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The depolarizing action of 5-hydroxytryptamine on rabbit vagal afferent and sympathetic neurones *in vitro* and its selective blockade by ICS 205-930

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1 Depolarizing responses to 5-hydroxytryptamine (5-HT) were recorded from rabbit nodose (NG) and superior cervical (SCG) ganglia using the sucrose-gap technique. The antagonist potency and selectivity of ICS 205-930 ([3α-tropanyl]-1H-indole-3-carboxylic acid ester) were investigated.

2 In NG, 5-HT (5 to 80 nmol) evoked depolarizations of graded amplitude. The ED_{50} was 18.2 (10.9–30.5) nmol (geometric mean, 95% confidence limits). Responses were blocked surmountably by ICS 205–930, 10^{-11} and 10^{-10} M, the threshold for blockade being below 10^{-11} M. Parallel, rightward shifts in dose-response curves were seen with these concentrations of antagonist, but at higher concentrations (10^{-9} and 10^{-8} M) there was a further rightward shift with reduction in slope and maximum of the curves.

3 In SCG, where 5-HT (20 to 320 nmol) evoked depolarizations of graded amplitude and the ED_{50} was 55.8 (22.3–139.6) nmol (geometric mean, 95% confidence limits), ICS 205-930 had a similar inhibitory effect to that observed in NG.

4 The apparent pA_2 values for the surmountable blockade produced by ICS 205-930 at concentrations of 10^{-11} and 10^{-10} M were 10.2 ± 0.2 for NG and 10.4 ± 0.1 for SCG (means ± s.e. mean).

5 ICS 205–930 was selective in its action since it had no effect on dimethylphenylpiperazinium (DMPP) responses in either ganglion or on GABA responses in NG.

6 This study provides quantitative evidence on the blocking action of ICS 205-930 at neuronal 5-HT receptors using a technique that allows the depolarizing responses evoked by the amine to be directly recorded.

Introduction

5-Hydroxytryptamine (5-HT) has a potent depolarizing action on vagal afferent somata in the nodose ganglion (NG) (Wallis *et al.*, 1982; Higashi & Nishi, 1982) and on sympathetic neurones in the superior cervical ganglion (SCG) (Wallis & Woodward, 1975; Wallis & North, 1978). This excitatory action of 5-HT is seen in a variety of neurones in the mammalian peripheral nervous system (Wallis, 1981; Fozard, 1984), but the receptors have not been completely characterized. Although cocaine and certain of its analogues (Fozard, 1979; Fozard *et al.*, 1979), metoclopramide (Fozard & Mobarok Ali, 1978) and quipazine (Lansdown *et al.*, 1980; Wallis *et al.*, 1982), show limited potency and selectivity at some neuronal receptors, only MDL 72222 (a tropanyl benzoic acid ester) has been shown to display a potent and selective blockade of the directly-recorded depolarizing action of 5-HT (Azami *et al.*, 1985). This antagonist appeared to be selective, since a concentration of 10^{-7} M reduced 5-HT responses but did not affect responses to γ-aminobutyric-acid (GABA) and noradrenaline in NG or responses to dimethylphenylpiperazinium (DMPP) in SCG. The 5-HT receptors in the two tissues appeared to be similar, since the apparent pA_2 value for MDL 72222 at the NG receptor was 7.7 and at the SCG receptor 7.8 (Round & Wallis, unpublished data).

Recently, the development from certain tropanyl indole carboxylic acid esters of extremely potent antagonists has been described (Richardson *et al.*, 1985). One of these, (3α-tropanyl)-1H-indole-3-carboxylic acid ester, ICS 205-930, has been shown to antagonize three kinds of 5-HT action: (i) the stimulant action on sympathetic nerve terminals of rabbit heart, (ii) the depression of rabbit vagus nerve compound action potential (Donatsch *et al.*, 1984), and (iii) the reflex bradycardia evoked by injection of 5-HT into the rat jugular vein (von Bezold-Jarisch

reflex) which is mediated by vagal afferents (Richardson *et al.*, 1985). The exact mechanisms by which 5-HT brings about these effects at the neuronal membrane are incompletely understood, although in each case a membrane depolarization may occur. In this paper we describe the effects of ICS 205-930 using a method which allows the depolarizations induced by 5-HT to be recorded directly. The membrane sucrose-gap technique was employed to measure membrane potential change from a population of neurones in NG and SCG.

A preliminary account of this work has been presented to the British Pharmacological Society (Round & Wallis, 1985).

Methods

Preparations

Nodose or superior cervical ganglia with short lengths of attached nerve were removed from young, adult New Zealand White rabbits of either sex, immediately after death induced by air embolism. Ganglia were transferred to a dish of ice-cold Krebs solution and desheathed using a binocular microscope. Details of the sucrose-gap method used for NG are given in Wallis *et al.* (1982) and for SCG in Wallis *et al.* (1975). In this version of the apparatus, which employs rubber membrane seals, diffusion potentials are minimized because the interface between solutions is almost entirely within the tissue. If the seals are adequate, the recorded d.c. signal stabilizes after an initial signal drift and remains stable for many hours when the apparatus is maintained at room temperature.

Responses to 5-hydroxytryptamine

Potential changes induced by 5-HT or other substances were amplified and displayed on a potentiometric chart recorder. Ganglia were superfused with Krebs solution at room temperature (20–22°C) at a rate of 2–3 ml min^{-1}. In our hands, superfusion of the tissue with a solution of 5-HT at an effective concentration produced a profound tachyphlaxis which made repeated determinations of dose-response curves impracticable. When injections of 5-HT in a small volume of Krebs solution were made into the superfusion stream (Wallis *et al.*, 1982; Azami *et al.*, 1985), reproducible responses could be obtained provided the flow of Krebs solution to the ganglion was controlled with a drop chamber and the rate of injection kept relatively constant (around 0.1 ml s^{-1}). Responses obtained by the two methods are shown in Figure 1a. Whereas superfusion of 5-HT (10^{-6} M) caused a modest depolarization which was sustained for the duration of the application, a ten fold higher concentration caused a depolarization which reached a peak and then declined while 5-HT was in contact with the tissue. The peak amplitude was similar to that of the responses evoked by a bolus of 40 nmol 5-HT. Superfusion of even 10^{-6} M 5-HT had a strong depressant effect on responses to bolus injections of 5-HT, as can be seen by comparing the response to 20 nmol 5-HT before and after superfusion. Bolus injections of 5-HT and other agonists were, therefore, adopted to ensure reproducibility of response, even though equilibrium between the receptors and a known concentration of agonist cannot be achieved with this method.

Several experiments of the kind illustrated in Figure 1 were performed in order to compare peak response amplitudes using the two methods. As can be seen from Figure 1b, the mean depolarization evoked by superfusion of 5-HT (10^{-6} M) corresponded to the response to bolus injection of about 7 nmol 5-HT (7.2 ± 0.6 nmol); the mean depolarization evoked by 5-HT (10^{-5} M) corresponded to the response to injection of 38 nmol (38 ± 4 nmol, means ± s.e. mean). It can be calculated that the injected material is dispersed in a volume of 6–7 ml of superfusate, which agrees well with the estimate made from injection of dye (about 5 ml). Dispersion of a bolus in 6–7 ml would result in ganglia being exposed to the amine for 2.5–3 min.

In SCG, in order to eliminate any effect on responses of an uptake system for 5-HT, the effect of the uptake inhibitor citalopram (Pawlowski *et al.*, 1981) was tested. In the rat SCG, the presence of a 5-HT uptake system (Ireland, 1984) leads to an underestimate of the apparent potency of competitive antagonists (Ireland *et al.*, 1983). In our experiments citalopram (5×10^{-9}, 10^{-7} or 10^{-6} M) did not potentiate responses to bolus injections of 5-HT and, therefore, was not routinely employed. A reduction in responses was seen at the highest concentration of citalopram.

Injections of 5-HT and other depolarizing agents were made from 1 ml syringes mounted in a Perspex block through which the superfusion medium flowed. The quantities of 5-HT used ranged from 5 to 5120 nmol, injected as volumes of 0.025–0.4 ml of 0.2, 0.8, 3.2 or 12.8×10^{-3} M solutions in Krebs. Dimethylphenylpiperazinium (DMPP) was used in quantities of 40–160 nmol (0.8×10^{-3} M solution) and GABA in quantities ranging from 50 to 800 nmol (2×10^{-3} M solution).

It proved advantageous to employ 2 or 3 conditioning doses of 5-HT (40 nmol in NG, 160 nmol in SCG) at the start of an experiment. In part this was done because response variability was much more marked during the first 2 or 3 responses elicited. Further, the partial desensitization this procedure induced, resulted in sub-maximal responses to 5-HT remaining

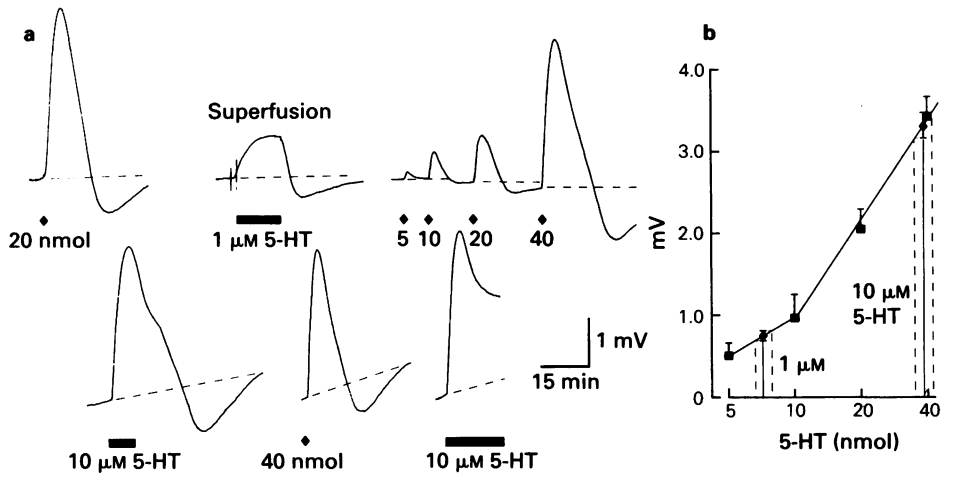

Figure 1 Responses to superfusion and bolus injection of 5-hydroxytryptamine (5-HT). (a) Chart records showing sequence of depolarizations elicited from the same preparation by superfusion of nodose ganglia (NG) with 5-HT (10^{-6} and 10^{-5} M) and by bolus injections of 5–40 nmol 5-HT. Black bars indicate duration of superfusion and black diamonds time of injection. Superfusion was maintained until a plateau response was attained. Note that the response to 20 nmol 5-HT was markedly attenuated by superfusion with 5-HT, 10^{-6} M, and that responses to 5-HT, 10^{-5} M, decayed from their peak during superfusion of the agonist. During the first superfusion at this concentration the inflexion in the decay phase indicates the onset of a faster repolarization due to removal of superfusate containing 5-HT. The time delay between this inflexion and termination of superfusion is due to the dead space of the system. (b) Pooled dose-response curves from three preparations in which responses to superfusion and bolus injection were evoked from the same ganglion. Ordinate scale, peak amplitude in mV; abscissa scale, quantity of 5-HT injected. (■) Responses to bolus injection (means ± s.e.mean). (◆) Responses to superfusion of 10^{-6} and 10^{-5} M 5-HT; the mean values have been interpolated onto the dose-response curve for injections. Projection from the mean amplitude values for superfusion and their standard errors allows an estimate of the bolus quantity required to generate a response of the same amplitude. 5-HT, 10^{-6} M, evoked a response equivalent to injection of 7.2 ± 0.6 nmol and 5-HT, 10^{-5} M, a response equivalent to 38 ± 4 nmol.

relatively constant in amplitude for several hours; two or three 4–5 point dose-response curves could be constructed using quantities of 5-HT which evoked near threshold to near maximal depolarizations. Quantities evoking maximal responses were normally avoided when constructing control curves in experiments in which a second curve was to be elicited in the presence of the antagonist.

Assessment of antagonist

Depolarization amplitude was measured from a projection of the trace preceding the response. ICS 205-930 was dissolved initially in distilled water before final dilution in Krebs solution. In all but preliminary experiments in which the onset of blockade was examined, the antagonist was superfused for 1 h before re-testing with depolarizing agents. Dose-response curves were constructed using 4 or 5 quantities of 5-HT, the order of testing varying in different preparations. Second dose-response curves were constructed after washing for 1 h in Krebs solution with or without antagonist. We have shown elsewhere (Azami *et al.*, 1985; Round & Wallis, unpublished data) that repeated dose-response curves can be obtained from both NG and SCG providing supramaximal amounts of 5-HT are avoided. Dose-ratios were measured on the linear portion of individual dose-response curves. In addition, normalized data were pooled to give overall dose-response curves and linear regression analysis performed on data contributing the linear portion of these curves.

Selectivity of blockade was assessed by using DMPP and GABA as control agonists on NG and DMPP on SCG. Repeated dose-response curves to these agents could be elicited from NG and SCG.

Statistical analysis

The measures of mean variation quoted are standard errors around the arithmetic mean except where indicated. The covariance of the pooled data contributing the linear portions of dose-response curves was determined to assess whether the slopes and

intercepts of the fitted lines for curves in the absence or presence of antagonist were significantly different. In the case of the NG but not the SCG, the 2nd control curve was slightly but significantly shifted to the right with respect to the initial control curve. Curves determined in the presence of antagonist were compared with the 2nd control curve.

Solutions and drugs

All solutions were made from distilled water which was also passed through a deionizer when isotonic sucrose solution was to be prepared. The Krebs solution had the following composition (mM): NaCl 118, KCl 4.75, $CaCl_2$ 2.54, KH_2PO_4 1.2, $NaHCO_3$ 25, $MgSO_4$ 1.2 and glucose 11. It was gassed with 5% CO_2 and 95% O_2. The concentration of the sucrose solution superfusing part of the vagus or internal carotid nerves was 315 mM and taken to be isotonic.

All drugs were dissolved in Krebs solution. The drugs used were 5-hydroxytryptamine creatinine sulphate (5-HT, Sigma), 1,1-dimethyl-4-phenylpiperazinium iodide (DMPP, Koch-Light), γ-aminobutyric acid (GABA, Sigma) and (3α-tropanyl)-1H-indole-3-carboxylic acid ester hydrochloride (ICS 205–930, Sandoz).

Results

Depolarizing responses in nodose ganglia

5-HT in amounts ranging from 5 to 80 nmol evokes depolarizations of graded amplitude from NG, 80 nmol evoking a maximal and 40 nmol 5-HT a near-maximal response (Azami *et al.*, 1985). The mean amplitudes of responses to 40 and 80 nmol 5-HT in these experiments were 3.81 ± 0.27 ($n=15$) and 4.18 ± 0.53 mV ($n=9$), respectively. A typical response to 5-HT (Figure 2) consisted of a relatively rapid depolarization and repolarization, followed by a slower after-hyperpolarization before a return to resting membrane potential occurred. The ED_{50} determined from 30 ganglia was 18.2 (10.9–30.5) nmol (geometric mean, 95% confidence limits).

Effect of ICS 205-930 on the responses of nodose ganglia to 5-hydroxytryptamine

ICS 205-930 was a potent antagonist of 5-HT responses in NG. There was some reduction in response amplitude at a concentration of 10^{-11} M and the threshold for antagonism was therefore below 10^{-11} M. Responses to 5-HT recorded in the presence of this agent (10^{-10} M) are shown in Figure 2. The character of the depolarizations evoked by 5-HT

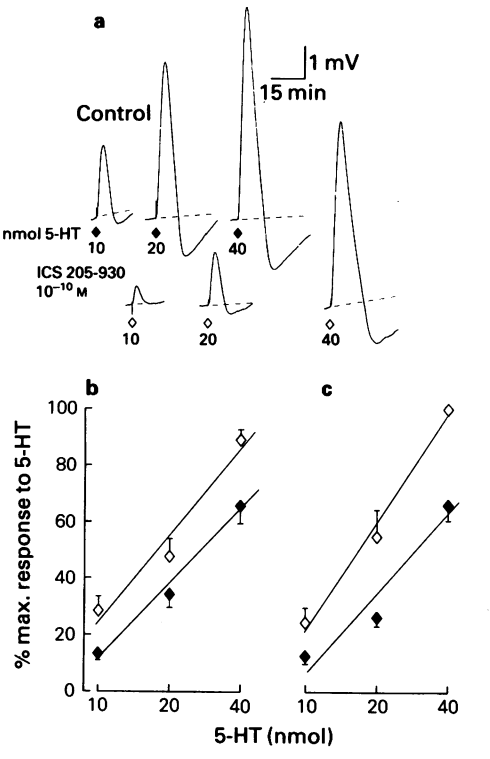

Figure 2 Effect of ICS 205-930 on responses of nodose ganglia (NG) to 5-hydroxytryptamine (5-HT). (a) Chart records showing dose-dependent depolarizations to bolus application of 5-HT before and after ICS 205-930 (10^{-10} M). A sequence of control responses to 5-HT (10–40 nmol) was elicited and the tissue exposed to the antagonist for 1 h before re-testing with 5-HT. (b and c) Pooled dose-response curves illustrating the effect of two concentrations of ICS 205-930 (b, 10^{-11} and c, 10^{-10} M) on responses to 5-HT (10–40 nmol). Ordinate scale, response amplitude expressed as percentage of maximum response to 5-HT elicited from each ganglion; abscissa scales, quantity of 5-HT. (◇) Control responses, (◆) responses in presence of ICS 205-930. Points show means with vertical lines indicating s.e.mean; 6 and 9 ganglia were used to test the effects of ICS 205-930, (b) 10^{-11} and (c) 10^{-10} M, respectively. The rightward shifts were parallel. Lines were fitted by the method of least squares.

appeared unaltered, except for reduced amplitude, and blockade appeared to be surmountable, since increasing the amount of 5-HT injected compensated for the blockade. In all but a few experiments ganglia were superfused with ICS 205-930 for 1 h before re-testing with 5-HT. This relatively long equilibration time was also used by Richardson *et al.* (1985) and in our earlier studies with MDL 72222 (Azami *et al.*, 1985). Dose-response curves of pooled data (Figure

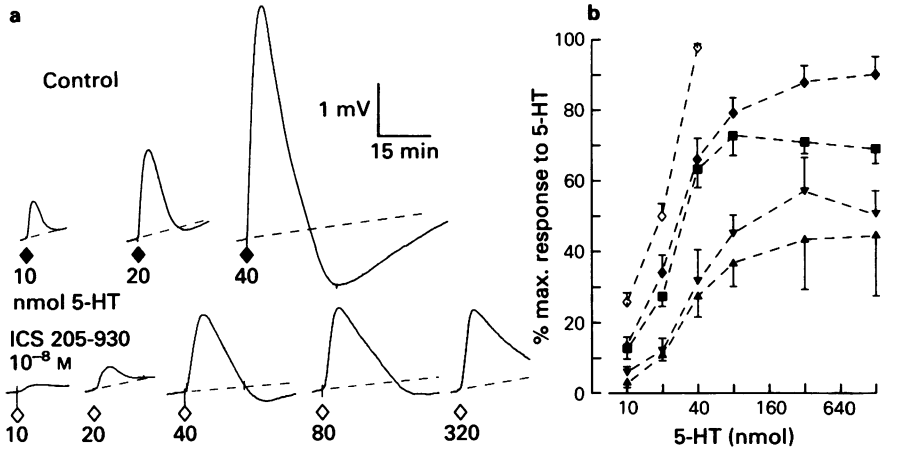

Figure 3 Effect of higher concentrations of ICS 205-930 on responses of nodose ganglia (NG) to 5-hydroxytryptamine (5-HT). (a) Chart records showing response to 5-HT (bolus injection of 10-320 nmol at diamonds) before and after ICS 205-930 (10^{-8} M). A sequence of control responses was elicited and the tissue then exposed to the antagonist for 1 h before re-testing with 5-HT. (b) Pooled dose-response curves showing the effects of ICS 205-930, 10^{-11} (◆), 10^{-10} (■), 10^{-9} (▼) and 10^{-8} (▲) M on responses to 5-HT in different series of experiments. (◇) Control responses to 5-HT. Ordinate and abscissa scales as in Figure 2. Points show means with vertical lines indicating s.e.mean. The curves showing the effects of 10^{-11} and 10^{-10} M ICS 205-930 from 6 and 9 ganglia, respectively, represent in part the same data presented in Figure 2b,c. Six and 5 ganglia were used to test the effects of 10^{-9} and 10^{-8} M ICS 205-930, respectively.

2b,c) from responses evoked by amounts of 5-HT, which induced near threshold to near maximal depolarizations in the absence of antagonist, were significantly shifted to the right with no reduction in slope by ICS 205-930 (10^{-11} and 10^{-10} M). The full data from these experiments are shown in Figure 3b. The maximum response (comparison was made with the maximum in a second control curve) was still obtained in the presence of 10^{-11} M ICS 205-930, while a 10 fold higher concentration appeared to reduce the maximum somewhat.

The effects of ICS 205-930 (10^{-9} and 10^{-8} M) were greater, but at these concentrations the maximum response was clearly depressed (Figure 3a). Figure 3b shows full dose-response curves for the effects of 5-HT in NG constructed for 4 concentrations of antagonist in four series of experiments. Concentrations of 10^{-9} and 10^{-8} M caused a rightward shift of the dose-response curves with reductions in slope and maxima. There appeared to be a tendency for the maximum response of NG cells to be progressively reduced by increasing concentrations of ICS 205-930.

Effect of ICS 205-930 on the responses of the superior cervical ganglion to 5-hydroxytryptamine

In SCG, 5-HT in amounts ranging from 20 to 320 nmol elicited depolarizations of graded amplitude. The mean amplitude of the response elicited by 320 nmol 5-HT was 1.7 ± 0.14 mV ($n=9$). Preliminary experiments showed that this was a near maximal response. Traces from a typical experiment are shown in Figure 4. The ED_{50} determined from 30 experiments was 55.8 (22.3–139.6) nmol (geometric mean, 95% confidence limits). ICS 205-930 had effects on 5-HT responses similar to those observed in NG. At a concentration of 10^{-10} M (Figure 4a,c), the reduction in response amplitude caused by the agent was surmounted by injecting a larger quantity of 5-HT. Note (Figure 4a) that SCG cells gave depolarizing responses of briefer duration than those recorded from NG in response to the same or smaller amounts of 5-HT. The responses consisted of a rapid depolarization followed by an almost equally rapid repolarization and a slower after-hyperpolarization. Concentrations of the antagonist of 10^{-11} and 10^{-10} M caused significant rightward, parallel shifts of the dose-response curve with no reduction in slope, as can be seen from the pooled data (Figures 4b,c and 5). A higher concentration (10^{-9} M) of the antagonist caused a further rightward shift but there was considerable variability in response amplitude with larger quantities of 5-HT (Figure 5).

Quantification of antagonism

Thus, in both NG and SCG, ICS 205-930 at concentrations of 10^{-10} M or less induced parallel rightward

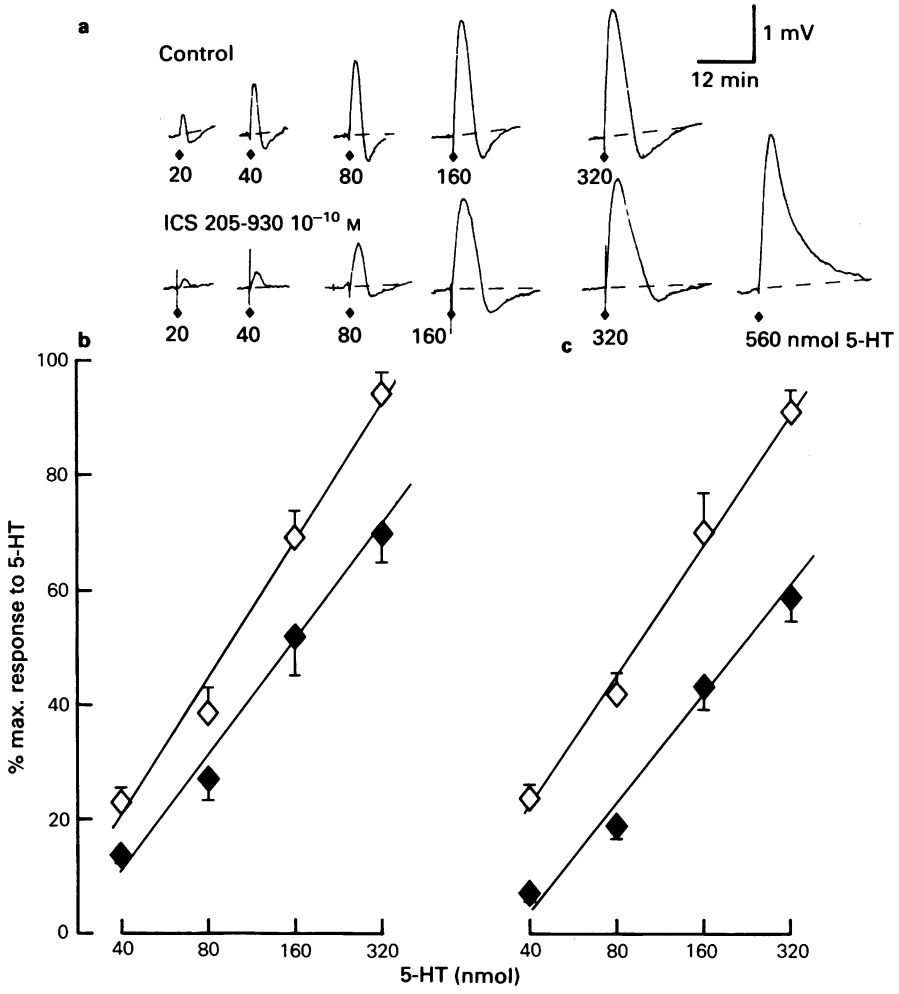

Figure 4 Effect of ICS 205-930 on responses of superior cervical ganglia (SCG) to 5-hydroxytryptamine (5-HT). (a) Chart records showing responses to 5-HT (bolus injection of 20-560 nmol 5-HT at ◆) before and after ICS 205-930 (10^{-10} M). A sequence of control responses was elicited and the tissue then exposed to the antagonist for 1 h before re-testing with 5-HT. (b,c) Pooled dose-response curves illustrating the effect of ICS 205-930 (b) 10^{-11} and (c) 10^{-10} M on responses to 5-HT (40–320 nmol) in different series of experiments. (◇) Control responses; (◆) responses in presence of ICS 205-930. Ordinate and abscissa scales as in Figure 2. Points show means with vertical lines indicating s.e.mean; 5 and 10 ganglia were used to test the effects of ICS 205-930, 10^{-11} and 10^{-10} M, respectively. Lines were fitted by the method of least squares. Rightward shifts were parallel.

shifts of the linear portion of the dose-response curves (Figures 2 and 4). The linear portions of the curves were based on a range of amounts of 5-HT which evoked near threshold to near maximal responses. Dose-ratios could be measured in these experiments at the ED_{50} level to quantify the antagonism. Dose-ratios were 1.7 and 2.1 for the action of 10^{-11} and 10^{-10} M ICS 205-930 on NG, respectively, and 1.8 and 2.8 for the action on SCG, respectively. Apparent pA_2 values were calculated in individual experiments from the relationship: $pA_2 = -\log[B] + \log(DR - 1)$, where [B] is the concentration of antagonist and DR the dose-ratio (see Arunlakshana & Schild, 1959). Over the limited concentration range, 10^{-11} to 10^{-10} M, an attempt was made to estimate the apparent pA_2 graphically from a Schild plot of log [B] against log (DR − 1). These concentrations gave values on either side of the horizontal line log (DR − 1) = 0. Because

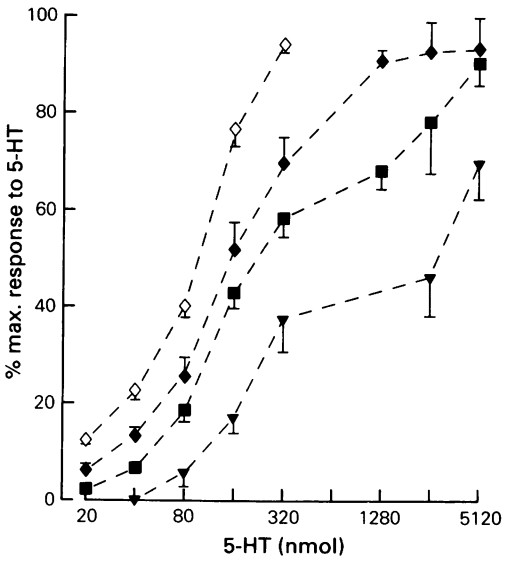

Figure 5 Dose-response curves showing the effect of ICS 205-930 on responses of the superior cervical ganglion (SCG) to 5-hydroxytryptamine (5-HT). Pooled curves show the effects of ICS 205-930, 10^{-11} (◆), 10^{-10} (■) and 10^{-9} (▼) M on 5, 10 and 5 ganglia, respectively. (◇) Control responses. The curves showing the effects of 10^{-11} and 10^{-10} M ICS 205-930 present in part the same data used in Figure 4b,c. Ordinate and abscissa scales as in Figure 2. Points show means with vertical lines indicating s.e.mean.

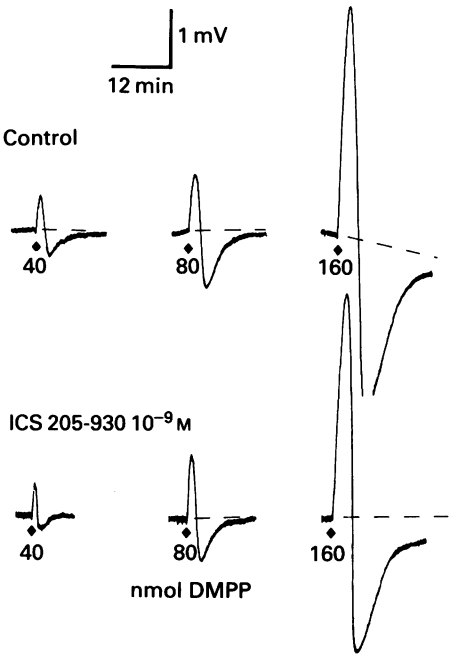

Figure 6 Effect of ICS 205-930 on responses of the superior cervical ganglion (SCG) to dimethylphenylpiperazinium (DMPP). Chart records showing responses to DMPP (bolus injection of 40–160 nmol at ◆) before and after ICS 205-930 (10^{-9} M). A sequence of control responses was elicited and the tissue then exposed to the antagonist for 1 h before re-testing with DMPP. Dashed lines indicate baseline drift. Note peak of after-hyperpolarization of control response to 160 nmol DMPP was truncated by chart recorder.

of the narrow dose band for parallel shift, it was not possible to measure the slope of the Schild plot and, thus, it cannot be established whether or not the slope of the plot was unity.

Apparent pA_2 values obtained by the two methods and for the two ganglia are shown in Table 1, but values derived from the formula have been taken as representative.

Selectivity of blockade by ICS 205-930

The selectivity of the blockade induced by ICS 205-930 was tested by using as control agonists DMPP and GABA on NG and DMPP on SCG. GABA gave poorly reproducible responses in the rabbit SCG. A typical series of responses to DMPP (40–160 nmol) recorded from SCG is shown in Figure 6. The response to the nicotinic agent characteristically consists of a large and rapid depolarization followed by an after-hyperpolarization as large or larger than the preceding depolarization. Superfusion of the SCG with ICS 205-930 nmol (10^{-9} M) for 1 h had no apparent effect on DMPP responses in this or other experiments. Similarly, DMPP responses from NG, although of smaller size and subject to variability, were not significantly altered by ICS 205-930. Responses under control conditions declined by 29 ± 6% (mean ± s.e.mean, $n = 14$) over the course of 3 h, while after equilibration with ICS 205-930, 10^{-10} M, they also declined by 29 ± 7% (mean ± s.e.mean, $n = 11$), with 10^{-9} M by 36 ± 11% (mean ± s.e.mean, $n = 10$) and with 10^{-8} M by 19 ± 13% (mean ± s.e.mean, $n = 9$). Dose-res-

Table 1 Apparent pA_2 values for antagonism of 5-hydroxytryptamine by ICS 205-930 at nodose and superior cervical ganglia

Nodose ganglion		*Superior cervical ganglion*	
Formula	*Schild plot*	*Formula*	*Schild plot*
10.2 ± 0.2 ($n = 14$)	9.8	10.4 ± 0.1 ($n = 14$)	10.6

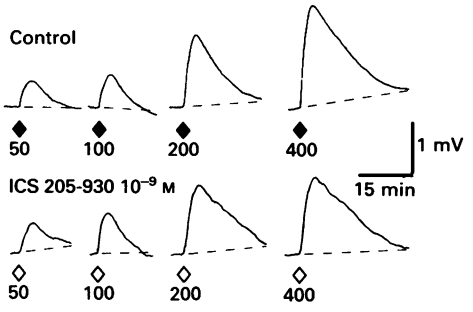

Figure 7 Effect of ICS 205-930 on responses of the nodose ganglion (NG) to GABA. Chart records showing responses to GABA (bolus injections of 50–400 nmol) (◆) before and (◇) after equilibration with ICS 205-930 (10^{-9} M). A sequence of control responses was elicited and the tissue then exposed to the antagonist for 1 h before re-testing with GABA.

ponse curves for the depolarizing action of GABA on NG were constructed before and after equilibration with ICS 205–930 (10^{-9} M) for 1 h. The responses from one of these experiments are shown in Figure 7. The antagonist at a concentration of 10^{-9} M had no depressant effect on responses to GABA. The decline in the maximal response (to 400 nmol) was also seen in most control experiments during a second control dose-response curve. The dose-response curve based on pooled data was not significantly altered by the antagonist.

ICS 205-930 did not itself alter the membrane potential of NG or SCG cells.

Discussion

In this paper we have described the blocking action of ICS 205-930 against 5-HT depolarizations of ganglion cell bodies in NG and SCG. The compound has been shown to be a potent and selective antagonist, the threshold concentration for blockade being around 10^{-11} M.

The high sensitivity to ICS 205-930 of soma membrane 5-HT receptors in NG parallels the sensitivity *in vivo* of receptors on rat vagal nerve endings, which are stimulated by 5-HT during the Bezold-Jarisch reflex (Richardson *et al.*, 1985). In addition the high sensitivity of 5-HT receptors on the soma of SCG cells parallels the sensitivity of receptors on the sympathetic nerve terminals in rabbit heart, which are stimulated by 5-HT to release noradrenaline (Richardson *et al.*, 1985). Thus, our more direct measure of 5-HT action confirms the antagonistic potency of ICS 205-930 described by these authors and suggests that both excitation of vagal sensory endings and noradrenaline release from sympathetic nerve terminals might be secondary to membrane depolarization. The close match in the pA_2 for NG soma membrane depolarization (10.2, this paper) and that against the action of 5-HT in reducing compound action potential amplitude in rabbit vagal axons (10.2, Richardson *et al.*, 1985) suggests that this latter effect is also secondary to membrane depolarization and may be mediated by the same 5-HT receptors.

The blocking action of ICS 205-930 against 5-HT in these two ganglia seems to be selective since the depolarizing actions of both DMPP and GABA were unaffected by the antagonist. Other depolarizing agents such as noradrenaline and histamine do not evoke sufficiently large or consistent responses in these ganglia in the rabbit to allow quantitative analysis. However, Richardson *et al.* (1985) have demonstrated that ICS 205-930 shows no appreciable affinity for H_1-histamine receptors or for α_1-, α_2-, β_1- or β_2-adrenoceptors.

Quantitative analysis of the blockade of 5-HT responses by ICS 205-930 yielded apparent pA_2 values. The values obtained afford a convenient measure of antagonism but do not imply any particular mechanism of agonist/antagonist interaction. Apparent pA_2 values obtained using the formula in experiments on NG and also on SCG were in reasonably close agreement; the pA_2 for NG (10.2) was not significantly different from that for SCG (10.4). These values are in good agreement with pA_2 values obtained by Richardson and his colleagues (1985) for rabbit vagus nerve (10.2) and nerve terminals of rabbit heart (10.6).

Thus, the excitatory 5-HT receptors present on visceral primary afferent (NG) neurones, whether on cell bodies, axons or sensory endings appear to be very similar to those on SCG cells (see also Wallis, 1981; Fozard, 1984). Whether they are homologous with the receptors on sympathetic nerve terminals remains an open question. Although the pA_2 for ICS 205-930 at SCG receptors is very similar to its pA_2 at terminals, the potency of other agents suggests that the terminal receptors are different in some respects from vagal axon receptors (Donatsch *et al.*, 1984; Richardson *et al.*, 1985) and, hence, from receptors on the somata of SCG cells (Round & Wallis, unpublished data). It should not be forgotten, of course, that the cardiac terminal receptors are located on stellate ganglion cells and not on SCG cells.

The neuronal 5-HT receptors of NG and SCG cells are different from those mediating neuronal excitation in the ileum (see Wallis, 1981; Fozard, 1984), the latter being the classical M-receptor of Gaddum & Picarelli (1957). ICS 205-930 is substantially less potent in blocking these ileal receptors (Richardson *et al.*, 1985).

The nature of the antagonism of 5-HT by

ICS 205–930 cannot be fully resolved. In NG, only concentrations of antagonist of 10^{-10} M or lower caused parallel shifts in the dose-response curves. In NG at least, it appeared that higher concentrations of the antagonist elicited a non-surmountable mode of blockade. Thus, ICS 205-930 appeared to cause a progressive reduction of the maximal response in NG with increasing concentrations (Figure 3). Further, we observed that exposure of NG to the antagonist at a concentration of 10^{-9} M for 3–4 h in a few experiments produced greater blockade than superfusion for 1–2 h, indicating that equilibrium at this concentration was incomplete at our standard exposure time. In general, however, very long equilibration times were impracticable with these preparations.

The effects of ICS 205-930 are in these respects very similar to those of MDL 72222 on NG cells (Azami *et al.*, 1985; Round & Wallis, unpublished data), although comparison of their apparent pA_2 values determined with low concentrations of the antagonists shows ICS 205–930 to be some 350 times more potent than MDL 72222. However, blockade of 5-HT responses in SCG by ICS 205-930 (this study) and by MDL 72222 (Round & Wallis, unpublished data) was more readily surmountable. It is possible that the interaction of agonists and antagonists in NG, particularly at high concentrations, may be distorted by the presence of more substantial connective tissue barriers and tight packing of the cells which hinders the removal of agents from the vicinity of the receptors (see Stansfeld & Wallis, 1984). Thus, the interaction may or may not be competitive at low concentrations of ICS 205-930. The results for NG, at least, are consistent with a non-competitive mode of interaction in a tissue where there is a substantial receptor reserve. It would be premature to conclude that this is the case, since the tachyphylaxis induced by the larger quantities of 5-HT may compromise the ability to surmount blockade because of reversible desensitization of receptors by 5-HT itself (Fozard & Mwaluko, 1976; Fozard & Mobarok Ali, 1978; Göthert & Duhrsen, 1979).

In conclusion, our results confirm and extend the recent findings of Richardson *et al.* (1985) and demonstrate that ICS 205-930 is a potent and selective antagonist of the membrane depolarizations evoked by 5-HT in vagal primary afferent and sympathetic ganglion cells.

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