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Origin of 5-hydroxytryptamine-induced hyperpolarization of the rat superior cervical ganglion and vagus nerve

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1 5-Hydroxytryptamine (5-HT)-induced membrane potential changes were recorded extracellularly from rat superior cervical ganglia (SCG) and cervical vagus nerves *in vitro*.

2 On the SCG, low concentrations of 5-HT (1×10^{-8}–3×10^{-7}M) induced concentration-related hyperpolarization responses. Higher concentrations of 5-HT (1×10^{-6}–1×10^{-4}M) induced complex responses which typically consisted of an initial hyperpolarization, followed by a depolarization and subsequent after-hyperpolarization. The depolarization, but not the initial hyperpolarization, was blocked by metoclopramide (3×10^{-5}M), quipazine (1×10^{-6}M) or MDL 72222 (1×10^{-5}M).

3 5-HT-induced hyperpolarization of the SCG was potentiated when the amount of calcium chloride added to the superfusion medium was reduced from 2.5 to 0.15 mmol l^{-1}. Hyperpolarization responses recorded from SCG preparations superfused with this low-calcium medium were unaffected by the substitution of lithium chloride for sodium chloride and were potentiated by the omission of potassium ions. Ouabain (1×10^{-3}M) abolished both the hyperpolarization and the depolarization induced by 5-HT.

4 On the vagus nerve, 5-HT (1×10^{-7}–3×10^{-5}M) did not induce initial hyperpolarization in either normal or low-calcium Krebs-Henseleit medium. However, in the latter solution only, depolarization responses induced by 5-HT at concentrations of 1×10^{-6}M or greater were followed by hyperpolarization. Both the depolarization and the post-5-HT hyperpolarization were blocked by metoclopramide (3×10^{-5}M) but were unaffected by spiperone (1×10^{-7}M).

5 On the vagus nerve, post-5-HT hyperpolarization responses were selectively and reversibly inhibited by ouabain, and by superfusion with Krebs-Henseleit medium that was either potassium-free or contained lithium chloride in place of sodium chloride.

7 These results demonstrate the generation in the rat SCG of a 5-HT-induced hyperpolarization response that is not mediated through 5-HT_3 receptors and is unlikely to be a consequence of depolarization. In contrast, on the rat vagus nerve, the post-5-HT hyperpolarization observed in the present study had the characteristics expected of depolarization-dependent activation of a sodium ion pump.

Introduction

5-Hydroxytryptamine (5-HT)-induced hyperpolarization can be recorded in rabbit isolated superior cervical ganglia (SCG) and nodose ganglia using either extracellular or intracellular techniques. The extracellularly-recorded 5-HT-induced hyperpolarization responses have been observed to follow, but not to precede, depolarization and on the SCG, have been attributed to the depolarization-dependent activation of a sodium ion pump (see Wallis & Woodward, 1975; Wallis *et al.*, 1982). The possibility that 5-HT may hyperpolarize some neurones in these rabbit ganglia via an additional and independent mechanism is suggested by the finding, in intracellular recording studies, that 5-HT occasionally caused hyperpolarization only (Wallis & North, 1978; Dun & Karczmar, 1981; Higashi & Nishi, 1982; Stansfeld & Wallis, 1982). Extracellular recordings of such 5-HT-induced hyperpolarization responses have been made from the cat SCG *in situ* (Haefely, 1974), and from bullfrog sympathetic ganglia in which 5-HT-induced depolarizations were abolished with nicotine (Watanabe & Koketsu, 1973).

In the present study, an examination has been made of the 5-HT-induced hyperpolarization responses that

can be recorded extracellularly from the rat isolated SCG and vagus nerve. Particular attention has been paid to two points: whether these responses were a direct consequence of the activation of a receptor, and if so, whether this receptor was of a different type from that which mediates 5-HT-induced depolarization in these tissues.

A preliminary account of some of the work described in this paper has been presented to the British Pharmacological Society (Fortune *et al.*, 1985).

Methods

Preparation of tissues

Male hooded rats weighing 200–300 g were stunned by a blow to the head and killed by cardiac puncture. Segments of cervical vagus nerve (VN) approximately 10 to 20 mm long and minus the nodose ganglion, or 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 or vagus nerve was then carefully removed.

Extracellular recording

Within one hour of dissection, de-sheathed superior cervical ganglia or vagus nerves were transferred to two-compartment Perspex baths to permit extracellular recording of 5-HT induced membrane potential changes. Each SCG was mounted with the ganglion lying in the first compartment and the internal carotid nerve containing postganglionic fibres (see Sacchi & Rossi, 1981) projecting through a greased slot (Dow-Corning high vacuum grease) into the second. Each VN was positioned so that approximately 50% lay in the first compartment, while the remainder projected through the greased slot into the second. The d.c. potential between the two compartments was recorded via silver-silver chloride electrodes connected to the tissue preparation via agar-saline/filter paper bridges and was displayed on a potentiometric chart recorder (Servogor 220 or SE 130). Each compartment of the bath was perfused continuously at a constant rate of approximately 1 ml per min with Krebs-Henseleit medium dripped directly onto the tissue. Drugs were applied at a known concentration via the superfusion stream into the first compartment only. Modified Krebs-Henseleit media were also applied to this compartment only.

The temperature of each preparation was maintained at 27 ± 1°C. This temperature was chosen since, in preliminary experiments, recorded base-lines were found to be more stable at 27°C than at the more physiological level of 37°C. This was considered particularly important for recording 5-HT-induced hyperpolarizations, which are of small amplitude (see Results).

Design of experiments

5-HT concentration-response curves were constructed using non-cumulative applications. The period of exposure to each concentration of 5-HT was sufficient for the evoked potential change (hyperpolarization or depolarization for a monophasic response, depolarization for a biphasic or triphasic response) to appear to have stabilized. Contact times were generally three min or less. Preparations were allowed to repolarize fully between 5-HT applications.

The effects of antagonists were measured only once apparent equilibrium was attained. This was taken to have occurred when the response to two successive applications of an approximate EC_{50} of 5-HT, in the presence of the antagonist, had stabilized. The same criterion was applied when measuring the effects of changes to the ionic composition of the superfusion medium, although in this case, re-attainment of a stable recorded base-line was a prerequisite.

The maximum and EC_{50} of each 5-HT concentration-response curve were estimated by direct, computer-aided fit of a logistic curve to the experimental data (see Ireland & Tyers, 1987).

Drugs and solutions

In all the experiments described in this paper, the creatinine sulphate salt of 5-HT was used. Creatinine sulphate alone (1×10^{-8}–1×10^{-4}M) did not cause any significant changes to the extracellularly-recorded resting membrane potential of SCG or vagus nerve preparations superfused with either normal or low-calcium Krebs-Henseleit medium (results not shown). Both 5-HT creatinine sulphate and creatinine sulphate were purchased from the Sigma Chemical Company Ltd. Other drugs used were: 1,1-dimethyl-4-phenylpiperazinium iodide (DMPP) (Sigma), metoclopramide hydrochloride (Beecham), quipazine maleate (Miles), MDL 72222 (1αH, 3α, 5αH-tropan-3-yl-3, 5-dichlorobenzoate) (Merrell-Dow), spiperone (Janssen), ouabain (Strophanthin G) (Sigma) and atropine sulphate (Sigma).

Drugs were dissolved in Krebs-Henseleit medium, unless otherwise stated, to give final concentrations of 1×10^{-3}–1×10^{-2}M. Solutions were prepared immediately before use. Spiperone was dissolved to give a 1×10^{-2}M solution in 0.1 M (±)-tartaric acid. This was subsequently diluted with normal or modified Krebs-Henseleit medium, without causing visible precipitation.

The composition of the normal Krebs-Henseleit

medium used in the present study was (in mmol l^{-1}): NaCl 118, $NaHCO_3$ 25, KH_2PO_4 1.18, KCl 4.7, $MgSO_4$ 1.18, $CaCl_2$ 2.5, glucose 11. Low-calcium medium contained $CaCl_2$ 0.15 mM, the small reduction in osmolarity being ignored. In potassium-free medium, NaH_2PO_4 1.18 mM was substituted for KH_2PO_4, and NaCl 4.7 mM was substituted for KCl; this solution contained $CaCl_2$ 0.15 mM. In lithium medium, LiCl 118 mM was substituted for NaCl; this solution contained $CaCl_2$ 0.15 mM. All media were prepared in glass-distilled water and reagents, which were A.R. grade, were obtained from commercial sources. All solutions were gassed with 95% O_2 and 5% CO_2.

Results

5-HT-induced potential changes recorded in normal Krebs-Henseleit medium

On the SCG, brief (2–3 min) applications of low concentrations of 5-HT (1×10^{-8}–3×10^{-7}M), caused concentration-dependent hyperpolarization. The maximum response for a given tissue preparation was small (typically less than 200 μV) (Figure 1). Higher concentrations of 5-HT (1×10^{-6}–1×10^{-4}M) induced complex responses that often comprised an initial hyperpolarization followed by a depolarization and subsequent after-hyperpolarization. In some cases, especially in the presence of higher concentrations of 5-HT (1×10^{-5}–1×10^{-4}M), the initial hyperpolarization was not observed (Figure 1).

On the vagus nerve 5-HT (1×10^{-7}–3×10^{-5}M) induced rapid concentration-related depolarization responses, but no hyperpolarization.

Effects of reducing the external calcium concentration

On both the SCG and vagus nerve, application of low-calcium Krebs-Henseleit medium caused a rapid maintained apparent hyperpolarization of 300–500 μV. On the SCG, 5-HT-induced hyperpolarization was potentiated in the low-calcium medium. This effect was approximately maximal within one hour of changing the perfusion medium, at which time the amplitude of the hyperpolarizations induced by 5-HT (3×10^{-7}M) was found to be increased to a mean ($\pm$ s.e.mean) of 302 $\pm$ 29% of the control ($n = 4$). 5-HT-induced depolarization of the SCG was not potentiated under these conditions.

In contrast, on the vagus nerve, the amplitude of 5-HT-induced depolarization responses was markedly potentiated on changing from normal to low-calcium solution (Figure 4); this effect appeared maximal within one hour. The degree of potentiation did not change significantly with the concentration of 5-HT ($P > 0.05$, analysis of variance). In addition, in the low-calcium medium, depolarization induced by 5-HT at concentrations of 1×10^{-6}M, or greater, was usually followed by an after-hyperpolarization. On the vagus nerve, hyperpolarization was never seen to precede 5-HT-induced depolarization.

Low-calcium media were used in all of the experiments described in the remainder of this paper.

Effect of lithium medium

On both the SCG and vagus nerve, application of Krebs-Henseleit medium in which lithium chloride was substituted for sodium chloride, caused a maintained apparent depolarization of 1 to 2 mV, that reached peak amplitude within 40 to 60 min. The effects of this ionic substitution were therefore measured after at least 60 min exposure to the modified solution.

On the SCG, 5-HT-induced depolarization was markedly attenuated in the lithium medium although the hyperpolarization responses appeared to be either unaffected or potentiated. Some recovery of depolarization amplitude was noted approximately 60 min after changing back to superfusion with low-calcium Krebs-Henseleit medium (Figure 2).

In vagus nerve preparations superfused with lithium medium, 5-HT-induced depolarization was attenuated, but the principal effect was a slowing of the repolarization rate on washout of 5-HT, and reversible abolition of the after-hyperpolarization. The attenuation of depolarization amplitude, but not the abolition of after-hyperpolarization, could be overcome by the application of higher concentrations of 5-HT (see Figure 2).

Effect of ouabain

Brown *et al.* (1972) demonstrated that, in the rat SCG, a very high concentration of ouabain (approximately 1×10^{-3}M) was required to block completely post-carbachol hyperpolarization. In the present study, ouabain (1×10^{-3}M) caused a slow depolarization of both the SCG and vagus nerve, that increased in amplitude over the first 30 to 40 min of drug application, and reached a peak of 2 to 5 mV.

On the SCG, ouabain inhibited reversibly both the hyperpolarization and the depolarization induced by 5-HT (Figure 3). In contrast, ouabain (1×10^{-3}M) had no effect on depolarization of this tissue induced by the nicotinic agonist DMPP even though it abolished post-DMPP hyperpolarization ($n = 4$; result not shown).

On the vagus nerve, the principal effect of ouabain, like that of superfusion with sodium-free medium, was to prolong the period of repolarization following washout of 5-HT, and to block the after-hyper-

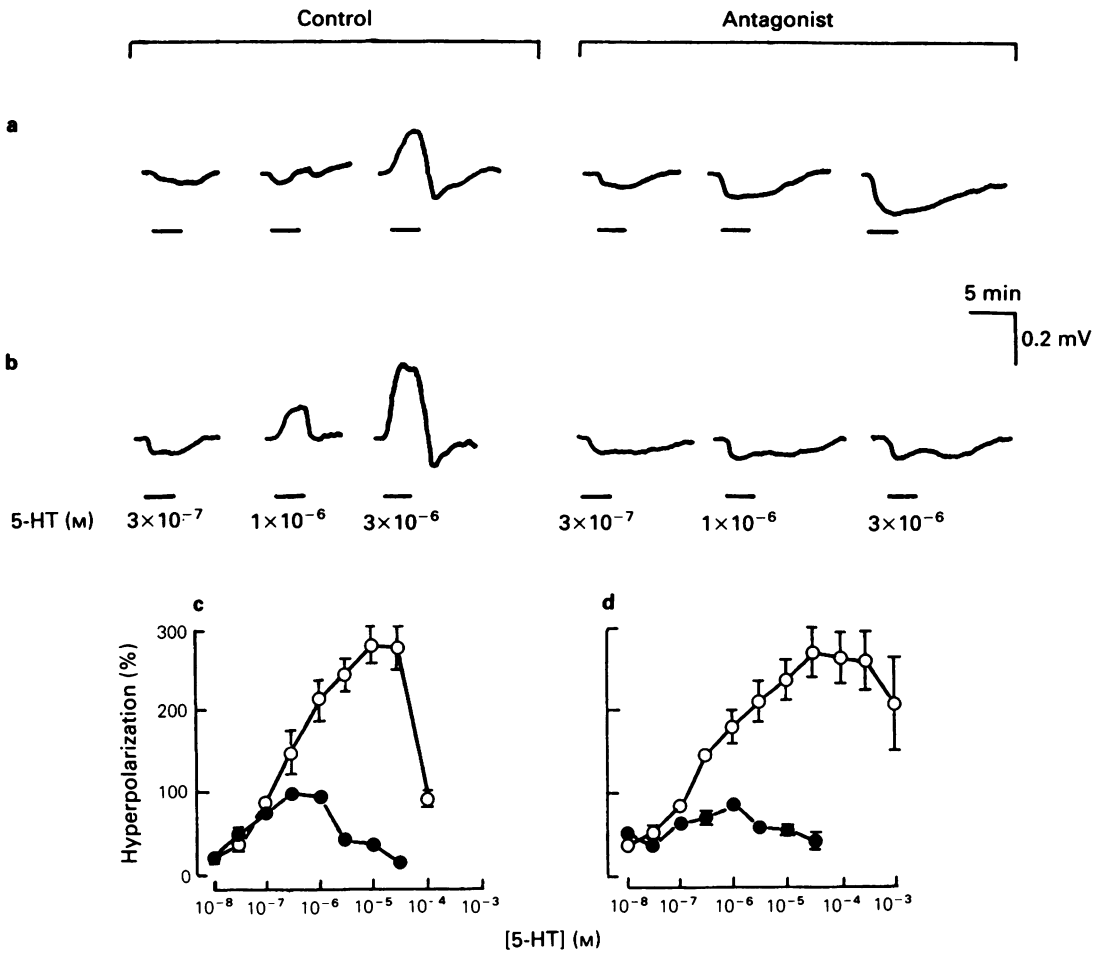

Figure 1 Effects of antagonists on 5-hydroxytryptamine (5-HT)-induced responses in rat isolated superior cervical ganglion (SCG). Metoclopramide (3×10^{-5}M) (a) or quipazine (1×10^{-6}M) (b) were applied to separate SCG preparations superfused with Krebs-Henseleit medium containing $CaCl_2$ 2.5 mM. Upward deflection indicates depolarization; the bar under each response indicates the approximate duration of the 5-HT application. The responses shown are sequential records from single SCG preparations; similar effects were obtained when these antagonists were applied to a further three SCG preparations each. (c and d) The effects of metoclopramide (3×10^{-5}M) and MDL 72222 (1×10^{-5}M), respectively, on the amplitude of 5-HT-induced initial hyperpolarization responses recorded in modified Krebs-Henseleit medium containing $CaCl_2$ 0.15 mM. Results are expressed as a percentage of the estimated control maximum. Each point is the mean of single determinations in 4 (c) or 7 (d) individual ganglia with vertical lines indicating the s.e.mean. (●) Control responses, (○) data obtained in the presence of antagonist. The approximate threshold concentrations of 5-HT required to induce depolarization were: 3×10^{-6}M in control preparations, 1×10^{-4}M in the presence of metoclopramide and 1×10^{-3}M in the presence of MDL 72222.

polarization. Both effects were readily reversible on washing with ouabain-free medium (Figure 3).

Effect of potassium-free medium

Application of potassium-free Krebs-Henseleit medium to SCG and vagus nerve preparations caused an apparent hyperpolarization that reached a peak amplitude of 2 to 3 mV within about 30 min. After this period, the recorded base-line was sufficiently stable to allow 5-HT-induced potential changes to be measured. On the SCG, 5-HT-induced hyperpolarization was potentiated in potassium-free medium (Figure 4); in SCG preparations superfused with potassium-free medium for 30 min, the amplitude of the hyperpolarization induced by 5-HT, 3×10^{-7}M,

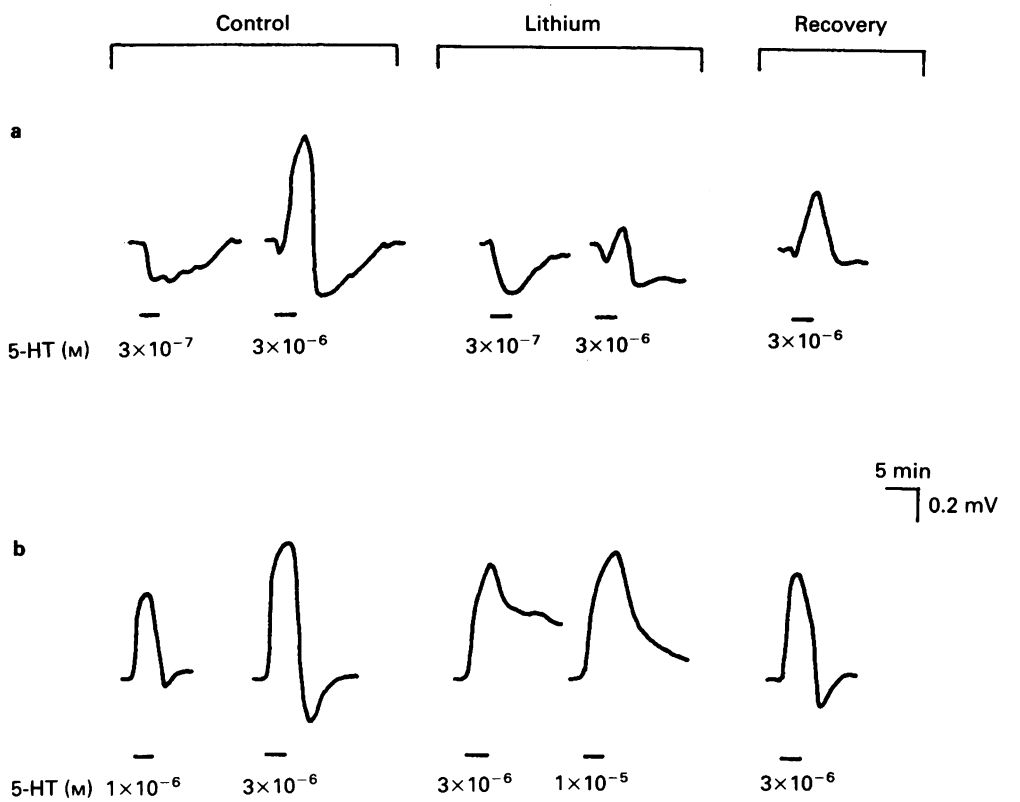

Figure 2 Effect of substituting LiCl (118 mM) for NaCl (118 mM) on 5-hydroxytryptamine (5-HT)- induced responses in the rat isolated superior cervical ganglion (SCG) (a) and vagus nerve (b). The responses shown are sequential records from single tissue preparations; similar results were obtained in one further SCG and one further vagus nerve. Experiments were performed using modified Krebs-Henseleit media containing $CaCl_2$ 0.15 mM. The responses shown were recorded no sooner than 60 min after altering the ionic composition of the superfusion medium. Upward deflection indicates depolarization; the line under each response indicates the approximate duration of the 5-HT application.

was increased to a mean (± s.e.) of 201 ± 21% of the control value recorded in low-calcium Krebs-Henseleit medium ($n = 4$). The time-course of this effect was not examined.

On the vagus nerve, superfusion with potassium-free medium had no significant effect ($P > 0.05$, t test) on the amplitude of the depolarization induced by 5-HT (3×10^{-6}M), but abolished reversibly the after-hyperpolarization ($n = 4$; Figure 4).

Re-addition of potassium ions to SCG or vagus nerve preparations previously superfused with potassium-free medium caused a further hyperpolarization of between 1 and 5 mV, followed by a slow depolarization.

Effects of antagonists on 5-HT-induced hyperpolarization of the SCG

On the SCG, hyperpolarization responses induced by low concentrations of 5-HT (1×10^{-8}–3×10^{-7}M) were not antagonized by metoclopramide (3×10^{-5}M) quipazine (1×10^{-6}M) or MDL 72222 (1×10^{-5}M). However, these compounds, applied individually, did appear to increase both the amplitude and duration of the initial hyperpolarization occurring at higher concentrations of 5-HT (1×10^{-6}–3×10^{-4}M). It was considered likely that this effect was due to selective blockade of 5-HT-induced depolarization (see Figure 1). Of the three compounds tested, MDL 72222

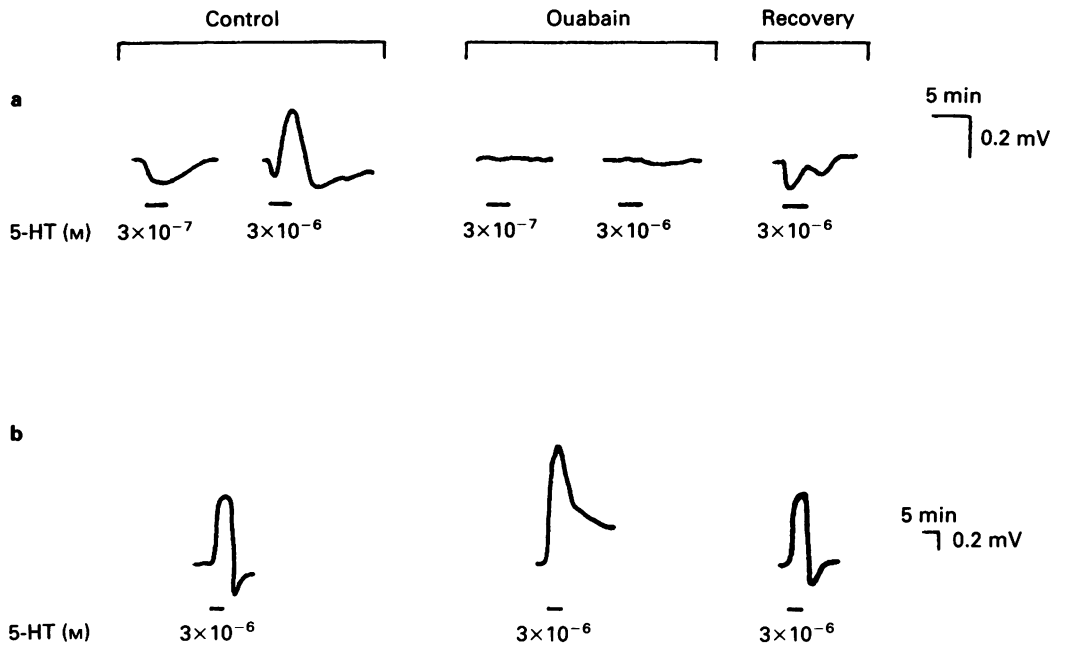

Figure 3 Effect of ouabain (1×10^{-3}M) on 5-hydroxytryptamine (5-HT)-induced responses of the rat isolated superior cervical ganglion (SCG) (a) and rat vagus nerve (b). These experiments were performed using modified Krebs-Henseleit medium containing $CaCl_2$ 0.15 mM. The responses shown are sequential records from single tissue preparations; similar results were obtained in one further SCG preparation and three further vagus nerve preparations. The effects of ouabain were recorded after an equilibration period of approximately 60 min; the recovery responses were obtained after superfusion with ouabain-free medium for at least 60 min. Upward deflection indicates depolarization; the bar under each response indicates the approximate duration of the 5-HT application.

(1×10^{-5}M) was the most effective in antagonizing 5-HT-induced depolarization. Atropine (1×10^{-5}M) did not block either the hyperpolarization or depolarization responses induced by 5-HT (result not shown).

Effects of antagonists on post-5-HT hyperpolarization of the vagus nerve

On the vagus nerve both the depolarization, and the after-hyperpolarization responses induced by 5-HT (1×10^{-6}–3×10^{-5}M) were abolished in the presence of metoclopramide (3×10^{-5}M). Under these conditions, hyperpolarization responses could be induced by applying higher concentrations of 5-HT (3×10^{-4}–1×10^{-3}M) (Figure 5). As in the absence of metoclopramide, these followed, but did not precede 5-HT-induced depolarization. On the vagus nerve, spiperone (1×10^{-7}M) had no effect on either the depolarization or the after-hyperpolarization induced by 5-HT (Figure 5), although this compound antagonizes 5-HT-induced hyperpolarization of the SCG (Ireland & Jordan, 1987).

Discussion

The results obtained in the present study suggest that 5-HT can hyperpolarize *in vitro* preparations of rat peripheral neuronal tissue via two distinct mechanisms.

Hyperpolarization of the superior cervical ganglion

On the SCG, low concentrations of 5-HT induced hyperpolarization with no preceding depolarization. It is unlikely that this hyperpolarization was mediated either directly or indirectly via 5-HT_3 receptors since it was not antagonized by metoclopramide, quipazine or MDL 72222 (see Ireland & Tyers, 1987). This conclusion is supported by the observation that phenylbiguanide does not hyperpolarize the rat SCG (Fortune *et al.*, 1985) even though it mimics the 5-HT_3 receptor-mediated depolarizing activity of 5-HT on the rat vagus nerve (Ireland & Tyers, 1987).

5-HT-induced hyperpolarization of the rat SCG was markedly potentiated in low-calcium Krebs-Hen-

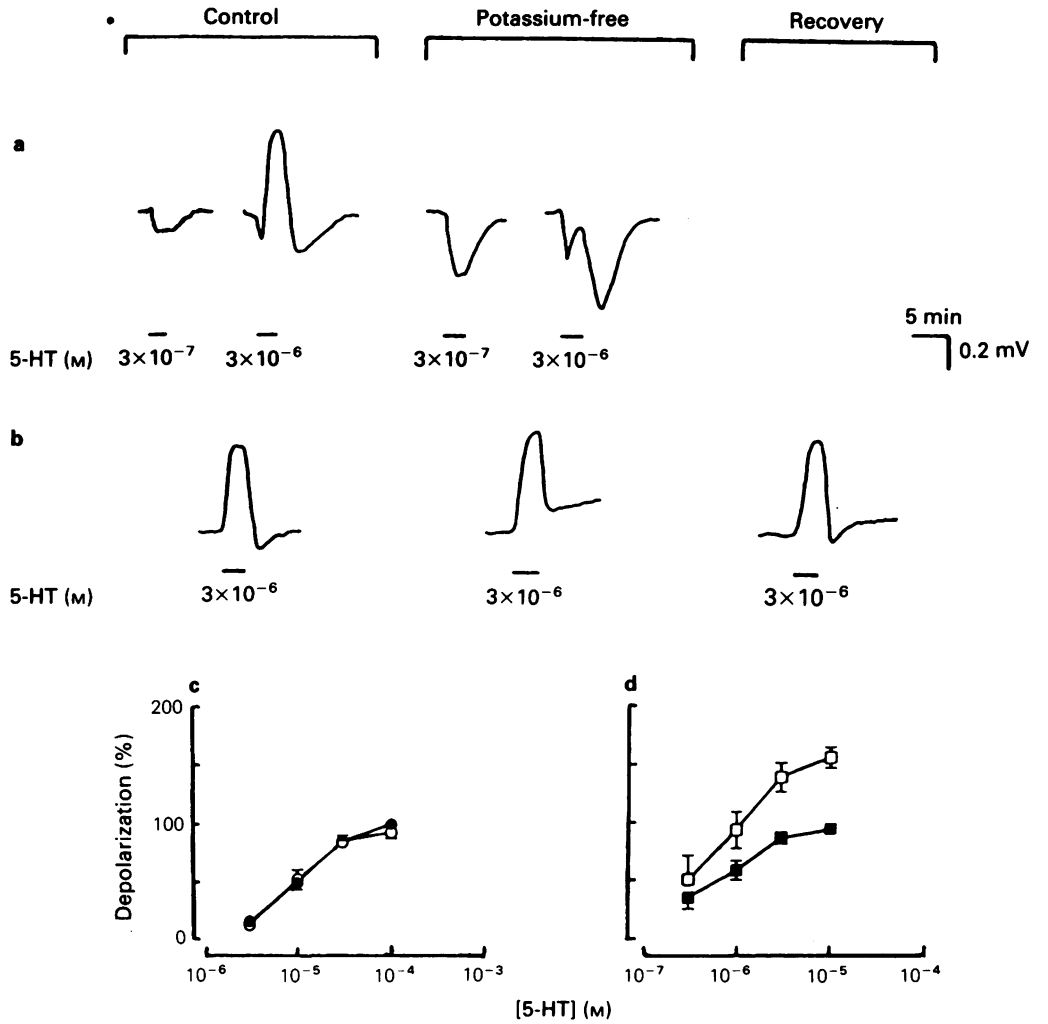

Figure 4 Effects of changing the potassium ion or calcium ion content of the superfusion medium on 5-hydroxytryptamine (5-HT)-induced responses. (a and b) Sequential records from a single superior cervical ganglion (SCG) (a) and a single vagus nerve (b) showing the effect of the omission of potassium ions. Preparations were superfused throughout with modified Krebs-Henseleit medium containing $CaCl_2$ 0.15 mM. Effects similar to those shown were obtained in a further three examples of each preparation. Upward deflection indicates depolarization; the bar under each response indicates the approximate duration of the 5-HT application. (c and d) Effect of reducing the $CaCl_2$ concentration from 2.5 mM (●, ■) to 0.15 mM (○, □) on 5-HT-induced depolarization of the rat isolated SCG (c) and vagus nerve (d). Results are expressed as a percentage of the estimated control maximum, each point is the mean of single determinations in four separate preparations, with the vertical line indicating the s.e.mean. All results were recorded at least 60 min after altering the ionic composition.

seleit medium. Unfortunately, no insight was gained into how calcium might influence the receptor-effector chain for 5-HT-induced hyperpolarization. Both α_2-adrenoceptor- and muscarinic receptor-mediated hyperpolarizations of the rat SCG are potentiated in low-calcium medium (Brown & Caulfield, 1979; Brown *et al.*, 1980). It is unlikely that 5-HT-induced hyperpolarization was mediated via the release of either noradrenaline or acetylcholine since it was not blocked by phentolamine (Ireland & Jordan, 1987) or atropine (this paper).

The Na^+K^+-ATPase inhibitor ouabain (Skou,

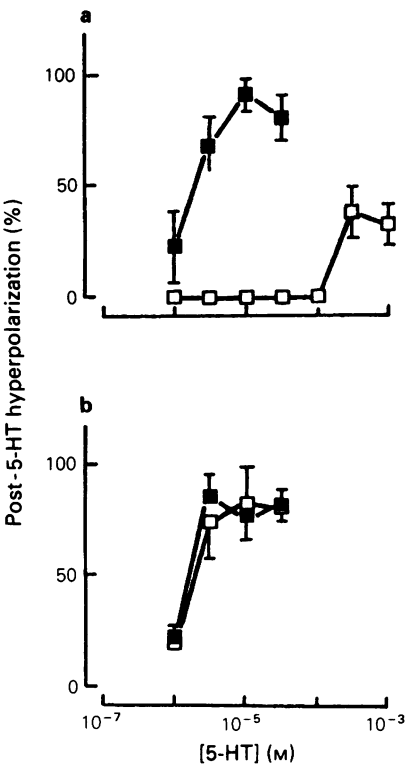

Figure 5 Effect of metoclopramide (3×10^{-5}M) (a) and spiperone (1×10^{-7}M) (b) on post-5-hydroxytryptamine (5-HT) hyperpolarization of the rat isolated vagus nerve. Each panel illustrates a separate experiment. Symbols indicate control responses (■), or presence of antagonist (□). Results are expressed as a percentage of the estimated control maximum. Each point is the mean of single determinations in 4 separate vagus nerves and the vertical lines show the s.e.mean.

1965) abolished the hyperpolarization induced by 5-HT on the rat SCG, indicating that this response may result directly from activation of the enzyme. However, this conclusion seems inconsistent with the observation that 5-HT-induced hyperpolarization was not blocked in SCG preparations superfused with Krebs-Henseleit medium in which lithium chloride was substituted for sodium chloride: lithium ions are a poor substrate for Na^+K^+-ATPase (Wespi, 1969) and prevent its activation by extracellular potassium ions (Baker & Connelly, 1966). Watanabe & Koketsu (1973) found that both ouabain and substitution of lithium ions for sodium ions inhibited 5-HT-induced hyperpolarization of bullfrog sympathetic ganglia; they suggested that this hyperpolarization is electrogenic in origin. An alternative explanation for the inhibitory effect of ouabain on 5-HT-induced hyperpolarization of the rat SCG is that it was an indirect result of the inhibition of Na^+K^+-ATPase — possibly secondary to a fall in membrane potential. Further, because rat Na^+K^+-ATPase seems rather insensitive to ouabain (see Aldridge, 1962; Akera *et al.*, 1969; Brown *et al.*, 1972; Brown & Scholfield, 1974) a very high concentration of the drug (1×10^{-3}M) was used in the present study. Therefore the effects of ouabain described here may have been due to some action other than inhibition of this enzyme. 5-HT-induced hyperpolarization was potentiated when SCG preparations were superfused with potassium-free Krebs-Henseleit medium. A simple explanation for this influence of potassium ion concentration is that this response is generated through an increase in membrane potassium ion conductance. A similar conclusion was reached by Higashi & Nishi (1982) for intracellularly-recorded 5-HT-induced hyperpolarization of the rabbit nodose ganglion.

Hyperpolarization of the vagus nerve

5-HT-induced hyperpolarization was observed in vagus nerve preparations superfused with low-calcium Krebs-Henseleit medium, but not those superfused with normal medium. The reason for this was not determined. These hyperpolarization responses were observed to follow, but not to precede, 5-HT-induced depolarizations. Hyperpolarization was blocked surmountably, along with depolarization, by metoclopramide, consistent with the involvement of 5-HT_3 receptors in the generation of both responses.

Analysis of the ionic basis of 5-HT-induced depolarization of the rat vagus nerve has not been published; 5-HT-induced depolarization of the rabbit SCG and nodose ganglion is probably generated through an increase in sodium ion conductance (see Wallis & North, 1978; Higashi & Nishi, 1982). In the present study on the rat vagus nerve, post-5-HT hyperpolarization had the characteristics expected of the activity of a sodium ion pump. Thus it was abolished in the presence of ouabain although the preceding 5-HT-induced depolarization was unaffected. Post-5-HT hyperpolarization was also abolished when vagus nerves were superfused with Krebs-Henseleit medium in which lithium chloride was substituted for sodium chloride. This was consistent with the observation (Wespi, 1969) that lithium is a poor substrate for the sodium ion pump in mammalian non-myelinated nerve even though it can permeate membrane sodium ion channels (see Armett & Ritchie, 1963a, b) and thus carry depolarizing current in these tissues. Finally, post-5-HT hyperpolarization was selectively inhibited in potassium-free Krebs-Henseleit medium, consistent with the requirement for extracellular potassium ions for the activation of the sodium ion pump (see Brown & Scholfield, 1974). A

similar inhibitory effect of potassium-free media has been described for post-carbachol and post-DMPP hyperpolarization of the rat and rabbit SCG, respectively (Brown *et al.*, 1972; Lees & Wallis, 1974). In contrast, post-5-HT hyperpolarization of the rabbit SCG was shown to be potentiated in the absence of external potassium ions (Wallis & Woodward, 1975). The reason for this discrepancy is unclear.

Depolarization responses

One further aspect of the present results deserves comment: 5-HT-induced depolarization responses of the rat SCG and vagus nerve seemed to differ in their sensitivity to ouabain and to variation of the ionic composition of the superfusion medium. This might indicate significant differences in the mechanisms responsible for 5-HT depolarization of these two preparations. In low-calcium medium, 5-HT-induced depolarization of the SCG was not potentiated even though both 5-HT-induced hyperpolarization of this preparation and 5-HT-induced depolarization of the vagus nerve were markedly increased in amplitude under these conditions. Such potentiation is also observed in the SCG and nodose ganglion of the rabbit (Nash & Wallis, 1981; Stansfeld & Wallis, 1981). The mechanism underlying this apparent difference was not elucidated. However, it might be pertinent that unlike the rat SCG, hyperpolarization does not precede depolarization induced by 5-HT in the rat vagus nerve (this paper) or in either of the rabbit tissues (Wallis & Woodward, 1975; Wallis *et al.*, 1982). Further, in the present study, no examination was made of the time-course of the changes in responsiveness caused by reducing the concentration of calcium ions. Therefore, the possibility remains that apparent differences in absolute sensitivity were actually differences in rate of change. 5-HT-induced depolarization of the rat SCG appeared more sensitive to inhibition by ouabain or lithium ions than did this response on the rat vagus nerve. The inhibitory effect of ouabain against 5-HT on the SCG was particularly unexpected since, in agreement with the results of Brown *et al.* (1972), ouabain had no effect on depolarization of this preparation induced by a nicotinic agonist, despite causing complete blockade of post-nicotinic hyperpolarization. However, Skok & Selyanko (1979) have suggested that within the rabbit SCG, 5-HT and nicotinic agonists do not depolarize the same population of neurones. Therefore the present results with both lithium ions and ouabain might be explained if following inhibition of Na^+K^+-ATPase, the membrane potential did not decline at a uniform rate in all SCG cells, but changed most rapidly in those sensitive to 5-HT.

Conclusions

The present study was undertaken to characterize the 5-HT-induced hyperpolarization responses that can be recorded extracellularly from the rat isolated SCG and vagus nerve. On the rat SCG, 5-HT was capable of inducing hyperpolarization responses independent of the 5-HT-induced depolarization that could also be recorded extracellularly from this preparation. In contrast to the depolarization, this hyperpolarization was not mediated through 5-HT_3 receptors. An attempt to identify the receptors involved is the subject of the companion paper (Ireland & Jordan, 1987). On the vagus nerve, 5-HT-induced hyperpolarization followed depolarization induced by the agonist and had the characteristics expected of the depolarization-dependent activation of a sodium ion pump; no 5-HT-induced hyperpolarization occurred independent of depolarization on this tissue.

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