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MEMBRANE POTENTIAL CHANGES INDUCED BY 5-HYDROXYTRYPTAMINE IN THE RABBIT SUPERIOR CERVICAL GANGLION

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1 Changes in resting membrane potential induced by 5-hydroxytryptamine (5-HT) have been measured in the excised ganglion by the sucrose-gap technique.

2 5-HT produced a rapid depolarization, the threshold concentration for depolarization being around 10 μM. With concentrations of 100 μM or greater, repolarization began during the course of the superfusion; this was followed by prolonged tachyphylaxis.

3 Tachyphylaxis was largely avoided by making injections into the superfusion stream. Standard injections of 0.2 μmol 5-HT dissolved in 0.2 ml of Krebs solution were used routinely and could be given at 20-30 min intervals to evoke relatively constant responses.

4 The response to an injection consisted of a rapid depolarization, followed by a rapid repolarization and subsequent after-hyperpolarization. The threshold quantity for depolarization was around 0.01 μmol, while the ED_{50} estimated from 6 dose-response curves was 0.12 ± 0.02 μmol (mean ± s.e. mean).

5 Injections of 5-HT (0.2 μmol), choline (10 μmol) and acetylcholine (9.9 μmol) produced depolarizations of similar magnitude.

6 Monoamine oxidase inhibitors failed to alter substantially the amplitude of depolarizations to 5-HT.

7 5-HT depolarizations were unaltered in amplitude when the impermeant anion benzenesulphonate was substituted for the chloride ion in Krebs solution, but were initially markedly reduced in amplitude in a sodium-deficient medium; some recovery of the response subsequently occurred. The depolarization which persisted in sodium-deficient solutions was much reduced or abolished when calcium ions were then removed from the superfusion medium. Removal of either calcium ions alone or potassium ions from the superfusion fluid did not reduce depolarization amplitude.

8 The after-hyperpolarization was abolished in sodium-deficient solutions, usually increased in potassium-free solutions, reduced or abolished by ouabain or nicotine, but unaffected by calcium-free solutions.

9 A depolarizing action of 5-HT on presynaptic terminals in the ganglion appears probable.

Introduction

The excitatory action of 5-hydroxytryptamine (5-HT) on the guinea-pig ileum described by Gaddum (1953) was subsequently ascribed to activation of 5-HT receptors located on neurones (Gaddum & Picarelli, 1957). The receptors were designated M receptors and it was noted that tachyphylaxis was readily induced. These receptors could be differentiated from the 5-HT receptors of smooth muscle in terms of the antagonists which affected them. More recently there have been a number of reports of the excitatory action of 5-HT on ganglion cells of the autonomic nervous system (see Haefely, 1972, 1974); occasionally, inhibitory actions have also been observed (see Wallis & Woodward, 1974). de Groat & Volle (1966), Jaramillo & Volle (1968), Machova & Boska (1969) and Haefely (1974) described changes in ganglionic surface potential induced by injection of the amine into the blood supply of the cat superior cervical ganglion, while Wallis & Woodward (1973, 1974) reported that 5-HT evoked a rapid depolarization in the excised superior cervical ganglion of the rabbit. Recent studies on the cat superior cervical ganglion have suggested that 5-HT may mediate three distinct

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responses involving different 5-HT receptors: a depressant action and two types of excitation (de Groat & Lalley, 1973). Haefely (1974) also described separate depressant and excitatory actions in the same ganglion. He suggested that 5-HT receptors may be located both presynaptically and postsynaptically.

Despite these studies the nature of ganglion 5-HT receptors and the ionic basis of the membrane potential changes are still far from clear, while quantitative analysis of the potential changes induced by 5-HT has not usually been attempted.

This paper describes experiments in which the effects of 5-HT on membrane potential were examined in the excised superior cervical ganglion by the sucrose-gap technique. The aim of the experiments was to characterize more fully some of the properties of peripheral neuronal 5-HT receptors. A preliminary account of this work has been given to the British Pharmacological Society (Wallis & Woodward, 1973).

Methods

Preparation

Rabbits were anaesthetized with urethane (1.5-2 g/kg, i.p. as a 50% w/v solution). The superior cervical ganglion was removed, together with several cm of the cervical sympathetic nerve and as great a length as possible of the internal carotid nerve. The ganglia were prepared for insertion into the sucrose-gap apparatus, in which the sucrose compartment was separated by membranes, by the method previously described (Kosterlitz & Wallis, 1966; Kosterlitz, Lees & Wallis, 1968; Wallis, Lees & Kosterlitz, 1975). With this method, stable recordings may be made over periods of up to 5 hours. Potential changes were displayed on a potentiometric chart recorder (Servoscribe R.E. 511.20). The amplitude of the potential change was measured from a projection of the baseline preceding the response. Baseline drift was variable but tended to be greater in those experiments in which changes were made in the ionic composition of the superfusion medium. Flow rate over the ganglion was 2-3 ml/minute.

Solutions

All solutions were made up from distilled water passed through a deionizer. The Krebs solution used to superfuse the ganglion 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. In experiments in which the effects of changes in ionic composition of the superfusion medium on 5-HT depolarizations were examined, the following solutions were used: (1) low-chloride Krebs in which NaCl was replaced with an equivalent amount of sodium benzenesulphonate ($C_6H_5 . SO_3Na$); (2) low-sodium/low-chloride Krebs in which all NaCl was replaced with an equivalent amount of sucrose; (3) sodium-free solution in which the NaCl and $NaHCO_3$ were omitted from Krebs solution and replaced with an equivalent amount of Tris HCl, pH being adjusted to 7.4 by the addition of HCl; (4) calcium-free Krebs in which the $CaCl_2$ was omitted from normal Krebs and the small change in osmolarity ignored; (5) calcium-rich Krebs in which the $CaCl_2$ concentration was raised from 2.54 to 5.08 mM and the small change in osmolarity ignored; (6) potassium-free Krebs in which KCl was replaced with an equivalent amount of NaCl and (7) potassium-rich Krebs in which KCl replaced equivalent amounts of NaCl (see Wallis *et al.*, 1975). The concentration of the sucrose solution superfusing part of the internal carotid nerve was 315 mM and taken to be isotonic. Experiments were carried out at temperatures between 20 and 22° C.

Drugs

The drugs used were 5-hydroxytryptamine creatinine sulphate (5-HT), acetylcholine chloride (ACh), choline chloride, physostigmine (eserine) sulphate, hexamethonium bromide, nicotine hydrogen tartrate, iproniazid phosphate, harmine hydrochloride, strophanthin G (ouabain) and dinitrophenol. The concentrations (μM) and quantities of drugs injected (μmol) refer to the salts.

Results

Depolarization by continuous superfusion or by injections of 5-hydroxytryptamine into the superfusion stream

In confirmation of a number of previous reports (see introductory section), we have found that 5-HT is a potent depolarizing agent of sympathetic ganglia. When a solution of 5-HT (100 μM) superfused the ganglion, a rapid depolarization occurred (Figure 1c). In the experiment illustrated, a concentration of 10 μM also induced a depolarization (Figure 1b), while a concentration of 1 μM appeared to be below threshold for a membrane potential change (Figure 1a). In most experiments the threshold concentration for

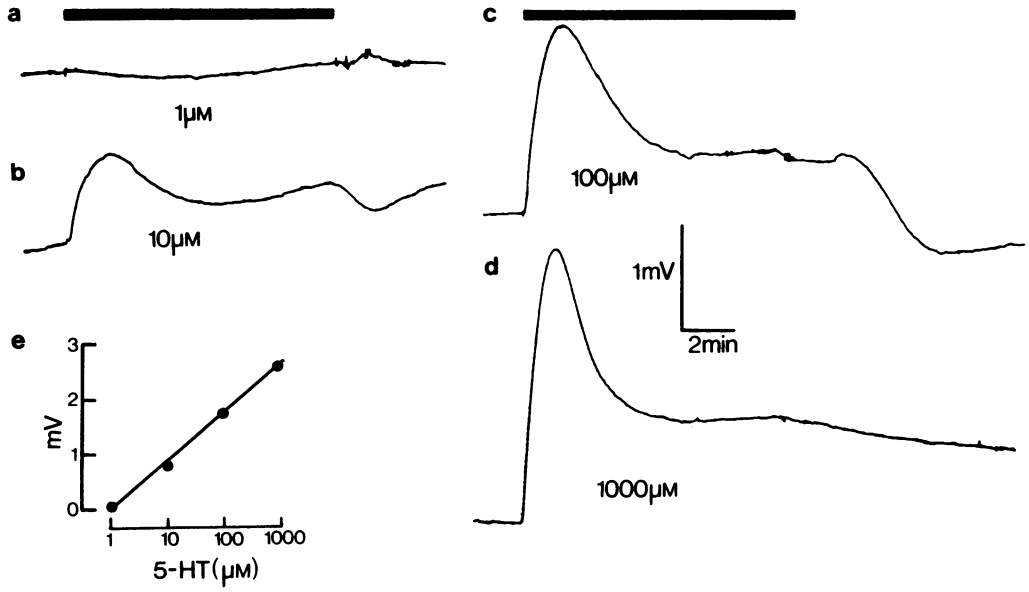

Figure 1 Effect on resting potential of rabbit superior cervical ganglion of superfusion with various concentrations of 5-hydroxytryptamine (5-HT). (a-d) Chart records, depolarization upwards. Solid bars, superfusion with Krebs solution containing 5-HT: (a) 1 μM, (b) 10 μM, (c) 100 μM, (d) 1000 μM. Initial exposure was to 10 μM; 1 μM was tested after washing for 75 min, then 100 μM after washing for 35 min and finally 1000 μM after washing for 45 minutes. In (e) amplitude of peak depolarization (mV) is plotted on a semilogarithmic scale against concentration (μM) of 5-HT.

depolarization was around 10 μM, while a concentration of 100 μM consistently produced depolarization. With the latter concentration and with higher concentrations, it was noticeable that repolarization began during the course of superfusion with the drug. On returning to superfusion with normal Krebs solution, there were often indications that an after-hyperpolarization ensued (e.g. Figure 1c). As reported previously (Wallis & Woodward, 1974), the transmitted ganglionic action potential was depressed during depolarization by 5-HT. Although the amplitude of the peak depolarization evoked by 5-HT was concentration-dependent (Figure 1e), in most experiments the tachyphylaxis which followed an initial exposure made subsequent determinations of the depolarizing action difficult, unless long periods of washing with Krebs solution intervened. However, tachyphylaxis was largely avoided if injections of 5-HT were made into the superfusion stream. This method makes estimation of the concentration of 5-HT at the ganglion hazardous; injection of 0.2 ml of dye at the end of the experiment showed that the material was dispersed in a volume of about 5 ml. Preliminary experiments suggested that in any particular preparation an injection of 0.2 μmol (81 μg) 5-HT dissolved in a volume of 0.2 ml Krebs solution produced a depolarization which was 50% maximal or larger. Injections of 5-HT were given at 20 or 30 min intervals and elicited depolarizations which were relatively constant (Figure 2), although some decline occurred over a period of hours. The response consisted of a depolarization of rapid onset and an almost equally rapid repolarization, followed by an after-hyperpolarization. Injections of 0.2 μmol 5-HT produced relatively consistent potential changes, as can be seen from the standard errors in Table 1, first column. Further, successive responses from the same preparation were well maintained, even though there was a slight decline in depolarization amplitude and in the other parameters of the response (Table 1). Comparing the first with the third response, these differences are statistically significant.

Changes in the volume of the Krebs solution in which a given amount of 5-HT was dissolved had little effect on the magnitude of the depolarization, except that small volumes (i.e. less than 0.1 ml) were proportionately less effective. To construct dose-response curves, the smaller quantities of 5-HT were injected at greater dilution. In two such experiments it was possible to obtain curves based on at least eight responses

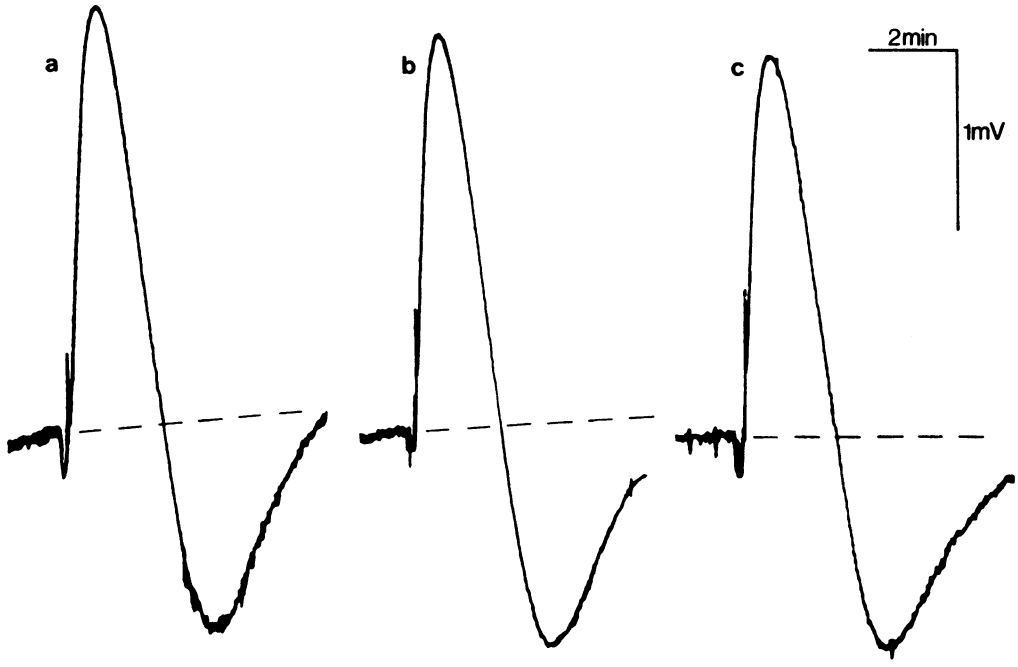

Figure 2 Resting potential changes of rabbit superior cervical ganglion in response to injections of 5-hydroxytryptamine (5-HT) into the superfusion stream. 5-HT 0.2 μmol (81 μg) dissolved in 0.2 ml Krebs solution injected at 30 min intervals. Chart records, depolarization upwards; note initial downward deflection of pen is injection artefact. (a) second, (b) third and (c) fourth response to 5-HT. The dashed lines in this and subsequent figures indicate baseline drift estimated over several min prior to the response.

(Figure 3). The amplitude and the area of the depolarization have been plotted against the quantity of 5-HT injected. An approximately linear relationship exists between depolarization amplitude and the logarithm of the quantity of 5-HT over a range of 5-HT values; for larger quantities of 5-HT the amplitude reached a plateau (Figure 3a and b). The area of the depolarization, however, tended to increase without clearly reaching a plateau because, for larger quantities of 5-HT, the duration of the response also increased. The threshold quantity for the depolarizing action of 5-HT appeared to be around 0.01 μmol (5 μg). In some preparations, it proved difficult because of the time involved to obtain sufficient responses for a full curve and, in general, it was not possible

Table 1. Characteristics of 1st, 2nd and 3rd responses to injections of 0.2 μmol 5-hydroxytryptamine

Injection	*Depolarization (mV)*	*Rate of depolarization (mV/min)*	*Duration of depolarization (min)*	*Rate of repolarization (mV/min)*	*Hyperpolarization (mV)*
1st $n = 28$	1.91 ± 0.10	3.20 ± 0.28	2.73 ± 0.10	1.66 ± 0.14	0.97 ± 0.10
2nd $n = 30$	1.83 ± 0.12	2.86 ± 0.23	3.13 ± 0.14	1.29 ± 0.09	0.87 ± 0.09
3rd $n = 25$	1.59 ± 0.12 $P < 0.01$	2.72 ± 0.27 $P < 0.05$	3.07 ± 0.14 $P < 0.01$	1.13 ± 0.10 $P < 0.001$	0.77 ± 0.11 $P < 0.001$

Values show mean ± s.e. mean; where baseline instability occurred the response was not analysed, hence the variability in *n*. Values of *P* refer to comparison of 1st and 3rd responses. Successive injections at 20-30 min intervals.

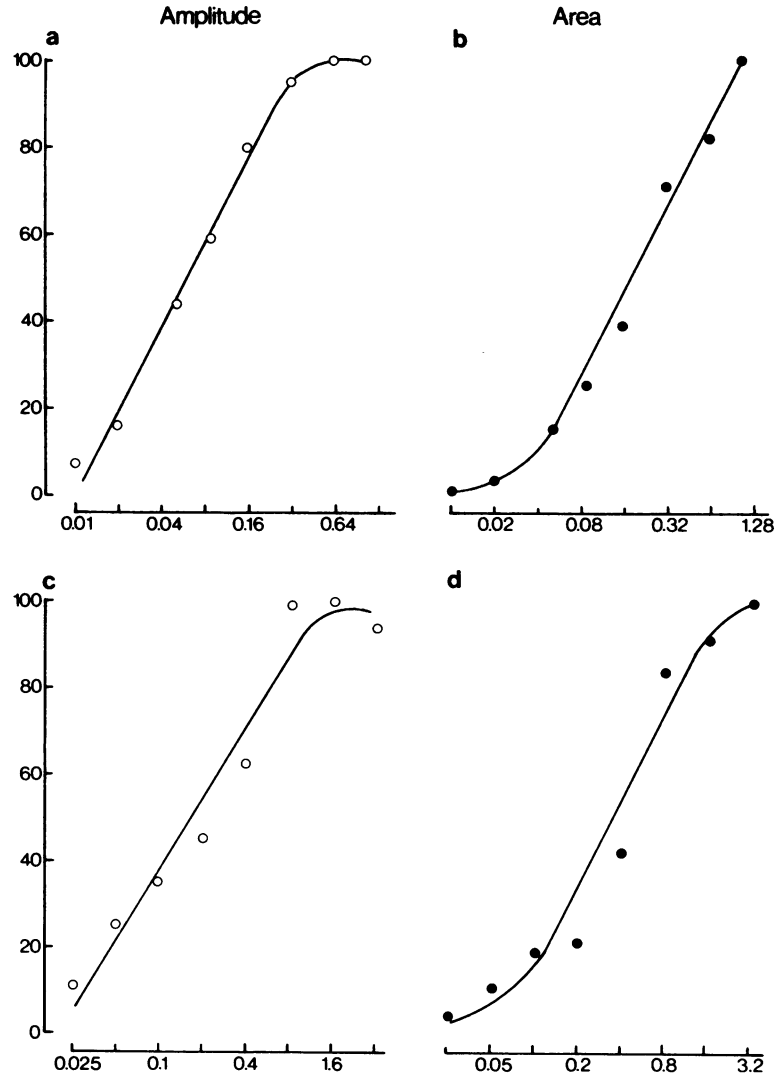

Figure 3 Relationship between quantity of 5-hydroxytryptamine (5-HT) and amplitude and area of the depolarization in two experiments. Abscissae: μmol 5-HT injected into the superfusion stream in a volume ranging from 0.1-0.65 ml, logarithmic scale; ordinates: depolarization amplitude or depolarization area expressed as a percentage of the maximal response. (a) and (b) are curves from one preparation, (c) and (d) curves from another.

to repeat observations for a second curve on the same preparation. However, in 6 preparations the ED_{50} values for the depolarizing action of 5-HT could be estimated and ranged from 0.06-0.22 μmol (24-88 μg) with a mean of 0.12 (49 μg) ± 0.02 μmol (mean ± s.e. mean).

The after-hyperpolarizations which follow depolarization were also concentration-dependent, at least over a certain range, but the tendency for the after-hyperpolarizations to decline with time made it difficult to construct full dose-response curves.

Comparison with the depolarizing action of acetylcholine and choline

The depolarizing action of 5-HT on ganglion cells appeared to be comparable to that of acetyl-

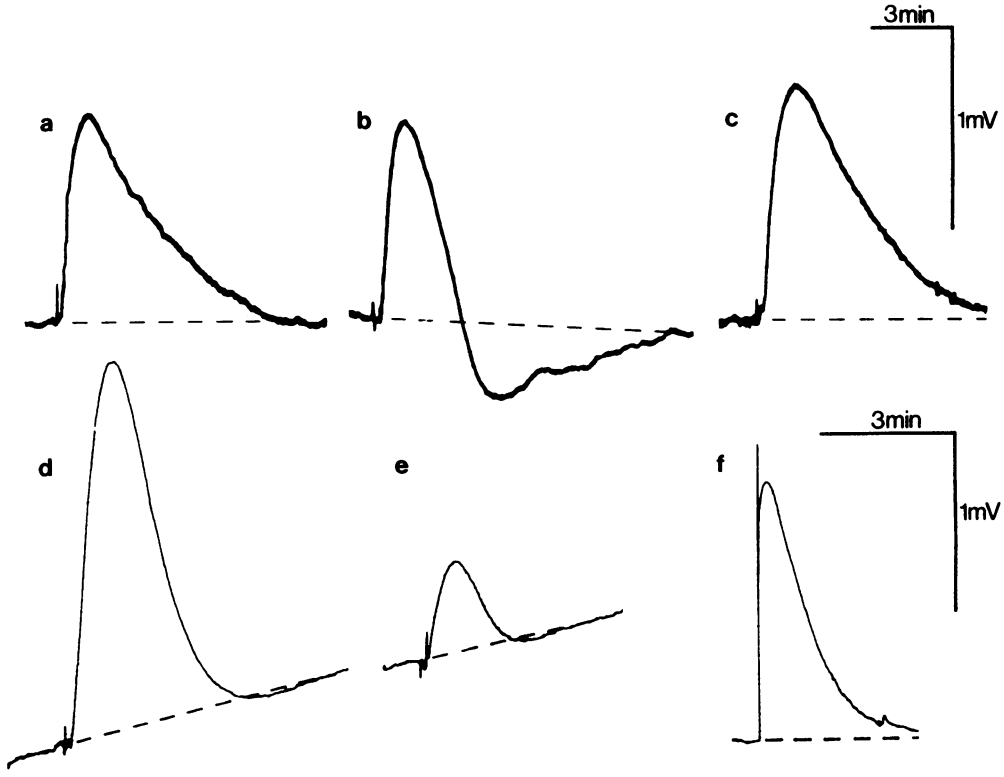

Figure 4 Depolarizations by 5-hydroxytryptamine (5-HT), acetylcholine (ACh) and choline and the effect of an anticholinesterase. (a) injection of 9.9 μmol ACh, (b) injection of 0.2 μmol 5-HT, (c) injection of 10 μmol choline, (a-c) from same preparation, (d-f) in the presence of 30 μM physostigmine in a second preparation, (d) injection of 0.2 μmol ACh, (e) injection of 0.2 μmol 5-HT, (f) LN wave following a 1 s train of stimuli at a frequency of 10 Hz to the preganglionic nerve. Spike amplitude is attenuated by the recording system.

choline (ACh) or choline (Figure 4), although the amplitude of a maximal depolarization produced by 5-HT was generally smaller than a maximal depolarization induced by ACh. ACh applied by superfusion is largely hydrolysed to choline before it can depolarize the ganglion cells (Kosterlitz *et al.*, 1968). In a preparation untreated with anti-cholinesterase, injections into the superfusion stream of ACh (9.9 μmol, 1800 μg), 5-HT (0.2 μmol, 81 μg) and choline (10 μmol, 1400 μg) produced depolarizations of similar magnitude displaying similar rates of depolarization (Figure 4a-c). After-hyperpolarizations were only consistently observed, however, in response to 5-HT. The large quantity of ACh required presumably reflects the high rate of hydrolysis of this substance, for in preparations treated with physostigmine (30 μM), much smaller amounts of ACh (e.g. 0.2 μmol) produced large depolarizations of rapid onset (Figure 4d), while the late depolarization (LN wave) which results from the muscarinic action of ACh released by orthodromic stimulation was also greatly potentiated (Figure 4f). In contrast, the sensitivity to 5-HT appeared to be depressed in the presence of physostigmine for 5-HT evoked only small depolarizations (Figure 4e).

It was of interest to know whether 5-HT might be destroyed enzymatically before the amine could act on the ganglion cells. In studies on the isolated rat stomach strip, Vane (1959) found that monoamine oxidase activity altered the relative potency of tryptamine and 5-HT, probably because of different rates of access to the enzyme. However, in the rabbit superior cervical ganglion, neither the amplitude of the depolarization induced by 5-HT nor the quantity required to produce a depolarization were much affected by pretreatment of the ganglion with iproniazid (2 mM), although the duration of the depolariza-

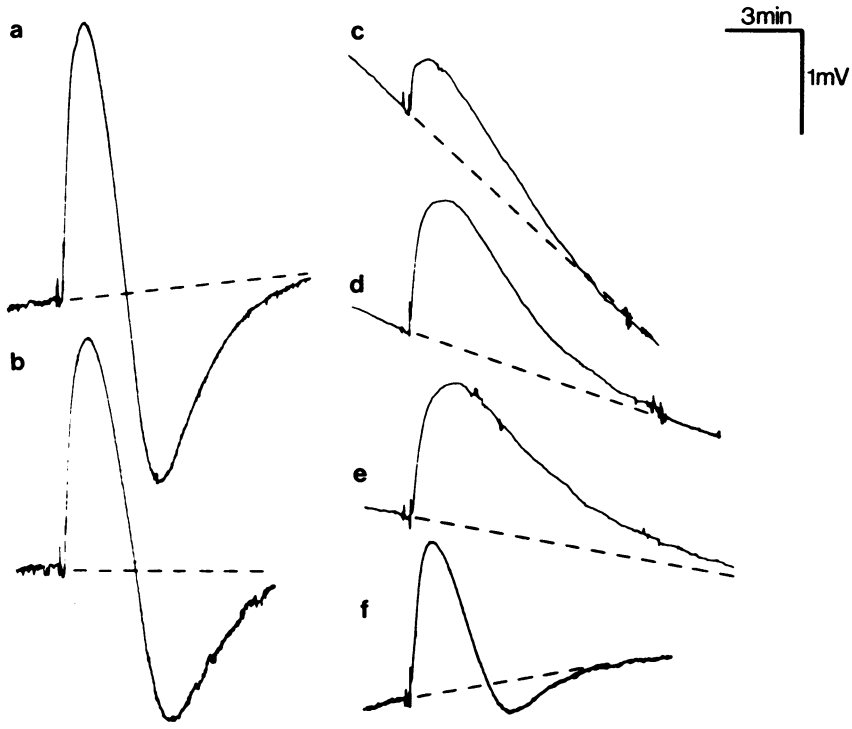

Figure 5 Effect of changes in sodium ion concentration of superfusion medium on 5-hydroxytryptamine (5-HT) depolarizations. (a-f) responses to injection of 0.2 μmol 5-HT. (a) and (b) controls, (c) 19 min, (d) 43 min and (e) 92 min after superfusing with sodium-free Krebs solution, (f) 41 min after returning to normal Krebs solution.

tions was increased. This concentration of iproniazid is 1.5 times that used by Gertner, Paasonen & Giarman (1959) in their studies on cat ganglia. Harmine (40 μM) also failed to alter the amplitude of the response to the standard injection of 5-HT (0.2 μmol).

Ionic basis of the depolarization

Both γ-aminobutyric acid (GABA) and 5-HT have actions on cat ganglia and picrotoxin, a GABA antagonist, blocks the excitatory effects of 5-HT (de Groat, 1970). It was therefore possible that the ganglionic actions of 5-HT and GABA might be due to activation of the same receptors. The potential change induced in the rat ganglion cell membrane by GABA has been studied in detail (Bowery & Brown, 1972, 1974; Adams & Brown, 1973) and is due to an increased chloride ion permeability. In our experiments, 5-HT depolarizations were of similar amplitude when the sodium chloride of the superfusion medium was entirely replaced with sodium benzenesulphonate, the anion of which is impermeant. Unlike the situation in rat ganglia, where successive GABA depolarizations in the absence of external chloride become progressively smaller as the intracellular concentration of chloride ions falls (Adams & Brown, 1973), successive 5-HT depolarizations showed no signs of diminution in low-chloride Krebs in our experiments.

The similarity of the depolarizations to ACh and 5-HT (Figure 4) suggested that the ionic basis of the two phenomena might be similar, i.e. an increased permeability to cations (cf. Koketsu, 1969). In a sodium-free superfusion medium (Figure 5), depolarizations evoked by 5-HT were initially very much reduced in amplitude. The rate of depolarization was slightly reduced and the rate of repolarization considerably reduced; the after-hyperpolarization was usually abolished. On removing the sodium ions from the superfusion medium, a rapid hyperpolarization was recorded followed by a prolonged further hyperpolarization lasting many minutes; 19 min after changing to the sodium-free medium (Figure 5c), the 5-HT

depolarization was superimposed on this slow hyperpolarization. Potential changes recorded on altering the ionic composition of the medium are partly due to changes in junction potentials (Wallis *et al.*, 1975) which could account for the rapid hyperpolarization observed; the slow hyperpolarization may be largely a membrane potential change induced by the altered ionic environment of the ganglion cells. In each of 8 experiments, reducing $[Na]_o$ led first to a reduction of the depolarization and the reduction was most pronounced in sodium-free solution; this was followed by a substantial recovery while still superfusing with the sodium-deficient medium (e.g. Figure 5, Table 2). In three of these experiments a low-sodium/low-chloride Krebs solution was used, when the initial diminution was less and the subsequent recovery more marked (expt. 3 in Table 2). On returning to normal Krebs solution, the potential change evoked by 5-HT resembled that seen initially and included an after-hyperpolarization. Response amplitude, however, was rarely restored fully (Figure 5f, Table 2).

The 5-HT depolarizations which persisted in sodium-deficient solutions were greatly reduced in amplitude, and in some cases abolished, when calcium ions were also omitted from the superfusion medium. The relative amplitudes of 5-HT depolarizations in 3 experiments in which ganglia were exposed sequentially to solutions deficient in sodium and deficient in sodium and calcium are given in Table 2 (1st control = 100%). Note that in sodium-free solution, removing calcium ions considerably reduced the amplitude or abolished the response (expt. 1, 2), while in the low-sodium medium (expt. 3) the reduction was less marked. Removal of $[Ca]_o$ led to some instability in the recorded baseline. In experiments 2 and 3 replacement of the calcium led to a partial recovery of the response, as did subsequent superfusion with Krebs solution in expts 1 and 2.

This apparent ability of calcium ions to support 5-HT depolarizations recalls Woolley's proposition (1958) that 5-HT might act by carrying calcium ions across the smooth muscle cell membrane. However, with ganglion cells, removal of only calcium ions from the superfusion fluid (Figure 6a) did not reduce depolarization amplitude. On the contrary, in 2 of 4 experiments (e.g. Figure 6a), there was a clear indication that the responses were of larger amplitude and area in the calcium-free medium. That enhancement of the responses had occurred was strengthened by the observation that on returning to normal Krebs solution response amplitude was reduced in all 4 experiments. In one further experiment, superfusion with calcium-rich Krebs solution reduced rather than enhanced response amplitude.

It should not be assumed that the effect of calcium ions is simply due to their presence or absence as charge carriers, for calcium ions are likely to alter membrane resistance (see Frankenhaeuser & Hodgkin, 1957; Brown, Brownstein & Scholfield, 1972; Lees & Wallis, 1974) and, thus, alter the potential resulting from the movement of other ions. It was noticeable that the high frequency noise, present to a variable degree from experiment to experiment in the baseline trace, was diminished in the presence of calcium-free media, perhaps suggesting a reduction

Table 2. Effect of removal of sodium ions and subsequent removal of calcium ions on the amplitude of 5-hydroxytryptamine depolarizations

Sequence of solutions (times indicate duration of exposure to solutions)		*Expt. 1*	*Expt. 2*	*Expt. 3*
Krebs	1st control	100%*	100%*	100%*
	2nd control	85%	84%	96%
	3rd control	81%	87%	83%
		Zero $[Na^+]_o$	Zero $[Na^+]_o$	25 mM $[Na^+]_o$
Low $[Na^+]_o$	20-30 min	41%	21%	53%
	50-100 min	64%	32%	83%
Low $[Na^+]_o$ / Zero $[Ca^{2+}]_o$	10-30 min	25%	0%	44%
	40-100 min	17%	0%	52%
Low $[Na^+]_o$	10-80 min	12%	20%	82%
Krebs	40-50 min	37%	24%	60%
	60-80 min	35%	36%	54%

* Depolarization amplitude expressed as a percentage, initial depolarization in Krebs solution taken as 100, controls at 30 min intervals. Each value is a single determination or the mean of 2 determinations.

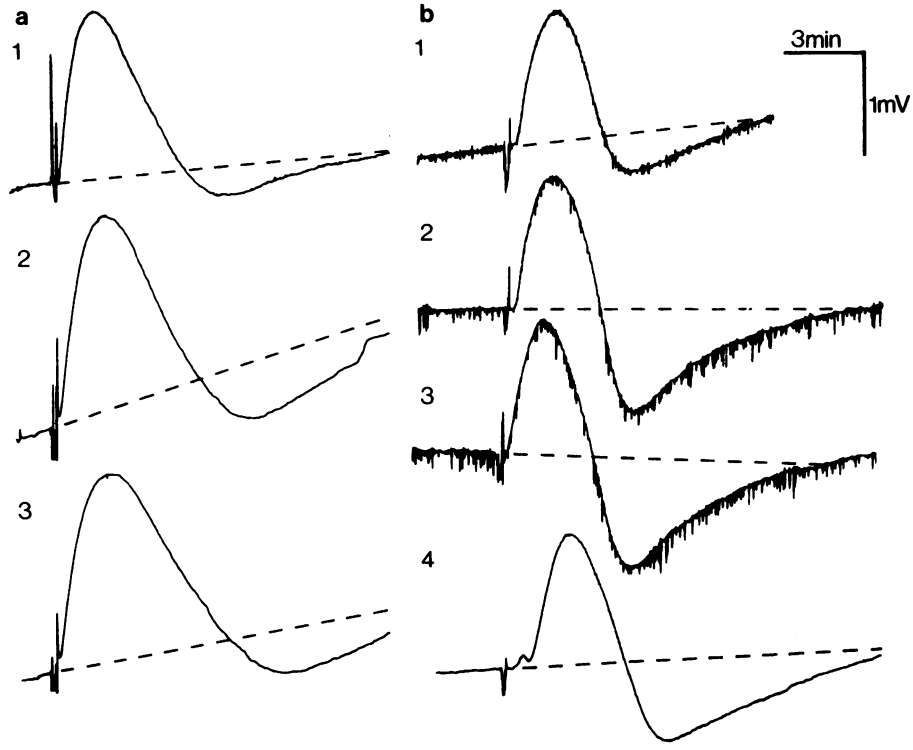

Figure 6 Effect of removing calcium ions (a) and potassium and calcium ions from the superfusion medium (b) on 5-hydroxytryptamine (5-HT) depolarizations. All responses to injection of 0.2 μmol 5-HT. (a) (1) Control, (2) 30 min and (3) 67 min in calcium-free Krebs solution. (b) Another preparation: (1) control, (2) 50 min and (3) 80 min in potassium-free Krebs solution, (4) 51 min in potassium-free/calcium-free Krebs solution.

in membrane resistance (e.g. Figure 6b). Removal of potassium ions from the superfusion fluid increased the baseline noise and usually increased the amplitude of both the depolarization and after-hyperpolarization (Figure 6b, 2 and 3). The response was maintained when the superfusion fluid was changed to one containing neither potassium nor calcium ions (Figure 6b, 4), but there was a great reduction in the high frequency noise. From the results of Figure 6b and similar experiments, it was clear that potassium ions in the external medium were not essential for either depolarization or after-hyperpolarization.

The nature of the after-hyperpolarization

The after-hyperpolarization, which follows depolarization of the superior cervical ganglion with ACh, is due to the activity of a ouabain-sensitive electrogenic sodium pump (Lees & Wallis, 1974). Characteristically, the rate of development and usually the amplitude of the after-hyperpolarization are reduced in the absence of external potassium ions. In the experiments described here, the after-hyperpolarization which followed a 5-HT depolarization was clearly increased in amplitude in 3 out of 4 experiments in potassium-free Krebs (e.g. Figure 6b). This increase was sustained even though superfusion with the potassium-free medium was prolonged. Ouabain (10 μM) initially reduced the amplitude of the 5-HT after-hyperpolarization and then abolished it (Figure 7). In one experiment, dinitrophenol (0.3 mM) also abolished the after-hyperpolarization but itself produced a prolonged depolarization of the ganglion. In sodium-deficient solutions, the after-hyperpolarizations were quickly abolished. Unlike the situation with ACh after-hyperpolarizations (Lees & Wallis, 1974), a calcium-free superfusion medium did not appear to alter greatly the amplitude of the after-hyperpolarization induced by 5-HT.

In the presence of nicotine, 5-HT induces a hyperpolarization and not a depolarization of

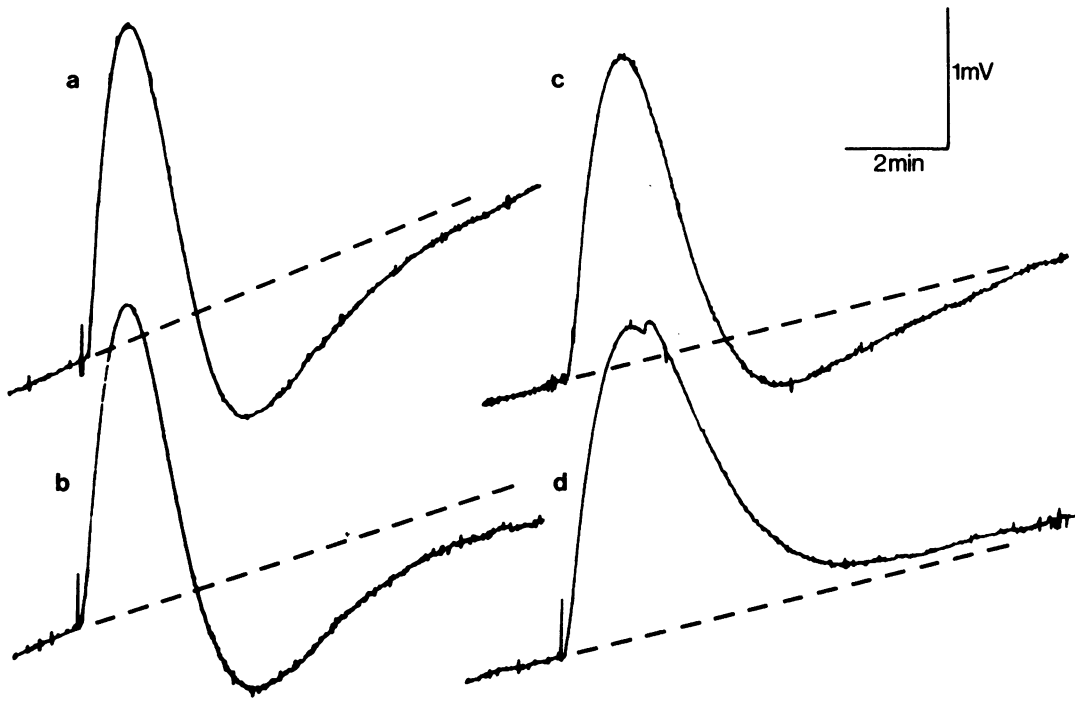

Figure 7 Effect of ouabain on 5-hydroxytryptamine (5-HT) depolarizations. (a-d) Responses to injection of 0.2 μmol 5-HT. (a) and (b) Controls, (c) 23 min and (d) 43 min after superfusion with ouabain (10 μM).

bullfrog ganglion cells (Watanabe & Koketsu, 1973). In two experiments where 5-HT evoked depolarizing responses with pronounced after-hyperpolarizations, nicotine (10 μM) depressed the amplitude of the depolarization and abolished the after-hyperpolarization of rabbit ganglia.

Possible presynaptic actions of 5-HT

If the rat superior cervical ganglion is arranged in the sucrose-gap so that the recorded potential differences arise between the proximal pole of the ganglion and the cervical sympathetic trunk, depolarizations induced on superfusion with ACh are thought to arise mainly in the preganglionic nerve terminals (Koketsu & Nishi, 1968). This experimental arrangement is shown diagrammatically in Figure 8. When injections of 5-HT were made into the superfusion stream, a depolarization was recorded (Figure 8), but the after-hyperpolarization was either absent or small in amplitude. The depolarization and after-hyperpolarization evoked by 5-HT in the same ganglion, but in the ganglion cells of the distal pole, are shown for comparison; note that the depolarization was larger and the after-hyperpolarization much more pronounced. Supporting evidence that records from the proximal pole were from structures other than ganglion cells comes from observations on the differential effects of hexamethonium. In a concentration (1.38 mM) which rapidly blocks ganglionic transmission, hexamethonium is known to potentiate the amplitude and particularly the area of 5-HT depolarizations of ganglion cells (Wallis & Woodward, 1974). Thus, in 5 experiments, ganglion cell depolarizations were 55 ± 14% (mean ± s.e. mean) larger in amplitude and 203 ± 81% (mean ± s.e. mean) larger in area in the presence of hexamethonium (1.38 mM) compared to control responses. In contrast, proximal pole responses evoked by 5-HT were not potentiated in amplitude in any of 4 experiments but reduced by 13 ± 7% (mean ± s.e. mean); their area was not significantly altered.

Discussion

An excitatory action of 5-HT on sympathetic ganglion cells detected by end-organ responses, discharge in post-ganglionic fibres or ganglion depolarization has been frequently observed

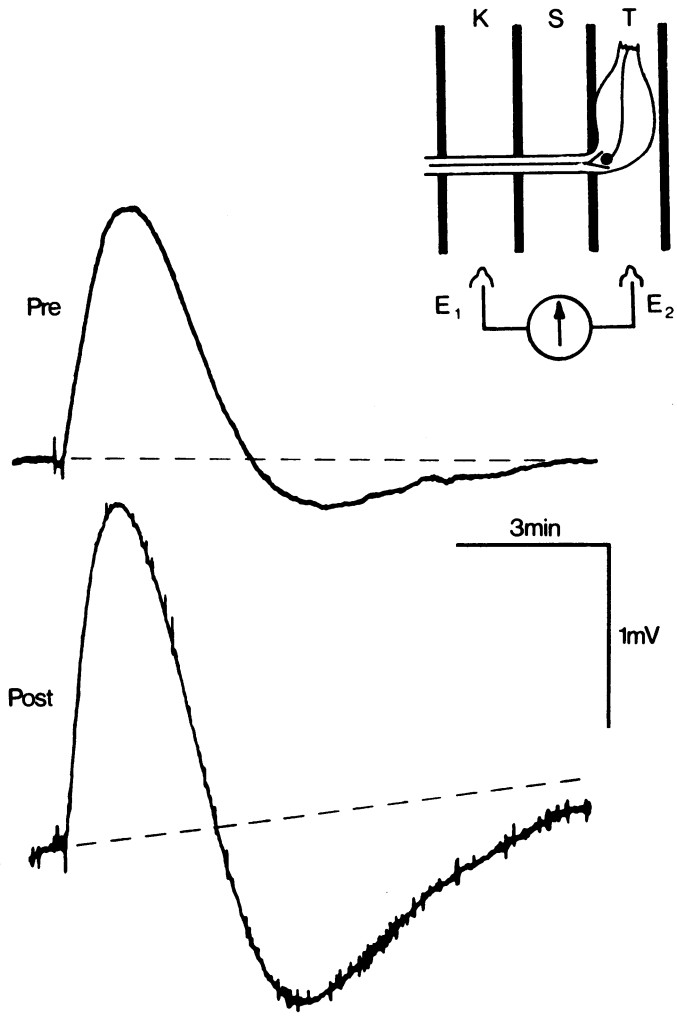

Figure 8 The effect of 5-hydroxytryptamine (5-HT) on the resting potential recorded from the proximal and distal poles of the ganglion. Inset: schematic drawing of experimental arrangement for recording from the proximal pole. The potential change is presumed to arise mainly in the preganglionic nerve terminals (see text). E_1 and E_2, calomel electrodes; K, S and T, Krebs solution, sucrose solution and test solution, respectively. Pre: record from proximal pole of ganglion. Post: record from distal pole of ganglion with the usual recording arrangement and from the same preparation. Responses to injection of 0.2 μmol 5-HT, depolarization upwards.

(Trendelenburg, 1956, 1957; Hertzler, 1961; Gyermek & Bindler, 1962; de Groat & Volle, 1966). In our experiments, 5-HT (100 μM) induced a rapid but unsustained depolarization, substantial repolarization occurring during exposure to the amine. This repolarization probably reflects the tachyphylaxis produced by relatively high concentrations or prolonged exposures to 5-HT. Tachyphylaxis was also observed by Haefely (1974) in the cat superior cervical ganglion, but not by Watson (1970) in the same ganglion of the rat. An alternative explanation for the repolarization might be that the amine evoked a hyperpolarization of slower onset. Haefely (1974) sometimes observed small hyperpolarizations on injection of 5-HT in amounts below threshold for depolarization; Watanabe & Koketsu (1973) found that 5-HT hyperpolarized bullfrog ganglion cells when the depolarizing action of the amine on these cells was blocked by nicotine.

However, in our experiments hyperpolarizations were only seen on removal of the 5-HT or secondary to the depolarization induced by injections into the superfusion stream; nicotine in fact abolished the after-hyperpolarizations.

By injecting small amounts of 5-HT dissolved in less than 1 ml of Krebs solution into the superfusion stream a series of depolarizing responses, relatively constant in magnitude, could be obtained or an ED_{50} value established. The quantities injected are not dissimilar to those employed for close arterial injection to the cat superior cervical ganglion by various authors, e.g. 0.01-0.1 μmol (de Groat & Volle, 1966), 0.002-0.025 μmol (Haefely, 1974), 0.74-1.23 μmol (Machova & Boska, 1969). In the cat inferior mesenteric ganglion, modification of the discharge frequency was induced by close arterial injection of 0.003-0.025 μmol (Gyermek & Bindler, 1962). Submaximal depolarizations evoked by injections of 5-HT, choline or ACh were similar in time course, but only those to 5-HT were consistently followed by an after-hyperpolarization. ACh (in the presence of physostigmine) and choline are known to evoke after-hyperpolarizations (Lees & Wallis, 1974), but continuous superfusion with the depolarizing agent for several minutes is necessary to induce this response. Monoamine oxidase inhibitors had very little effect on the responses to 5-HT, apart from causing some increase in their duration. This suggests that enzymatic destruction is not greatly affecting the amount of amine reaching the receptors and is in accord with the finding that monoamine oxidase inhibitors do not potentiate the action of 5-HT on the rat stomach strip (Vane, 1959).

The dependence of 5-HT depolarizations on external sodium ions suggests that the principal permeability change involved is an increase in sodium ion conductance (G_{Na}). The depolarizations were initially depressed but subsequently recovered to some extent in sodium-deficient solutions and the residual depolarization appeared to be dependent on calcium ions. Thus, 5-HT may induce a concomitant increase in G_{Ca}. Depolarizations to ACh are readily abolished in sodium-deficient solutions (Wallis & Woodward, unpublished observations), so that it appears that calcium ions may substitute for sodium ions as charge carriers during depolarization more readily in the case of responses to 5-HT than in the case of responses to ACh. Nevertheless, calcium ions can support an ACh-induced depolarization of the cat superior cervical ganglion at least (Pappano & Volle, 1966). In the rabbit ganglion, any depolarization resulting from entry of calcium did not make any appreciable contribution to the total depolarization induced by 5-HT when the extracellular sodium concentration was normal, for omitting calcium ions alone from the superfusion medium did not diminish the amplitude of the depolarization.

Alternative ionic mechanisms which might be considered include: (i) a reduced G_K (as suggested by Jéquier (1965) for 5-HT restoration of transmission and by Weight & Votava (1970) as the basis of the slow muscarinic depolarizing potential in frog ganglion cells); and (ii) an increased G_{Cl} (suggested as mediating GABA-induced depolarization of ganglion cells: Adams & Brown, 1973). In the absence of membrane resistance measurements, these cannot be definitely rejected, but appear unlikely for the following reasons: (i) the 5-HT depolarization persists in chloride-free solution, unlike that produced by GABA; (ii) the 5-HT depolarization is enhanced during DMPP-induced hyperpolarization (Jaramillo & Volle, 1968), whereas (assuming E_K is unchanged) it should be reduced if mediated by a decrease in G_K since the electrochemical gradient and net passive efflux of K^+ ions will be reduced (cf. Weight & Votava, 1970). In our experiments, 5-HT depolarization was sometimes increased in potassium-free solution (e.g. see Figure 6), but although this is compatible with a fall in G_K it is inconclusive. It may be noted that, in some snail neurones, 5-HT depolarization results from an increase in membrane conductance, probably of G_{Na} (Gerschenfeld & Stefani, 1968). However, depolarization and hyperpolarization as a result of reduced membrane conductances have also been reported (Paupardin-Tritsch & Gerschenfeld, 1973).

The after-hyperpolarization which followed a 5-HT-induced depolarization has also been observed in cat ganglia and some preparations also display a subsequent late depolarization (Machova & Boska, 1969; Haefely, 1974); we did not observe any late depolarization in our experiments. ACh after-hyperpolarizations are probably a consequence of active extrusion of sodium ions by an electrogenic pump (Lees & Wallis, 1974) and Watanabe & Koketsu (1973) have suggested that the hyperpolarization evoked by 5-HT in bullfrog sympathetic ganglia is also due to activation of an electrogenic sodium pump. That the after-hyperpolarization evoked by 5-HT is generated by electrogenic pumping of sodium ions is consistent with its abolition in sodium-deficient solutions and by ouabain and dinitrophenol, but not with the increase in amplitude in potassium-free solutions. Extracellular potassium ions are known to be necessary for activation of the pump (Lees & Wallis, 1974). Further, in contrast to the situation with after-hyperpolarizations evoked by 5-HT,

calcium-free solutions reduce or abolish ACh after-hyperpolarizations. These discrepancies may indicate that 5-HT induces conductance changes which outlast the phase of depolarization.

The depolarizations recorded from the proximal pole of the ganglion may tentatively be ascribed to the presynaptic terminals for two reasons. First, there are normally no or very few ganglion cells demonstrable electrophysiologically whose axons or axon collaterals project caudally along the cervical sympathetic trunk and, secondly, the 'presynaptic' depolarizations showed some differences from 5-HT-induced depolarization of ganglion cells, e.g. reduced or absent after-hyperpolarizations and no potentiation by hexamethonium.

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