

Pharmacological characterization of 5-hydroxytryptamine-induced hyperpolarization of the rat superior cervical ganglion

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- 1 A study has been made of the pharmacology of 5-hydroxytryptamine (5-HT)-induced hyperpolarization responses recorded extracellularly from the rat isolated superior cervical ganglion (SCG).
- 2 Hyperpolarization responses induced by 5-HT (1×10^{-8} – 1×10^{-4} M) in the presence of MDL 72222 (1×10^{-5} M) were not antagonized by phentolamine (1×10^{-6} M), prazosin (1×10^{-7} – 3×10^{-7} M), haloperidol (1×10^{-6} M) or ketanserin (1×10^{-7} – 1×10^{-6} M). However, the latter two compounds both potentiated and increased the persistence of the hyperpolarization induced by moderate to high concentrations of 5-HT. Spiperone (1×10^{-7} M) caused similar effects. All further experiments were performed in the presence of ketanserin (1×10^{-6} M) as well as MDL 72222.
- 3 8-Hydroxy-2(di-n-propylamino)-tetralin (8-OH-DPAT; 1×10^{-7} – 1×10^{-4} M) and ipsapirone (3×10^{-5} – 3×10^{-4} M) behaved as weak hyperpolarizing agonists on the SCG. However, at concentrations below those required to produce hyperpolarization, both compounds acted as unsurmountable antagonists of 5-HT-induced hyperpolarization.
- 4 5-Carboxamidotryptamine (5-CT; 1×10^{-9} – 1×10^{-5} M) mimicked the hyperpolarizing activity of 5-HT on the SCG. The EC_{50} for 5-CT was approximately 9 fold lower than that for 5-HT.
- 5 Spiperone (1×10^{-7} – 1×10^{-5} M) behaved as a reversible competitive antagonist of hyperpolarization responses induced by 5-HT with a pK_B value of 7.40 ± 0.09 . Spiperone (1×10^{-7} – 1×10^{-6} M) also caused concentration-dependent rightward displacement of the 5-CT concentration-hyperpolarization response curve. In this case, the pK_B was 7.80 ± 0.05 .
- 6 (\pm)-Cyanopindolol (3×10^{-7} – 3×10^{-6} M) caused non-parallel rightward displacements of the 5-HT concentration-response curve. Against 5-CT, (\pm)-cyanopindolol (3×10^{-7} – 3×10^{-6} M) caused a concentration-independent rightward displacement of the concentration-response curve, accompanied by a large increase in the maximum response. 5-CT-induced hyperpolarization recorded in the presence of (\pm)-cyanopindolol (3×10^{-7} M) was not significantly antagonized by methiothepin (1×10^{-6} M) or methysergide (1×10^{-6} M).
- 7 It is concluded that 5-HT-induced hyperpolarization of the rat SCG is mediated via a 5-HT₁-like receptor which resembles the 5-HT_{1A} binding site. However, a lack of selective drugs precludes more definitive characterization of this receptor.

Introduction

On the rat isolated superior cervical ganglion (SCG), 5-hydroxytryptamine (5-HT) induces concentration-related hyperpolarization responses. This hyperpolarization is not a consequence of 5-HT-induced depolarization and, unlike the depolarization, does not result from the activation of 5-HT₃ receptors since it is resistant to blockade by MDL 72222 and metoclopramide (Ireland, 1987; Ireland & Tyers, 1987). However, a more precise characterization of the receptor mediating the hyperpolarization response has not been published.

Early in the course of the present study, it was observed that 5-HT-induced hyperpolarization of the SCG was not antagonized by ketanserin and therefore could not result from the activation of 5-HT₂ receptors. This result prompted the hypothesis that if a 5-HT receptor did indeed mediate the hyperpolarization response, then it appeared to fall into the group of receptors described as '5-HT₁-like' by Bradley *et al.* (1986). Testing this hypothesis is complicated by the lack of good selective antagonists for the 5-HT₁-like receptors (see Charlton *et al.*, 1986; Bradley *et al.*,

1986). Nevertheless, the characterization of 5-HT-induced hyperpolarization of the SCG may be attempted using putative agonists and non-selective antagonists active at 5-HT₁-like receptors, ligands for 5-HT₁ binding sites (Pedigo *et al.*, 1981; Pazos *et al.*, 1984; Hoyer *et al.*, 1985) and selective antagonists for the 5-HT₂ and 5-HT₃ receptor types.

Methods

Preparation of tissues

Male hooded rats weighing 200–300 g were stunned by a blow to the back of the head and killed by cardiac puncture. Superior cervical ganglia were excised as rapidly as possible and placed in oxygenated Krebs-Henseleit medium (greater than 25 ml per tissue) at room temperature (approximately 21°C). The connective tissue sheath around each isolated SCG was then carefully removed.

Extracellular recording

Within one hour of isolation, de-sheathed superior cervical ganglia were transferred to two-compartment Perspex baths to permit extracellular recording of agonist-induced hyperpolarization. The method for mounting the preparations in the baths and the techniques for recording and drug application are described in the companion paper (Ireland, 1987). As in this previous study, the temperature of each preparation was maintained at $27 \pm 1^\circ\text{C}$.

Measurement of the effects of agonists and antagonists

Concentration-response curves for agonist-induced hyperpolarization were constructed non-cumulatively using serially-increasing concentrations. Each agonist application was continued until the evoked response appeared to have reached a peak. This generally took less than 3 min. Tissue preparations were always allowed to repolarize fully between agonist applications. For 5-HT this generally took 15–20 min, for 5-carboxamidotryptamine (5-CT) 30–50 min was required.

The EC_{50} and maximum response (E_{max}) for each concentration-response curve was estimated by direct computer-aided fit of a logistic curve (see Ireland & Tyers, 1987).

The effects of 5-HT and 5-CT were compared on eight superior cervical ganglia, each obtained from a different rat. Every preparation was dosed alternately with 5-HT and 5-CT. Four preparations were dosed in the sequence 5-HT–5-CT–5-HT, this was reversed for the remainder.

The effects of antagonists were quantified once

apparent equilibrium had been attained; details of the methodology are given elsewhere (Ireland & Tyers, 1987). The negative logarithm of the apparent dissociation constant for an antagonist (pK_B) was estimated by calculation of the mean of the individual results: $pK_B = \log_{10}(\text{dose-ratio} - 1) - \log_{10}(\text{concentration of antagonist})$. The effect of each concentration of antagonist was measured on at least four SCG preparations, each obtained from a different animal.

Drugs and solutions

Low-calcium Krebs-Henseleit medium was used in the present study (see Ireland, 1987). It had the following composition (in mmol l^{-1}): NaCl 118, NaHCO₃ 25, KH₂PO₄ 1.18, KCl 4.7, MgSO₄·7H₂O 1.18, CaCl₂ 0.15 and glucose 11.0. It was gassed with 95% O₂ and 5% CO₂. The medium was prepared in glass-distilled water and reagents, which were all A.R. grade, were purchased from commercial sources. For all the experiments described in this paper, the Krebs-Henseleit medium contained MDL 72222 (1×10^{-5} M) to suppress 5-HT₃-receptor-mediated depolarization.

The following drugs were used: 5-carboxamidotryptamine maleate (5-CT; Glaxo), chlorimipramine hydrochloride (Ciba), citalopram hydrobromide (Lundbeck), (\pm)-cyanopindolol (Sandoz), desmethylinipramine hydrochloride (Ciba), haloperidol (Janssen), (\pm)-8-hydroxy-2(di-n-propylamino)-tetralin hydrobromide (8-OH-DPAT; Research Biochemicals), 5-HT creatinine sulphate (Sigma), ketanserin tartrate (Janssen), MDL 72222 (1 α H, 3 α , 5 α H-tropan-3-yl-3,5-dichloro-benzoate) (tropanserin; Merrell-Dow), methiothepin maleate (Roche), methysergide hydrogenmaleate (Sandoz), paroxetine hydrochloride (Ferrosan), phentolamine mesylate (Ciba), (\pm)-pindolol (Sandoz), prazosin hydrochloride (Pfizer), spiperone (Janssen), and ipsapirone (TVX Q7821; Troponwerke). Drug solutions were prepared immediately before use. 5-HT, 5-CT, 8-OH-DPAT and phentolamine were dissolved in Krebs-Henseleit medium. (\pm)-Cyanopindolol, haloperidol, (\pm)-pindolol and spiperone were dissolved in 0.1 M (\pm)-tartaric acid to give 1×10^{-3} – 1×10^{-2} M solutions. The remaining drugs were dissolved in distilled water to give 1×10^{-3} – 1×10^{-2} M solutions. All these solutions were subsequently diluted with Krebs-Henseleit medium without causing visible precipitation.

Results

Preliminary characterization of 5-hydroxytryptamine-induced hyperpolarization

All results described in this paper were obtained from rat isolated superior cervical ganglia superfused with

low-calcium Krebs-Henseleit medium which contained MDL 72222 (1×10^{-5} M) to block 5-HT₃-receptor-mediated depolarization. Under these conditions, 5-HT (1×10^{-8} – 1×10^{-4} M) caused rapid concentration-dependent hyperpolarization responses with a maximum amplitude of 200 to 400 μ V. Responses induced by low concentrations of 5-HT (1×10^{-8} – 1×10^{-7} M) were well maintained during the period of application of the agonist. This was not the case for hyperpolarization responses induced by higher concentrations of 5-HT (1×10^{-6} – 1×10^{-4} M), there being a partial repolarization during this period (Figure 1). Full repolarization was always observed after returning to superfusion with 5-HT-free Krebs-Henseleit medium. 5-HT-induced hyperpolarization responses were highly reproducible: two concentration-response curves obtained at approximately one hour intervals on the same SCG preparations were virtually superimposable (Figures 1 and 2).

5-HT-induced hyperpolarization was not antagonized by prazosin (1×10^{-7} M and 3×10^{-7} M), phenolamine (1×10^{-6} M), ketanserin (1×10^{-7} and 1×10^{-6} M) or haloperidol (1×10^{-6} M) (Figure 2). However, the latter two compounds both potentiated and increased the persistence of the hyperpolarization responses induced by high concentrations of 5-HT (1×10^{-6} – 1×10^{-4} M) (Figures 1 and 2). Spiperone (1×10^{-7} M) caused similar effects, although in addition, it antagonized the responses to low concentra-

tions of 5-HT (Figure 2). None of these compounds caused significant changes in resting membrane potential.

All further experiments were performed using Krebs-Henseleit medium containing ketanserin (1×10^{-6} M) in addition to MDL 72222 (1×10^{-5} M). In the presence of these two drugs, 5-HT-induced hyperpolarization was both stable and reproducible (Figures 1 and 5).

Effects of monoamine uptake inhibitors

On the rat SCG, the presence of an inhibitor of 5-HT uptake causes a leftward shift of the concentration-response curve for 5-HT-induced depolarization and increases the apparent potency of metoclopramide as an antagonist of this response (Ireland *et al.*, 1987). It was therefore considered important to examine the effects of monoamine uptake inhibition on 5-HT-induced hyperpolarization of this preparation. It was found that the 5-HT uptake inhibitor paroxetine (at 1×10^{-6} but not 1×10^{-7} M) potentiated the amplitude of the maximum hyperpolarization response to 5-HT. However, it did not cause a leftward shift of the concentration-response curve since the degree of potentiation did not change significantly with the concentration of 5-HT ($P > 0.05$, analysis of variance; results not shown) making it unlikely that this effect was due to blockade of 5-HT uptake. In addition the 5-

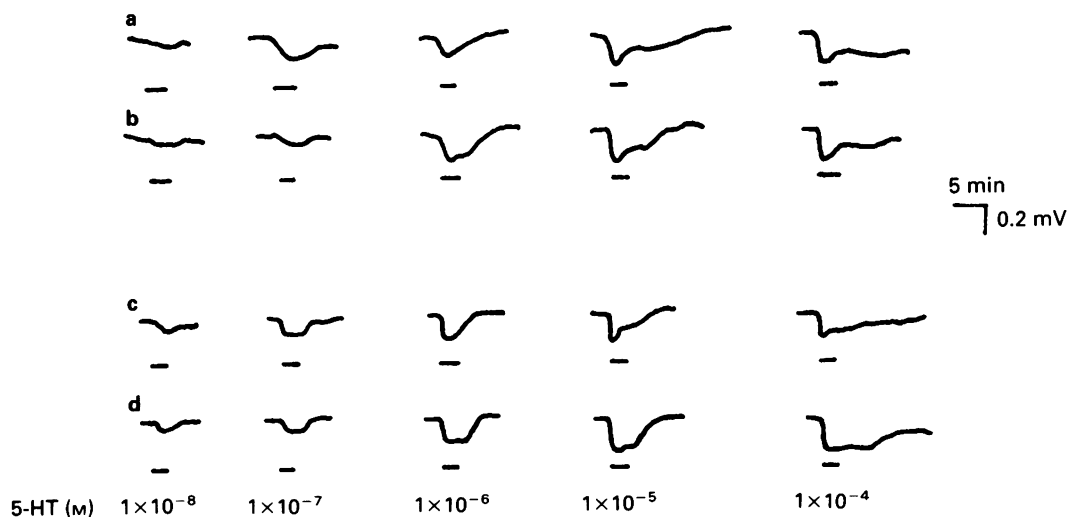


Figure 1 5-Hydroxytryptamine (5-HT)-induced hyperpolarization recorded from two rat superior cervical ganglion (SCG) preparations in the presence of MDL 72222 (1×10^{-5} M). (a and b) Responses constituting the first and second concentration-response curves, respectively, recorded from a single SCG preparation at an interval of approximately 1 h. (c and d) Responses obtained from a second SCG preparation before (c) and during (d) superfusion with ketanserin (1×10^{-6} M). Similar effects were observed in a further 7 ganglion preparations. The bar under each response indicates the approximate duration of the 5-HT application. Note that full recovery from the effects of 5-HT at 1×10^{-4} M did occur, although this is not shown.

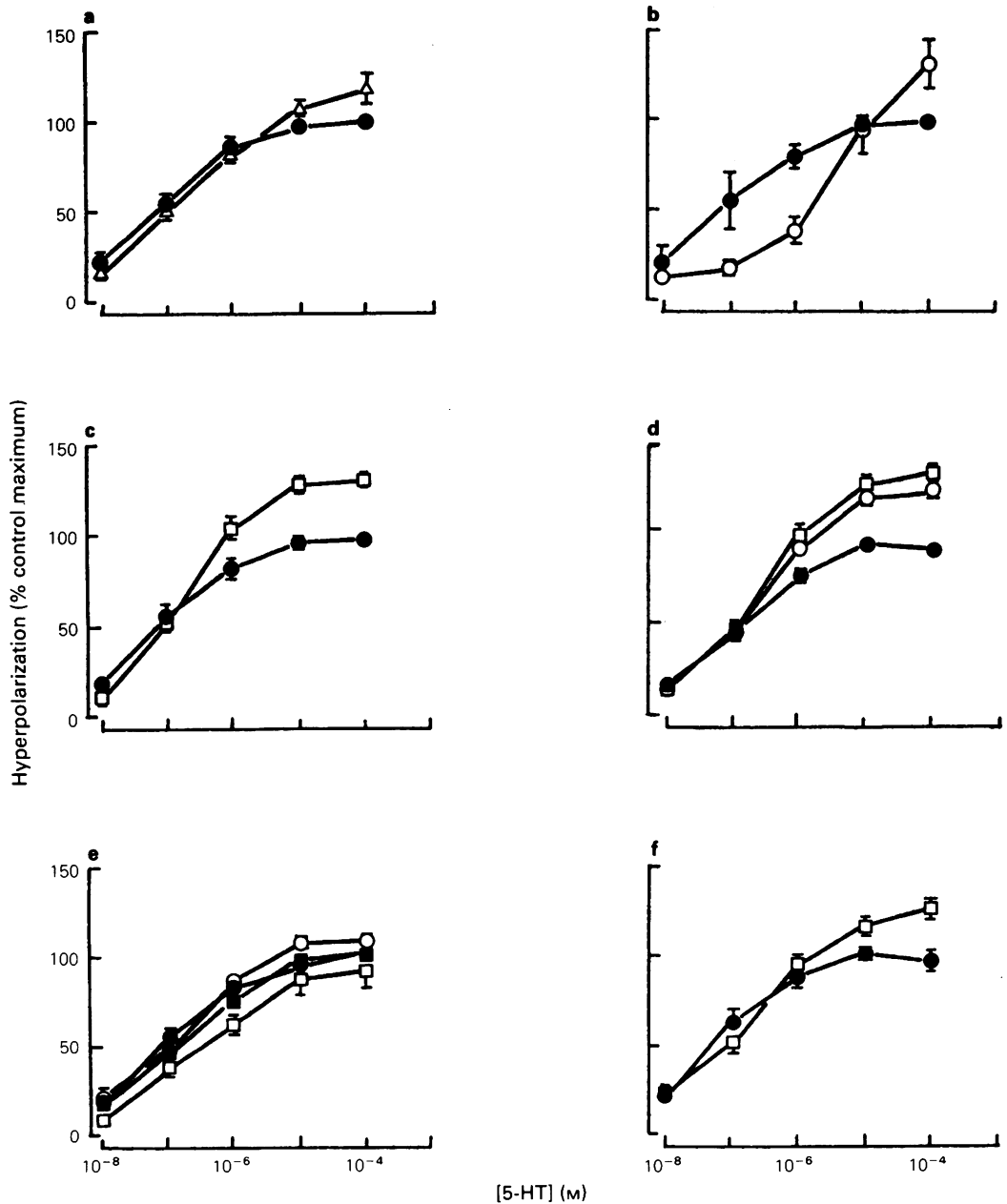


Figure 2 Effects of spiperone (b), haloperidol (c), ketanserin (d), prazosin (e) and phentolamine (f) on 5-hydroxytryptamine (5-HT)-induced hyperpolarization of the rat superior cervical ganglion (SCG). In (a), no drugs were applied; symbols indicate the control (●) and second (Δ) concentration-response curves to 5-HT obtained on the same SCG preparations at an interval of approximately 1h. In (b to f), symbols show control responses (●) and the effect of the test compound at 1×10^{-7} M (○), 3×10^{-7} M (■) or 1×10^{-6} M (□). Each point is the mean of single determinations in 4-8 individual SCG preparations, with vertical lines indicating the s.e.mean. All experiments were performed in the presence of MDL 72222 (1×10^{-5} M).

HT uptake inhibitors citalopram (1×10^{-6} M) or chlorimipramine (1×10^{-7} and 3×10^{-7} M), or the noradrenaline uptake inhibitor desmethylimipramine (1×10^{-6} M) had no effect on 5-HT-induced hyperpolarization. The concentrations of citalopram and chlorimipramine were chosen to produce a degree of inhibition of ganglionic 5-HT uptake equivalent to that caused by paroxetine (1×10^{-6} M; see Ireland *et al.*, 1987). In view of these results, it was considered unnecessary to inhibit monoamine uptake of the SCG in the present experiments.

8-OH-DPAT and ipsapirone

8-OH-DPAT (1×10^{-7} – 1×10^{-4} M) hyperpolarized the rat SCG. However, the responses were of very small amplitude: the maximum hyperpolarization induced by 8-OH-DPAT was only $19.9 \pm 3.1\%$ of that induced by 5-HT (1×10^{-4} M) ($n = 6$) (Figure 3). 8-OH-DPAT (1×10^{-8} and 1×10^{-7} M) also antagonized 5-HT-induced hyperpolarization, the principal effect being a concentration-dependent reduction in the amplitude of the maximum response (Figure 3).

Very high concentrations of ipsapirone (3×10^{-5} –

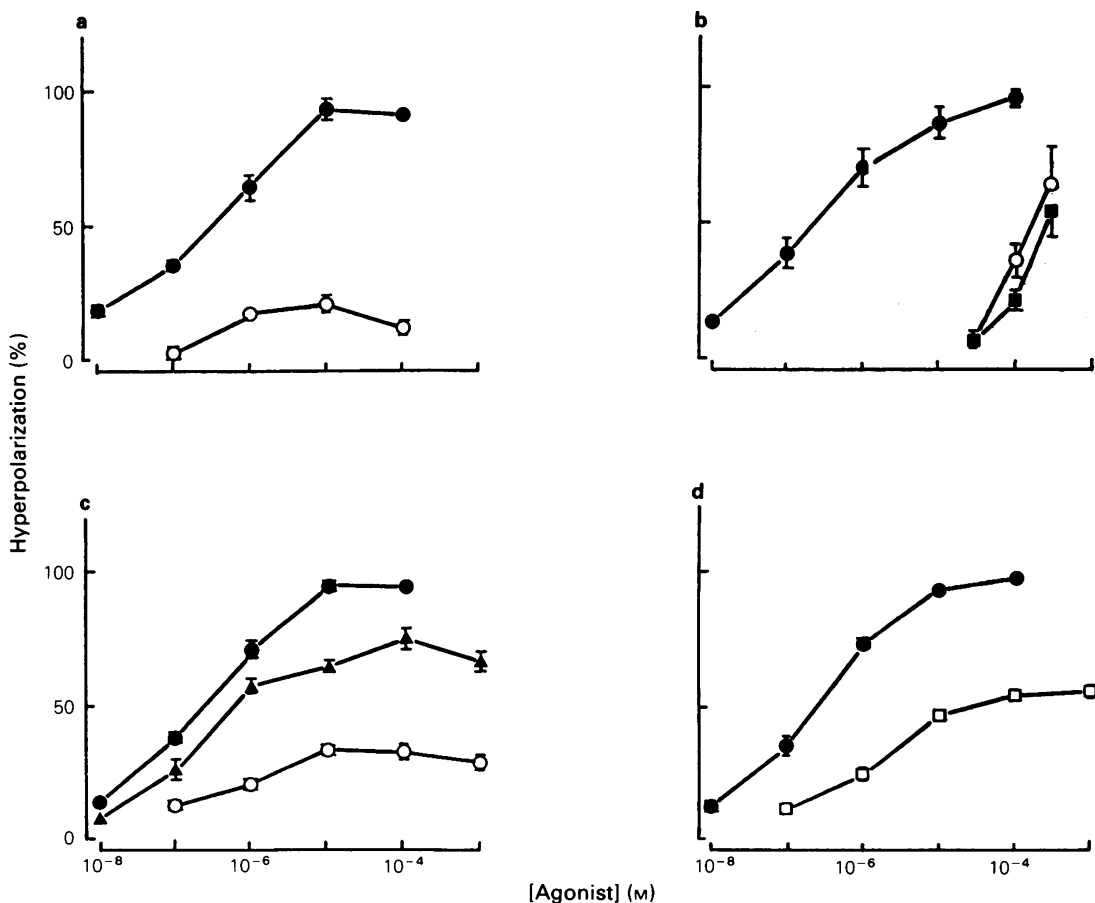


Figure 3 Effects of 8-hydroxy-2-(di-n-propylamino)-tetralin (8-OH-DPAT) and ipsapirone on the rat superior cervical ganglion. (a and b) Symbols indicate control responses to 5-hydroxytryptamine (5-HT) (●), the action of (a) 8-OH-DPAT or (b) ipsapirone (○) or the effect of the solvent control for ipsapirone (■); results are expressed as a percentage of the estimated maximum response to 5-HT. (c) The effects of 8-OH-DPAT and (d) ipsapirone as 5-HT antagonists. Symbols indicate control responses (●) or the presence of test compound at 1×10^{-8} M (▲), 1×10^{-7} M (○) or 1×10^{-6} M (□); results are expressed as a percentage of the estimated control maximum. Each point is the mean of single determinations from 5–8 individual SCG preparations; vertical lines indicate the s.e.mean. All responses were recorded in the presence of MDL 72222 (1×10^{-5} M) and ketanserin (1×10^{-6} M).

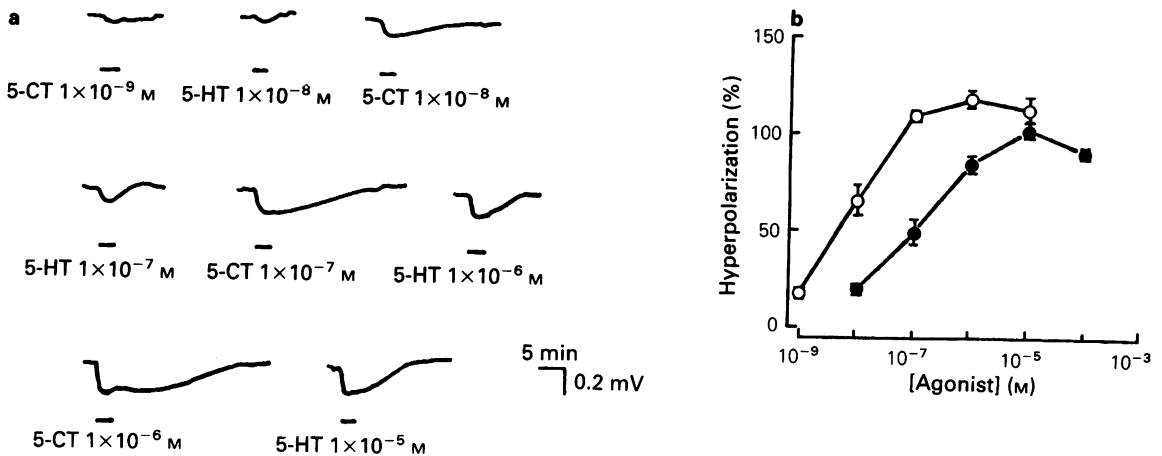


Figure 4 Comparison of the hyperpolarizing effects of 5-carboxamidotryptamine (5-CT) and 5-hydroxytryptamine (5-HT) on the rat superior cervical ganglion (SCG). (a) Hyperpolarization responses recorded sequentially from a single SCG preparation; the bar under each indicates the approximate duration of the agonist application. (b) Responses to 5-HT (●) and 5-CT (○); results are expressed as a percentage of the estimated maximum response to 5-HT. Each point is the mean of single determinations in 8 separate SCG preparations, with the vertical lines indicating the s.e.mean. All experiments were performed in the presence of MDL 72222 (1×10^{-5} M) and ketanserin (1×10^{-6} M).

3×10^{-4} M) were required to hyperpolarize that rat SCG. However, these hyperpolarization responses were difficult to distinguish from those induced by the appropriate solvent controls (Figure 3). A concentration of ipsapirone (1×10^{-6} M), much lower than that observed to cause overt changes in the resting membrane potential, was found to antagonize 5-HT-induced hyperpolarization. As with 8-OH-DPAT, this antagonism was characterized by a marked reduction in the amplitude of the maximum response to 5-HT (Figure 3).

5-Carboxamidotryptamine

5-CT (1×10^{-9} – 1×10^{-5} M) induced rapid concentration-dependent hyperpolarization responses that closely resembled those caused by 5-HT. However, hyperpolarization induced by 5-CT persisted for a considerable time after ceasing its application. This was in contrast to the effects of 5-HT (Figure 4). 5-CT was never observed to cause depolarization. The amplitude of the maximum response to 5-CT was estimated to be $117.2 \pm 4.4\%$ of that to 5-HT ($n = 8$). 5-CT was more active than 5-HT (Figure 4); the mean of the quotients 5-CT EC_{50} /5-HT EC_{50} (where both EC_{50} values were determined on the same SCG preparation) was 0.11 ± 0.04 ($n = 8$).

Hyperpolarization of the rat SCG induced by 5-CT was reproducible: two concentration-response curves determined at an interval of approximately 60 min on

the same SCG preparation showed little change in sensitivity (Figure 5).

Spiperone, (\pm)-cyanopindolol and (\pm)-pindolol

The resting membrane potential of SCG preparations was not significantly affected by spiperone (1×10^{-7} – 1×10^{-5} M), (\pm)-cyanopindolol (3×10^{-7} – 3×10^{-6} M) or (\pm)-pindolol (1×10^{-6} M).

Spiperone (1×10^{-7} – 1×10^{-5} M) caused a rightward displacement of the concentration-hyperpolarization response curves to both 5-HT and 5-CT. It achieved apparent equilibrium within 30 min of commencing application. Against 5-HT, low to moderate concentrations of spiperone (1×10^{-7} – 1×10^{-6} M) did not cause any significant change in the amplitude of the maximum response, although some reduction was observed in the presence of higher concentrations (3×10^{-6} and 1×10^{-5} M; Figure 5). A plot of the antagonism data according to the method of Arunlakshana & Schild (1959) yielded a straight line with a slope of 0.91 (95% confidence limits 0.73–1.09; Figure 5). The pK_B values calculated from the effects of each concentration of spiperone (1×10^{-7} – 1×10^{-5} M) did not change significantly with concentration ($P > 0.05$, analysis of variance); the mean was 7.40 ± 0.09 ($n = 20$).

The amplitude of the maximum response to 5-CT appeared to be potentiated by spiperone (1×10^{-7} – 1×10^{-6} M), although this effect was significant

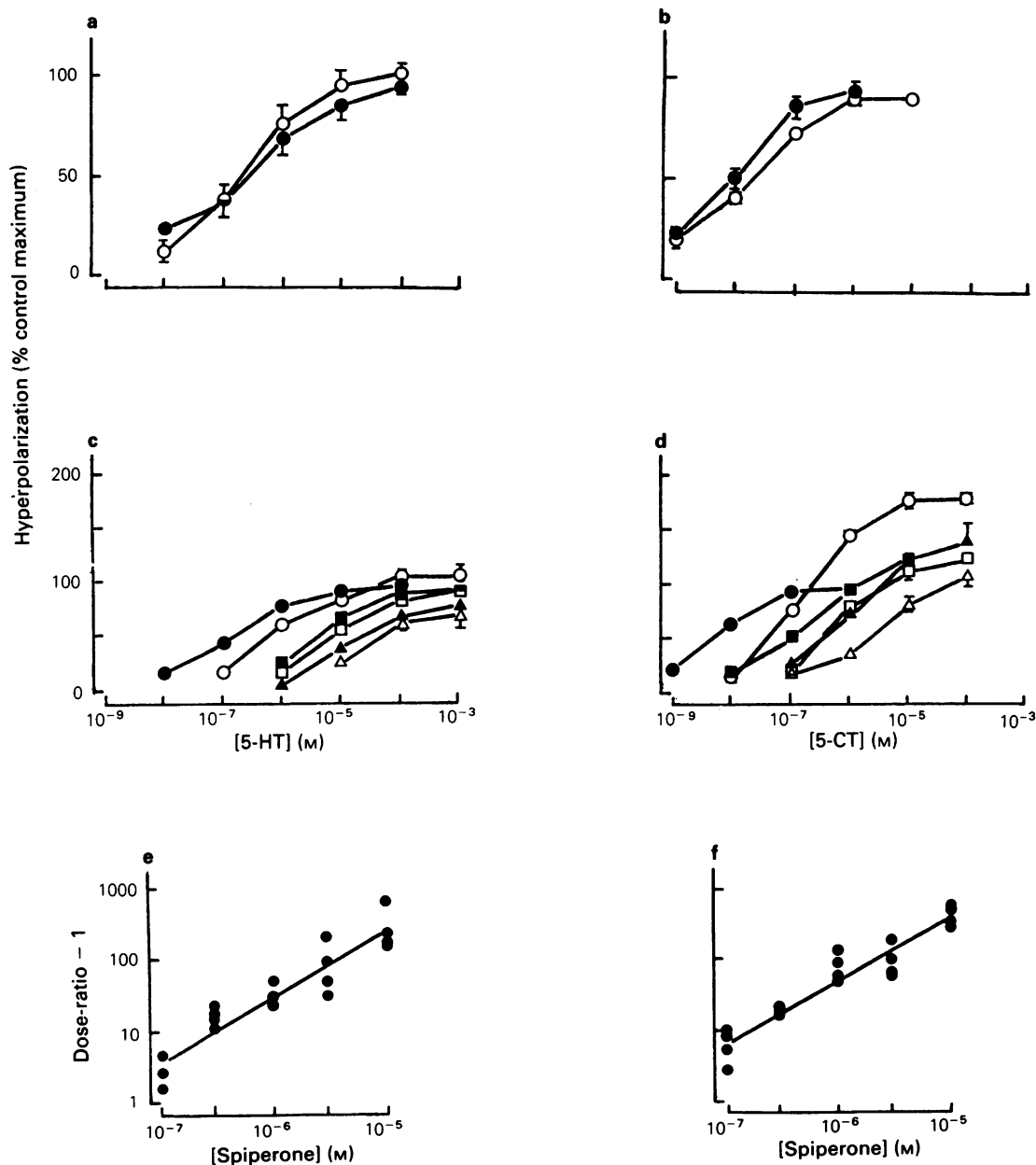


Figure 5 Antagonism by spiperone of 5-hydroxytryptamine (5-HT) and 5-carboxamidotryptamine (5-CT)-induced hyperpolarization of the rat superior cervical ganglion (SCG). (a and b) Two separate experiments in which SCG preparations were not exposed to spiperone. Symbols denote the control (●) and second (○) concentration-response curves to 5-HT (a) or 5-CT (b), determined on the same SCG preparations at an interval of approximately 1 h. (c and d) The effects of spiperone on agonist-induced hyperpolarization. Symbols indicate control responses (●), or the presence of spiperone at 1 × 10⁻⁷ M (○), 3 × 10⁻⁷ M (■), 1 × 10⁻⁶ M (□), 3 × 10⁻⁶ M (▲) or 1 × 10⁻⁵ M (△). In (a-d), points are the mean of single determinations in at least 4 separate SCG preparations, with vertical lines indicating the s.e.mean. (e and f) Schild plots of the effects of spiperone against 5-HT (e) and 5-CT (f). Each point is the result obtained on a separate SCG preparation; the straight lines were fitted by least-squares regression analysis. All the experiments were performed in the presence of MDL 72222 (1 × 10⁻⁵ M) and ketanserin (1 × 10⁻⁶ M).

($P < 0.05$, t test) only in the presence of low concentrations of the antagonist (1×10^{-7} and 3×10^{-7} M). The magnitude of the rightward displacement of the 5-CT concentration-hyperpolarization response curve by low concentrations of spiperone (1×10^{-7} – 1×10^{-6} M) appeared to be concentration-dependent. However, the effect of spiperone at 3×10^{-6} M was similar to that at 1×10^{-6} M (Figure 5). The Schild plot for the antagonist effects of spiperone (1×10^{-7} – 1×10^{-5} M) against 5-CT had a gradient of 0.87 (95% confidence limits 0.74–1.01). However, the pK_B values calculated from the effect of each of these concentrations of the antagonist changed significantly with

concentration ($P < 0.05$, analysis of variance). This was not the case when only the effects of low concentrations of spiperone (1×10^{-7} – 1×10^{-6} M) were analysed; from these, the mean pK_B value was 7.80 ± 0.09 ($n = 12$).

(\pm)-Cyanopindolol (3×10^{-7} – 3×10^{-6} M) took 60 to 90 min to achieve apparent equilibrium. It caused rightward displacement of the 5-HT concentration-hyperpolarization response curve, although the curves constructed in the presence of (\pm)-cyanopindolol were not parallel (Figure 6).

(\pm)-Cyanopindolol (3×10^{-7} – 3×10^{-6} M) also antagonized hyperpolarization responses induced by

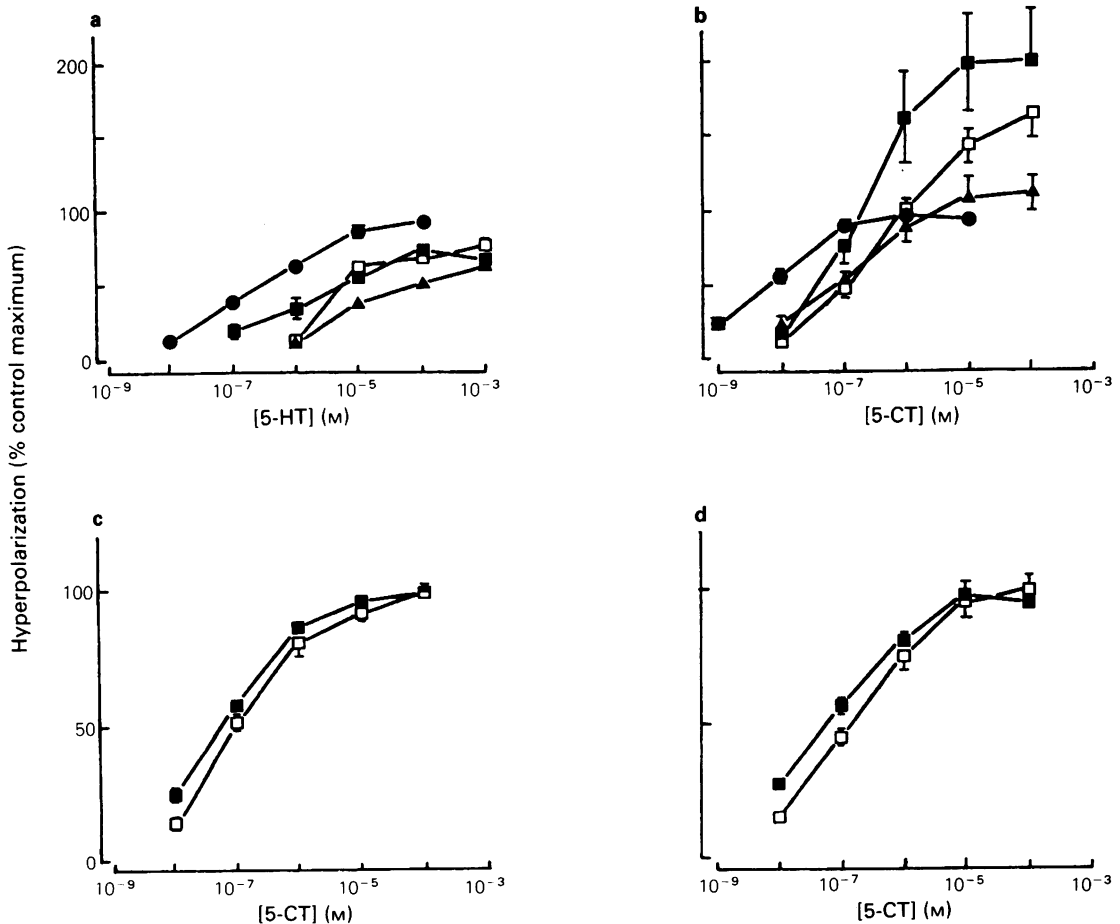


Figure 6 Antagonism by (\pm)-cyanopindolol of (a) 5-hydroxytryptamine (5-HT)- and (b) 5-carboxamidotryptamine (5-CT)-induced hyperpolarization of the rat isolated superior cervical ganglion (SCG). Symbols indicate control responses (\bullet) or the presence of (\pm)-cyanopindolol at 3×10^{-7} M (\blacksquare), 1×10^{-6} M (\square) or 3×10^{-6} M (\blacktriangle). (c) The effects of methysergide (1×10^{-6} M) (\square) and (d) methiothepin (1×10^{-6} M) (\square) on 5-CT-induced hyperpolarization recorded in the presence of (\pm)-cyanopindolol (3×10^{-7} M) (\blacksquare). Each point is the mean of single determinations in at least 4 separate SCG preparations and vertical lines indicate the s.e.mean. All experiments were performed in the presence of MDL 72222 (1×10^{-5} M) and ketanserin (1×10^{-6} M).

low concentrations of 5-CT (1×10^{-9} – 1×10^{-7} M). However, hyperpolarization induced by higher concentrations of the agonist (1×10^{-6} M and 1×10^{-5} M) were either unaffected or potentiated by (\pm)-cyanopindolol (Figure 6). The greatest degree of potentiation was observed in the presence of the antagonist at 3×10^{-7} M. EC₅₀ values were calculated for the 5-CT concentration-response curves constructed in the presence of (\pm)-cyanopindolol (3×10^{-7} – 3×10^{-6} M). These did not change significantly with increasing antagonist concentration ($P > 0.05$, analysis of variance). Hyperpolarization responses induced by 5-CT (1×10^{-8} – 1×10^{-4} M) in the presence of (\pm)-cyanopindolol (3×10^{-7} M) were essentially unaffected by methiothepin (1×10^{-6} M) or methysergide (1×10^{-6} M) (Figure 6).

(\pm)-Pindolol was tested for 5-HT antagonist activity at a single concentration (1×10^{-6} M). It achieved apparent equilibrium within 60 min and caused a parallel rightward displacement of the 5-HT concentration-hyperpolarization response curve, with no change in the amplitude of the maximum response (result not shown). The apparent pK_B value calculated from the effect of (\pm)-pindolol (1×10^{-6} M) was 6.81 ± 0.16 ($n = 6$).

Discussion

In this study, all experiments were performed on rat SCG preparations superfused with modified Krebs-Henseleit medium which contained MDL 72222 to block 5-HT₃ receptor-mediated depolarization. Under these conditions, the amplitude and persistence of hyperpolarization responses induced by moderate to high concentrations of 5-HT (1×10^{-6} – 1×10^{-4} M) were increased in the presence of ketanserin, haloperidol or spiperone, but not phentolamine or prazosin. These effects may have resulted from 5-HT₂ receptor blockade (see Maayani *et al.*, 1984; Fenuik, 1984) although the precise nature of the response(s) antagonized remains to be determined: one possibility is that it was an opposing depolarization. However, because of these results, ketanserin as well as MDL 72222 was added to the superfusion medium used in the remainder of the experiments described in this paper.

In the presence of ketanserin and MDL 72222, spiperone behaved as a reversible competitive antagonist of the 5-HT-induced hyperpolarization of the rat SCG. There was no change in the amplitude of the maximum response except for a small reduction in the presence of high concentrations of the antagonist. This latter effect may have been a consequence of depolarization mediated via incompletely blocked 5-HT₃ receptors, since very high concentrations of 5-HT were needed to produce approximately maximal

hyperpolarization responses in these experiments (see Ireland, 1987).

Spiperone has appreciable affinity at 5-HT_{1A}, 5-HT_{1B}, 5-HT_{1C}, 5-HT₂, noradrenaline α_1 , and dopamine D₁, D₂ and D₃ binding sites (Leysen *et al.*, 1978; 1981; Leff *et al.*, 1985; Hoyer *et al.*, 1985; Leff & Creese, 1985). However, ketanserin, haloperidol, phentolamine and prazosin did not antagonize 5-HT-induced hyperpolarization of the rat SCG. Taken together, these compounds have affinities at least comparable to those of spiperone at all the sites listed, with the exception of the 5-HT_{1A} binding site; here, they are all significantly less potent than spiperone (see Leysen *et al.*, 1978; 1981; Greengrass & Bremner, 1979; Engel & Hoyer, 1981; Norman *et al.*, 1984; Hoyer *et al.*, 1985; Leff *et al.*, 1985; Leff & Creese, 1985; Hoyer & Kalkman, 1986). Therefore, it is unlikely that spiperone antagonized 5-HT-induced hyperpolarizations of the rat SCG by acting at 5-HT_{1B}, 5-HT_{1C}, 5-HT₂, α_1 , D₁ or D₃ binding sites, since ketanserin has affinity greater than or equal to that of spiperone at each of these. Similarly, the high affinity of haloperidol at D₂ binding sites excludes their involvement. (–)-Noradrenaline and dopamine hyperpolarize the rat SCG via α_2 -adrenoceptors (Brown & Caulfield, 1979; see also Brown & Dunn, 1983). However, it is unlikely that 5-HT-induced hyperpolarization of this tissue was mediated either directly or indirectly via these sites, since it was unaffected by phentolamine. This compound is a potent antagonist (pA₂ 7.50, Drew, 1977) at the α_2 -adrenoceptor.

The possibility that 5-HT-induced hyperpolarization of the rat SCG resulted from interaction with a 5-HT_{1A}-like recognition site was further supported by the finding that it was antagonized by 8-OH-DPAT, ipsapirone, (\pm)-cyanopindolol and (\pm)-pindolol. These compounds are all potent at displacing radioligands from the 5-HT_{1A} binding site (Middlemiss & Fozard, 1983; Dompert *et al.*, 1985; Hoyer *et al.*, 1985; Engel *et al.*, 1986). In the present study, low concentrations of 8-OH-DPAT or ipsapirone caused marked reductions in the amplitude of the maximum response to 5-HT although neither produced significant change in the resting potential. Therefore, there is no reason to assume *a priori* that the antagonist effect of either compound was due to competition for the 5-HT recognition site. High concentrations of 8-OH-DPAT and ipsapirone did cause hyperpolarization of the SCG. The mechanisms underlying this action were not investigated. However, 8-OH-DPAT has been shown to mimic some of the agonist effects of 5-HT in the guinea-pig ileum (Fozard & Kilbinger, 1985), rat cerebellum (Raiteri *et al.*, 1986) and the hippocampus of the rat (Beck *et al.*, 1985) and guinea-pig (Shenker *et al.*, 1985). The antagonist effects of (\pm)-cyanopindolol also were

difficult to interpret. Thus, although the compound caused a concentration-related rightward displacement of the 5-HT concentration-response curve, this was non-parallel and the amplitude of the maximum response to 5-HT was reduced. In contrast, (\pm)-pindolol (1×10^{-6} M) behaved as a reversible competitive antagonist of 5-HT-induced hyperpolarization; the provisional pK_B value estimated from the effect of this single concentration was 6.81 ± 0.16 . ($-$)-Pindolol is a potent displacing agent at the 5-HT_{1A} binding site, where its pK_D value is 7.66, although the ($+$)-isomer is much weaker (pK_D 5.88; Engel *et al.*, 1986).

5-Carboxamidotryptamine (5-CT) induced stable and reproducible hyperpolarization responses that closely resemble those produced by 5-HT and was about nine times more active. These results fulfil one of the criteria for a response to be defined as being mediated through 5-HT₁-like receptors, in that it should be mimicked by 5-CT at concentrations equal to, or less than those of 5-HT (Bradley *et al.*, 1986). 5-CT-induced hyperpolarization of the rat SCG was antagonized by spiperone and by (\pm)-cyanopindolol. Spiperone (1×10^{-7} – 1×10^{-6} M) caused concentration-related rightward displacements of the 5-CT concentration-hyperpolarization response curve from which a mean pK_B of 7.80 ± 0.05 was calculated. This agreed reasonably well with the value of 7.40 ± 0.09 obtained with spiperone against 5-HT. Interestingly, spiperone antagonizes 5-HT- and 5-CT-induced responses with similar potency in other tissue preparations. For example, it antagonizes 5-CT-induced stimulation of adenylate cyclase in guinea-pig hippocampus with a pA_2 of 7.62 (Shenker *et al.*, 1985) and antagonizes both 5-HT- and 5-CT-induced inhibition of the amplitude of the CA1 population spike in rat hippocampus with pK_B values of 7.68 and 7.96, respectively (\log_{10} -transformation of data from Beck *et al.*, 1985). These values are also close to the pK_i for spiperone at the 5-HT_{1A} binding site (7.38–Hoyer *et al.*, 1985). In the present study, the antagonism of 5-CT-induced hyperpolarization caused by spiperone at 3×10^{-6} M was no greater than that observed in the presence of the antagonist at 1×10^{-6} M. Similarly

(\pm)-cyanopindolol produced a significant, but concentration-independent rightward displacement of the 5-CT concentration-response curve. These results may indicate that 5-CT can hyperpolarize the rat SCG via interaction with more than one type of receptor. In the rat isolated kidney, 5-HT induces inhibition of [³H]-noradrenaline release which is resistant to blockade by (\pm)-cyanopindolol, although it is antagonized by methiothepin (IC_{50} 4×10^{-9} M) and methysergide (IC_{50} 1.3×10^{-7} M) (Charlton *et al.*, 1986). However, this type of receptor does not appear to mediate the hyperpolarization of the SCG induced by 5-CT in the presence of (\pm)-cyanopindolol, since this response was not significantly antagonized by either methiothepin (1×10^{-6} M) or methysergide (1×10^{-6} M). Low concentrations of spiperone or (\pm)-cyanopindolol caused marked potentiation of the amplitude of the maximum response to 5-CT. Since such potentiation was not observed with 5-HT, these data suggest a further complexity of the action of 5-CT on the rat SCG.

In conclusion, the results obtained in the present study support the hypothesis that 5-HT-induced hyperpolarization of the rat SCG is mediated via a 5-HT₁-like receptor. The effects observed with spiperone suggest that this receptor resembles the 5-HT_{1A} binding site, although results obtained with other 5-HT_{1A} ligands ((\pm)-cyanopindolol, (\pm)-pindolol, 8-OH-DPAT and ipsapirone) were equivocal.

The results obtained with 5-CT were difficult to interpret. 5-CT was chosen for use in the present study since it shows appreciable selectivity for 5-HT₁-like receptors (see Bradley *et al.*, 1986). However, on the SCG, the effects of antagonists against the hyperpolarization responses induced by 5-CT were more complex than against those induced by 5-HT.

A more definitive analysis of the receptor(s) mediating 5-HT-induced hyperpolarization of the rat SCG must await the identification of selective agonists and antagonists.

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