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Evidence that the 5-HT_3 receptors of the rat, mouse and guinea-pig superior cervical ganglion may be different

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1 Using grease-gap recordings from the isolated superior cervical ganglion of mouse, rat and guinea-pig, we have compared the depolarization evoked by 5-hydroxytryptamine (5-HT) with that evoked by the selective 5-HT_3 receptor agonist 2-methyl-5-HT (2-Me-5-HT).

2 The maximum depolarization induced by 2-Me-5-HT was smaller than that induced by 5-HT in all three species, and particularly in the guinea-pig.

3 The 5-HT_2 receptor antagonist ketanserin (1 μM) caused a clear rightward shift of the dose-response curve to 5-HT on the guinea-pig ganglion, but not on the mouse or rat ganglion. Spiperone (0.03 μM) had a quantitatively similar action to ketanserin (0.1 μM) on the 5-HT dose-response curve of the guinea-pig ganglion. Ketanserin had no significant effect on the dose-response curve to 2-Me-5-HT on any of these ganglia.

4 Using 2-Me-5-HT as the agonist, we determined the pA_2 values for two 5-HT_3 receptor antagonists. The potency of ICS 205-930 varied by approximately 100 fold between the species and that of (+)-tubocurarine varied by over 1000 fold. The differences in the pA_2 values of these compounds varied independently among the species.

5 We conclude that 5-HT_3 receptors are present on the superior cervical ganglion from the rat, mouse and guinea-pig, but these receptors may be pharmacologically distinct from each other. In addition, the depolarization of the guinea-pig superior cervical ganglion by low concentrations of 5-HT is largely mediated by ketanserin-sensitive receptors.

Introduction

The actions of 5-hydroxytryptamine (5-HT) on mammalian sympathetic ganglia were among its first effects on neurones to be reported (e.g. Trendelenburg, 1956). On the rabbit superior cervical ganglion, a 5-HT-induced fast depolarization was shown to be associated with the opening of channels permeable to sodium and potassium ions (Wallis & Woodward, 1975; Wallis & North, 1978). The fast depolarization of such ganglia is generally considered to be mediated by 5-HT_3 receptors given its sensitivity to the antagonists MDL 72222 (Fozard, 1984b) and ICS 205-930 (Richardson *et al.*, 1985). 5-HT_3 receptors also occur on parasympathetic, sensory and enteric neurones (Fozard, 1984a; Bradley *et al.*, 1986; Richardson & Engel, 1986), and there have been a number of recent reports of the presence of 5-HT_3 binding sites in the central nervous system (Kilpatrick *et al.*, 1987; Watling, 1988).

A number of reports has indicated that 5-HT_3 receptors may be heterogeneous (Bradley *et al.*, 1986; Round & Wallis, 1987; Richardson & Engel, 1986). The principal reasons for such conclusions are the differing pA_2 values of particular 5-HT_3 receptor antagonists on different tissues. For instance, MDL 72222 had a pA_2 of approximately 9 on the rabbit heart (Fozard, 1984b), about 8 on both the rabbit vagus and rabbit superior cervical ganglion (Azami *et al.*, 1985; Donatsch *et al.*, 1984), but on the guinea-pig ileum it was lower than 6 (Fozard, 1984b). Another antagonist ICS 205–930 had a pA_2 of approximately 10 on the heart, superior cervical ganglion and vagus nerve of the rabbit (Donatsch *et al.*, 1984; Round & Wallis, 1986), but between 7 and 8 on the guinea-pig ileum (Donatsch *et al.*, 1984; Richardson *et al.*, 1985; Lattimer *et al.*, 1989). The above evidence was cited by Richardson & Engel (1986) when they proposed three subtypes of 5-HT_3 receptor (5-HT_{3A}, 5-HT_{3B} and 5-HT_{3C}).

Since (+)-tubocurarine antagonizes 5-HT responses (Gerschenfeld & Paupardin-Tritsch, 1974; Nash *et al.*, 1984), including those of the rat superior cervical ganglion (Newberry & Gilbert, 1989), it was interesting to note that (+)-tubocurarine antagonized the 5-HT_3 response of NG 108-15 and N1E-115 cells (Yakel & Jackson, 1988; Peters *et al.*, 1990) in nanomolar concentrations, but 10–500 μM was needed to antagonize the fast depolarization of guinea-pig submucous plexus neurones (Surprenant & Crist, 1988) and guinea-pig coeliac ganglion cells (Wallis & Dun, 1988).

It is not clear whether the different sensitivities of the above 5-HT responses to the 5-HT_3 receptor antagonists were due to the use of different tissues and/or different species. We have studied the action of 5-HT and 2-methyl-5-HT (2-Me-5-HT) on a neuronal preparation isolated from three species with the aim of detecting possible species differences in 5-HT_3 receptors.

Methods

Male Sprague-Dawley rats (150–180 g, Bantin and Kingman), Swiss-Webster mice (18–30 g, Bantin and Kingman) and Duncan-Hartley guinea-pigs (300–450 g, Interfauna or Graystone) were killed by a blow to the head followed by exsanguination. Each superior cervical ganglion (SCG, sometimes called the cranial cervical ganglion in the guinea-pig) was desheathed and submerged in a three compartment bath with the ganglion body in the central compartment and the pre- and postganglionic trunks (the cervical sympathetic trunk and the internal carotid nerve, respectively) protruding through greased gaps into the outer two compartments (Newberry & Priestley, 1987; Newberry, 1988). The central compartment (volume ca. 0.5 ml) was continually superfused at 2.0–2.5 ml min^{-1} with an aqueous medium, at 25°C. The same medium in the outer two compartments was static. The medium, pre-equilibrated with 5% CO_2/95% O_2, contained (in mM): NaCl 125, $NaHCO_3$ 25, (+)-glucose 10, $CaCl_2$ 2.5, KCl 2, KH_2PO_4 1 and $MgSO_4$ 1. The ganglionic potential was recorded between two Ag/AgCl electrodes, one in the central compartment and the other in the compartment containing the internal carotid nerve.

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5-Hydroxytryptamine or 2-Me-5-HT was applied to the ganglia for 1 min periods via the superfusing medium. At the start of each experiment, 100 μM 5-HT was applied 2 to 3 times, at 30 min intervals, to obtain two consecutive responses of a similar size. All subsequent response amplitudes (measured at the peak of the fast depolarization) were normalised to the amplitude of the last of these applications (given the arbitrary value of 1). Approximately 30 min after the 'last' application of 100 μM 5-HT (see above) the agonist dose-response curve was determined by superfusing 5-HT in increasing concentrations at intervals of 10–20 min (the longer intervals being used for the higher concentrations). The antagonist was added to the superfusate 15 min after the agonist application which evoked the maximum response. The antagonist was superfused for 1 h and the dose-response curve was re-determined in its presence; one concentration of antagonist was used for each ganglion. The same protocol was used for controls except that the antagonist was not added to the medium. On the guinea-pig ganglion, however, the determination of the 5-HT dose-response curve (10 nM–1 mM) took a considerable amount of time. Therefore, in order to investigate the action of ketanserin or spiperone against 5-HT on this preparation (e.g. Figure 2), the following protocol was used. The antagonist superfusion was started 30 min after the 'last' application of 100 μM 5-HT. After 1 h in the antagonist the agonist dose-response curve was determined. The effect of the antagonist was judged by comparing such dose-response curves with those determined on other ganglia where the same protocol had been used but no antagonist added to the medium. To be consistent on the guinea-pig SCG (for Figure 2), we used the same protocol to study the action of ketanserin on the dose-response curve to 2-Me-5-HT. However, full dose-response curves to 2-Me-5-HT were determined before and after the 5-HT_3 antagonists on all SCGs.

The potency of an agonist on a given preparation was expressed as its pEC_{50} value. The potency of an antagonist was expressed as a pA_2 value (Schild, 1947), the negative logarithm (base 10) of the molar concentration of the antagonist that would give a dose-ratio of 2. This was routinely estimated by the equation: $pA_2 = \log_{10}(DR - 1) - \log_{10}$ (molar concentration of the antagonist). Since in the majority of cases (see Figure 4) the maximum response to 2-Me-5-HT was reduced in the presence of the antagonist, the pA_2 value provides only a convenient means of quantifying the potency of an antagonist; however, by using the term 'pA_2' we neither imply that the antagonist acted competitively nor claim that this value is an accurate representation of the true binding affinity of the compound for the receptor (like a pK_B). Dose-ratios (DR) were obtained at the level of half the maximum response in the second dose-response curve. The dose-ratio values used in the calculation were usually between 2 and 10, but they were not used if the maximum response to 2-Me-5-HT was reduced by more than 60%. Dose-ratios were not possible with the methodology used for 5-HT on the guinea-pig ganglion. For these studies, apparent dose-ratios were obtained from the increase in the geometric mean of the concentration that produced half the response of the standard 100 μM 5-HT application i.e. a relative depolarization level of 0.5.

Statistical analyses of ratios (relative depolarizations and dose-ratios) were carried out with geometric ($\log_{10}$) values, since ratios are more normally distributed using this transformation. Comparisons were made by Student-Neuman-Keuls multiple range test or the unpaired t test (BMDP Statistical Software Inc.), with significance at $P < 0.05$. The number of determinations refers to the number of experiments on different ganglia.

2-Me-5-HT hydrobromide and ICS 205-930 hydrochloride ((3α-tropanyl)-1H-indole-3-carboxylic acid ester) were prepared at Merck Sharp & Dohme, Terlings Park by Clare Kneen. (+)-Tubocurarine chloride and 5-hydroxytryptamine hydrochloride were obtained from Sigma, 1-phenylbiguanide from Aldrich and spiperone and ketanserin tartrate from Research Biochemicals Inc. Stock solutions (1–100 mM) of each compound were prepared and diluted to the required concentrations on each experimental day. Stock solutions were prepared in distilled water, except for spiperone which was dissolved in 5 mM HCl.

Results

Agonists

At a concentration which was maximal on the rat SCG, 5-HT (100 μM) reproducibly depolarized the isolated SCG from all three species. This response was much larger on the mouse SCG than on SCG from the rat or guinea-pig (Figure 1). Following the 5-HT-induced fast depolarization of the rat and mouse SCG, but not the guinea-pig SCG, there was a hyperpolarization.

Two putative selective 5-HT_3 receptor agonists were also tested on these ganglia, initially at 100 μM since this concentration evoked a maximal response on the rat SCG. 2-Me-5-HT (Richardson *et al.*, 1985; Bradley *et al.*, 1986) depolarized the SCG from each species, but the response of the guinea-pig SCG was much smaller than that to 100 μM 5-HT (Figure 1). At 100 μM, phenylbiguanide (Fortune *et al.*, 1983) depolarized three mouse SCG by 0.88, 0.82 and 0.95 of the response to 100 μM 5-HT. On four rat SCG it evoked relative depolarizations of 1.21, 0.76, 0.93 and 0.93. By contrast, phenylbiguanide evoked no detectable depolarization of three guinea-pig

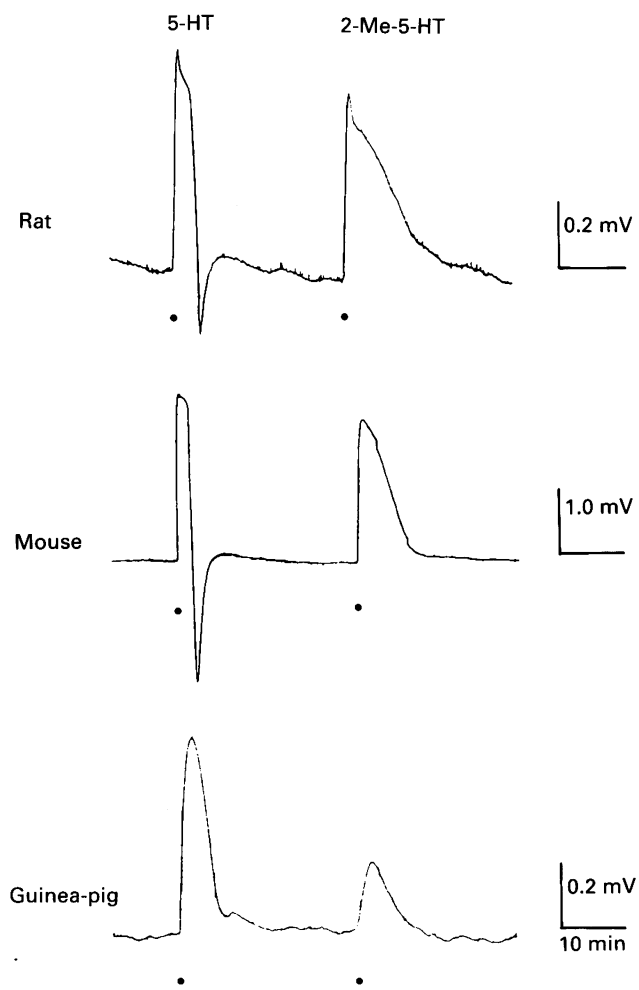

Figure 1 Chart recordings of the depolarizing responses to 5-hydroxytryptamine (5-HT) and 2-methyl-5-HT (2-Me-5-HT) on the isolated superior cervical ganglion from the indicated species. Each agonist was superfused at 100 μM for 1 min at the time indicated by the dot. The time calibration is the same for all records.

Table 1 The potencies and relative maximum responses to 5-hydroxytryptamine (5-HT) and 2-methyl-5-HT (2-Me-5-HT) on the superior cervical ganglia from three species

Agonist pharmacology of ganglionic depolarization

	Response to 100 μM 5-HT (mV)	*Relative maxima* 5-HT	*Relative maxima* 2-Me-5-HT	pEC_{50} 5-HT	pEC_{50} 2-Me-5-HT	*Repeat dose-ratios* 5-HT	*Repeat dose-ratios* 2-Me-5-HT
Rat							
Mean	**0.56**	**1.07**	**0.91**	**5.26**	**5.39**	**1.04**	**0.69**
+ s.d.	0.21	0.07	0.08	0.10	0.12	0.26	0.19
− s.d.	0.21	0.07	0.07	0.10	0.12	0.21	0.15
n	19	16	19	16	19	8	4
Mouse							
Mean	**2.44**	**1.10**	**0.90**	**5.32**	**5.16**	**1.13**	**0.81**
+ s.d.	1.00	0.09	0.08	0.26	0.15	0.48	0.29
− s.d.	1.00	0.08	0.07	0.26	0.15	0.34	0.21
n	39	11	28	11	28	7	8
Guinea-pig							
Mean	**0.62**	**1.27**	**0.81**	**5.08**	**4.17**	**ND**	**0.95**
+ s.d.	0.30	0.13	0.13	0.16	0.11	—	0.11
− s.d.	0.30	0.12	0.11	0.16	0.11	—	0.10
n	59	6	11	6	11	—	4

Potencies are expressed as pEC_{50} values ($-\log_{10}$ of the molar concentration required to evoke a half-maximal response). Relative maxima are in arbitrary units compared to the amplitude of the last response to 100 μM 5-HT at the start of the experiment (given the value of 1, see Methods). The range of absolute values of this particular response is indicated. The reproducibility of the 5-HT and 2-Me-5-HT dose-response curves are indicated by the dose-ratios obtained by repeating the dose-response protocol in the absence of an antagonist. ND = not done because of the different protocol (see Methods). Values are arithmetic means for *Response to 5-HT* and *pEC_{50}* and geometric means for *Relative maxima* and *Repeat dose-ratios*, hence the differences in the +s.d. and −s.d. values. The relative maxima and pEC_{50} values for 2-Me-5-HT and 5-HT, when compared for the ganglia from each individual species, were all significantly different.

SCG at this concentration. The phenylbiguanide responses of the rat and mouse SCG were both biphasic: a depolarization followed by a hyperpolarization (see Figure 6 of Newberry & Gilbert, 1989).

Dose-response curves to 5-HT and 2-Me-5-HT were determined on the SCG from each species. Their potencies in evoking depolarization and their relative maximum responses are shown in Table 1. The reproducibility of these dose-response curves was demonstrated by repeating the dose-response curve 1.25 h after the first determination. The 5-HT dose-response curve on the guinea-pig SCG occurred between 10 nM and 1 mM which was clearly a greater concentration-range than on the rat or mouse SCG. The maximum response to 2-Me-5-HT was significantly smaller than that to 5-HT on all SCG, especially that from the guinea-pig. In each species, the potency of 5-HT was significantly different from that of 2-Me-5-HT.

5-HT_2 receptor antagonists

We investigated the action of 1 μM ketanserin on the 5-HT dose-response curves of these ganglia. Its only clear action was against the 5-HT dose-response curve on the guinea-pig SCG where it caused a rightward shift of the dose-response curve (Figure 2). The resultant curve was steeper and had a reduced maximum. In contrast, the dose-response curves to 2-Me-5-HT on each SCG were not significantly affected by ketanserin.

Although not studied in great detail, the effect of ketanserin (1 μM) on the dose-response curve to 5-HT on the guinea-pig SCG was mimicked by spiperone (0.3 μM, $n = 5$, not shown). This similarity of action was also apparent at lower concentrations of spiperone and ketanserin (Figure 3). The geometric mean concentration (± s.d.) of 5-HT necessary to evoke a response level of 0.5 was 4.0 μM (+0.6, −0.5; $n = 6$) in the absence of an antagonist. This value increased to 34 μM (+14, −11; $n = 5$) in 0.1 μM ketanserin and to 32 μM (+11, −7; $n = 4$) in 0.03 μM spiperone.

5-HT_3 receptor antagonists

The antagonist potencies of two structurally unrelated 5-HT_3 receptor antagonists, ICS 205-930 and (+)-tubocurarine, were evaluated on the SCG from each species, with 2-Me-5-HT as agonist. Except on the guinea-pig SCG, tubocurarine produced a parallel shift of the dose-response curve. In contrast, ICS 205-930 flattened the dose-response curve on all SCGs. This type of action precluded the study of a wide range of concentrations. The calculated pA_2 values (see Methods) of both antagonists varied with species (Table 2). In a given species, however, the pA_2 values did not change significantly over the effective concentration-range. Real differences in the threshold concentration of an antagonist were apparent as the concentration-ranges tested often included a concentration that gave no significant shift of the dose-response curve. For instance, 1 nM ICS 205-930 had no effect on a mouse SCG, but it reproducibly antagonized 2-Me-5-HT on the rat SCG. The differences in potency of these compounds are clearly suggested in Figure 4 which shows graphs from individual experiments. There is a potency difference between the three species of approximately 100 fold for ICS 205-930 and greater than 1000 fold for (+)-tubocurarine. Since these differences could be due to the antagonist reaching equilibrium conditions at different rates on the three preparations, we tested the possibility on the rat and mouse SCG where ICS 205-930 had a relatively high pA_2. On these preparations a 2 h incubation of ICS 205-930 resulted in no greater antagonism compared to that observed after a 1 h pretreatment.

The presence of a ketanserin-sensitive response would have affected the calculated pA_2 values for a 5-HT_3 receptor antagonist against 5-HT or 2-Me-5-HT. This was clearly demonstrable on the guinea-pig SCG where 1 μM ICS 205-930 had no effect on the dose-response curve to 5-HT ($n = 2$) unless the preparation was superfused with ketanserin (1 μM) throughout the experiment. In three experiments in the presence of ketanserin, ICS 205-930 (1 μM) produced dose-ratios of 17, 15 and 7. From the data in Figure 2, it was possible that the response to 2-Me-5-HT on the guinea-pig SCG was

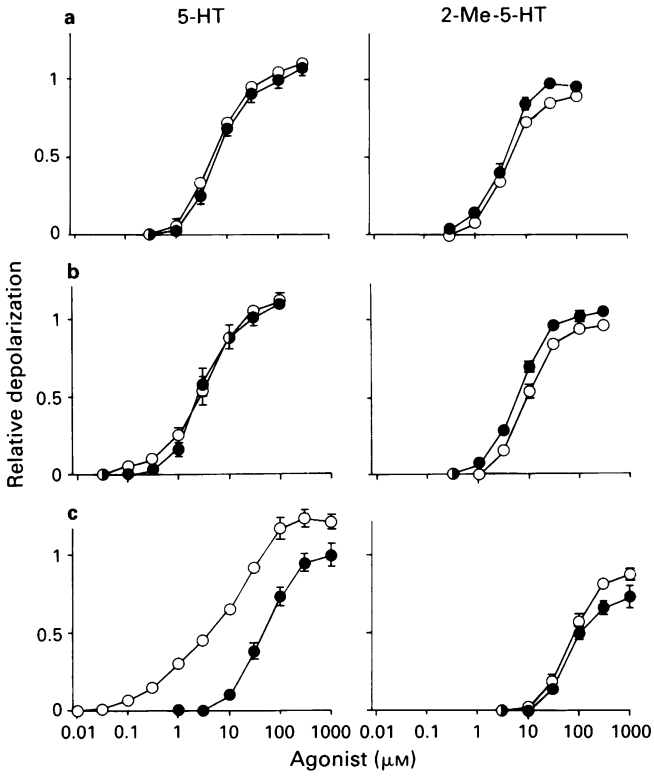

Figure 2 The effect of 1 μM ketanserin (1 h) on the dose-response curves to 5-hydroxytryptamine (5-HT) and 2-methyl-5-HT (2-Me-5-HT) on the superior cervical ganglia (SCG) from (a) rat, (b) mouse and (c) guinea-pig. Control (○); ketanserin (●). Since the agonist dose-response curves were reproducible (Table 1), the dose-response curves before (controls) and after ketanserin were determined on the same rat and mouse SCG. However, for reasons highlighted in the Methods, the control dose-response curves for the guinea-pig SCG were obtained from different animals. All responses are expressed as the relative depolarization compared to the depolarization of the last application of 100 μM 5-HT at the beginning of each experiment (see Methods). Relative depolarization data are the geometric means with s.e.mean shown by vertical bars. The number of determinations was the same for both curves in each graph: (a) left 8, (a) right 4; (b) left 4, (b) right 4; (c) left 6 and (c) right 7. Ketanserin did not significantly reduce the response to 10 μM–1 mM 2-Me-5-HT on the guinea-pig SCG (with the exception of the response to 300 μM). Ketanserin significantly reduced the response to 0.3 μM 5-HT on the mouse SCG (by 50–80%). Unfortunately, the magnitude of this effect is masked by the size of the symbols and the scale of the y axis, but further experiments are necessary before any clear conclusions can be reached.

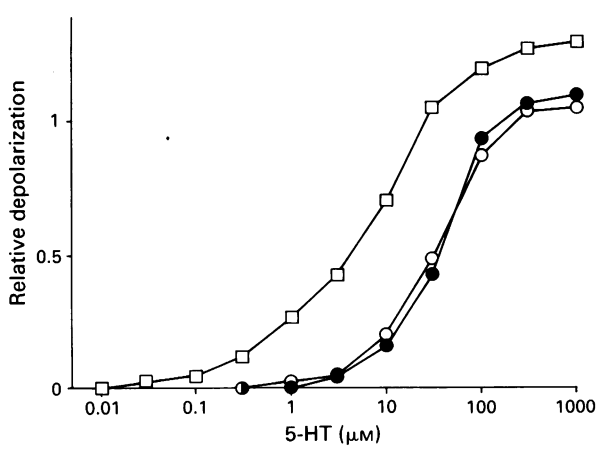

Figure 3 The effect of spiperone (0.03 μM, ●) and ketanserin (0.1 μM, ○), both for 1 h, on the 5-hydroxytryptamine (5-HT) dose-response curve of the guinea-pig superior cervical ganglion. Data from individual ganglia: those treated with spiperone or ketanserin were from the same guinea-pig, the untreated ganglion (□) from another.

Table 2 The pA_2 values of ICS 205-930 and (+)-tubocurarine against 2-methyl-5-hydroxytryptamine (2-Me-5-HT) on the superior cervical ganglion from three species

	Antagonist pharmacology of ganglionic depolarization to 2-methyl-5-HT			
	ICS 205-930		*(+)-Tubocurarine*	
	Concn range	*pA_2*	*Concn range*	*pA_2*
	(low, high) *n*	Mean (± s.d.) *n*	(low, high) *n*	Mean (± s.d.) *n*
Rat	0.3 nM	9.4	300 nM	7.1
	1.0 nM	0.2	3 μM	0.1
	7	5	6	6
Mouse	1 nM	8.7	3 nM	8.1
	10 nM	0.2	100 nM	0.1
	7	6	7	6
Guinea-pig	100 nM	7.2	1 μM	4.8
	300 nM	0.1	100 μM	0.2
	6	6	9	7

The pA_2 values were calculated from the Schild equation (1947, see Methods). The pA_2 values of each compound are significantly different between all species. The concentration-range often includes a concentration which produced no significant shift of the dose-response curve i.e. no greater than the repeat dose-ratios from Table 1. Consequently, the *n* value for this column is larger than that for the number of times that a pA_2 was calculated. The pA_2 values have been rounded to one decimal place but they have not been corrected for the small sensitivity shifts indicated in Table 1.

reduced by ketanserin. In the presence of 1 μM ketanserin, however, the mean pA_2 value for (+)-tubocurarine against 2-Me-5-HT was 4.7 ± 0.2 ($n = 4$), which was not significantly different from that determined in the absence of ketanserin (see Table 2).

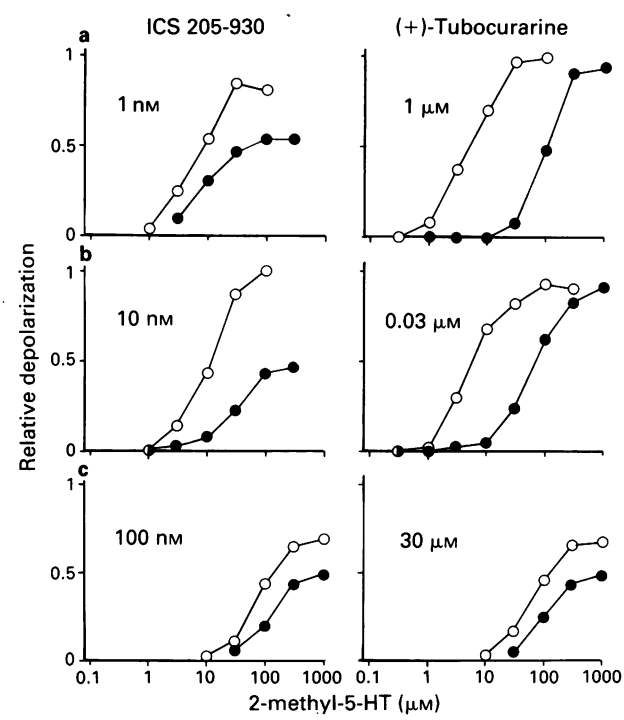

Figure 4 Graphs from individual experiments showing the effect of the indicated concentrations of the two $5\text{-}HT_3$ receptor antagonists (1 h, ●) on the dose-response curve to 2-methyl-5-hydroxytryptamine (2-Me-5-HT, ○) on the superior cervical ganglion from (a) rat, (b) mouse and (c) guinea-pig.

At the concentrations tested, none of the 5-HT_2 or 5-HT_3 receptor antagonists evoked a noticeable change in the ganglionic potential.

Discussion

The principal finding of this study is that the 5-HT_3 receptors of the rat, mouse and guinea-pig SCG appear to be pharmacologically distinguishable with the antagonists ICS 205-930 and (+)-tubocurarine. For these experiments, we used 2-Me-5-HT as the agonist of choice since there was a ketanserin-sensitive response to 5-HT on the guinea-pig SCG.

The 5-HT induced depolarization of the mouse SCG was followed by two other potentials, as seen on the rat SCG (Newberry & Gilbert, 1989). By analogy, therefore, it is possible that 5-HT can evoke on the mouse SCG a 5-HT_3 receptor-mediated fast depolarization with an underlying 5-HT_{1A} receptor-mediated hyperpolarization, followed by a slow depolarization that is mediated by an unidentified receptor. In contrast, the 5-HT depolarization of the guinea-pig SCG was not followed by a hyperpolarization.

2-Me-5-HT (Richardson *et al.*, 1985; Bradley *et al.*, 1986) evoked a smaller depolarization than 5-HT in all three species, particularly in the guinea-pig. A smaller maximum response to this agonist has also been seen on the rat isolated vagus nerve (Ireland & Tyers, 1987). Phenylbiguanide (100 μM) depolarized the SCG from the rat and mouse, but not the guinea-pig. This compound has been reported to be a partial agonist of 5-HT_3 receptors on the rat vagus (Ireland & Tyers, 1987), but it failed to depolarize the guinea-pig vagus (Burridge *et al.*, 1989). A species, rather than a preparation, difference in 5-HT_3 receptors was therefore suspected. It is possible, however, that marked variations in the substrate specificity of the 5-HT uptake systems of the SCG of these species (cf. Ireland *et al.*, 1987) could contribute to the different potencies of these agonists.

The depolarization of mammalian sympathetic ganglia by 5-HT is generally considered to be mediated by 5-HT_3 receptors because of its sensitivity to antagonists such as MDL 72222 and ICS 205-930 (Fozard, 1984b; Azami *et al.*, 1985; Richardson *et al.*, 1985; Round & Wallis, 1986; 1987). Moreover, the 5-HT-induced depolarization of the rabbit superior cervical ganglion (Nash *et al.*, 1984) and its preganglionic axons (Elliot & Wallis, 1988) is resistant to the 5-HT_2 receptor antagonists, ketanserin (Leysen *et al.*, 1981) and cyproheptadine. However, ketanserin shifted the dose-response curve to 5-HT on the guinea-pig SCG to the right (see Figures 2 and 3). Since ketanserin also has affinity for 5-HT_{1C} binding sites (Hoyer *et al.*, 1985), we investigated the action of spiperone (spiroperidol) which has a high affinity for 5-HT_2 receptors, but a relatively low affinity for 5-HT_{1C} receptors (Hoyer *et al.*, 1985). The similar and highly potent action of spiperone and ketanserin on the 5-HT dose-response curve of the guinea-pig ganglion (Figure 3) indicates that a large part of the 5-HT dose-response curve on this preparation may be mediated by 5-HT_2, rather than 5-HT_{1C}, receptors. This conclusion is not unreasonable given that other workers have suggested that 5-HT_2 receptors mediate neuronal depolarization and/or excitation (Davies *et al.*, 1987; 1988; North & Uchimura, 1989). The possible presence of 5-HT_2 and 5-HT_3 receptors on the guinea-pig SCG may correspond to the fast and slow depolarizations evoked by 5-HT on another sympathetic ganglion from this species, the coeliac ganglion (Wallis & Dun, 1988).

The effects of the 5-HT_3 receptor antagonists, ICS 205-930 and (+)-tubocurarine, were determined with 2-Me-5-HT as the agonist, given that the ganglionic depolarization to this agonist was demonstrated to be resistant to ketanserin in all three species. There were differences in the pA_2 values obtained with ICS 205-930 and (+)-tubocurarine between these species, although there was not a common trend i.e. the antagonist potency of ICS 205-930 ranked rat > mouse > guinea-pig, whereas for (+)-tubocurarine it ranked mouse > rat > guinea-pig.

Our data add to other reports showing a marked similarity of pA_2 values for ICS 205-930 as an antagonist of 5-HT_3 receptor-mediated responses on different tissues from the guinea-pig, e.g. it has a pA_2 of between 7 and 8 on the superior cervical ganglion (this report), vagus (Lattimer *et al.*, 1989; Burridge *et al.*, 1989) and ileum (Donatsch *et al.*, 1984; Lattimer *et al.*, 1989). Other antagonists, e.g. MDL 72222 and GR38032F, also have similar pA_2 values on various tissues from the guinea-pig (Burridge *et al.*, 1989; Grossman *et al.*, 1989; Lattimer *et al.*, 1989). It may therefore be concluded that the 5-HT_3 receptors at many sites in this species are similar. From the pA_2 values for ICS 205-930 on the vagus of the guinea-pig and the rat, it has recently been suggested that the 5-HT_3 receptors in these species are different (Burridge *et al.*, 1989; Lattimer *et al.*, 1989). Our work with ICS 205-930 on the SCG from those species would support such a suggestion.

Tubocurarine provided more conclusive evidence for species differences in 5-HT_3 receptors. The separation of its antagonist potencies between the species was considerably greater than for ICS 205-930. In contrast to ICS 205-930, it produced a parallel shift of the dose-response curve to 2-Me-5-HT on the rat and mouse (but not the guinea-pig) SCG, indicating that it may act competitively. Evidence supporting this type of action comes from work on neuroblastoma cells where its antagonism of the 5-HT_3 receptor-induced fast inward current was not voltage- or agonist-dependent (Peters *et al.*, 1990). In addition to a competitive action, (+)-tubocurarine may antagonize non-competitively since on rabbit nodose ganglion cells (Higashi & Nishi, 1982) it produced a parallel shift of the 5-HT dose-response curve, followed at higher concentrations, by a depression of the maximum response.

It should be noted that on guinea-pig submucous plexus neurones (Surprenant & Crist, 1988) 5-HT and 2-Me-5-HT evoked a ketanserin-resistant depolarization, associated with an increase in membrane resistance. This response was unaffected by (or indeed better observed in the presence of) ICS 205-930 (2 μM) or tubocurarine (200 μM). The pharmacology of this response would appear to be similar to that evoked by 2-Me-5-HT on the guinea-pig SCG. However, we do not believe that these responses are mediated by the same receptor since 2 μM ICS 205-930 or 200 μM tubocurarine would have substantially antagonized the response to 2-Me-5-HT on the guinea-pig SCG.

Recent electrophysiological experiments have shown that different types of channel can be activated by 5-HT_3 receptors. Thus, the channels activated by 5-HT on guinea-pig submucous plexus neurones have conductances of 9 and 15 pS (Derkach *et al.*, 1989), whereas the 5-HT_3-induced channel conductance on N1E-115 cells is 0.3 pS (Lambert *et al.*, 1989). Such work provides additional evidence for heterogeneity among 5-HT_3-receptor ion channel complexes.

In conclusion, superior cervical ganglia from rat, mouse and guinea-pig respond differently to agonists and antagonists of 5-HT receptors. The ganglionic depolarization to 5-HT cannot be assumed to be mediated solely by 5-HT_3 receptors since a 5-HT_2 receptor mediated depolarization may dominate the response to low concentrations of 5-HT on the guinea-pig SCG. The action of the selective 5-HT_3 receptor agonist, 2-Me-5-HT, showed that 5-HT_3 receptors do appear to mediate sympathetic ganglionic depolarization in each of these species. The affinities of the 5-HT_3 receptor antagonists, ICS 205-930 and (+)-tubocurarine, varied dramatically and independently among these species. These results suggest that the 5-HT_3 receptors in the rat, mouse and guinea-pig superior cervical ganglion may be pharmacologically distinct.

Note added in proof

Our conclusion that 5-HT_3 receptors may differ between species is further supported by recent data from two other research groups. The pharmacology of the 5-HT_3 receptor on the guinea-pig vagus clearly differs from that of the rat vagus (Butler *et al.*, 1990; *Br. J. Pharmacol.*, **101**, 591–598). In addition, the 5-HT_3 receptors mediating an inward current in rabbit and guinea-pig dissociated nodose ganglion cells are pharmacologically distinguishable (Peters, Malone & Lambert, personal communication). Like us, these groups detected differences in the potencies of 5-HT_3 antagonists on the same preparation taken from separate species. Interestingly, Peters *et al.* noted that (+)-tubocurarine was 90 times more potent on cells taken from rabbits as compared to guinea-pigs, its pIC_{50} on guinea-pig cells (5.0) being comparable to our pA_2 (4.8).

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