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Effects of adrenaline on nerve terminals in the superior cervical ganglion of the rabbit

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Summary

1. Adrenaline decreases the release of transmitter from the presynaptic nerves in the superior cervical ganglion of the rabbit. The presynaptic mechanism of adrenaline action was investigated with micro-electrode techniques.

2. Adrenaline (10^{-5}M) did not change the threshold of the presynaptic nerve terminals to a current stimulus.

3. The effect of adrenaline on the excitatory postsynaptic potential (e.p.s.p.) was decreased when the calcium concentration of the perfusing solution was increased to 10 mM.

4. The effect of adrenaline on a train of e.p.s.ps (30–50 Hz) was analysed. Adrenaline decreased the readily available stores of transmitter, but only slightly changed the probability of release.

5. The probability of release was increased when the calcium concentration was increased to 5 mM.

6. It was concluded that adrenaline acts directly on excitation release coupling in the presynaptic terminals.

Introduction

Adrenaline decreases the excitatory postsynaptic potential (e.p.s.p.) in the superior cervical ganglion by decreasing the release of transmitter from the presynaptic nerves (Christ & Nishi, 1970); however, little is known about the presynaptic mechanism of action. This investigation was undertaken to clarify the mechanism by which adrenaline decreases the release of transmitter.

Methods

All experiments were performed on the isolated superior cervical ganglion of the rabbit. Isolation of the ganglion and recording of intracellular potentials have been described in previous papers (Nishi & Koketsu, 1960; Christ & Nishi, 1970). The ganglion was perfused with an oxygenated Tyrode solution which was maintained at a constant temperature between 36° and 38° C.

Stimulation threshold of presynaptic nerve terminals was measured with micro-electrodes (Hubbard & Schmidt, 1963). A stimulating micro-electrode containing 1 M NaCl (resistance less than 1 MΩ) was placed near the intracellular recording micro-electrode (3 M KCl). Rectangular pulses (0·1–0·5 ms) were passed through

the stimulating electrode, and this electrode was manoeuvred until a direct action potential of the ganglion cell was recorded. Hyperpolarizing currents were passed through the recording electrode, thereby suppressing the ganglion cell spike. The stimulating electrode was then moved closer to the presynaptic nerve endings, until an e.p.s.p. was recorded after each stimulus. Threshold was described as the current necessary to initiate an e.p.s.p. The e.p.s.p. occurred in an all or none manner, determined by the all or none characteristic of the presynaptic nerve.

Changes in the membrane potentials of preganglionic nerve terminals were recorded with the sucrose-gap technique (Koketsu & Nishi, 1968). A short portion of the preganglionic nerve near the ganglion was perfused with an isotonic sucrose solution. Potential changes were recorded between Tyrode channels on each side of the sucrose channel.

Single e.p.s.ps were induced when the presynaptic nerve was stimulated with a supramaximal pulse of 0·1 ms in duration at a frequency of 0·2 Hz. Trains of e.p.s.ps were induced with submaximal pulses, because submaximal pulses usually excited only a single nerve in synaptic contact with the recording cell. Each train (1 per min) included 30 e.p.s.ps or more at a frequency of 30–50 Hz. Five trains of e.p.s.ps were recorded under each experimental condition. The amplitude of each e.p.s.p. was measured and was corrected for non-linear summation (Martin, 1955). Amplitudes of corresponding e.p.s.ps in the heads (initial ten e.p.s.ps) of the five trains were averaged (V). The tails of the five trains were divided in blocks of ten e.p.s.ps. The quantum size for each block of ten was calculated from the variance of the e.p.s.p. amplitudes and thus a mean quantum size (q) was obtained. The quantal content of each e.p.s.p. was determined (V/q). All values were calculated with a Controller Data 6400 computer.

The modified Tyrode solution contained: 136·9 mM NaCl, 5 mM $CaCl_2$, 0·5 mM $MgCl_2$, 12 mM $NaHCO_3$, 1 mM NaH_2PO_4 and 11 mM glucose (Eccles, 1955). When the calcium ion concentration was increased to 5 or 10 mM, the $NaHCO_3$ concentration was decreased to 1·2 mM. Thus $CaCO_3$ did not precipitate from the solution. The pH was always near pH 7·0.

Adrenaline bitartrate (10^{-5}M) was used in all experiments.

Results

Presynaptic membrane polarity

Adrenaline decreases the release of transmitter from the presynaptic nerves (Christ & Nishi, 1970). As depolarization and hyperpolarization have very prominent effects on transmitter release (del Castillo & Katz, 1954 ; Liley, 1956), it is necessary to determine if adrenaline is hyperpolarizing or depolarizing the presynaptic nerve terminals. Any effect of adrenaline on membrane potential can be observed with the technique of Hubbard & Schmidt (1963). It is possible with this technique to measure drug induced changes in the threshold of the nerve terminal to a current stimulus.

The determination of threshold for the nerve terminal with an extracellular stimulating electrode relies on the basic assumption that the site of stimulation is the same as the site of release (Hubbard, Schmidt & Yokota, 1965). This assumption was justified by establishing two criteria. First, the stimulating electrode was located in

such a manner that the delay (interval between stimulus and onset of e.p.s.p.) was very short. The shorter the delay, the closer the site of stimulation should be to the releasing sites. The delay in the experiment of Fig. 1 was less than 2 ms which is very near the synaptic delay suggested by previous workers (Brown, 1934; Eccles, 1935). Second, the stimulating electrode had to stimulate directly the ganglion cell body before the experiment was continued. The occurrence of direct action potentials indicates that the stimulating electrode was very near the cell body and therefore near the presynaptic terminals.

The e.p.s.p. was recorded after stimulation of the terminal, and occurred as an all or nothing response when the stimulus was near threshold for the presynaptic nerve. If the stimulus was increased, the amplitude of the e.p.s.p. was augmented (Fig. 1). This augmentation was probably due to recruitment of adjacent terminals. Stimuli near threshold were used in all experiments with adrenaline. Adrenaline (10^{-5}M) did not change the current threshold of the terminals (Fig. 2), although the e.p.s.p. (Fig. 2b, c) was always depressed.

Potential changes in the nerve terminal can also be indirectly recorded with the sucrose-gap technique of Koketsu & Nishi (1968). Adrenaline did not depolarize or hyperpolarize the nerve terminals in any of four ganglion preparations, even after the ganglia were exposed to adrenaline for 5 minutes. Nicotine (10^{-4}M) depolarized the terminals in three of the four ganglia in which adrenaline was ineffective.

These results indicate that adrenaline does not depress the release of transmitter by changing the membrane potential of the presynaptic nerve terminals.

Caffeine

The actions of adrenaline on skeletal muscle are readily influenced by caffeine (Bowman & Nott, 1969). In contrast to its effect at the neuromuscular junction, caffeine (10^{-4}–10^{-2}M) had no effect on the e.p.s.p. and did not block initiation of the orthodromic action potential in any of the ganglion cells tested. It appeared to have no action on ganglionic transmission. Furthermore, caffeine did not alter the effectiveness of adrenaline.

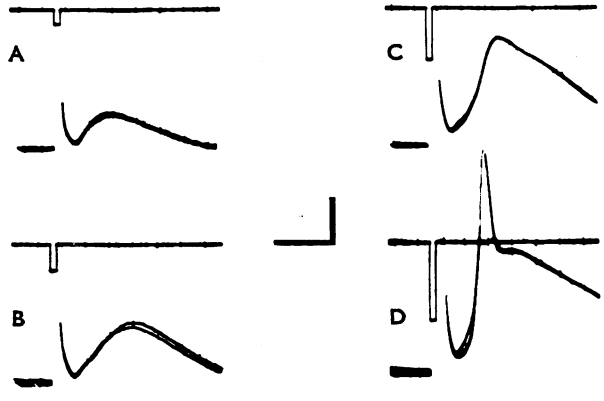

FIG. 1. Postsynaptic potentials from direct stimulation of the nerve terminal with a NaCl micro-electrode. Potentials were recorded intracellularly. Upper trace, current recording; lower trace, postsynaptic recording. A, Threshold current stimulus; B, 2×threshold; C, 3×threshold; D, 4×threshold. (The ganglion cell was hyperpolarized to eliminate the direct spike.) Calibrations: 20 mV (4×10^{-5} A) and 5 ms.

E.p.s.p. in 5 and 10 mM $CaCl_2$

When the calcium concentration in the perfusing solution was increased from 2 to 10 mM (control—2 mM), the amplitude of the e.p.s.p. was increased between 10% and 100% (probably due to increased quantal release and increased post-synaptic membrane resistance). Adrenaline was tested in the presence of 10 mM

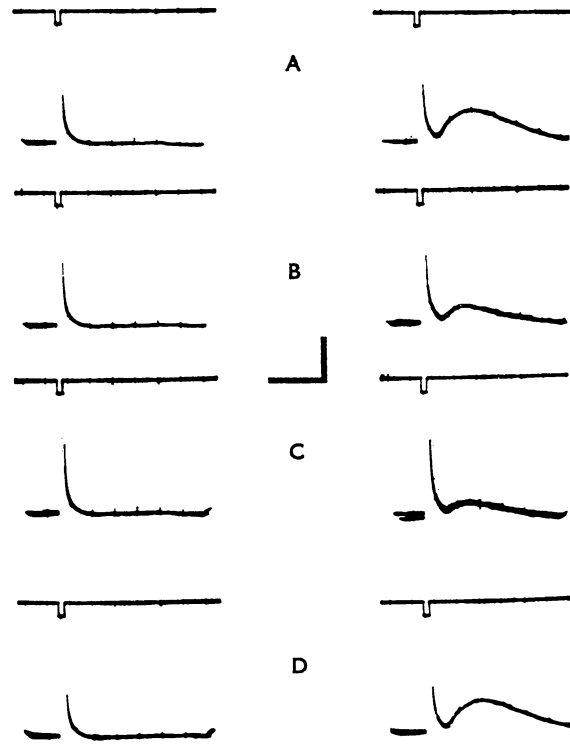

FIG. 2. Effect of adrenaline on the current threshold of presynaptic nerve terminals. Left vertical column, responses at approximately 0·9×threshold; right vertical column, responses at approximately 1·0×threshold. A, Control; B, adrenaline (10^{-5}M), 1 min; C, adrenaline (10^{-5}M), 2 min; D, control, 5 min after adrenaline. Calibrations: 20 mV (4×10^{-5}A) and 5 ms.

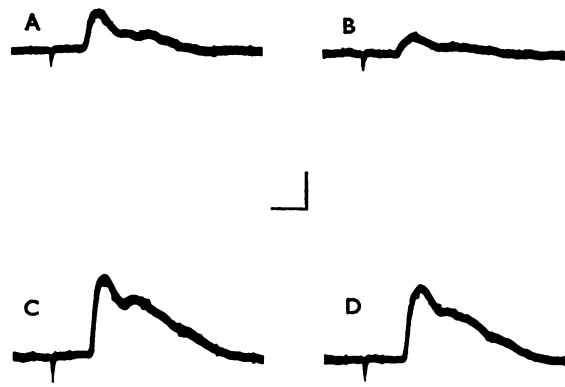

FIG. 3. Effect of adrenaline (10^{-5}M) in 2 mM $CaCl_2$ and in 10 mM $CaCl_2$. A, Control (2 mM $CaCl_2$); B, adrenaline; C, 10 mM $CaCl_2$; D, 10 mM $CaCl_2$ and adrenaline. Calibrations: 10 mV and 10 ms.

$CaCl_2$. As shown in Fig. 3 adrenaline was very effective in the control medium, but much less effective in a medium containing 10 mM $CaCl_2$. In four cells adrenaline depressed the e.p.s.p. by an average of 68% in control, as compared with only 16% in 10 mM $CaCl_2$. Adrenaline was considerably more effective in 5 mM $CaCl_2$ than in 10 mM $CaCl_2$. The depression in control (44%) and in 5 mM $CaCl_2$ (37%) was not significantly different.

Tetanus

Quantal content (m) of the e.p.s.p. is dependent on the number of quanta readily available for release (n) and the probability of release for each quantum (p), according to the relationship, $m=np$. Adrenaline decreases m, and it is interesting whether this action is due to a decrease in n, p or both. The e.p.s.ps declined in amplitude when the presynaptic nerve was stimulated at frequencies of 30–50 Hz. After the initial decline (first five e.p.s.ps), the e.p.s.p. amplitude levels off to a constant value. This is probably the point at which mobilization equals release. The initial decline is due to depletion of n (Liley & North, 1953 ; Takeuchi, 1958 ; Thies, 1965). When m of each e.p.s.p. in the head of the train is plotted against depletion (sum of previous m in a train), the slope of the line is equal to p and the intercept of the X-axis is equal to n (Elmqvist & Quastel, 1965).

These calculations are based on two assumptions. First, p is constant during the tetanus. In several cells the second e.p.s.p. was larger in amplitude than the first e.p.s.p. After this initial increase, the e.p.s.ps decreased linearly ; therefore p must be constant after the first e.p.s.p. in each train. Second, q=(variance of e.p.s.ps)/(mean e.p.s.p.). This relationship applies only if the e.p.s.ps are Poisson distributed. Release follows a Poisson distribution at the neuromuscular junction and appears to follow a Poisson distribution at the ganglion (see Fig. 8, Christ & Nishi, 1970). Furthermore the potential changes from single quantum must follow a normal distribution. The multiple innervation of single ganglion cells will result in a polymodal distribution for the amplitudes of single quantum. However, when only a single preganglionic nerve is stimulated, as with submaximal stimulation, the quantum amplitudes should be normally distributed. Thus the values calculated for m, n and p should be very good estimates.

Control values for p were consistently near 0·2, but values for n were quite variable between cells. Adrenaline did not significantly change q (0·25–0·24 mV). This confirms the conclusion that adrenaline acts through a presynaptic mechanism. m was decreased by adrenaline to 65% of control (Table 1). This decrease in m was primarily due to a decrease in n (71% of control) ; p was not significantly decreased (96% of control).

The effect of calcium ions was tested on the ganglion (Table 2). In addition to a postsynaptic action (increased membrane resistance, resulting in an increased q), 5 mM $CaCl_2$ exhibited potent effects on quantal release. Calcium increased m. This change of m was due primarily to an increase in p.

Discussion

There are many theoretical mechanisms whereby adrenaline can decrease the release of transmitter from the preganglionic neurons. The most obvious possibility involves a change in the resting membrane potential of the nerve terminal. The

absence of any effect by adrenaline on the threshold of the nerve terminal indicates that adrenaline does not alter the membrane potential of the nerve terminal.

The absence of a presynaptic potential after perfusion with adrenaline in the sucrose-gap experiments supports this conclusion. Furthermore results in our previous paper (Christ & Nishi, 1970) are not consistent with a mechanism involving a change of membrane potential. Adrenaline decreased m.e.p.s.p. frequency and decreased e.p.s.p. amplitude. These effects are probably not due to a change in presynaptic membrane potential, as changes in the presynaptic membrane potential should affect m.e.p.s.p. frequency and e.p.s.p. amplitude in a reciprocal manner (del Castillo & Katz, 1954; Liley, 1956; Hubbard & Willis, 1962, 1968; Vladimirova, 1964).

TABLE 1. *Changes in the release parameters* (m, n & p) *induced by adrenaline* (10^{-5}M)

Expt.	Control	Adrenaline		% Control
		m (quanta)		
1	39	27		69
2	99	66		67
3	246	152		62
4	213	135		63
			Mean	65†
		p		
1	0·23	0·22		96
2	0·20	0·17		85
3	0·22	0·16		73
4	0·13	0·17		131
			Mean	96
		n (quanta)		
1	170	123		72
2	507	376		74
3	1090	953		87
4	1575	798		51
			Mean	71*

* Significant at $P=0{\cdot}1$; † significant at $P=0{\cdot}05$.

TABLE 2. *Changes in the release parameters* (m, n & p) *induced by* 5 mM $CaCl_2$

Expt.	Control	Ca^{++}		% Control
		m (quanta)		
1	271	420		155
2	54	47		87
3	64	109		170
4	116	160		138
			Mean	138
		p		
1	0·22	0·33		150
2	0·14	0·19		136
3	0·21	0·40		191
4	0·26	0·49		188
			Mean	166†
		n (quanta)		
1	1260	1269		101
2	377	254		67
3	304	276		91
4	447	329		74
			Mean	83

† Significant at $P=0{\cdot}05$.

The inability of adrenaline to change the threshold of the nerve terminal also eliminates the possibility that adrenaline produces its effect by a local anaesthetic action (decrease of sodium activation). Such an action should cause a significant increase in threshold.

Krnjevic & Miledi (1958) observed that adrenaline decreased failure of propagation in the motor nerve terminals during tetanic stimulation. An increase of propagation failure rate would produce blockade at the ganglion. This blockade would not be an all or none phenomenon due to the multiple synaptic input to a single postganglionic cell. Yet this mechanism does not appear to be occurring, as adrenaline was effective even when the nerve terminal was directly stimulated. If it is assumed that the site of stimulation was distal to the branching of the terminal, this result indicates that adrenaline is acting at a site distal to the branching.

The decrease of quantal release, without potential changes in the nerve terminal membrane, indicates that adrenaline acts directly on the release mechanism. So far, the steps involved in excitation-release coupling have not been completely elucidated. The best known step in the release mechanism is the influx of calcium ions during the action potential (Katz & Miledi, 1968). Adrenaline could produce blockade by decreasing the entry of calcium ions. This hypothesis is supported by the observation that adrenaline is less effective in 10 mM $CaCl_2$, than in the control solution. The release sites may be completely activated in 10 mM $CaCl_2$; thus a decrease in calcium entry by adrenaline cannot alter significantly the quantal release.

Alternatively, this result may be a non-specific effect. High concentrations of calcium can be expected to block any drug induced depressant action, if the site of drug action is previous to the utilization of calcium in the release mechanism. A further argument against the calcium mechanism can be presented. The actions of calcium and adrenaline on release are quite different. Calcium appears to regulate the probability of release, but adrenaline acts primarily by decreasing the available stores of transmitter.

The role of sodium ions in excitation release coupling is still controversial (Kelly, 1965; Katz & Miledi, 1967). In the presence of a low calcium concentration, a decrease in the extracellular sodium concentration results in an increase of transmitter release (Rahamimoff & Colomo, 1967; Colomo & Rahamimoff, 1968). Alternatively, increasing the sodium concentration should decrease release. Adrenaline may potentiate the actions of sodium ions, thereby producing blockade. This hypothesis would be strengthened if it could be observed that sodium ions are involved in the regulation of the size of the available store. Sodium ions are involved in synthesis of acetylcholine (Birks, 1963), and may also regulate transmitter stores.

Adrenaline has a potent facilitatory action at the neuromuscular junction, and may be producing its effects at neuromuscular and ganglionic synapses by a similar mechanism, although the end result is opposite. Bowman & Nott (1969) hypothesized that adrenaline unbinds calcium through a mechanism involving cyclic 3′,5′-AMP. This hypothesis is supported by the observation that caffeine potentiates the action of adrenaline at the neuromuscular junction. However, caffeine has no action at the ganglion. This indicates that adrenaline action at the ganglion does not involve cyclic 3′,5′-AMP.

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