Br. J. Pharm. (1974), **51**, 521-531

THE FACILITATORY ACTIONS OF 5-HYDROXYTRYPTAMINE AND BRADYKININ IN THE SUPERIOR CERVICAL GANGLION OF THE RABBIT

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1 The effects of 5-hydroxytryptamine (5-HT) on ganglionic transmission and on intrinsic modulation of transmission have been re-examined and compared with the effects of bradykinin by means of electrophysiological techniques.

2 Early facilitation, which is maximal 40-75 ms after a conditioning stimulus, was considerably enhanced by 5-HT. This enhancement was concentration-dependent, the threshold concentration lying between 0.1 and 1 μM. With concentrations of 5-HT 10 μM or greater, there was some depression of the Sa response to the conditioning stimulus.

3 5-HT reduced or abolished the inhibition of a test response induced by a conditioning response 100-300 ms earlier. Facilitation was observed at these intervals at concentrations of 5-HT of 25 μM or greater.

4 Late facilitation, which is maximal 700-2000 ms after a conditioning stimulus, was increased by 5-HT, but the effect was not as great as on early facilitation and was not always seen with a concentration of 1 μM.

5 Bradykinin reduced early facilitation but increased the amplitude of the transmitted action potential in response to a single stimulus. The threshold concentration producing these effects was between 1 and 2 μM.

6 5-HT produced a rapid depolarization of the ganglion cell membrane which was followed by an after-hyperpolarization.

7 Bradykinin either produced no measurable change in ganglion cell resting potential or only very small, transient depolarizations.

8 The depression of transmission, enhancement of intrinsic facilitation and the depolarization of the ganglion cell membrane induced by 5-HT may indicate more than one mode of action of this amine at the ganglionic synapse.

Introduction

It is now well established that transmission through sympathetic ganglia may be modulated by intrinsic processes initiated by previous activity in the ganglion or by a variety of humoral agents. This paper attempts to clarify the ways in which one particular humoral agent, 5-hydroxytryptamine (5-HT), can alter intrinsic modulation of transmission. Previous reports have not been in agreement over the action of this amine at ganglionic synapses. Trendelenburg (1956a, b; 1957) reported a facilitatory action of 5-HT on the cat superior cervical ganglion and Hertzler (1961) described facilitation of transmission in the rat stellate ganglion. Other authors have observed a depression of transmission by 5-HT (Jéquier, 1965; Machová & Boska, 1969), while de Groat & Lalley (1973) found that low doses injected intra-arterially towards the cat superior cervical ganglion produced a depression of transmission and larger doses induced discharge of ganglion cells and an initial enhancement followed by a depression of transmission. Our results on the rabbit superior cervical ganglion show that 5-HT affects both the transmission of single volleys through the ganglion and also the intrinsic modulatory processes in the ganglion. The rather complex effects on transmission prompted us to make a comparison with bradykinin, another humoral agent reported to affect the ganglionic

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synapse in low concentrations. The ability of bradykinin to stimulate the cells of the cat superior cervical ganglion has been reported by Lewis & Reit (1965), who showed that this action could occur after chronic denervation of the ganglion, and by Trendelenburg (1966).

In the superior cervical ganglion, there are intrinsic modulatory mechanisms of presynaptic and postsynaptic origin which induce a complex temporal pattern of facilitation and inhibition (Eccles, 1935; Larrabee & Bronk, 1947; Eccles & Libet, 1961; Brimble, Wallis & Woodward, 1972). Of the two major components of the post-ganglionic compound action potential, the Sa component is elicited by stimulation of pre-ganglionic B fibres while the Sb component is elicited by stimulation of preganglionic C fibres. The Sa and Sb components do not represent entirely distinct cell groups, since there is evidence that Sb activity is in part due to convergence of B and C fibres onto Sa cells (Brimble *et al.*, 1972). The facilitation of the Sa response following a conditioning stimulus (CS) is separable into phases of early facilitation, occurring 40-75 ms after CS, and late facilitation, occurring 700-2000 ms after CS. Early and late facilitation are most clearly separable when the CS excites the C fibres and a phase of inhibition occurs 100-300 ms after CS (Brimble *et al.*, 1972). In this paper, the effects of 5-HT on early and late facilitation and on the inhibition of transmission have been examined. A preliminary account of this work was communicated at the Fifth International Congress on Pharmacology (Wallis & Woodward, 1972).

Methods

Preparation

Rabbits were anaesthetized with urethane (1.5-2 g/kg i.p. as a 50% w/v solution), which was chosen because Larrabee & Posternak (1952) had shown that, in anaesthetic concentrations, it had no depressant action on ganglionic transmission. 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. With the preparation in cold Krebs solution, the ganglion was de-sheathed under a microscope.

Recording methods

Ganglionic and postganglionic compound action potentials, evoked by stimulation of the cervical sympathetic trunk, and changes in resting potential were recorded from excised ganglia. Three techniques employing extracellular recording were used: (1) A periodic immersion bath in which the electrode assembly and support posts pivot so that the ganglion and associated nerves are suspended in a moist chamber at 37°C; tilting allows immersion in a physiological solution at set intervals (Eccles, 1952; Brimble *et al.*, 1972). (2) A continuous superfusion bath, constructed from a single block of perspex and containing heating ducts in its base. Krebs solution at 37°C superfuses the ganglion, while the cervical sympathetic and internal carotid nerves pass through partitions sealed with stopcock grease (Scientific Industries, Inc., Springfield, Mass.) to lie in chambers containing liquid paraffin. (3) Change in resting membrane potential was recorded by means of a sucrose-gap apparatus, as described by Kosterlitz & Wallis (1966a) and Kosterlitz, Lees & Wallis (1968); records were displayed on a potentiometric chart recorder (Servoscribe RE 511.20).

Rates of stimulation were employed which were low enough to avoid the intrinsic facilitation which results from repetitive stimulation at even low frequencies, e.g. 1 Hz. In most experiments, responses were evoked at 0.017 Hz (1 per min) or 0.033 Hz, except where rapid changes following injection were investigated when the rate was 0.1 Hz.

Facilitation or inhibition of transmission was estimated from the amplitude of the post-ganglionic compound action potential, recorded from the internal carotid nerve at least 3 mm from the ganglion. Relative amplitude as a percentage of control values was determined, as described in Brimble *et al.* (1972). In these experiments the periodic immersion or the continuous superfusion baths were used with RC amplification of the signals, time constant 1 second. Facilitation or inhibition was estimated by a bracketing method where an unconditioned response (1) was followed by two determinations (2 and 3) of the conditioned response, i.e. CS followed by test stimulus, the latter eliciting the test response; this in turn was followed by an unconditioned response (4). The control value against which facilitation or inhibition was calculated was the mean amplitude of (1) and (4).

Solutions

Krebs solution was used, having the following composition (mM): NaCl 118, KCl 4.75, $CaCl_2$ 2.54, KH_2PO_4 1.2, $MgSO_4$ 1.2, $NaHCO_3$ 25 and glucose 11; it was gassed with 5% carbon dioxide and 95% oxygen.

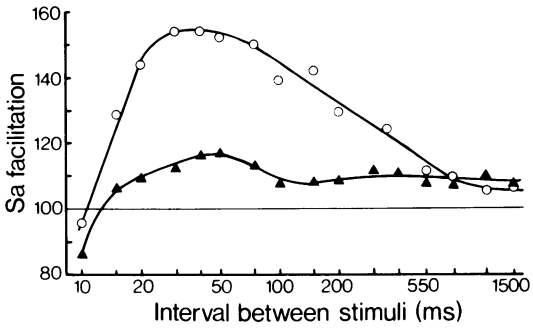

Fig. 1 The effect of 5-hydroxytryptamine (5-HT) on the magnitude and time course of the facilitation induced by a single conditioning stimulus. Conditioning and test stimuli submaximal for Sa response, at varying intervals apart, paired responses so evoked separated by at least 1 min rest periods. Periodic immersion bath, preparation removed from bathing fluid until facilitation at each interval had been determined. Abscissae: interval between conditioning and test stimuli in ms. Ordinates: facilitation expressed as relative amplitude, percentage control (test response alone taken as 100%). (▲) control values; (○) after approximately 30 min exposure to 5-HT (10 μM).

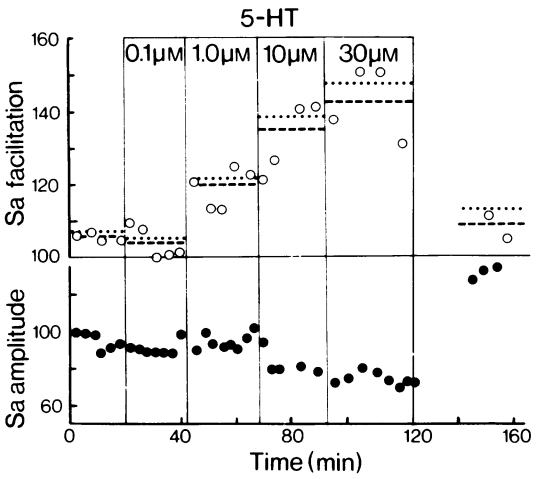

Fig. 2 The effect of 5-hydroxytryptamine (5-HT) on early facilitation of Sa responses and on the amplitude of the transmitted action potential. Conditioning and test stimuli submaximal for Sa responses, paired responses so evoked separated by at least 1 min rest periods. Periodic immersion bath, preparation dipped into bathing solution for 30 s between each determination. The concentration of 5-HT in the bathing medium was varied as shown in the upper part of the figure. Upper graph, facilitation to test stimulus 50 ms after a conditioning stimulus. Ordinates: facilitation expressed as relative amplitude, percentage control (test response alone taken as 100%). Points show means of 2-4 determinations, dashed lines mean facilitation for the duration of the line and dotted lines s.e. mean. Lower graph, amplitude of post-ganglionic Sa compound action potential in response to conditioning stimulus. Ordinates, relative amplitude, initial control taken as 100%.

Drugs

5-hydroxytryptamine creatinine sulphate (5-HT) and bradykinin were kindly supplied by Sandoz Products Ltd. The concentrations (μM) refer to the bases.

Results

Effects of 5-hydroxytryptamine on transmission and early facilitation of transmission

The temporal pattern of facilitation of a submaximal Sa response, following a single submaximal conditioning stimulus to the preganglionic B fibres, is shown in Figure 1 (triangles). Peak early facilitation occurred at an interval of around 50 ms, but a long tail of facilitation extended to intervals of 1500 ms after the conditioning stimulus. The effect of 5-HT (10 μM) was greatly to enhance early facilitation which increased from a control value of around 15% to 55%, while facilitation at intervals of 1000-1500 ms was, in this experiment, unaltered. The experiment illustrated in Fig. 2 was one of a number which attempted to detect the threshold concentration of 5-HT increasing early facilitation. This concentration lies between 0.1 and 1 μM. Facilitation increased to a mean value of around 40% during exposure to 5-HT (30 μM); the dashed lines indicate mean facilitation for the duration of the line. There was no consistent change in the latencies of the facilitated responses compared to the controls. It was noticeable that the degree of early facilitation during the control period varied quite markedly from one experiment to another; the value in Fig. 2 was a low one. In another experiment early facilitation during the control period was 29.2 ± 2.2%, but increased to 43.4 ± 2.4% during exposure to 5-HT (1 μM). On washing out the drug, early facilitation fell to 31.7 ± 2.8% (n = 10). As indicated by Fig. 2, the increase in facilitation was, at least to some extent, concentration dependent. However, with concentrations of 10 μM or more, there was some depression of the Sa response to the conditioning stimulus (lower graph, Figure 2).

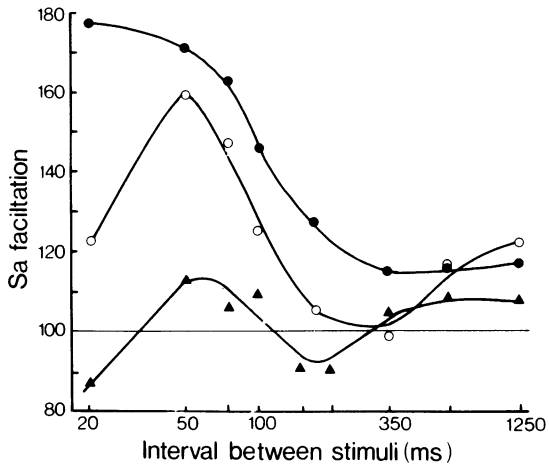

Fig. 3 The effect of two concentrations of 5-hydroxytryptamine (5-HT) on facilitation and inhibition following a maximal conditioning stimulus. Conditioning stimulus maximal test stimulus sub-maximal for Sa response at varying intervals apart, paired responses so evoked separated by at least 1 min rest periods. Periodic immersion bath, preparation removed from bathing fluid until facilitation at each interval had been determined. Abscissae: interval between conditioning and test stimuli in ms. Ordinates: facilitation expressed as relative amplitude, percentage control (test response alone taken as 100%). Each point represents a single determination or the mean of two determinations. (▲) control values; (○) 15 min exposure to 5-HT (25 μM); (●) 60 min exposure to 5-HT (99 μM).

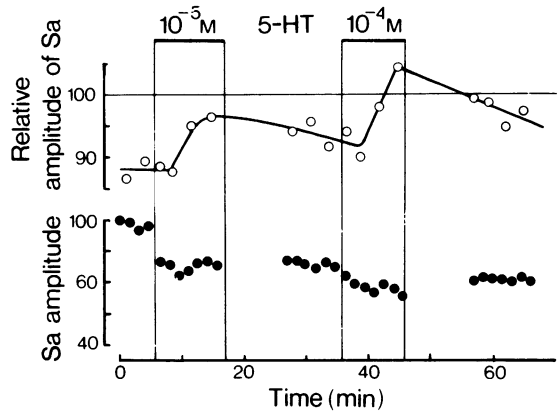

Fig. 4 The effect of 5-hydroxytryptamine (5-HT) on inhibition and on the amplitude of the transmitted action potential. Conditioning and test stimuli supra-maximal for Sa response, paired responses so evoked separated by at least 30 s rest periods. Ganglion superfused with Krebs solution, 5-HT present during periods marked by the black bars. Upper graph, inhibition of test response 200 ms after conditioning stimulus. Ordinates: relative amplitude as percentage control (test response alone taken as 100%). Each point is the mean of two determinations. Lower graph, amplitude of postganglionic Sa compound action potential in response to conditioning stimulus. Ordinates: relative amplitude, initial control taken as 100%. Each point is the mean of two determinations.

The onset of the increase in early facilitation was rapid, as determined in a number of experiments in which the preparation was re-immersed in the bathing solution for 30 s of each minute (cf. Hertzler, 1961); the increase in facilitation was well developed after the first 30 s exposure to 5-HT. This rapid increase can be seen on exposure to the 5-HT (1 μM) solution in Figure 2.

The effects of 5-HT on early facilitation and on action potential amplitude were reversible on washing (Figure 2). In an experiment where the ganglion was exposed to 5-HT (100 μM), maximum early facilitation was increased from 25% to almost 100%; the effects of this relatively high concentration were reversible, but only gradually disappeared on washing. After 75 min in a solution free of 5-HT, the facilitation curve had reached values similar to those of the mean curve in normal ganglia, cf. Fig. 3 in Brimble *et al.* (1972).

Effects of 5-hydroxytryptamine on inhibition and late facilitation

Early facilitation of both submaximal and maximal Sa responses is also seen following a conditioning stimulus maximal for the B fibres, providing preganglionic C fibres are not also maximally excited (Brimble *et al.*, 1972). The temporal pattern then revealed phases of early and late facilitation, separated by a phase of inhibition (triangles, Figure 3). In the presence of 5-HT (25 μM) the inhibition 150-350 ms after the conditioning stimulus disappeared, although a diminution in the facilitation curve was still observed (open circles, Figure 3). Early facilitation was again much increased, but late facilitation was affected to a much smaller extent. 5-HT (99 μM) (filled circles, Fig. 3) caused a further increase in early facilitation and some degree of facilitation was seen at all intervals after the conditioning stimulus.

Values for facilitation and inhibition at a particular interval after a conditioning stimulus showed rather less variability in apparatus allowing continuous superfusion of the ganglion. In Fig. 4 a

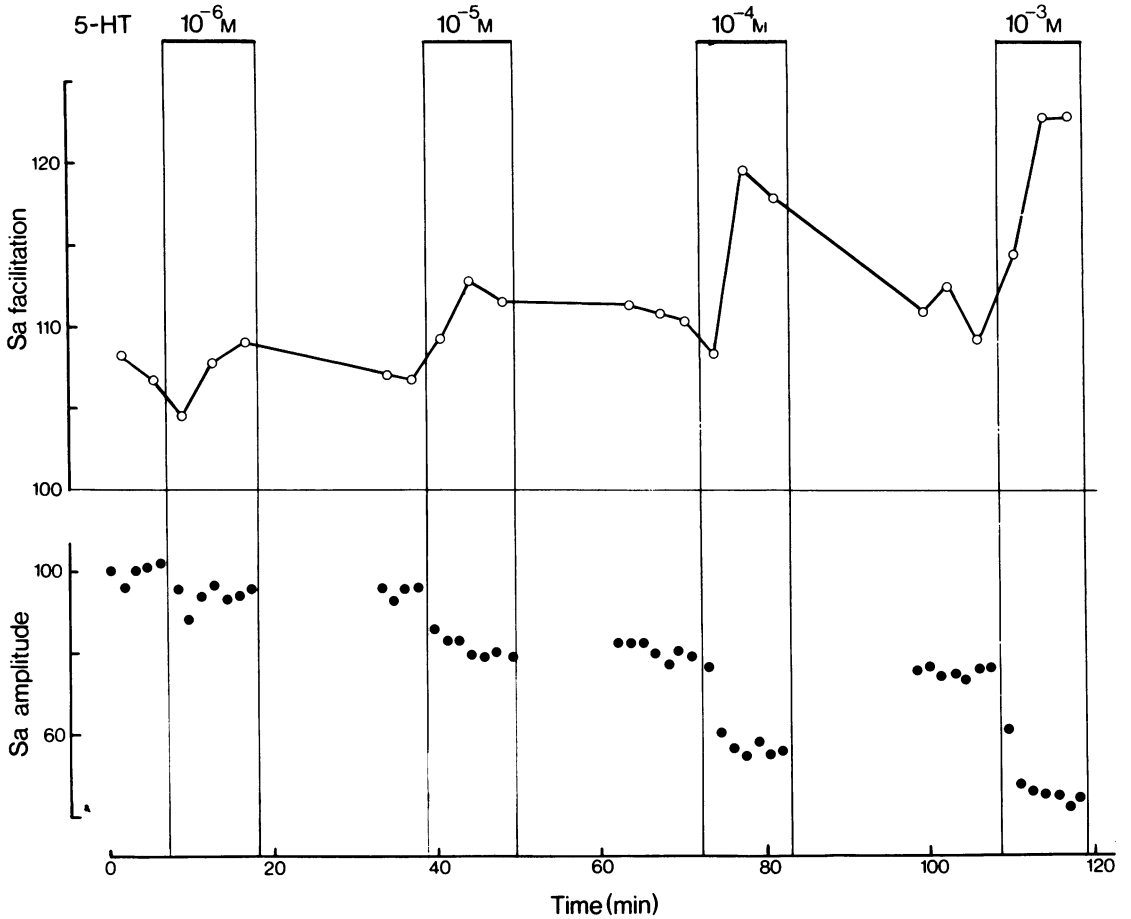

Fig. 5 The effect of 5-hydroxytryptamine (5-HT) on late facilitation and on the amplitude of the transmitted action potential. Conditioning and test stimuli supramaximal for Sa response, paired responses so evoked separated by at least 10 s rest periods. Ganglion superfused with Krebs solution. 5-HT present during periods marked by black bars. Upper graph, facilitation of test response 1500 ms after conditioning stimulus. Ordinates: relative amplitude as percentage control (test response alone taken as 100%). Each point is the mean of two or three determinations. Lower graph, amplitude of postganglionic Sa compound action potential in response to conditioning stimulus. Ordinates: relative amplitude, initial control taken as 100%. Each point is the mean of two determinations.

test response 200 ms after a conditioning stimulus was inhibited by more than 10%. 5-HT (10 μM) lessened the degree of inhibition; this effect was partially reversed on superfusion with normal Krebs solution. During superfusion with 5-HT (100 μM) (Fig. 4), the inhibition disappeared and was replaced by slight facilitation, an effect reversible on washing. However, these changes in the degree of inhibition in the presence of 5-HT occurred at the same time as the unconditioned response was diminished (Figure 4, lower graph). In this experiment the unconditioned response was evoked by maximal stimulation of the B fibre pathway. In general, responses to maximal stimulation were as readily depressed by 5-HT as responses to submaximal stimulation, 10 μM usually, and 100 μM always, causing depression of the response.

Experiments of the kind illustrated in Fig. 3 suggested that late facilitation might also be increased in the presence of 5-HT, but the changes were not marked. Results with the superfusion bath confirmed that such an increase occurred (Figure 5). The late facilitation of a maximal test response, 1500 ms after a CS maximal for the preganglionic B fibres, is normally around 10%

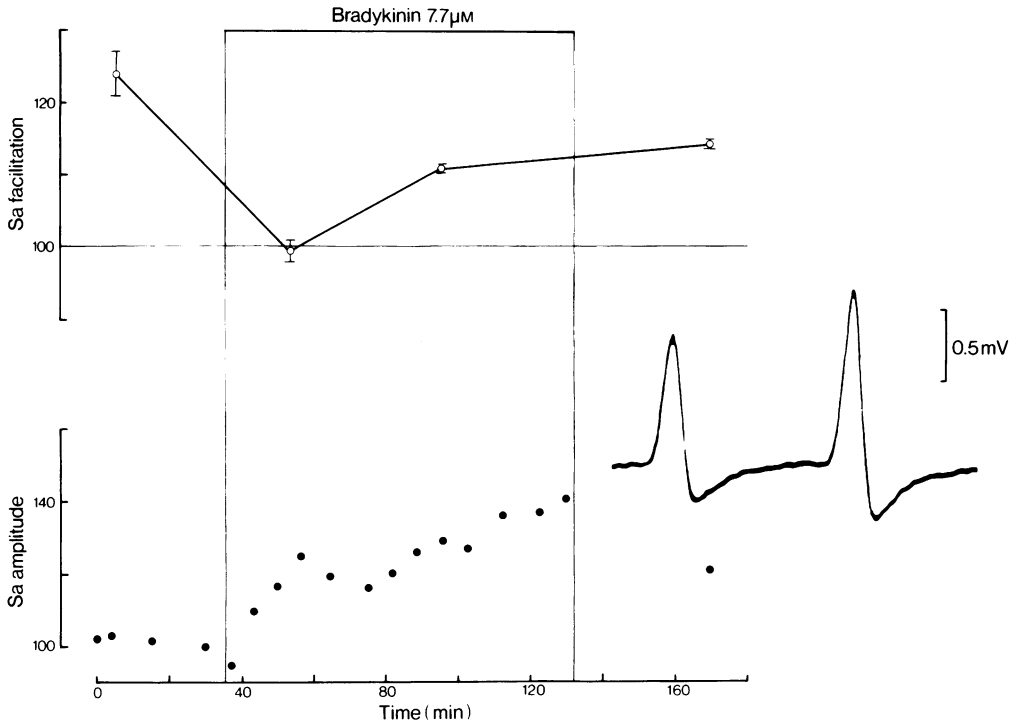

Fig. 6 The effect of bradykinin on early facilitation and on the amplitude of the transmitted action potential. Conditioning and test stimuli submaximal for Sa response, paired responses so evoked separated by at least 1 min rest periods. Periodic immersion bath, preparation dipped into bathing solution for 30 s between each determination, bradykinin (7.7 μM) present during the period indicated by the black bar. Upper graph, early facilitation of test response after conditioning stimulus. Ordinates: relative amplitude as percentage control (test response alone taken as 100%, mean and s.e.). Inset: sample record during control period of response to conditioning stimulus followed by response to test stimulus 60 ms later. Lower graph, amplitude of postganglionic Sa compound action potential in response to conditioning stimulus. Ordinates: relative amplitude, final control taken as 100%. Each point is the mean of two or three determinations.

(Brimble *et al.*, 1972). A similar value (7-8%) was obtained in the experiment illustrated in Fig. 5 but this was little affected by 5-HT (1 μM). In other experiments a small but distinct increase occurred with 5-HT (1 μM). As can be seen from Fig. 5, 5-HT (10 μM) distinctly increased late facilitation and 5-HT (100 μM) increased it further to a value of 18%. These increases were only partly reversed by washing. However, 10 μM and, to a greater extent, 100 μM reduced the amplitude of the transmitted compound action potential evoked by the CS. In general, it appeared that the increases in late facilitation induced by 5-HT were not as great as the increases in early facilitation.

Comparison with the action of bradykinin

The action of bradykinin at the ganglionic synapse seems to be quite different from 5-HT, for the polypeptide reduced early facilitation (Figs. 6 and 8) but increased the amplitude of the transmitted compound action potential evoked by a single stimulus. Results from five experiments suggested the threshold for this action was between 1 and 2 μM. In Fig. 6 (upper graph), bradykinin (7.7 μM) was able, at least for a period, to abolish early facilitation of submaximal Sa responses; early facilitation of the test response was 24% during the control period. Typical responses to conditioning and test stimuli, 60 ms apart and during the control period, are shown in the inset; facilitation of the second response is readily apparent. Some recovery of early facilitation occurred during the prolonged exposure to bradykinin, perhaps indicating tachyphylaxis. The increase in amplitude of the unconditioned response (Fig. 6, lower graph) occurred gradually during exposure to bradykinin, reaching 140% of control values.

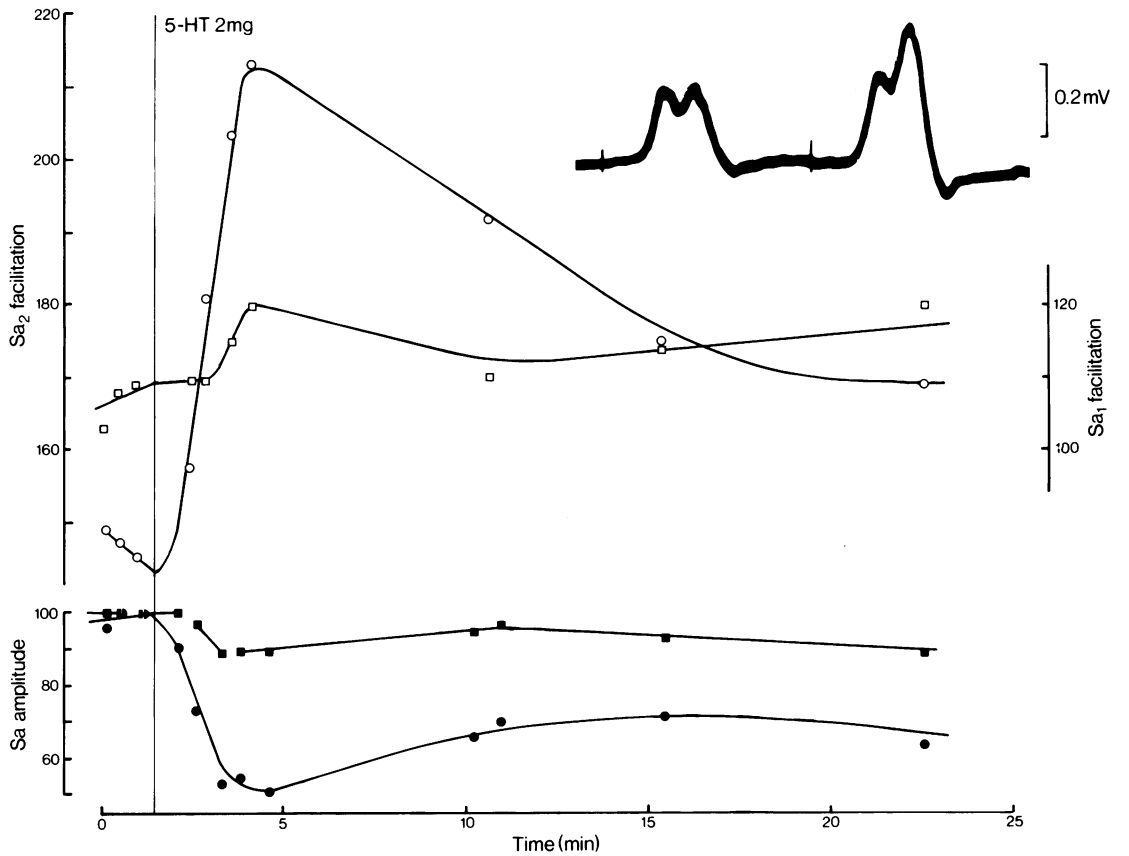

Fig. 7 The effects of an injection of 5-hydroxytryptamine (5-HT) on early facilitation and amplitude of the Sa_1 and Sa_2 components of the postganglionic compound action potential. Conditioning and test stimuli submaximal for Sa_1 and Sa_2 responses, paired responses so evoked separated by at least 10 s rest periods. Ganglion superfused with Krebs solution. 5-HT (2 mg) injected into perfusion stream to the ganglion at the vertical line. Upper graph, facilitation of Sa_1 and Sa_2 responses 50 ms after conditioning stimulus. Ordinates (left): relative amplitude of Sa_2 response (○) as percentage controls; ordinates (right). relative amplitude of Sa_1 response (□), test response alone taken as 100%. Each point is the mean of two determinations. Inset: sample record during maximum 5-HT action; response to conditioning stimulus followed by response to test stimulus 50 ms later, showing Sa_1 and Sa_2 components of the response. Lower graph, amplitude of Sa_1 (■) and Sa_2 components (●) of postganglionic compound action potential in response to conditioning stimulus. Ordinates: relative amplitude, initial control taken as 100%.

This effect was only partially reversed on washing.

In one experiment the effects of 5-HT and bradykinin were compared on the same preparation and, to avoid tachyphylaxis, the drugs were injected into the perfusion stream. Initially 5-HT (2 mg) was injected; estimates of dilution by the perfusion stream suggest an injection was dispersed in a volume of about 10 ml. The effects on early facilitation of submaximal Sa responses are shown in the upper graph of Figure 7. In this experiment, as in a few others, two Sa subcomponents were apparent (Kosterlitz & Wallis, 1966b). The Sa_1 contributes the axons of fastest conduction velocity to the internal carotid nerve and probably controls structures in the orbit, while the Sa_2 component may represent mainly vasoconstrictor neurones (Bishop & Heinbecker, 1932). The results (Fig. 7) demonstrate that the Sa_2 component responded with changes of greater magnitude than the Sa_1 component to 5-HT, the only data obtained which suggested a differential sensitivity between functionally different pathways through the ganglion. The effect on the amplitude of the unconditioned responses is

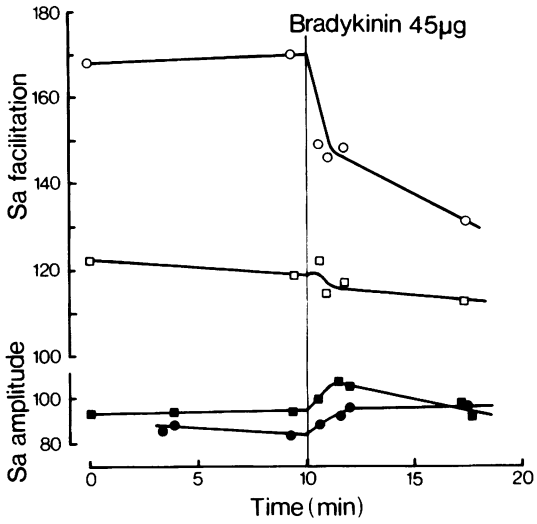

Fig. 8 The effects of an injection of bradykinin on early facilitation and amplitude of the Sa_1 and Sa_2 components of the postganglionic compound action potential. Same experiment as Figure 7. Bradykinin (45 μg) injected into the perfusion stream at vertical line. Upper graph, facilitation of Sa_1 and Sa_2 responses 50 ms after conditioning stimulus. Ordinates: relative amplitude of Sa_1 response (□) and Sa_2 response (○) as percentage controls (test response alone taken as 100%). Each point is the mean of two determinations. Lower graph, amplitude of Sa_1 (■) and Sa_2 (●) as in Figure 7. Ordinates: relative amplitude, initial control at start of experiment (see Fig. 7) taken as 100%.

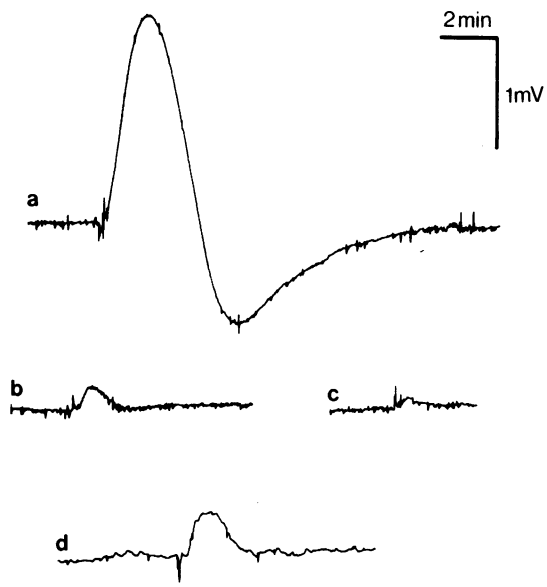

Fig. 9 Changes in resting potential in response to injections of 5-hydroxytryptamine (5-HT) and bradykinin into the perfusion stream to the ganglion. Depolarization upwards. (a) response to 5-HT (81 μg), (b) response of the same preparation to bradykinin (30 μg) 30 min later; (c) response to bradykinin (30 μg), 25 min after (b); (d) response to bradykinin (150 μg), 128 min after (c). Note injection artefacts. Estimates of dilution by the perfusion stream suggest an injection was dispersed in a volume of about 5 ml.

shown in the lower graph of Figure 7. The Sa_2 response was more sensitive to the depressant action of 5-HT as well as displaying much greater early facilitation. Note that to allow Sa_1 and Sa_2 facilitation to be plotted on the same scale, the left-hand ordinate scale refers to Sa_2 facilitation, the right-hand to Sa_1 facilitation. Sa_2 facilitation changed from around 50% to over 110% during the peak action of 5-HT at a time when the amplitude of the unconditioned response was diminished by 50%. Typical responses to conditioning and test stimuli, 50 ms apart and during peak 5-HT action, are shown in the inset (Figure 7). The large change in Sa_2 is readily apparent. Bradykinin produced results which were quite different in the same preparation. The effects of an injection of bradykinin (45 μg) were to reduce early facilitation of Sa_2 (upper graph, Fig. 8) while the amplitude of the unconditioned responses was increased (lower graph, Figure 8). The facilitation of the Sa_2 component was affected to a much greater extent than that of the Sa_1, which was scarcely altered, but the amplitude of the unconditioned Sa_1 and Sa_2 responses was increased to a similar extent.

In several experiments, the Sb component of the postganglionic compound action potential was examined. The Sb component was little affected by 5-HT, although in some experiments it was slightly depressed. Similarly, the pattern of facilitation of this component was little, if at all, altered. In view of the finding that the component results, not from activation of a distinct pool of ganglion cells, but partly from Sa cells firing when their C fibre input is activated (Brimble *et al.*, 1972), no further analysis of Sb responses was attempted.

Effect of 5-hydroxytryptamine and bradykinin on resting potential

We have attempted to establish whether the radically different actions of 5-HT and bradykinin could be correlated with their depolarizing actions

on the ganglion cell membrane. Ganglia, superfused with Krebs solution, were set up in a sucrose-gap apparatus and drugs injected into the perfusion stream to the ganglion. Whereas 5-HT was a potent depolarizing agent, causing marked changes in membrane potential which consisted of a rapid depolarization followed by an after-hyperpolarization (Fig. 9a), bradykinin caused no measurable changes in membrane potential in several experiments. In some experiments, very small, transient depolarizations occurred in response to bradykinin (Fig. 9b, c, and d), but these showed much variability (cf. Fig. 9b and c). In an attempt to establish whether the presynaptic nerve terminals were also depolarized by 5-HT, we adopted the strategy of Koketsu & Nishi (1968). If the ganglion is arranged in the sucrose-gap in such a manner that the recorded potential differences arise between the proximal pole of the ganglion and a point on the cervical sympathetic trunk, it is claimed that the potential changes recorded arise mainly in the preganglionic nerve terminals (Koketsu & Nishi, 1968). When 5-HT was then injected into the perfusion stream to the ganglion, relatively large and rapid depolarizations were recorded. However, in general depolarizations were smaller than those recorded from ganglion cells and after-hyperpolarizations were either absent or less pronounced.

Discussion

Changes in the amplitude of the postganglionic compound action potential have been used as a measure of facilitation and depression on the assumption that amplitude is approximately proportional to the number of cells firing. Clearly, this assumption would be incorrect for ganglionic compound action potentials, since drug-induced changes in membrane potential would alter the absolute amplitude of the action potential of individual ganglion cells. However, postganglionic compound action potentials were led off at a point at least 3 mm distal to the ganglion since alterations in ganglion cell resting potential induced by 5-HT or bradykinin would have little effect on the resting potential and, hence, on the absolute amplitude of the action potential of individual postganglionic fibres (cf. Libet 1967). The results suggest that 5-HT in concentrations as low as 1 μM can modulate transmission in the rabbit superior cervical ganglion and alter the total number of cells discharging action potentials on excitation of the preganglionic nerve. Whereas the transmitted action potential was depressed, the early and late facilitation of the response after a conditioning stimulus was increased and the inhibition of the response after a conditioning stimulus was reduced. The 5-HT-induced depression indicates a reduction in the number of cells discharging to a single stimulus, while the increase in early and late facilitation indicates that there is a larger cell pool available for recruitment by a second stimulus. At least at the higher 5-HT concentrations, the 5-HT action may be explained by supposing that the subliminal fringe is increased at the expense of cells discharging to a single stimulus. Similarly, the reduction by 5-HT of the inhibition 200 ms after a CS suggests that recruitment by the test stimulus is able to predominate over inhibition at this interval. However, other evidence discussed below suggests that 5-HT has more than one action at the ganglionic synapse. If this is the case, depression and increased intrinsic facilitation may reflect these different actions.

A facilitatory action of 5-HT on normal transmission has been reported for the cat superior cervical ganglion (Trendelenburg, 1957) and for the rat stellate ganglion (Hertzler, 1961). These authors did not report the depression of transmission we observed with single stimuli, nor that facilitation and inhibition following conditioning stimuli were modified. However, Jéquier (1965) working on the rat superior cervical ganglion and Machová & Boska (1969) using the same ganglion of the cat both reported a depression of transmission with 5-HT. Trendelenburg's experiments, it should be noted, involved prolonged stimulation at 10 Hz, so that his results are not directly comparable with those reported here. Repetitive stimulation leads to recruitment of cells not recruited after a single conditioning stimulus and thus increases the size of the demonstrable subliminal fringe (Brimble & Wallis, 1973). Any situation that brings about an increase in the subliminal fringe of cells might, in the light of our results, be expected to unmask the facilitatory action of 5-HT. Hertzler's results, on the other hand, seem inconsistent with our findings since his rates of stimulation were very similar (i.e. 0.017 Hz). Presumably, this is the result either of the species difference or of the different ganglion investigated. De Groat & Lalley (1973) recently showed that 5-HT can have complex actions on the cat superior cervical ganglion; depression was observed with low concentrations of 5-HT, but in ganglia conditioned by repetitive stimulation 5-HT elicited both short latency and long latency discharge of action potentials.

There are several lines of evidence which suggest that 5-HT has at least two actions at the ganglionic synapse. First, the increase in intrinsic facilitation was apparent at lower concentrations than the depression of transmission. The higher concentrations apparently necessary for the latter

action may be associated with the higher concentrations required to depolarize ganglion cells. Detectable depolarizations were not usually evoked by concentrations of 5-HT lower than 10 μM. This is in agreement with Trendelenburg's findings (1956b) that the concentration of 5-HT which facilitated transmission in the cat was very much lower than that necessary to produce direct stimulation of the ganglion cells and elicit end-organ effects. Secondly, while both early and late facilitation were affected by 5-HT, the processes underlying them may be different (Brimble *et al*, 1972). Late facilitation may be presynaptic in origin, while early facilitation may result from postsynaptic summation of e.p.s.p.s. Thirdly, de Groat & Lalley (1973) concluded, partly on the grounds that picrotoxin blocks the excitatory but not the depressant actions of 5-HT, that these actions were mediated through different receptors. They also found that picrotoxin blocks the 5-HT-induced depolarization of cat ganglion cells, as it does that of rabbit ganglion cells (Wallis & Woodward, 1973). Fourthly, our results suggest that 5-HT depolarizes not only ganglion cells but perhaps preganglionic nerve terminals as well. However, the technique for demonstrating terminal depolarization has the weakness that ganglion cells whose axons or axon collaterals project caudally along the cervical sympathetic trunk would contribute to the recorded potentials. Presynaptic actions of 5-HT have been demonstrated on other tissues, for Dudel (1965) has reported facilitation of transmitter release by 5-HT at the crayfish neuromuscular junction due to an increase in quantal content. In other invertebrates the action of 5-HT may be extremely complex; Paupardin-Tritsch & Gerschenfeld (1973) maintain that 5-HT has five different actions on certain molluscan neurones, some effects involving increases, others decreases, in conductance.

Are ganglionic synapses *in situ* ever exposed to 5-HT concentrations of 1 μM and is modulation of transmission by 5-HT likely under any physiological conditions? Gertner, Paasonen & Giarman (1959) detected small amounts of 5-HT in the perfusate from cat ganglia in the presence of a monoamine oxidase inhibitor. This was presumably released from non-neural structures as preganglionic stimulation had no effect on release; it may be related to the high levels of 5-hydroxytryptophane decarboxylase found in sympathetic ganglia (Gaddum & Giarman, 1956). Serum levels of 5-HT in man may be in excess of 1 μM, i.e. 0.02-0.2 μg/ml (Franzen & Eysell, 1969) and may be considerably higher in rabbit serum, 0.4-4 μg/ml (Garattini & Valzelli, 1965). In addition, plasma levels in man are raised in association with cigarette smoking (Schievelbein & Werle, 1962), in certain allergies and due to tumours of argentaffin cells of the digestive tract (carcinoid syndrome). The last condition can also be associated with an increase in plasma bradykinin and related polypeptides, for the carcinoid tissue produces a specific kallikrein (Oates, Melmon, Sjoerdsma, Gillespie & Mason, 1964).

The action of bradykinin on the rabbit superior cervical ganglion was quite different from that of 5-HT. The amplitude of the response to stimulation of the preganglionic nerve was increased, while the intrinsic facilitation induced by a conditioning stimulus was reduced. It appears that the size of the subliminal fringe and thus recruitment are reduced in the presence of bradykinin. Presumably, cells formerly in the subliminal fringe now discharge to a single stimulus to the preganglionic nerve. Our findings contrast with those of Haefely, Hürlimann & Thoenen (1965), who reported that bradykinin produced a depression of transmission in the cat superior cervical ganglion. However, the failure of bradykinin to induce any substantial change in ganglion cell resting potential is in agreement with Haefely's results (1970) for the cat superior cervical ganglion. This may indicate that the action of bradykinin is a presynaptic one, but postsynaptic actions involving minimal change in resting potential but subtle changes in ionic conductances cannot be ruled out. Further, a direct activation of cat ganglion cells by bradykinin can occur (Lewis & Reit, 1965), although this direct stimulant action may be inconsistent and small compared to its action in facilitating transmission and enhancing acetylcholine-induced stimulation (Haefely, Hürlimann & Thoenen, 1966; Trendelenburg, 1966). The effects of both bradykinin and 5-HT on acetylcholine release in sympathetic ganglia remain to be evaluated.

This work was supported by the Medical Research Council, Grant G 970/346/B. We wish to thank Mr D.H. Brown and Mr A. Mordecai for invaluable help with the design and construction of the baths.

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(Received June 3, 1973)