffected (see also Figure 5a). A similar effect on the 5-CT CRC was observed with spiperone (30 nM, $n=5$, not shown). Such actions may be explained because high concentrations of 5-CT can activate 5-HT_{2A} receptors (Bond *et al.*, 1989). Consequently, 5-CT could activate a 100 nM ketanserin-resistant response with an EC_{50} of 0.1–0.3 μM and a maximum response at ≈10 μM.

We determined the pharmacology of the 5-CT response in two ways. Firstly, we determined the CRC to 5-CT in the presence of an antagonist or secondly, we assessed whether an antagonist (superfused for 30–75 min) would reduce the response to 1 μM 5-CT. We used both selective and non-selective antagonists at concentrations which were 10–1000 times their apparent affinities for 5-HT receptors reported in the literature. The effects of the antagonists tested are listed in Table 1.

In contrast to ketanserin (0.1 μM), other 5-HT_2 receptor antagonists reduced the responses induced by low concentrations of 5-CT (<1 μM). The effects of mesulergine, mianserin and methiothepin on the CRC to 5-CT were similar to that of LSD (Figure 4b). For comparison, the action of LSD on the 5-HT CRC in the presence of granisetron, mediated by 5-HT_{2A} receptors, is also shown (Figure 4c). Individual responses to 1 μM 5-CT were inhibited by LSD (75 min) in a concentration-dependent manner with an IC_{50} of ≈30 nM and almost complete inhibition at 1 μM LSD. It should be noted that the response to 1 μM 5-CT was not completely resistant to ketanserin and spiperone as it was reduced by 40–60% in the presence of 1 μM of these compounds. A large range of antagonists of other 5-HT receptors failed to reduce significantly the response to 1 μM 5-CT, as compared to repeating the application of 5-CT in the absence of an antagonist. At the concentration tested, none of these antagonists produced a direct action by themselves.

Could 5-CT be activating a novel 5-HT receptor?

In order to address the possibility that 5-CT might be activating a 5-HT receptor, it was necessary to show that 5-HT could evoke a pharmacologically similar response. We considered that the key characteristics of the 5-CT response were its resistance to tropisetron (1 μM) and ketanserin (100 nM) (Figure 5a) and its sensitivity to LSD. To reduce further the possibility of 5-HT activating 5-HT_{2A} receptors, we superfused 1 μM ketanserin (Figures 5b,c). In its presence, 5-HT activated a biphasic depolarizing response comprising a fast response, mediated by 5-HT_3 receptors, and a slower response which was reduced by 1 μM LSD (Figure 5b, $n=4$). The slow LSD-sensitive response to 5-HT could also be recorded in the presence of both tropisetron and ketanserin (both at 1 μM, Figure 5c, $n=3$). This response to 5-HT was concentration-dependent, although, compared to 5-CT, higher concentrations of 5-HT were needed to activate the LSD-sensitive response. It should be noted that LSD (1 μM) had no effect on the 5-HT_3 receptor-mediated response to 2-methyl-5-HT (see above).

Discussion and conclusions

We have found that exogenously applied 5-HT directly activates three 5-HT receptor subtypes in the guinea-pig isolated superior cervical ganglion. Each receptor mediates a depolarizing response. We have extended previous findings on the pharmacology of 5-HT_3 receptors in this tissue and we can

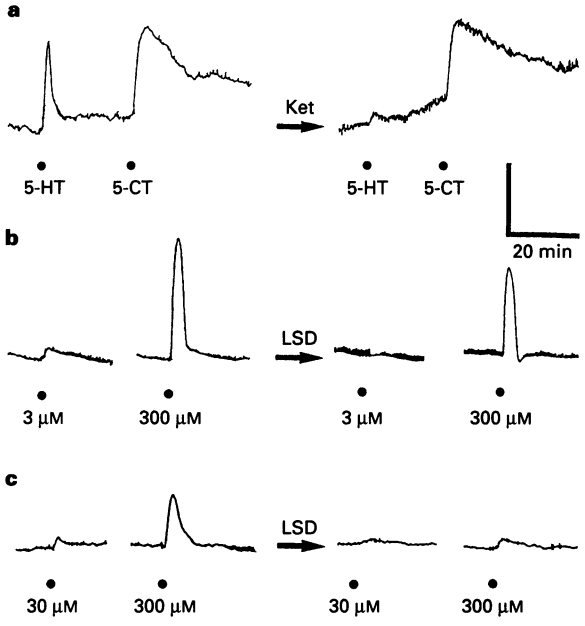

Figure 5 Antagonism of the responses evoked by 5-HT on the guinea-pig SCG: (a) in the presence of tropisetron (1 μM), shows that the response to 5-HT (1 μM), but not 5-CT (1 μM), was antagonized by ketanserin (0.1 μM, 70 min). (b) In the presence of 1 μM ketanserin, shows that 5-HT evoked a slow depolarizing response at 3 μM but a biphasic response at 300 μM: only the slower response was abolished by LSD (1 μM) (the fast response being mediated by 5-HT_3 receptors). (c) In the presence of tropisetron and ketanserin (both 1 μM), shows that LSD (1 μM) antagonized the response to 3 and 300 μM 5-HT. The vertical calibration bar represents 0.2 mV in (a) and 0.5 mV in (b) and (c).

suggest that the depolarization to low concentrations of 5-HT is largely mediated by 5-HT_{2A} receptors; but these 5-HT_{2A} receptors do not appear to be down-regulated by chronic treatment with mianserin. Another pharmacologically distinct depolarizing response was activated by 5-CT. Under certain conditions, we showed that 5-HT could also activate this receptor. The receptor mediating the 5-CT induced response could not be classified according to the published subtypes of 5-HT receptor but its pharmacology was similar to that of members of the 5-HT_2 receptor family.

We have shown that the 5-HT_3 receptors activated by 2-methyl-5-HT were antagonized in a surmountable manner by granisetron and mianserin, but in an insurmountable way by BRL 46470 (cf. Newberry *et al.*, 1993). The relatively low apparent affinity of granisetron ($pA_2 = 8.1$) and BRL 46470 ($pA_2 = 7.5$) in the guinea-pig ileum have been previously reported (Sanger & Nelson, 1989; Blackburn *et al.*, 1992). Likewise the low affinity of mianserin ($pK_{app} = 5.4$) in the guinea-pig SCG contrasts dramatically with its high affinity for rat 5-HT_3 receptors (Wood *et al.*, 1993). The low affinity of 5-HT_3 receptor antagonists for guinea-pig 5-HT_3 receptors is therefore confirmed and extended by this study. The use of several 5-HT_3 receptor antagonists on the guinea-pig SCG has supported the contention that the depolarizing responses to high concentrations of 5-HT ($\geqslant 10$ μM) are largely mediated by 5-HT_3 receptors (Newberry *et al.*, 1991).

We investigated the pharmacology of that part of the concentration-response curve to 5-HT which was not mediated by 5-HT_3 receptors. This was done by determining the effect of antagonists on the responses to low concentrations of 5-HT or occasionally in the presence of granisetron, a selective 5-HT_3 receptor antagonist. Low concentrations of ketanserin, spiperone, mianserin, DOI and LSD antagonized this response indicating that it was probably mediated by 5-HT_{2A} receptors. Although guinea-pig 5-HT_{2A} receptors have not been extensively studied, it has been shown that 5-HT_{2A} receptor stimulation of phosphoinositide metabolism in the guinea-pig central nervous system (CNS) is inhibited by similar concentrations of 5-HT_{2A} receptor antagonists to those found to be effective in rat tissue (Cadogan *et al.*, 1993).

The antidepressant drug, mianserin, has been shown to interact with a variety of 5-HT receptors (Wood *et al.*, 1993). In the guinea-pig SCG, we were able to show that mianserin could selectively antagonize 5-HT_{2A} receptors in preference to 5-HT_3 receptors. This selectivity, which is less apparent in rat tissues, reflects a change between species in the affinity of mianserin for 5-HT_3 receptors, rather than 5-HT_{2A} receptors (see Wood *et al.*, 1993). The affinity of mianserin for 5-HT_{2A} receptors in rat and guinea-pig tissues may be similar but we have evidence that the regulation of these receptors might differ. Although we showed that the mianserin acts as an antagonist of 5-HT_{2A} receptors in the guinea-pig SCG at low concentrations (30 nM), we have failed to show a down-regulation of electrophysiological responses mediated by these receptors following 10 days of oral treatment. A down-regulation of the numbers of rat 5-HT_{2A} and 5-HT_{2C} receptors is induced with chronic *in vivo* treatment with 5-HT_2 receptor antagonists (Sanders-Bush, 1990). In this regard, these receptors are unusual since chronic treatment with antagonists generally induces a compensatory up-regulation of receptor numbers. The unusual effect on 5-HT_2 receptors may be explained by differences in receptor regulation. In cultured C6 glioma cells of the rat, the down-regulation of endogenous 5-HT_{2A} receptors by mianserin has been shown to involve a regulatory site on the 5′ end or the mRNA for the receptor (Toth & Shenk, 1994). It is possible, therefore, that the apparent difference in antagonist-induced down-regulation of rat and guinea-pig 5-HT_{2A} receptors is due to their different mRNA sequences (compare Julius *et al.*, 1990; Watts *et al.*, 1994).

A possible novel 5-HT receptor in the guinea-pig SCG was revealed when studying the pharmacology of the slow response activated by 5-CT. Although 5-CT may activate 5-HT_{2A} receptors above 1 μM, the response evoked by $\leqslant 1\mu$M was clearly not mediated by such receptors given its resistance to ketanserin, spiperone and clozapine (30–100 nM, Figure 5a, Table 1). The range of 5-HT receptor antagonists shown in Table 1 which failed to reduce the response to 1 μM 5-CT indicated that it was pharmacologically unlike the 16 published 5-HT receptors including 5-HT_{1P}. Note that 5-HT_{1B} receptors are not considered present in guinea-pig tissues (Heuring & Peroutka, 1987). The non-selective antagonists LSD, mesulergine, methiothepin, mianserin and methysergide did antagonize the response to 5-CT as did high concentrations of ketanserin and spiperone. This indicated that the receptor involved was pharmacologically most similar to receptors in the 5-HT_2 family, although the ineffectiveness of SB 200646 and the small effect of a high concentration of methysergide suggested that it was not mediated by 5-HT_{2B} or 5-HT_{2C} receptors, respectively. The demonstration that 5-HT could activate an LSD-sensitive response in the presence of a 5-HT_{2A} and a 5-HT_3 receptor antagonist strengthened the case that the receptor was likely to be a 5-HT receptor, rather than another neurotransmitter receptor. It is possible that the response to 5-CT could be mediated by two receptors which would complicate the pharmacology. We think that this is unlikely since both of these receptors would have to be resistant to the antagonist tested. Although we believe that this receptor is pharmacologically distinct from the classified receptors, we could not exclude that it is a species homologue of a published receptor. Examples of species differences include 5-HT_3 receptors and 5-HT_{1B}/5-HT_{1D} receptors. Alternatively, the receptor could be one of the so-called 'orphan' receptors such as those mediating the depolarization of rat motoneurones (Connell & Wallis, 1989; Larkman & Kelly, 1991) or the dopaminergic neurones of the guinea-pig substantia nigra (Nedergaard *et al.*, 1991), but the lack of action of sumatriptan would rule out some vascular tissue receptors (e.g. Sahin-Erdemli *et al.*, 1991). From the data in Table 1, we believe that this receptor is pharmacologically most similar to receptors in

the 5-HT_2 receptor family. Of 5-HT_{2A}, 5-HT_{2B} and 5-HT_{2C} receptors, the evidence in the literature suggests that the 5-HT_{2B} receptor differs the most between species and, furthermore, there are probably further subtypes within this group (Baxter *et al.*, 1995). Unfortunately, we can find no data in the literature on the effects of these antagonists on guinea-pig 5-HT_{2B} and 5-HT_{2C} receptors. At the present time, therefore, the potential influence of species differences in receptors leaves open the question of whether 5-HT_{2B}, 5-HT_{2C} or some novel receptor is involved.

We have shown that 5-HT, in the presence of a 5-HT_3 and a 5-HT_{2A} receptor antagonist, can probably activate the same LSD-sensitive receptor as does 5-CT. Could this have any physiological relevance? Sympathetic ganglia have been shown to contain small intensely fluorescent cells and 5-HT containing nerve fibres. It has also been shown that 5-HT can evoke two pharmacologically distinct depolarizing responses in guinea-pig sympathetic neurones (Wallis & Dun, 1988): a fast response sensitive to blockade by MDL 72222 and a slow response sensitive to methysergide. In two cells, fast and slow depolarizations were recorded where the latter were resistant to methysergide. Cultures of sympathetic ganglion cells also have cells which contain 5-HT and cells which give a variety of electrophysiological responses to 5-HT (Knoper *et al.*, 1992; Matsumoto *et al.*, 1993). In these cells, slow responses to 5-HT were not inhibited by methysergide. It seems likely that the fast response recorded by Wallis & Dun (1988) was mediated by the same 5-HT_3 receptor that we have been studying, but the receptor mediating the slow response is less clear. Methysergide is known to be a potent antagonist of 5-HT_{2A} and 5-HT_{2C} receptors but we have also shown that it is a weak antagonist of the novel response activated by 5-CT. It is therefore possible that some of the slow depolarizations in guinea-pig sympathetic neurones may be mediated by this novel 5-HT receptor. It remains to be seen whether the electrophysiological mechanism underlying the depolarizing responses mediated by 5-HT_{2A} receptors and this novel receptor are similar. The pharmacology we describe here should help to address this issue.

We conclude that 5-HT can evoke three pharmacologically distinct depolarizing responses on the guinea-pig isolated SCG: the 5-HT_3 receptor, the 5-HT_{2A} receptor and a novel 5-HT receptor. We have extended the pharmacology of the 5-HT_3 receptor in this preparation and found that the antidepressant, mianserin, also exhibits a lower affinity for guinea-pig than for rat 5-HT_3 receptors. With a larger range of antagonists we have been able to demonstrate that the depolarizing responses activated by low concentrations of 5-HT are mediated by 5-HT_{2A} receptors. Although these receptors are pharmacologically similar to those in the rat CNS, we were unable to detect any down-regulation in the responses mediated by them after 10 days of oral treatment with mianserin. The depolarization activated by relatively low concentrations of 5-CT was mediated by a 5-HT receptor unlike those classified in the literature. The identity and physiological relevance of this receptor have yet to be determined.

We are grateful to Drs Gordon Baxter and Derek Middlemiss for their comments on the manuscript.

References

AZAMI, J., FOZARD, J.R., ROUND, A.A. & WALLIS, D.I. (1985). The depolarizing action of 5-HT on rabbit vagal primary afferent and sympathetic neurones and its selective blockade by MDL 72222. *Naunyn Schmied. Arch. Pharmacol.*, **328**, 423–429.

BAXTER, G., KENNETT, G., BLANEY, F. & BLACKBURN, T. (1995). 5-HT_2 receptor subtypes: a family reunited. *Trends Pharmacol. Sci.*, **16**, 105–110.

BLACKBURN, T.P., BAXTER, G.S., KENNETT, G.A., KING, F.D., PIPER, D.C., SANGER, G.J., THOMAS, D.R., UPTON, N. & WOOD, M.D. (1992). BRL46470: a highly potent, selective and long acting 5-HT_3 receptor antagonist with anxiolytic-like properties. *Psychopharmacology*, **110**, 257–264.

BOESS, F.G. & MARTIN, I.L. (1994). Molecular biology of 5-HT receptors. *Neuropharmacology*, **33**, 275–317.

BOND, R.A., ORNSTEIN, A.G. & CLARKE, D.E. (1989). Unsurmountable antagonism to 5-hydroxytryptamine in rat kidney results from pseudoirreversible inhibition rather than multiple receptors or allosteric receptor modulation. *J. Pharmacol. Exp. Ther.*, **249**, 401–410.

CADOGAN, A.K., MARSDEN, C.A., TULLOCH, I. & KENDALL, D.A. (1993). Evidence that chronic administration of paroxetine or fluoxetine enhances 5-HT_2 receptor function in the brain of the guinea-pig. *Neuropharmacology*, **32**, 249–256.

CONNELL, L.A. & WALLIS, D.I. (1989). 5-Hydroxytryptamine depolarises neonatal rat motoneurones through a receptor unrelated to an identified binding site. *Neuropharmacology*, **28**, 625–634.

CRAVEN, R., GRAHAME-SMITH, D. & NEWBERRY, N. (1994). WAY-100635 and GR127935: effects on 5-hydroxytryptamine-containing neurones. *Eur. J. Pharmacol.*, **271**, R1–R3.

FLETCHER, A., BILL, D.J., CLIFFE, I.A., FORSTER, E.A., JONES, D. & REILLY, Y. (1994). A pharmacological profile of WAY-100635, a potent and selective 5-HT_{1A} receptor antagonist. *Br. J. Pharmacol.*, **112**, 91P.

GERSCHENFELD, H.M. & PAUPARDIN-TRITSCH, D. (1974). Ionic mechanisms and receptor properties underlying the responses of molluscan neurones to 5-hydroxytryptamine. *J. Physiol.*, **243**, 427–456.

GILBERT, M.J. & NEWBERRY, N.R. (1987). A 5-HT_1-like receptor mediates a sympathetic ganglionic hyperpolarisation. *Eur. J. Pharmacol.*, **144**, 385–388.

GLENNON, R.A., SEGGEL, M.R., SOINE, W.H., HERRICK-DAVIS, K., LYON, R.A. & TITELER, M. (1988). [125I]-1-(2,5-dimethoxy-4-iodophenyl) 2-aminoproprane: an iodinated radioligand that specifically labels the agonist high affinity state of 5-HT_2 serotonin receptors. *J. Med. Chem.*, **31**, 5–7.

GOZLAN, H., EL MESTIKAWY, S., PICHAT, L., GLOWINSKI, J. & HAMON, M. (1983). Identification of presynaptic serotonin autoreceptors using a new ligand: ^{3}H-PAT. *Nature*, **305**, 140–142.

HEURING R.E. & PEROUTKA, S.J. (1987). Characterisation of a novel [^{3}H]5-HT binding site subtype in bovine brain membranes. *J. Neurosci.*, **7**, 894–903.

HOYER, D., CLARKE, D.E., FOZARD, J.R., HARTIG, P.R., MARTIN, G.R., MYLECHARANE, E.J., SAXENA, P.R. & HUMPHREY, P.P.A. (1994). International Union of Pharmacology Classification of Receptors for 5-Hydroxytryptamine (Serotonin). *Pharmacol. Rev.*, **46**, 157–203.

HYTTEL, J. (1994). Pharmacological characterization of selective serotonin reuptake inhibitors (SSRIs). *Int. Clin. Psychopharmacol.*, **9** Suppl.1, 19–26.

IRELAND, S.J. & JORDAN, C.C. (1987). Pharmacological characterization of 5-hydroxytryptamine-induced hyperpolarisation of the rat superior cervical ganglion. *Br. J. Pharmacol.*, **92**, 417–427.

IRELAND, S.J., STRAUGHAN, D.W. & TYERS, M.B. (1987). Influence of 5-hydroxytryptamine uptake on the apparent 5-hydroxytryptamine antagonist potency of metoclopramide in the rat isolated superior cervical ganglion. *Br. J. Pharmacol.*, **90**, 151–160.

JULIUS, D., HUANG, K.N., LIVELLI, T.J., AXEL, R. & JESSEL, T.M. (1990). The 5-HT_2 receptor defines a family of structurally distinct ionally conserved serotonin receptors. *Proc. Natl. Acad. Sci. U.S.A.*, **87**, 928–932.

KENNETT, G.A., WOOD, M.D., GLEN, A., GREWAL, S., FORBES, I., GADRE, A. & BLACKBURN, T.P. (1994). *In vivo* properties of SB 200646A, a 5-$HT_{2C/2B}$ receptor antagonist. *Br. J. Pharmacol.*, **111**, 797–802.

KNOPER, S.R., MATSUMOTO, S.G. & KREULEN, D.L. (1992). Response to 5-hydroxytryptamine on neurones of guinea pig coeliac and inferior mesenteric ganglia in primary culture. *Life Sci.*, **51**, 703–710.

LARKMAN, P.M. & KELLY, J.S. (1991). Pharmacological characterisation of the receptor mediating 5-HT evoked motoneuronal depolarization *in vitro*. In *Serotonin: Molecular Biology, Receptors and Functional Effects*. ed. Fozard, J.R. & Saxena, P.R. pp. 310–321. Basel, Switzerland: Birkhauser Verlag.

LEYSEN, J.E., AWOUTERS, F., KENNIS, L., LADURON, P.M., VANDENBERK, J. & JANNSSEN, P.A.J. (1981). Receptor binding profile of R 41 468, a novel antagonist at 5-HT_2 receptors. *Life Sci.*, **28,** 1015–1022.

MATSUMOTO, S.G., GRUENER, R.P. & KREULEN, D.L. (1993). Neurotransmitter properties of guinea-pig sympathetic neurons grown in dissociated cell culture - 1. Adult neurons. *Neuroscience*, **7,** 1135–1145.

MIDDLEMISS, D.N. & FOZARD, J.R. (1983). 8-Hydroxy-2(di-n propylamino)-tetralin discriminates between subtypes of the 5-HT_1 recognition site. *Eur. J. Pharmacol.*, **90,** 151–153.

NEDERGAARD, S., FLATMAN, J.A. & ENGBERG, I. (1991). Excitation of substantia nigra pars compacta neurones by 5-hydroxytryptamine *in vitro*. *Neuroreport*, **2,** 329–332.

NEWBERRY, N.R., CHESHIRE, S.H. & GILBERT, M.J. (1991). Evidence that the 5-HT_3 receptors of the rat, mouse and guinea-pig superior cervical ganglion may be different. *Br. J. Pharmacol.*, **102,** 615–620.

NEWBERRY, N.R. & GILBERT, M.J. (1989). 5-Hydroxytryptamine evokes three distinct responses on the rat superior cervical ganglion *in vitro*. *Eur. J. Pharmacol.*, **162,** 197–205.

NEWBERRY, N.R., WATKINS, C.J., SPROSEN, T.S., BLACKBURN, T.P., GRAHAME-SMITH, D.G. & LESLIE, R.A. (1993). BRL 46470 potently antagonizes neural responses activated by 5-HT_3 receptors. *Neuropharmacology*, **32,** 729–735.

PEROUTKA, S.J. & SNYDER, S.H. (1979). Multiple serotonin receptors: differential binding of [^{3}H]-5-hydroxytryptamine, [^{3}H]-lysergic acid diethylamide and [^{3}H]-spiroperidol. *Mol. Pharmacol.*, **16,** 687–699.

RICHARDSON, B.P., ENGEL, G., DONATSCH, P. & STADLER, P.A. (1985). Identification of serotonin M-receptor subtypes and their specific blockade by a new class of drug. *Nature*, **316,** 126–131.

ROBERTS, K.E. & NEWBERRY, N.R. (1990). A pharmacological study of the responses induced by muscarinic agonists on the isolated superior cervical ganglion of the guinea pig. *Eur. J. Pharmacol.*, **186,** 257–265.

SAHIN-ERDEMLI, I., HOYER, D., STOLL, A., SEILER, M.P. & SCHOEFFTER, P. (1991). 5-HT_1-like receptors mediate 5-hydroxytryptamine-induced contraction of guinea pig isolated ileac artery. *Br. J. Pharmacol.*, **102,** 386–390.

SANDERS-BUSH, E. (1990). Adaptive regulation of central serotonin receptors linked to phosphoinositide hydrolysis. *Neuropsychopharmacology*, **3,** 411–416.

SANGER, G.J. & NELSON, D.R. (1989). Selective and functional 5-hydroxytryptamine$_3$ receptor antagonism by BRL 43694 (granisetron). *Eur. J. Pharmacol.*, **159,** 113–124.

STARKEY, S.J. & SKINGLE, M. (1994). 5-HT_{1D} as well as 5-HT_{1A} autoreceptors modulate 5-HT release in the guinea pig dorsal raphe nucleus. *Neuropharmacology*, **33,** 393–402.

TOTH, M. & SHENK, T. (1994). Antagonist-mediated down-regulation of 5-hydroxytryptamine type 2 receptor gene expression: modulation of transcription. *Mol. Pharmacol.*, **45,** 1095–1100.

TRENDELENBURG, U. (1956). The action of 5-hydroxytryptamine on the nictitating membrane and on the superior cervical ganglion of the cat. *Br. J. Pharmacol. Chemother.*, **11,** 74–80.

TSOU, A., KOSKA, A., BACH, C., ZUPPAN, P., YEE, C., TOM, L., ALVAREZ, R., RAMSAY, S., BONHAUS, D.W., STEFANICH, E., JAKEMAN, L., EGLEN, R.M. & CHAN, H.W. (1994). Cloning and expression of a 5-hydroxytryptamine$_7$ receptor positively coupled to adenylyl cyclase. *J. Neurochem.*, **63,** 456–464.

WADE, P.R., TAMIR, H., KIRCHGESSNER, A.L. & GERSHON, M.D. (1994). Analysis of the role of 5-HT in the enteric nervous system using anti-idiotopic antibodies to 5-HT receptors. *Am. J. Physiol.*, **266,** G403–G416.

WALLIS, D.I. & DUN, N.J. (1988). A comparison of fast and slow depolarisations evoked by 5-HT in guinea pig coeliac ganglion cells *in vitro*. *Br. J. Pharmacol.*, **93,** 110–120.

WALLIS, D.I. & NORTH, R.A. (1978). The action of 5-hydroxytryptamine on single neurones of the rabbit superior cervical ganglion. *Neuropharmacology*, **17,** 1023–1028.

WARDLE, K.A., ELLIS, E.S., BAXTER, G.S., KENNET, G.A., GASTER, L.M. & SANGER, G.J. (1994). The effects of SB 204070, a highly potent and selective 5-HT_4 receptor antagonist, on guinea-pig distal colon. *Br. J. Pharmacol.*, **112,** 789–794.

WATTS, S.W., COHEN, M.L., MOONEY, P.Q., JOHNSON, B.J., SCHOEPP, D.D. & BAEZ, M. (1994). Disruption of potential α-helix in the G loop of the guinea pig 5-hydroxytryptamine$_2$ receptor does not prevent receptor coupling to phosphoinositide hydrolysis. *J. Neurochem.*, **62,** 934–943.

WOOD, M.D., THOMAS, D.R., WATKINS, C.J. & NEWBERRY, N.R. (1993). Stereoselective interaction of mianserin with 5-HT_3 receptors. *J. Pharm. Pharmacol.*, **45,** 711–714.

ZIFA, E. & FILLION, G. (1992). 5-Hydroxytryptamine receptors. *Pharmacol. Rev.*, **44,** 401–458.

(Received June 28, 1995
Revised September 7, 1995
Accepted September 12, 1995.)