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Effect of amphetamine on the transmission of repetitive impulses through the isolated superior cervical ganglion of the rat

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Summary

1. The cervical sympathetic trunk of the isolated rat superior cervical ganglion was stimulated with short bursts of repetitive pulses. At room temperature with rates of stimulation of 4 Hz and above, the ganglionic action potentials were reduced in size.

2. Amphetamine ($2{\cdot}7 \times 10^{-5}$M), which caused some depression of transmission during stimulation at 0·1 Hz, caused a partial reversal of the depression of transmission occurring with rates of stimulation above 4 Hz.

3. This action of amphetamine was mimicked by adrenaline (3×10^{-5}M) and noradrenaline ($9{\cdot}6 \times 10^{-5}$M) but not by isoprenaline ($1{\cdot}8 \times 10^{-5}$M) and was unaffected by propranolol ($1{\cdot}4 \times 10^{-5}$M) but was abolished by prior application of phenoxybenzamine ($5{\cdot}8 \times 10^{-6}$M). Furthermore, this action of amphetamine was unaltered in ganglia taken from rats pretreated with reserpine (single dose of 6 mg/kg, 16 h before dissection).

4. Amphetamine had no effect on the surface potentials of the ganglion or on changes in these potentials produced by concentrations of carbachol ($5{\cdot}5 \times 10^{-6}$M to $5{\cdot}5 \times 10^{-3}$M).

5. It is concluded that amphetamine has a direct action on α-adrenoceptors situated at presynaptic sites.

Introduction

The first report of an action of amphetamine on autonomic ganglia was by Kewitz & Reinert (1954), who showed that it stimulated the perfused superior cervical ganglion of the cat, stimulation being followed by a depression in the sensitivity to injected acetylcholine (ACh), an action later reported to be due to a 'nicotine like' depolarization of the ganglion cells (Gold & Reinert 1960). Reinert (1960) suggested that a similar depolarizing action might account for some of the central actions of amphetamine.

The work below describes an action by amphetamine of facilitating transmission through the isolated superior cervical ganglion of the rat when transmission is depressed during repetitive stimulation of the preganglionic nerve.

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Methods

Experiments were performed on isolated superior cervical ganglia of the rat. For transmission studies the technique was essentially similar to that described for the isolated rabbit superior cervical ganglion by Elliott (1965).

Wistar strain albino rats of either sex (150–250 g) were anaesthetized with an intraperitoneal injection of a 20% (W/V) solution of urethane (1·0–1·2 g/kg).

The right superior cervical ganglion was removed together with approximately 10–15 mm of cervical sympathetic nerve and 2–3 mm of internal carotid nerve. The connective tissue sheath was then removed from the entire preparation with the ganglion bathed in Krebs solution previously equilibrated with 95% O_2 and 5% CO_2 (pH7·4) at room temperature (19–25° C).

For transmission studies, the preparation was mounted horizontally in a 50 ml bath of Krebs solution and 'bubbled' with the same gas mixture used for equilibration.

Rectangular pulses of 0·5 ms duration were applied to the cervical sympathetic trunk from an electronic stimulator (Attree 1950) which was isolated from the preparation by a 1:1 transformer (Gardener 30570). For most experiments, the cervical sympathetic trunk was stimulated with 4·5 s bursts of stimuli, at 30 s intervals in ascending order of frequency at 1, 2, 4, 6, 8 and 10 Hz. For stimulation and recording, the preparation was raised to just above the level of the bath fluid. A stimulation voltage was chosen which was supramaximal for the main (Sa) spike of the ganglionic action potential.

Preganglionic and ganglionic action potentials were recorded from the surface of the preparation by two pairs of silver/silver chloride/saline agar electrodes with balsa wood wicks. One pair recorded potentials from the preganglionic nerve, the other pair recorded potentials between the body of the ganglion and the crushed end of the internal carotid nerve. The responses were amplified by the d.c. preamplifiers of a Solartron C.D. 1183 oscilloscope and photographed on stationary film.

Drugs were added to the bath fluid in cumulative concentrations 15 min contact being allowed for each concentration before recordings were made.

Depolarization of the ganglion by carbachol was measured with the preparation mounted vertically, postganglionic nerve uppermost, using the moving fluid electrode technique of Fatt (1950) as adapted to the ganglion by Pascoe (1956) and modified by Brown (1966).

The drugs used were (±)-amphetamine sulphate, (−)-noradrenaline hydrogen tartrate, (−)-adrenaline hydrogen tartrate, (±)-isoprenaline sulphate, phenoxybenzamine hydrochloride, propranolol hydrochloride, carbachol chloride and reserpine.

Results

Transmission: 'single' stimuli

With a stimulation rate of 0·1 Hz to the preganglionic nerve, amphetamine caused a depression of the ganglionic action potentials with concentrations of $6{\cdot}5 \times 10^{-6}$M–$8{\cdot}6 \times 10^{-4}$M. Transmission was blocked with concentrations above 4×10^{-4}M, as judged by a lack of any spike component of the ganglionic action potential. A

significant depression of preganglionic action potentials was seen with concentrations above $1{\cdot}1 \times 10^{-4}$M (Fig. 1).

Repetitive stimuli

As the rate of stimulation of the preganglionic nerve was increased, the height of the ganglionic action potentials decreased with little effect on preganglionic action potentials (Fig. 2A). At the highest rate of stimulation used (10 Hz) the ganglionic action potentials were usually reduced to within 10% of the control at 1 Hz.

Typical results obtained with a low concentration of amphetamine ($2{\cdot}7 \times 10^{-5}$M) are shown in Fig. 2B.

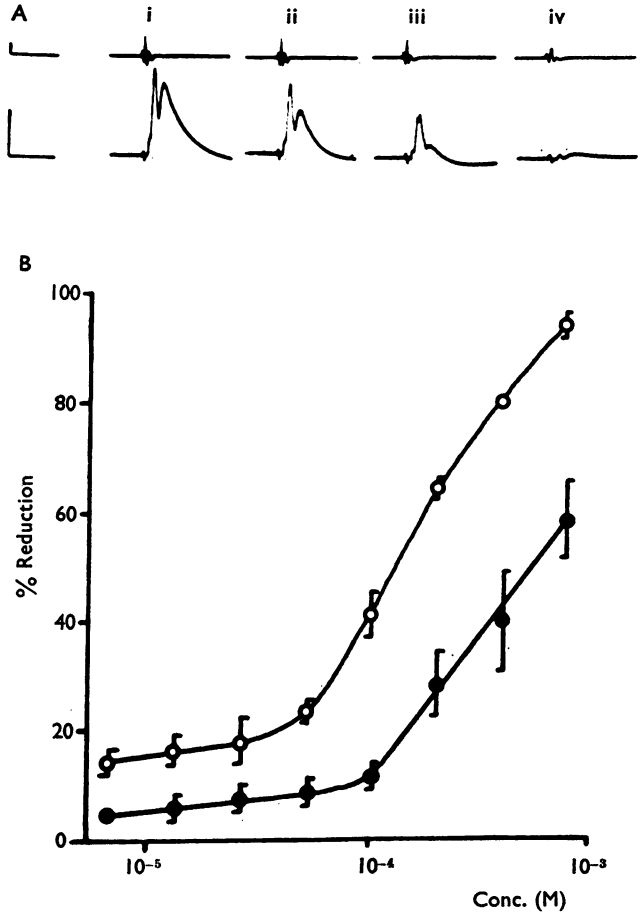

FIG. 1. Effect of amphetamine on preganglionic and ganglionic action potentials. The preganglionic nerve was stimulated with supramaximal shocks at 0·1 Hz. (A), Typical records obtained after contact for 15 min with various concentrations of amphetamine; upper record, preganglionic action potential; lower record, ganglionic action potential; (i), control; (ii), $2{\cdot}7 \times 10^{-5}$M; (iii), $2{\cdot}2 \times 10^{-4}$M; (iv), $8{\cdot}6 \times 10^{-4}$M amphetamine. Horizontal and vertical calibrations are 200 ms and 2 mV respectively. (B), Combined results; ordinate, percentage reduction of action potential height; abscissa, concentration of amphetamine. (○——○), Ganglionic action potentials; (●——●), preganglionic action potentials. Values are the mean ± standard error (S.E.M.). (n=5.)

Amphetamine caused a partial reversal of the depression of transmission seen with rates of stimulation above 4 Hz with no effects on the conduction of preganglionic action potentials.

A similar reversal of the depression of transmission seen with high rates of stimulation was observed with low concentrations of noradrenaline ($9{\cdot}6 \times 10^{-5}$M) and adrenaline (3×10^{-5}M) but not by isoprenaline ($1{\cdot}8 \times 10^{-5}$M). (Fig. 3). These concentrations were chosen since they caused a similar reduction of single (0·1 Hz) ganglionic action potentials.

When the concentration of amphetamine, adrenaline or noradrenaline was raised above that necessary to depress single ganglionic action potentials by more than 50%, the salutory effect of the drugs on transmission during repetitive stimulation became progressively less marked. Furthermore, low concentrations of the drugs which had no depressant effect on single transmitted action potentials were without action on the transmission of repetitive stimuli.

The effects of pretreating the preparation with selective α-(phenoxybenzamine) and β-(propranolol) adrenoceptor blocking agents were studied on these ganglionic actions of amphetamine, adrenaline and noradrenaline. For these experiments stimulation rates of 0·1, 1, 6 and 8 Hz only were used.

Propranolol, in concentrations of up to $1{\cdot}4 \times 10^{-5}$M given 20 min before amphetamine, caused up to 20% reduction of single ganglionic action potentials (0·1 Hz) but had no effect on the action of amphetamine. On the other hand, phenoxybenzamine ($5{\cdot}8 \times 10^{-6}$M) given 20 min before amphetamine, caused no reduction of single ganglionic action potentials, but prevented the partial improvement in repetitive transmission produced by amphetamine (Fig. 4A, B). Similar results were obtained with propranolol and phenoxybenzamine against adrenaline (3×10^{-5}M) and noradrenaline ($9{\cdot}6 \times 10^{-5}$M).

In order to determine whether this action of amphetamine was due to a direct action or to a release of catecholamines from within the ganglion, five rats were pretreated with a single dose of reserpine (6 mg/kg i.p.), 16 h before dissection, which caused complete ptosis in all animals.

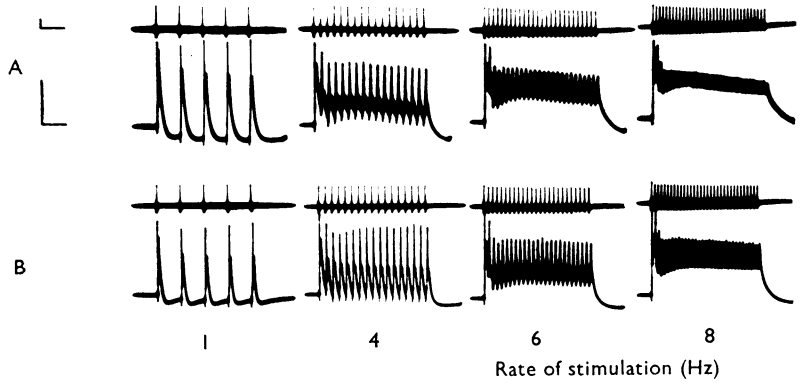

FIG. 2. Typical records showing the effect of amphetamine on preganglionic (upper beam) and ganglionic (lower beam) action potentials. The preganglionic nerve was stimulated with short bursts of repetitive pulses at rates shown. (A), Control responses. (B), Responses after application of amphetamine ($2{\cdot}7 \times 10^{-5}$M) for 15 minutes. Horizontal and vertical calibrations are one second and 2 mV respectively.

Amphetamine had actions on ganglia taken from these rats similar to those observed on ganglia taken from untreated rats (c.f. Fig. 4A & 4C).

Depolarization

The moving-fluid electrode technique was used to investigate the action of amphetamine on the ganglion cells. No changes in the demarcation potential were observed with amphetamine alone in concentrations of up to $8{\cdot}6 \times 10^{-4}$M, kept in contact with the tissue for 15 minutes.

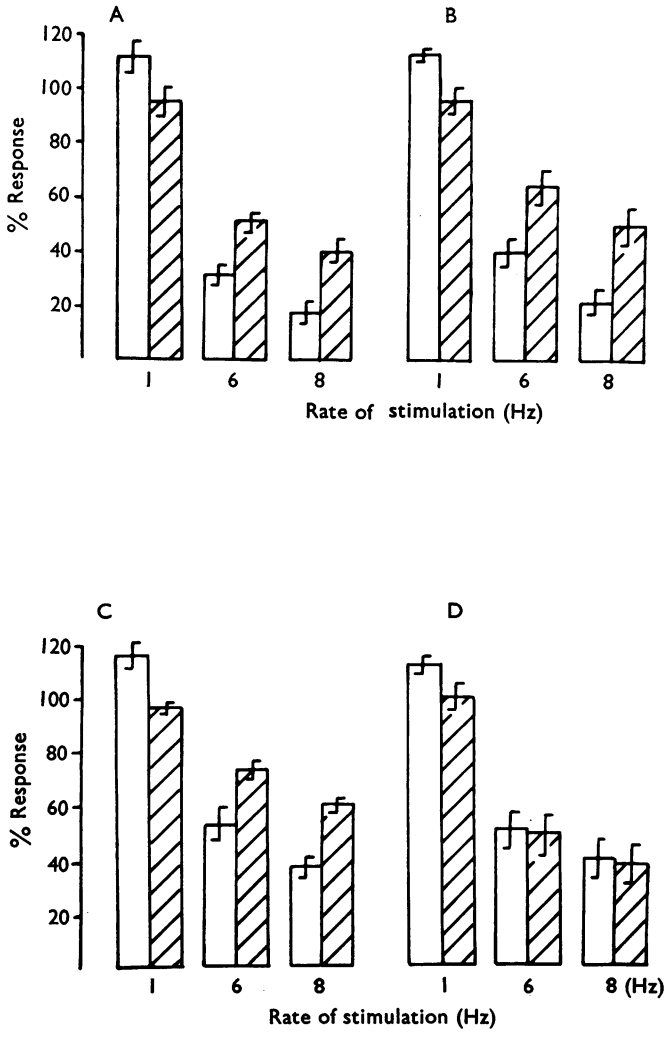

FIG. 3. Effect of drugs on ganglionic action potentials when the preganglionic nerve was stimulated with short burst of repetitive pulses at rates shown. (A), Amphetamine ($2{\cdot}7 \times 10^{-5}$) (17·5%). (B), Adrenaline (3×10^{-5}) (22·0%). (C), Noradrenaline ($9{\cdot}6 \times 10^{-5}$) (23·0%). (D) Isoprenaline ($1{\cdot}8 \times 10^{-5}$) (13·0%). Ordinate, the magnitude of the fifth action potential at each stimulation rate is expressed as a percentage of the first action potential at a stimulation rate of 1 Hz. Open columns, control responses; cross hatched columns, responses after contact for 15 min with drug in concentrations given. Percentages in parentheses are the mean reduction of single (0·1 Hz) ganglionic action potentials with these concentrations. Vertical bars are S.E.M., for each drug. $n=5$.

In order to study the possible interactions of amphetamine with cholinergic agonists on the ganglion cell receptors, dose/depolarization curves to carbachol were constructed. Four concentrations of carbachol were used for each curve ($5{\cdot}5 \times 10^{-6}$, $5{\cdot}5 \times 10^{-5}$, $5{\cdot}5 \times 10^{-4}$ and $5{\cdot}5 \times 10^{-3}$M). A concentration of $5{\cdot}5 \times 10^{-4}$M carbachol is sufficient to cause a maximum depolarization of the ganglion (Brown, 1966); a higher concentration ($5{\cdot}5 \times 10^{-3}$M) was included here to cause some desensitization of the receptors as demonstrated by the inability of the ganglion to maintain a constant level of depolarization over several minutes with this concentration (Fig. 5B).

Amphetamine ($2{\cdot}7 \times 10^{-5}$M) did not cause any significant change in the dose response curves of depolarization or hyperpolarization (after washing) to carbachol

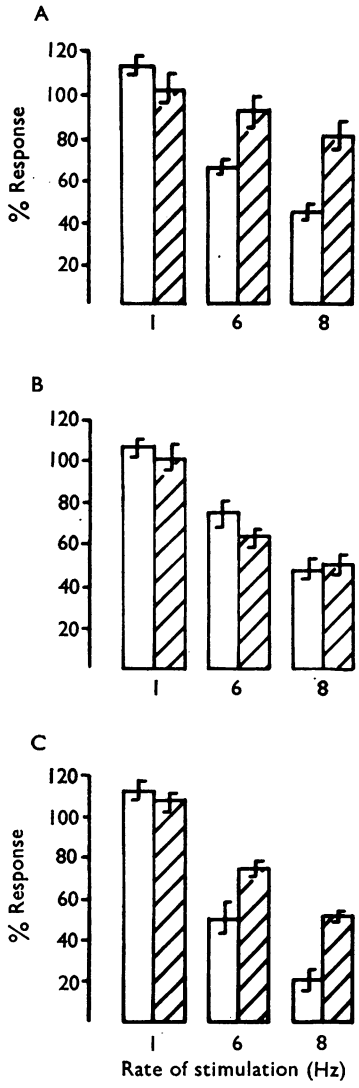

FIG. 4. Influence of phenoxybenzamine and reserpine on the effect of amphetamine. Results presented in the same way as in Fig. 3. (A), Control response to amphetamine ($2{\cdot}7 \times 10^{-5}$M). (B), Amphetamine ($2{\cdot}7 \times 10^{-5}$M) after contact with phenoxybenzamine ($5{\cdot}8 \times 10^{-6}$M) for 20 minutes. (C), Amphetamine ($2{\cdot}7 \times 10^{-5}$M) on ganglia taken from rats pretreated with reserpine (6 mg/kg. i.p.; single dose). Vertical bars are S.E.M. In each case $n=5$.

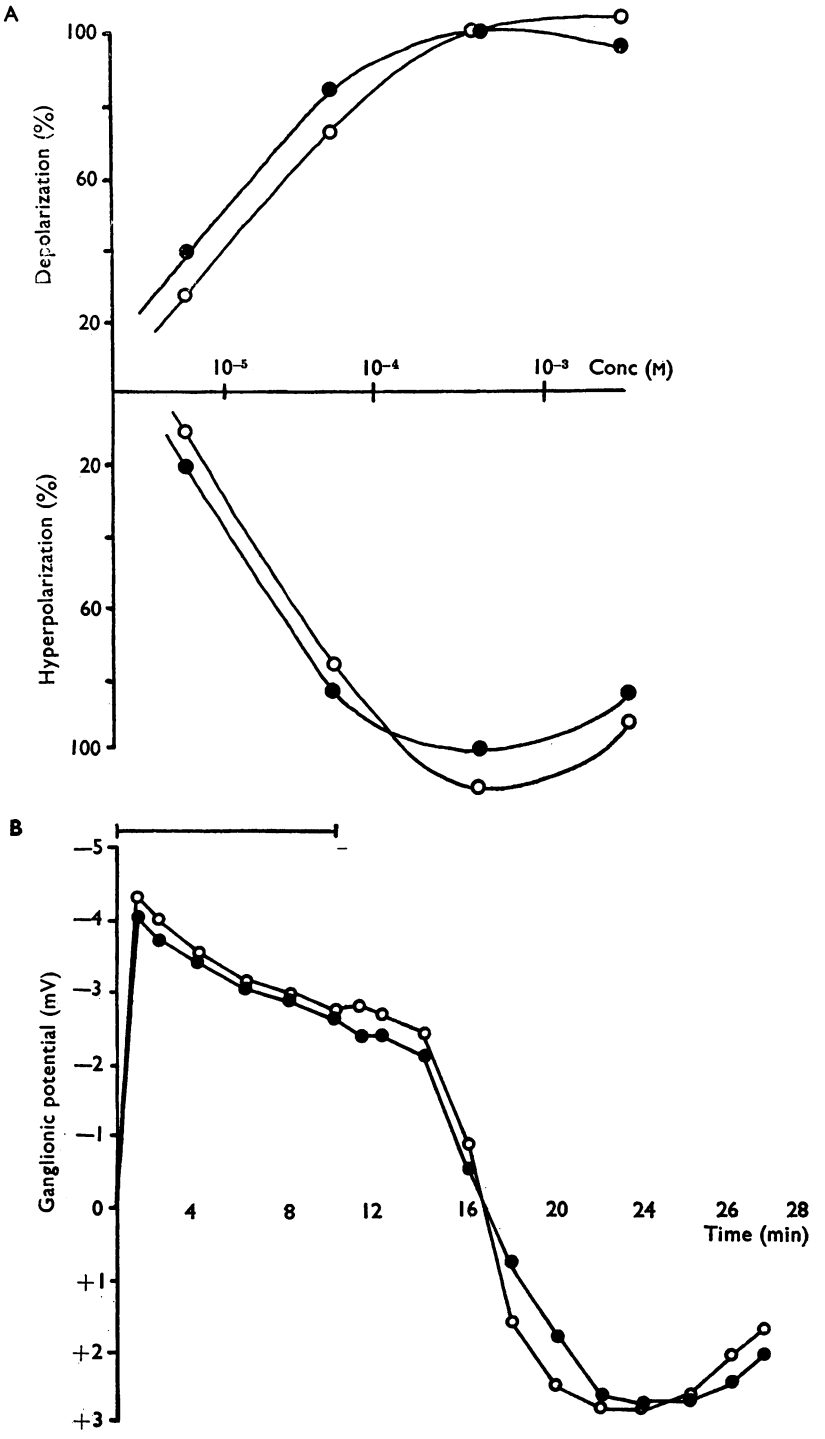

FIG. 5. Effect of amphetamine ($2{\cdot}7\times10^{-5}$M) on changes in surface potentials of the ganglion produced by carbachol. (A), Mean of three experiments showing the lack of effect of amphetamine (○——○) on control (●——●) dose-response curves of depolarization and hyperpolarization (after washing) produced by the concentrations of carbachol shown. Ordinate expresses the maximum response obtained with each concentration of carbachol as a percentage of the maximum values obtained with $5{\cdot}5\times10^{-4}$M carbachol. (B), Typical effect of amphetamine ($2{\cdot}7\times10^{-5}$M) (○——○) on the time course of depolarization and hyperpolarization produced by $5{\cdot}5\times10^{-3}$M carbachol (●——●) in contact with the preparation for 10 min as shown by the horizontal bar above the record.

(Fig. 5A), and furthermore, amphetamine had no effect on the time course of depolarization and hyperpolarization seen with $5{\cdot}5 \times 10^{-3}$M carbachol (Fig. 5B). Adrenaline (3×10^{-5}M) and noradrenaline ($9{\cdot}6 \times 10^{-5}$M) were similarly without effect.

Discussion

The effect of amphetamine in causing a partial reversal of the depression of ganglionic transmission occurring during repetitive stimulation of the preganglionic nerve, appears likely to be due to an action on α-adrenoceptors since this effect is (1) mimicked by adrenaline and noradrenaline but not isoprenaline, and (2) is abolished by phenoxybenzamine but unaffected by propranolol. A direct action of the amphetamine is suggested by an apparently unaltered action in ganglia taken from reserpinized rats. Because amphetamine only increases transmission, when transmission is depressed during repetitive stimulation, the mechanism of amphetamine action and the cause of the depression may be related.

The mechanisms of transmission failure in autonomic ganglia during repetitive stimulation are still largely unknown. Birks & MacIntosh (1961) measured ACh output from the perfused superior cervical ganglion of the cat and considered that a reduction of 'volley output' was unlikely following prolonged high frequency stimulation. Using less direct experimental criteria from the isolated superior cervical ganglion of the rabbit, Elliott (1965) considered it probable that there was a diminished output of ACh during the first few seconds of stimulation.

The mechanisms underlying transmission failure in autonomic ganglia during repetitive stimulation of the preganglionic nerve may be similar to those responsible for transmission failure at the skeletal neuromuscular junction under similar experimental conditions. At the latter site initial failure may arise from a desensitization of end-plate receptors (Thesleff, 1959), and more protracted failure may be due to a reduction of ACh release (Straughan, 1960). A presynaptic site of action for amphetamine is indicated in the present study because of a lack of any demonstrable action on the surface potentials of the ganglion or on changes in these potentials produced by carbachol.

Amphetamine may act by increasing the amount of ACh released, since Birks & MacIntosh (1961) showed that low doses of adrenaline could increase the output of ACh from repetitively stimulated superior cervical ganglia of the cat.

A potentiation of ACh release by an action on presynaptic α-adrenoceptors, has been suggested as the probable mechanism of action of adrenaline in enhancing skeletal neuromuscular transmission when this is depressed during repetitive stimulation (Bowman & Raper, 1966 ; Jenkinson, Stamenović & Whitaker, 1968 ; Hidaka & Kuriyama, 1969).

This action of adrenaline on the skeletal neuromuscular junction was suggested by Bowman & Raper (1966) to be due to a weak hyperpolarization of the nerve endings.

A similar action by amphetamine on the presynaptic terminals in the ganglion seems likely since the superior cervical trunk of the rat is largely composed of C fibres (Dunant, 1967) and Goffart & Holmes (1962) have shown that adrenaline can hyperpolarize C fibres in the cat.

On the other hand, Christ & Nishi (1971a, b) were unable to demonstrate a hyperpolarizing action of adrenaline on nerve terminals in the isolated superior cervical

ganglion of the rabbit with concentrations which depressed transmission by a selective action on presynaptic α-adrenoceptors.

If the depression of transmission in the rat ganglion during brief periods of repetitive stimulation were due to a desensitization of the postsynaptic cholinergic receptors caused by a high local concentration of ACh, then transmission might be enhanced if amphetamine acted to reduce ACh release.

This latter hypothesis is in keeping with the observations of Christ & Nishi (1971a & b) on the rabbit and the depressant effects of amphetamine on preganglionic conduction observed in the present study ; furthermore, this hypothesis assumes only a single (depressant) action of amphetamine irrespective of the rate of stimulation.

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