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SITE OF ADRENALINE BLOCKADE IN THE SUPERIOR CERVICAL GANGLION OF THE RABBIT

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SUMMARY

1. The blocking action of adrenaline on the superior cervical ganglion of the rabbit was investigated with intracellular recording techniques.

2. Adrenaline (10^{-5} M) blocked initiation of post-synaptic action potentials and decreased the amplitude of excitatory post-synaptic potentials (e.p.s.p.s), but did not hyperpolarize the post-synaptic membrane.

3. The depressant action of adrenaline was antagonized by phenoxybenzamine and dihydroergotamine.

4. Acetylcholine depolarizations from iontophoretic ACh were not affected by adrenaline.

5. Adrenaline decreased the frequency of miniature excitatory post-synaptic potentials (m.e.p.s.p.s) and decreased the quantal content of e.p.s.p.s in a low $[Ca^{2+}]:[Mg^{2+}]$ media.

6. It was concluded that adrenaline blocks ganglionic transmission by acting at an alpha-adrenoceptive site in the presynaptic nerve terminals.

INTRODUCTION

The blocking action of adrenaline on ganglionic transmission, initially reported by Marrazzi (1939), has been described by many investigators (Bülbring, 1944; Matthews, 1956; Eccles & Libet, 1961; Pardo, Cato, Gijon & Alonso de Florida, 1963). Adrenaline can shift the surface potential of cat ganglia in the hyperpolarizing direction (Lundberg, 1952), thus De Groat & Volle (1966) concluded that catecholamine-induced inhibition and ganglionic hyperpolarization were mediated by a single site of action. Presently, there is little direct evidence to support this hypothesis. Thus this investigation was undertaken to clarify the site of action at which adrenaline produces blockade of ganglionic transmission. This site of action is of special interest as adrenaline is released from an activated

ganglion (Bülbring, 1944) and has been suggested to have a physiological role in ganglionic transmission (Eccles & Libet, 1961).

The results have led to the conclusion that adrenaline blocks ganglionic transmission by acting at a presynaptic site. Preliminary reports of the present findings have been published (Christ & Nishi, 1969*a*, *b*).

METHODS

All experiments were performed on the isolated superior cervical ganglion of the rabbit. The ganglion and approximately 2 cm of the preganglionic nerve trunk were isolated and mounted in a 1 ml. chamber. The preganglionic nerve extended into a second chamber, containing mineral oil, where the nerve was placed over a pair of platinum electrodes. In most experiments the nerve was stimulated at a frequency of 0·2 Hz (pulse duration 0·1 msec). The ganglion was perfused with a modified Tyrode solution (Eccles, 1955) which was oxygenated and was maintained at a constant temperature between 36 and 38° C. The flow rate was 100–200 ml./hr.

Glass capillary microelectrodes were filled with 3 M-KCl (resistance greater than 25 MΩ) and were inserted into ganglion cells for recording purposes. Calomel electrodes were used as indifferent electrodes. The methods of recording and producing electrotonic potentials by passage of current through the recording electrode have been previously described (Nishi & Koketsu, 1960). When ganglion cells were successfully impaled, their resting potential (−50 to −70 mV) and action potential (60–90 mV) were well maintained for periods as long as 3 hr.

A micro-electrode with 2·7 M acetylcholine (resistance of 30–100 MΩ) was used for iontophoretic application of acetylcholine (ACh). The ACh electrode was placed near the ganglion cell in which the recording electrode had been inserted. Application of a short (10–50 msec) outward current through the ACh electrode induced a transient depolarization (ACh potential) with a rise time less than 200 msec. Diffusion of ACh from the electrode was prevented by passing a constant inward current ($1–5 \times 10^{-9}$ A) through the ACh electrode. The current intensity was determined from the voltage change across a resistor inserted in series with the ACh electrode.

The drugs used were adrenaline bitartrate, noradrenaline hydrochloride, isoprenaline hydrochloride, propranolol hydrochloride, phenoxybenzamine hydrochloride, dichloroisoprenaline hydrochloride, dihydroergotamine methanesulphonate and acetylcholine chloride.

RESULTS

Synaptic potential

When the preganglionic nerve to the superior cervical ganglion was stimulated, multiple potentials were recorded at the post-synaptic membrane. A typical response (Fig. 1*A*) was composed of several e.p.s.p.s, and an action potential, if one of the e.p.s.p.s was suprathreshold. This is similar to responses reported previously (Eccles, 1955; Erulkar & Woodward, 1968). When adrenaline was added to the perfusing solution at a final concentration of 10^{-5} M, the amplitudes of the subthreshold e.p.s.p.s were decreased in all the cells studied, although the decrease of e.p.s.p. amplitude varied considerably among different cells (average decrease in twenty-one cells was 49 %, S.D. ± 22 %). The effects of adrenaline were

reversed within 5 min after adrenaline perfusion was discontinued. Adrenaline also depressed the suprathreshold e.p.s.p.s sufficiently in a majority of the cells to block initiation of a post-synaptic action potential. In a few cells the post-synaptic action potential after indirect stimulation was unaffected by adrenaline (10^{-5} M), although subthreshold e.p.s.p.s of the same cell were depressed (Fig. 1*A*, *B*). The inability of adrenaline to block spike initiation in these cells was probably due to a high safety factor of transmission.

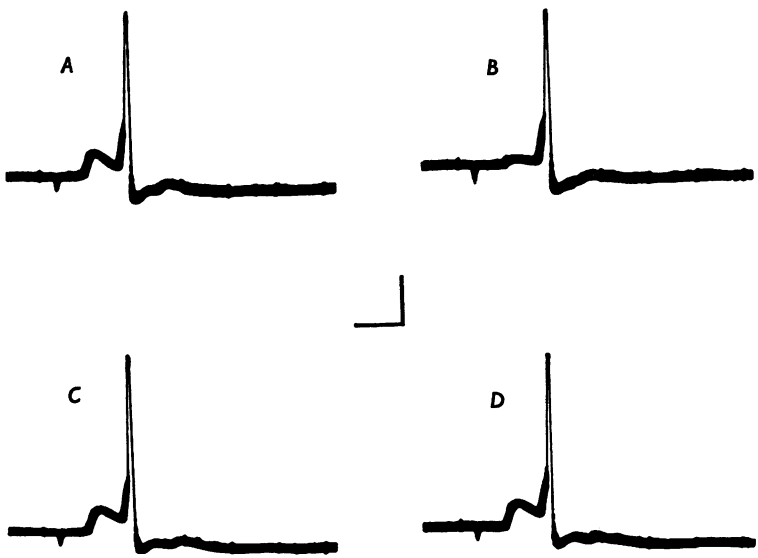

Fig. 1. Effect of adrenaline and dihydroergotamine on post-synaptic potentials from indirect stimulation. *A*: control. *B*: adrenaline (10^{-5} M). *C*: dihydroergotamine (10^{-5} M). *D*: dihydroergotamine and adrenaline. Calibration: 10 mV and 10 msec.

Concentrations of adrenaline between 10^{-6} and 10^{-3} M decreased the e.p.s.p. amplitude. 10^{-5} M was used in most experiments, as this concentration was consistently effective and readily reversible.

Noradrenaline (10^{-5} M) also decreased the e.p.s.p. amplitude in each of the three cells in which it was tested. Isoprenaline (10^{-5} M) decreased the e.p.s.p. amplitude in only one of three cells. Furthermore, in one experiment in which all three catecholamines were applied consecutively to a single cell, the order of effectiveness was: adrenaline, noradrenaline and isoprenaline. This result indicates that the adrenoceptive site is an alpha-receptor.

When the ganglion was continuously perfused with phenoxybenzamine (10^{-5} M) or dihydroergotamine (10^{-5} M), adrenaline was ineffective (Fig.

1*D*). These concentrations of alpha-blockers had no effect on the e.p.s.p. (Fig. 1*C*). The alpha-blocking action of phenoxybenzamine and dihydroergotamine developed very slowly; thus they had to be given for at least 10 min before adrenaline. Furthermore, they were relatively irreversible, as adrenaline was ineffective for several hours after perfusion of the alpha-blocker was discontinued.

In contrast propranolol (3×10^{-5} M) or dichloroisoprenaline (10^{-5} M) did not affect the blocking potency of adrenaline. These concentrations of beta-blockers were the highest concentrations which did not change the e.p.s.p.

Membrane potential

The membrane potential of ganglion cells was not altered at the time when the e.p.s.p.s were initially depressed by adrenaline. When the adrenaline (10^{-5} M) perfusion was continued for periods up to 4 or 5 min, the membrane potential tended to shift in the depolarizing direction. Only two of eleven cells were hyperpolarized by adrenaline. The amplitude of these depolarizations were quite variable, even in a single cell and did not correspond to the blocking action of adrenaline. Only when the ganglion was perfused with concentrations of adrenaline as high as 10^{-3} M did the changes of membrane potential become consistent. Three cells in which adrenaline (10^{-3} M) was tested responded with a prolonged depolarization (5–10 mV), as illustrated in Fig. 2. This cell was the only one of the three to respond with a brief hyperpolarization before the long depolarization.

The inability of adrenaline to hyperpolarize the ganglion cell membrane at a concentration at which blockade of transmission occurs, or even at much higher concentrations, indicates that hyperpolarization is not the primary mechanism of adrenaline blockade.

Electrical membrane properties

There was no change in the amplitude and time course of the electrotonic potential (Fig. 3*A*, *B*), when the amplitude of the e.p.s.p. was considerably decreased by adrenaline. Furthermore, there was no change in the amplitude of the catelectrotonic potential necessary to initiate a direct ganglionic spike (Fig. 3*C*, *D*). These experimental results indicate that the electrical properties of the post-synaptic membrane were not altered by adrenaline.

Post-synaptic membrane sensitivity

ACh depolarizations of the cell membrane were observed before and after applying adrenaline to determine the effect of adrenaline on the sensitivity of the post-synaptic cholinoceptive receptors. Proper location of an ACh electrode resulted in a rapid ACh potential. An example of the effect of adrenaline on the e.p.s.p. and ACh potential is shown in Fig. 4. Adrenaline

rapidly decreased the e.p.s.p. amplitude by 40 % but did not significantly change the amplitude of the ACh potential. Similar results were obtained in five other cells in which ACh was applied iontophoretically and in four cells in which ACh was applied by addition to the bathing solution at a final concentration of 10^{-3} M. The presence of atropine ($3{\cdot}4 \times 10^{-5}$ M) did not alter the results.

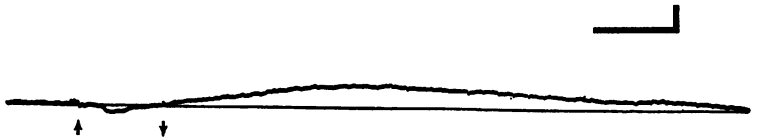

Fig. 2. Effect of adrenaline (10^{-3} M) on the resting membrane potential. Adrenaline was perfused between the arrows. Upward deflexion is positive. The resting membrane potential before adrenaline was −58 mV. Calibration: 10 mV and 30 sec.

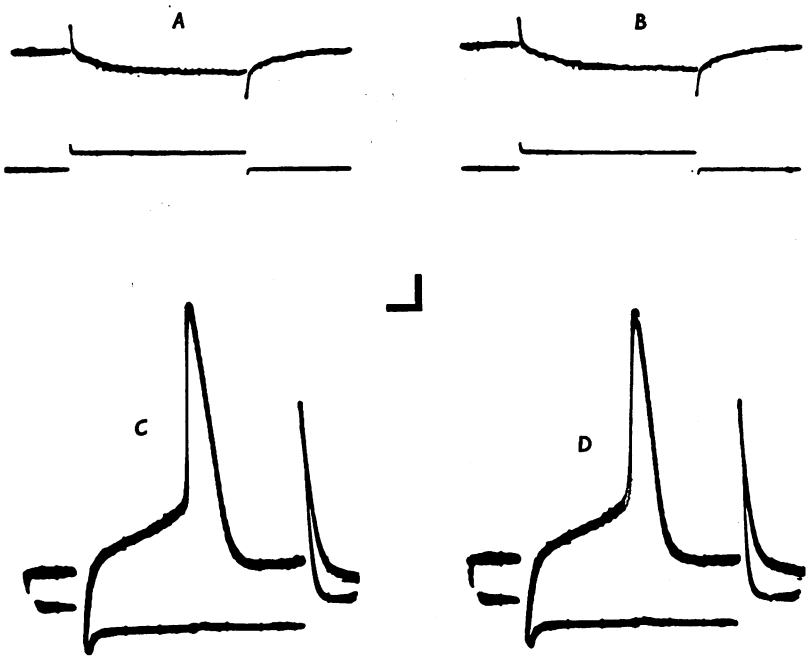

Fig. 3. Effect of adrenaline (10^{-5} M) on the electrical properties of the postsynaptic membrane. Anelectrotonic potentials (upper traces) induced by a current pulse ($2{\cdot}8 \times 10^{-10}$ A, lower traces) in control (*A*) and in the presence of adrenaline (*B*). Spike responses (upper traces) induced by a cathodal current pulse ($1{\cdot}8 \times 10^{-9}$ A, lower traces) in control (*C*) and in the presence of adrenaline (*D*). Anelectrotonic potentials and spike responses were recorded in different cells. Vertical calibration: 10 mV for the upper traces of all records. Horizontal calibration: 10 msec for *A* and *B*, and 2 msec for *C* and *D*.

The observation that adrenaline did not depress the post-synaptic response to ACh when the e.p.s.p. was depressed indicates that the site of action of adrenaline at the rabbit superior cervical ganglion is presynaptic.

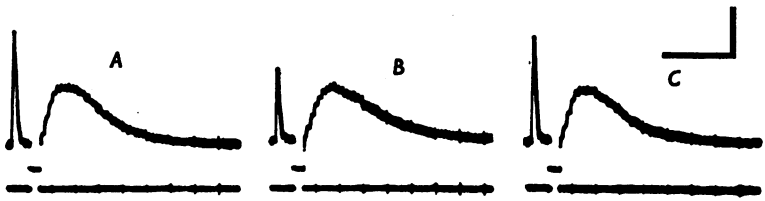

Fig. 4. E.p.s.p.s and ACh potentials in control (*A* and *C*) and in the presence of adrenaline (10^{-5} M) (*B*). ACh was ejected iontophoretically from a micro-electrode during the current pulse (8×10^{-8} A) shown in the lower trace. Calibrations: 20 mV and 300 msec.

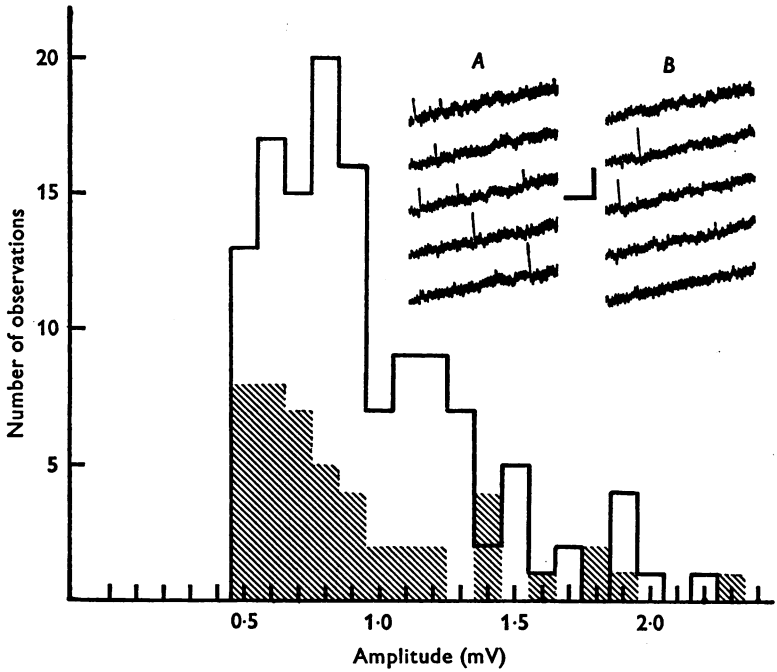

Fig. 5. Histograms of m.e.p.s.p. amplitudes recorded in 20 mM-KCl. Each histogram describes the m.e.p.s.p.s recorded during a period of 80 sec. There were 129 m.e.p.s.p.s in the control and forty-seven m.e.p.s.p.s in the presence of adrenaline (10^{-5} M, cross-hatched). Inset: records of m.e.p.s.p.s before (*A*) and during (*B*) adrenaline perfusion. Calibrations: 2 mV and 200 msec.

Transmitter release

Spontaneous release. M.e.p.s.p.s in the superior cervical ganglion occurred at such a slow frequency, it was difficult to measure their frequency accurately. If the ganglion was immersed in 15 or 20 mM-KCl, the frequency of m.e.p.s.p.s increased in most ganglion cells to the range of 1–10/ sec. The effects of adrenaline on m.e.p.s.p.s were observed in the

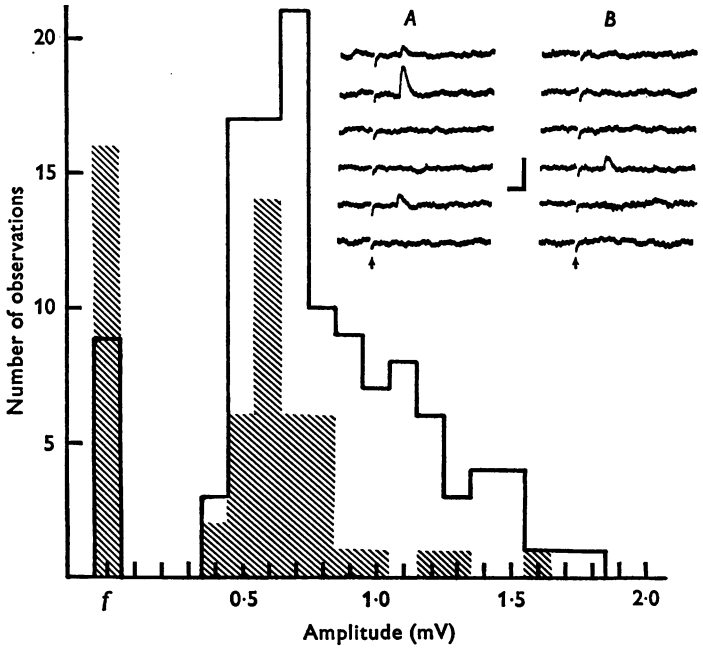

Fig. 6. Histograms of e.p.s.p. amplitudes recorded in 0·5 mM-$CaCl_2$ and 5·5 mM-$MgCl_2$. Failures (*f*) are plotted at 10 × the ordinate values. Adrenaline (10^{-5} M), cross-hatched. Inset: Records of e.p.s.p.s before (*A*) and during (*B*) adrenaline perfusion. Indirect stimulation occurs at the arrow. Calibrations: 2 mV and 10 msec.

presence of these high concentrations of KCl. Results from a typical experiment are shown in the histogram in Fig. 5. The amplitude distribution of the control m.e.p.s.p.s does not appear to follow a normal distribution curve. There are several peaks, which is indicative of the dispersion of synaptic sites on a single ganglion cell. (In view of this observation, a decrease of mean m.e.p.s.p. amplitude may not involve a post-synaptic event. It may be due to a decrease in the frequency of the larger m.e.p.s.p.s.) Adrenaline decreased the m.e.p.s.p. frequency in the five cells tested (1·6 to 0·6; 1·0 to 0·9; 0·9 to 0·7; 6·2 to 2·0; 1·7 to 0·7/sec). The frequency of the experiment illustrated in Fig. 5 was decreased from 1·6/sec to 0·6/sec

(there was only a small decrease in average m.e.p.s.p. amplitude from 1·04 to 1·01 mV). The decrease in frequency occurred at all amplitudes, as would be expected if adrenaline was acting at a presynaptic site.

Quantal content of e.p.s.p. Further evidence concerning the site of action of adrenaline was obtained from experiments in which the quantal content of the e.p.s.p. was decreased by a low calcium-magnesium concentration ratio in the perfusion solution. Fig. 6 is the amplitude distribution of e.p.s.p.s from a single cell in 0·5 mM-$CaCl_2$ and 5·5 mM-$MgCl_2$. With this technique it is possible to calculate the average quanta of transmitter released per stimulus by observing the number of failures in a series of stimuli. There were eightynine failures in 200 stimuli in the control records. Assuming the release follows a Poisson distribution, the average quantal content of the e.p.s.p. was 0·81 quanta/stimulus. Adrenaline increased the failure rate to 161 failures in 200 stimuli. Thus the average quantal release was decreased by adrenaline from 0·81 to 0·22 quanta/stimulus. Decreases were also observed in three other cells (0·86 to 0·30; 0·57 to 0·25; 1·08 to 0·10).

This decrease in quantal content occurred without change in the quantal size. Quantal size obtained from the ratio of the average e.p.s.p. amplitude and the average quantal content was 0·58 mV in the control and 0·62 mV in the presence of adrenaline. Furthermore, the first peak is indicative of the amplitude of a single quantum of transmitter. This peak occurred at approximately 0·5–0·7 mV in the control and at 0·6 mV in the experimental situation. Thus it may be concluded that adrenaline has no prominent post-synaptic actions at 10^{-5} M.

DISCUSSION

These results demonstrate that adrenaline has a presynaptic action which decreases the e.p.s.p. in the rabbit superior cervical ganglion. It has been previously observed that adrenaline can decrease the ACh output (Birks & MacIntosh, 1961; Paton & Thompson, 1953). A presynaptic adrenergic site has been demonstrated for other synapses. Adrenaline and noradrenaline decrease the release of ACh from the guinea-pig ileum (Kosterlitz & Lydon, 1968; Vizi, 1968; Paton & Vizi, 1969) and guinea-pig colon (Beani, Bianchi & Crema, 1969). Adrenaline (Krnjevic & Miledi, 1958) and noradrenaline (Jenkinson, Stamenović & Whitaker, 1968) increase the end-plate potential at the neuromuscular junction by increasing the release of transmitter. The presynaptic adrenoceptive site in the ganglion appears to be an alpha receptor, as at the neuromuscular junction (Bowman, Goldberg & Raper, 1962; Bowman & Raper, 1966). It is blocked by phenoxybenzamine and dihydroergotamine, but not by

propranolol and dichloroisoprenaline. Furthermore, the order of potency (adrenaline, noradrenaline, isoprenaline) is what would be anticipated for an alpha receptor.

This presynaptic site must be responsible for blockade of ganglionic transmission by adrenaline. Hyperpolarization does not appear to have more than a minor role in adrenaline blockade. First, no hyperpolarization was recorded in the presence of adrenaline (10^{-5} M), even though adrenaline decreased the amplitude of the e.p.s.p. Secondly, hyperpolarization may have occurred, but could not be recorded (this would be possible if the adrenoceptive hyperpolarizing sites are located on remote dendritic branches). However, as noradrenaline hyperpolarization and inhibitory potentials occur with no change in the membrane conductance (Nishi & Koketsu, 1968; Libet & Kobayashi, 1969), hyperpolarization by adrenaline should have caused an augmentation, not a depression of the e.p.s.p. Other post-synaptic mechanisms, such as a decrease in effective membrane resistance, a decrease in the threshold membrane potential and a competitive blocking action at the post-synaptic cholinoceptive receptor do not appear to be involved in adrenaline blockade.

The origin of facilitation of transmission by adrenaline is more difficult to establish. Facilitation of post-ganglionic activity by adrenaline has been reported in a normal medium (Bülbring & Burn, 1942; Bülbring, 1944; Trendelenburg, 1956), and in the presence of alpha-blocking drugs (De Groat & Volle, 1966). The results of this paper indicate that adrenaline was unable to augment the e.p.s.p., even in the presence of phenoxybenzamine or dihydroergotamine. However, adrenaline did depolarize many of the cells. A possible explanation, as suggested by De Groat & Volle (1966), is that facilitation is due to depolarization. The e.p.s.p. would not be augmented during the depolarization. Alternatively, a small augmentation (less than 20 %) may be quite difficult to demonstrate with this technique. The high variability of the e.p.s.p. amplitude could mask small increases necessary for facilitation.

Catecholamines may be physiologically involved in ganglionic transmission (Eccles & Libet, 1961; Costa, Revzin, Kuntzman, Spector & Brodie, 1961; Jacobowitz & Woodward, 1968). Eccles & Libet (1961) suggested that an adrenergic transmitter substance is responsible for the slow inhibitory potential at the ganglion. Results from this paper neither confirm nor contradict their hypothesis. The only observation, which is inconsistent with the hypothesis that adrenaline is the transmitter for the inhibitory potential, is that adrenaline was unable to hyperpolarize the ganglion cell membrane consistently, even at 10^{-3} M. In this regard noradrenaline may be a better candidate for the transmitter, as noradrenaline has been shown to hyperpolarize the ganglion cell membrane

consistently and this hyperpolarization has characteristics similar to the inhibitory potential (De Groat & Volle, 1966; Libet & Kobayashi, 1969).

A physiological role of the presynaptic adrenergic receptors must also be considered. Adrenaline and noradrenaline are present in the ganglion and are released after preganglionic stimulation (Bülbring, 1944). The observation that adrenaline (and probably noradrenaline) is 10 to 100 times as potent at the presynaptic receptor as compared to the postsynaptic receptor does not in itself designate a physiological role for the presynaptic receptor. The physiological effectiveness of a transmitter at any receptor depends not only on the potency of the transmitter at that receptor, but also upon the spatial distribution of transmitter releasing and receptor sites. If the catecholamines are released diffusely, as from a chromaffin cell, the presynaptic site will be of considerable significance.

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