Br. J. Pharmac. Chemother. (1967), **29**, 436–444.

EFFECTS OF EXOGENOUS CHOLINE ON ADRENERGIC RESPONSES OF THE GUINEA-PIG VAS DEFERENS

BY

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(*Received March* 13, 1967)

During pharmacological studies of the guinea-pig vas deferens it was observed that high concentrations of choline potentiated the contractile responses to post-ganglionic nerve stimulation.

Choline has been reported to possess weak cholinergic stimulatory activity at both nicotinic and muscarinic sites (Dale, 1914 ; Le Heux, 1921 ; Fatt, 1950 ; Gebber & Volle, 1965). Anti-curare (Hutter, 1952) and anticholinesterase (del Castillo & Katz, 1957) actions have also been attributed to it, and it enhances the release of acetylcholine from preganglionic nerve endings (Brown & Feldberg, 1936 ; Matthews, 1963 ; Desiraju, 1966).

In the present paper the effects of choline on contractile responses of the vas deferens to nerve stimulation and applied noradrenaline have been correlated with its effects on transmission to single smooth muscle cells.

METHODS

In experiments concerned with measurement of contractile responses the isolated guinea-pig hypogastric nerve vas deferens preparation was mounted in a 50 ml. bath containing modified Krebs solution (Huković, 1961). The bathing solution was aerated with 95% oxygen and 5% carbon dioxide and maintained at 36° C. Contractions of the vas deferens were recorded with a frontal point writing lever on a smoked drum. Post-ganglionic nerve stimulation was normally elicited by means of shielded platinum ring electrodes placed around the hypogastric plexus approximately 1 mm from its junction with the vas deferens. Square wave pulses of 2 msec duration were delivered with a Grass S5 stimulator at a frequency of 10–20 pulses/sec for 10 sec every 90 sec. Responses produced by this method of stimulation were abolished by cutting the hypogastric plexus distal to the electrodes, indicating that no direct muscle stimulation was involved. Experiments concerned with responses to applied noradrenaline were performed using vasa deferentia stripped of mesenteric investment (Bentley & Sabine, 1963). In some of these experiments intramural nerve stimulation was elicited by means of shielded platinum electrodes placed around the base of the vas deferens.

In electrophysiological experiments the isolated hypogastric nerve–vas deferens preparation was pinned down on a Perspex block in a 10 ml. bath. Modified Krebs solution maintained at 35.5–36° C was run through the bath at a rate of approximately 2 ml./min. Postganglionic nerve stimulation was elicited with shielded platinum electrodes placed around the hypogastric plexus, and square wave pulses of 2 msec duration were delivered with a Grass S4 stimulator—at frequencies of 1–2/sec. Intracellular electrical events in the longitudinal musculature were recorded with capillary microelectrodes filled with 2 M KCl and having a tip resistance of 20–80 M Ω. The criterion used for successful impalement of a cell was an action potential with an overshoot of at least 10 mV.

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Drugs used were: choline chloride, hyoscine bromide, noradrenaline bitartrate and physostigmine sulphate. Concentrations cited refer to the final concentration of these salts in the bath. In organ bath experiments the drugs were dissolved in distilled water and added to the bath in volumes of not more than 0.5 ml. In electrophysiological experiments the drugs were flowed into the bath with the bathing medium. Contractile responses of the stripped vas deferens to noradrenaline were measured using a 30 sec contact time and a 5–7 min cycle.

The statistical significance of differences was tested using Student's t test (Fisher, 1936) on the assumption that the difference was not significant.

RESULTS

Organ bath experiments

Choline (10^{-4}–5×10^{-4} g/ml.) produced 10–100% potentiation of the contractile response to post-ganglionic nerve stimulation (Fig. 1a). The mean potentiation obtained with 5×10^{-4} g choline/ml. was 35% (7 experiments). The response to applied noradrenaline (10^{-6}–2×10^{-6} g/ml.) was more extensively potentiated (60–400%), the mean potentiation obtained with 5×10^{-4} g choline/ml. being 160% (7 experiments) (Fig. 1b). The potentiating effect of choline on contractile responses appeared rapidly, was rapidly reversed on washing out the bath, and was reproducible over up to four successive applications of choline.

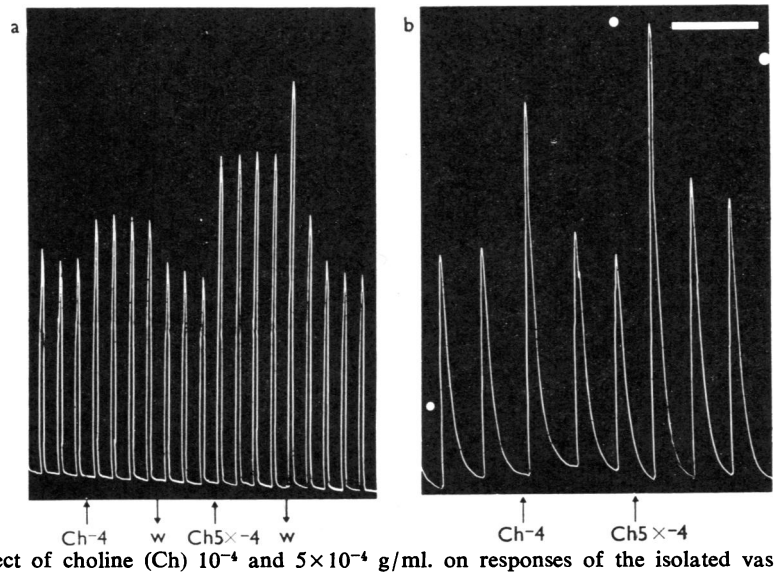

Fig. 1. Effect of choline (Ch) 10^{-4} and 5×10^{-4} g/ml. on responses of the isolated vas deferens to (a) post-ganglionic nerve stimulation (20 pulses/sec) and (b) applied noradrenaline 10^{-6} g/ml. W denotes wash. Time marker: 10 min.

Pretreatment of the tissue with hyoscine (10^{-6} g/ml.) for 10 min completely abolished the potentiating effect of 10^{-4} g choline/ml. on responses to both nerve stimulation and applied noradrenaline, and completely or nearly completely abolished the effect of 5×10^{-4} g choline/ml. (Fig. 2).

Following treatment of the tissue with physostigmine (2×10^{-6} g/ml.) for 30 min, the potentiating effect of 5×10^{-4} g choline/ml. on the nerve-mediated response was reversed to a slight depression (Fig. 3). In these circumstances it was still possible to potentiate the response by addition of 10^{-6} g noradrenaline/ml., indicating that the lack of poten-

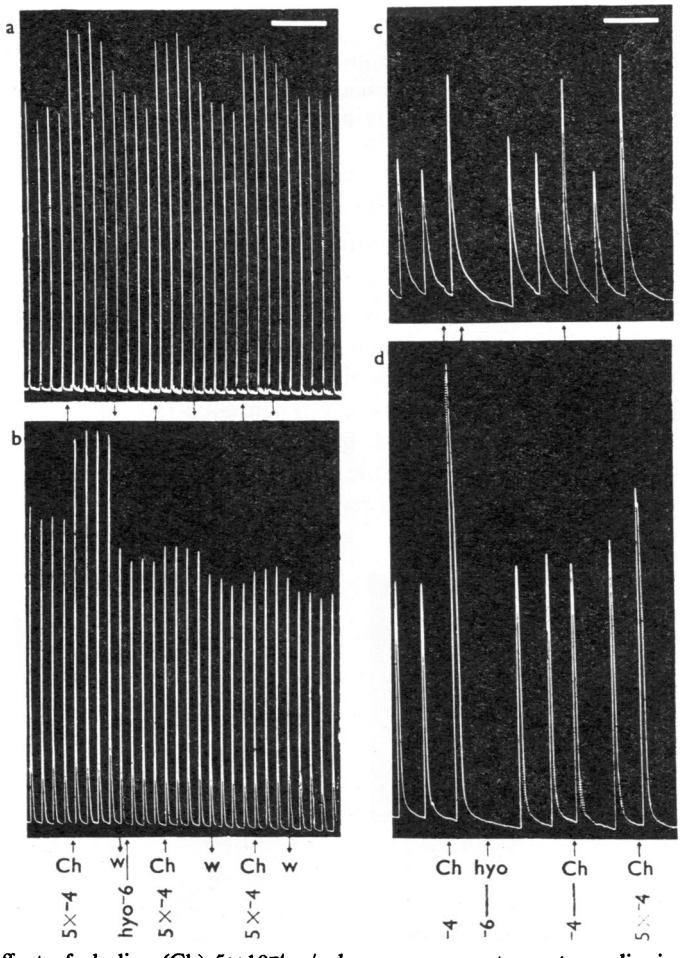

Fig. 2. (a, b) Effect of choline (Ch) 5×10^{-4} g/ml. on responses to post-ganglionic nerve stimulation (20 pulses/sec) of paired vasa deferentia. At (hyo), hyoscine 10^{-6} g/ml. was added to (b). W denotes wash. Time marker: 10 min. (c, d) Effect of choline (Ch) 10^{-4} and 5×10^{-4} g/ml. on responses to applied noradrenaline 10^{-6} g/ml. of paired stripped vasa deferentia. At (hyo), hyoscine 10^{-6} g/ml. was added to (d). Time marker: 10 min.

tiation by choline was not due to the fact that the tissue was already contracting maximally.

Physostigmine treatment did not alter the response of the stripped vas deferens to applied noradrenaline. In these preparations choline potentiated the response to noradrenaline just as it did before physostigmine treatment. However when the intramural nerves were stimulated intermittently subsequent to exposure of the tissue to physostigmine, the responses to applied noradrenaline were potentiated in 4 out of 5 experiments performed. The degree of potentiation caused by this procedure was roughly the same as the degree of potentiation caused by 5×10^{-4} g choline/ml. prior to treatment with physostigmine. In these preparations no further potentiation of the response to noradrenaline was induced by choline (Fig. 4).

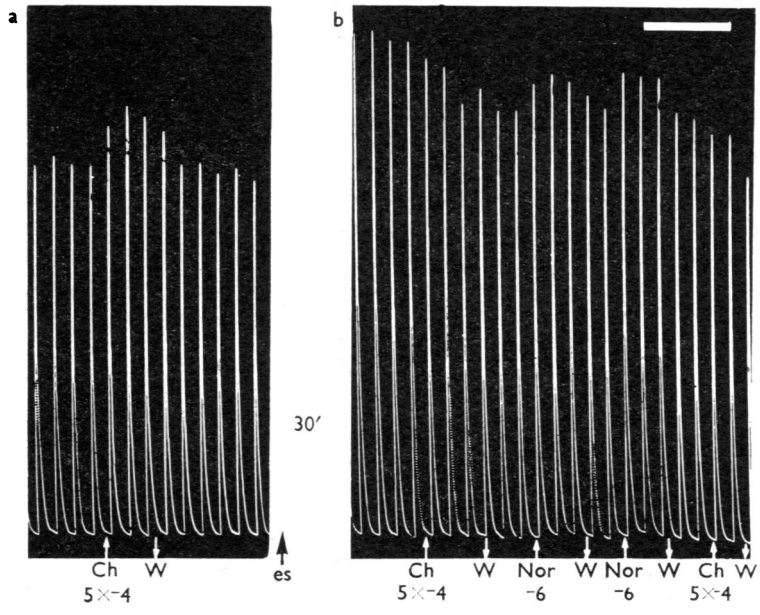

Fig. 3. Effect of choline (Ch) 5×10^{-4} g/ml. on responses to post-ganglionic nerve stimulation (20 pulses/sec) under normal conditions (a) and 40 min after addition of physostigmine (es) 2×10^{-6} g/ml. to the bath (b). Note the reversal of the choline-induced potentiation to a slight depression, and the ability of noradrenaline (Nor) 10^{-6} g/ml. to induce potentiation after physostigmine treatment. W denotes wash. Time marker: 10 min.

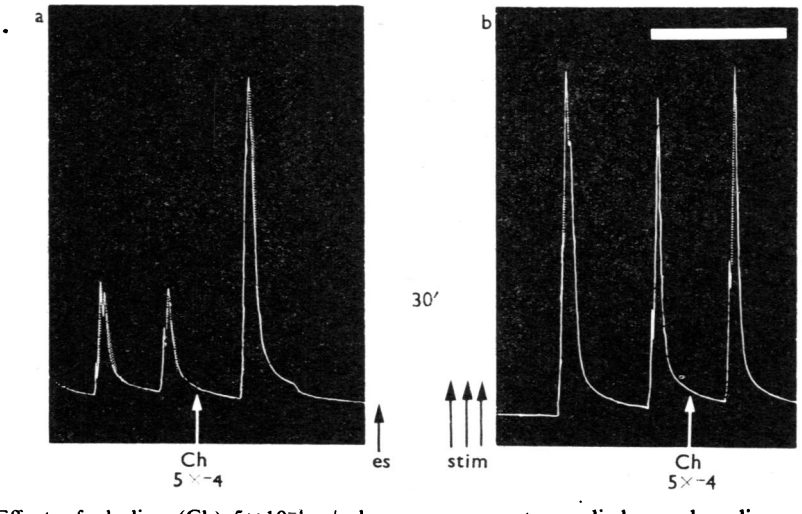

Fig. 4. Effect of choline (Ch) 5×10^{-4} g/ml. on responses to applied noradrenaline under control conditions (a) and 40 min after addition of physostigmine (es) 2×10^{-6} g/ml. to the bath and intermittent intramural nerve stimulation (b). Note the potentiation of control responses to noradrenaline following this procedure. Time marker: 10 min.

Electrophysiological experiments

In the presence of 5×10^{-4} g choline/ml. the mean resting membrane potential of the smooth muscle cells of the vas deferens was reduced by 10 mV (Table 1). This reduction was highly significant ($P<0.001$). Some cells impaled possessed resting membrane potential lower than 40 mV (Fig. 5b).

As a consequence of the low resting membrane potential, the membrane depolarization necessary for initiation of a propagated action potential was reduced in the presence of choline. Figure 5 shows the result of low frequency nerve stimulation at constant voltage before and in the presence of 5×10^{-4} g choline/ml. Under control conditions, stimulation at 1 pulse/sec required several excitatory junction potentials (EJP's) to be fired before the muscle membrane was sufficiently depolarized to cause initiation of a propagated action potential (Fig. 5a). The threshold membrane potential for initiation of an action potential was about -35 to -40 mV, as previously reported by Burnstock & Holman (1961).

Figure 5b shows the result of nerve stimulation at the same voltage 30 min after addition of 5×10^{-4} g choline/ml. to the bath. Under these conditions the resting membrane potential was markedly lowered, and the first EJP was of sufficient amplitude to lead to initiation of an action potential.

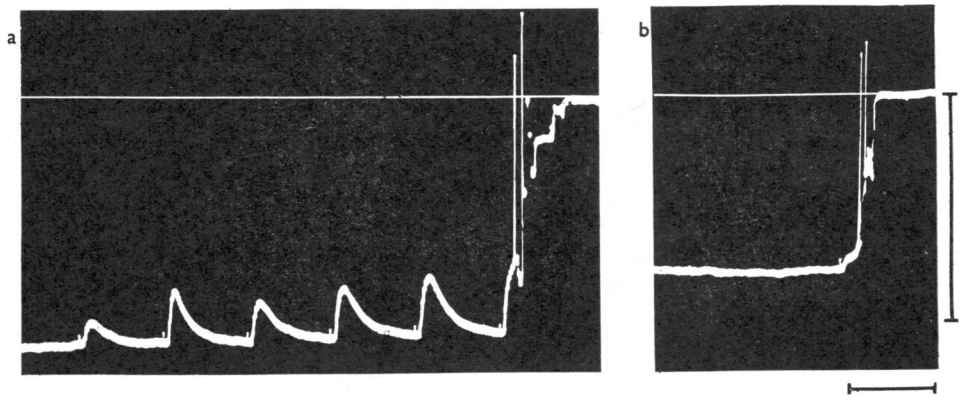

Fig. 5. (a) Excitatory junction potentials (EJP's) and action potentials recorded from a smooth muscle cell in response to post-ganglionic nerve stimulation at 1 pulse/sec and 4.2 V. Membrane potential 56 mV. (b) After 30 min exposure of the tissue to 5×10^{-4} g choline/ml. this record was taken from a neighbouring cell, using the same stimulation parameters. Under these conditions the first EJP led to initiation of an action potential. Membrane potential 39 mV. Upper trace represents extracellular potential. Action potentials retouched. Calibrations: 50 mV and 1 sec.

The depolarizing effect of 5×10^{-4} g choline/ml. was prevented by pretreatment of the vas deferens with hyoscine (10^{-6} g/ml.) for 30 min (Table 1).

In three experiments, the frequency of nerve stimulation was adjusted so as to be just below that necessary for summation of successive EJP's under control conditions, and the voltage was adjusted so that this frequency of stimulation was just insufficient to lead to initiation of an action potential. Under these conditions, the time course of the EJP's

was slightly slowed down by 5×10^{-4} choline/ml. in 7 out of 20 cells impaled, resulting in summation of successive EJP's (Fig. 6). In the remaining 13 cells no change in time course was observed.

TABLE 1

EFFECT OF CHOLINE (5×10^{-4} G/ML.) ON THE RESTING MEMBRANE POTENTIAL OF THE SMOOTH MUSCLE CELLS OF THE GUINEA-PIG VAS DEFERENS

Treatment	Cells (No.)	Preps (No.)	Range (mV)	Mean ± SE (mV)	Signif. of difference from control
Control	26	2	50–73	58·1 ± 1·2	—
Choline (5×10^{-4} g/ml.)	27	2	37–60	48·0 ± 1·5	$P<0{\cdot}001$
Choline (5×10^{-4} g/ml. after pretreatment with hyoscine (10^{-6} g/ml.)	14	1	48–70	59·3 ± 1·6	$P>0{\cdot}1$

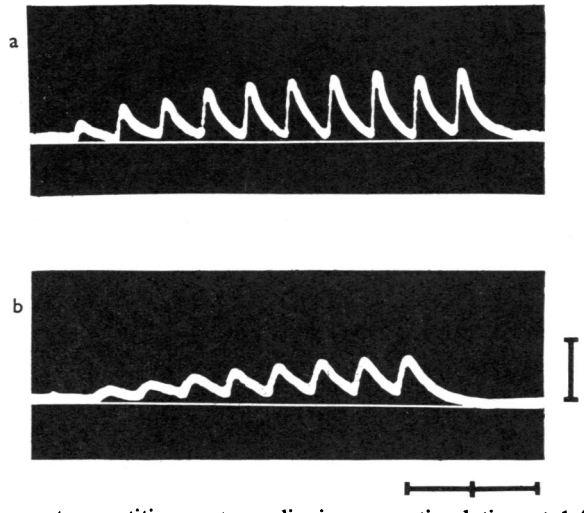

Fig. 6. EJP's in response to repetitive post-ganglionic nerve stimulation at 1.4 pulses/sec (a) under control conditions, (b) in the presence of 5×10^{-4} g choline/ml. Stimulation voltages: (a) 3.8 V, (b) 0.8 V. Note summation in (b). Calibrations: 10 mV and 1 sec.

DISCUSSION

Choline potentiated the contractile responses of the vas deferens to both post-ganglionic nerve stimulation and applied noradrenaline. This potentiation was correlated with marked lowering of the resting membrane potentials of the smooth muscle cells. Both the potentiation of contractile responses and the depolarization due to choline were prevented by the muscarinic blocking agent hyoscine. Thus these effects must have been due to an action at muscarinic receptors, either by choline itself or by an enhanced effect of endogenous acetylcholine.

The effects of choline on the vas deferens were somewhat similar to those of physostigmine as described by Bell (1967). Both compounds potentiated the contractile response to nerve stimulation, depolarized the muscle membrane and caused prolongation of the

EJP's in some cells. Furthermore the potentiating effect of choline on nerve-mediated contractions was prevented by treatment of the tissue with physostigmine. These results suggest that choline may have exerted its effects on the vas deferens by an anti-cholinesterase action. Del Castillo & Katz (1957) noted that iontophoretic application of choline in the vicinity of the skeletal motor end-plate increased the amplitude of the depolarization due to subsequent application of acetylcholine, and that this effect was prevented by treatment of the muscle with the cholinesterase inhibitor neostigmine. These workers concluded that the potentiating effect of choline was due to a rapid anticholinesterase action.

However, certain results in the present study argue against the effects of choline on the vas deferens being due at least in the main to anticholinesterase activity. Although the contractile response to noradrenaline was strongly potentiated by choline, it was not potentiated by physostigmine at a time when the nerve-mediated response was potentiated. Furthermore, although the potentiation by choline of the nerve-mediated response was prevented by physostigmine treatment, choline still potentiated the response to noradrenaline after this procedure. On the other hand, intermittent nerve stimulation subsequent to physostigmine treatment resulted in potentiation of control responses to noradrenaline and in prevention of further potentiation by choline.

A more likely explanation for the results obtained is that choline acted by occupation and weak stimulation of cholinergic receptors on the muscle, resulting in depolarization of the muscle membrane and increased excitability of the tissue. In the presence of nerve stimulation, physostigmine treatment of the vas deferens has been demonstrated to cause a lowering of the resting muscle membrane potential which has been attributed to the persistence of nervously released acetylcholine in the vicinity of cholinergic muscle receptors (Bell, 1967). In these conditions the effect of choline would be abolished because of prior occupation of the receptors by acetylcholine, and the depolarized state of the muscle membrane would result in potentiation of the response to noradrenaline. However, in the presence of physostigmine without nerve stimulation it can be envisaged that insufficient spontaneous acetylcholine release might occur to exert a marked effect on the membrane potential. Under these conditions control responses to noradrenaline would not be potentiated and the effects of choline would not be abolished.

Choline caused slight prolongation of the EJP's in 7 out of 20 cells impaled. This effect in addition to the depolarization may have contributed to potentiation of the nerve-mediated response. It is possible that this effect was due to a weak anticholinesterase action of choline at the post-junctional membrane. On the other hand, it was conceivably due to an increased time course of transmitter liberation in response to nerve stimulation. Brown & Feldberg (1936), Matthews (1963), and Desiraju (1966) have previously reported that choline enhances acetylcholine liberation from preganglionic endings in response to nerve stimulation. It is not yet certain to what extent the time course of junction potentials in smooth muscle tissues is controlled by the duration of pre- and post-junctional events (Bennett & Merrillees, 1966 ; Burnstock & Holman, 1966).

The results of this study raise an interesting problem with regard to the use of hemicholinium as a pharmacological tool. The hemicholiniums, and in particular hemicholinium-3 (HC_3), have been claimed specifically to block cholinergic fibres. The mechanism of action of HC_3 appears to be prevention of incorporation of extracellular

choline into the acetylcholine synthesis pathway leading to depletion of neuronal acetylcholine and failure in intermittently stimulated cholinergic fibres (Schueler, 1960; MacIntosh, 1961). Addition of exogenous choline re-establishes acetylcholine synthesis and leads to partial or complete restoration of the nerve-mediated response.

In addition to its specific effect on acetylcholine synthesis, HC_3 has been shown to exert curare-like effects at the motor endplate (Thies & Brooks, 1961; Martin & Orkand, 1961) and to depress the reactivity of smooth muscle (Bentley & Sabine, 1963). The chief criterion used to determine that failure of a nerve-mediated response in the presence of HC_3 is not due to such a non-specific action is restoration of the response with choline (see Burn, 1966, for discussion). However, the present results suggest that in a tissue which possesses muscarinic receptors an increase in the nerve-mediated response by choline following HC_3 treatment may be due to a post-synaptic action of choline and not necessarily indicate a specific antagonism of HC_3. It is possible, therefore, that choline would produce partial restoration of responses reduced by HC_3 regardless of whether the reduction was due to specific or non-specific actions of HC_3.

SUMMARY

1. Choline (10^{-4}–5×10^{-4} g/ml.) potentiated the responses of the guinea-pig vas deferens to both post-ganglionic nerve stimulation and applied noradrenaline.

2. Choline also caused marked depolarization of the resting membrane potentials of the smooth muscle cells.

3. All these effects were prevented by treatment of the tissue with hyoscine (10^{-6} g/ml.).

4. Potentiation of the nerve-mediated contraction by choline was prevented by treatment of the tissue with physostigmine (2×10^{-6} g/ml.). When, and only when, treatment with physostigmine was followed by intermittent nerve stimulation, the response to applied noradrenaline was potentiated and no further potentiation was induced by choline.

5. It is suggested that choline occupied and weakly stimulated muscarinic receptors on the muscle.

6. Choline also slightly increased the duration of the excitatory junction potentials (EJP'S) elicited by hypogastric nerve stimulation in 7 out of 20 cells impaled. This may have been due to either a pre- or a post-junctional action.

This work was supported by the National Heart Foundation of Australia, by Public Health Research Grant (NB 09202) from the National Institute of Neurological Diseases and Blindness and by the National Health and Medical Research Council of Australia. I should like to thank Professor G. Burnstock and Dr. G. Campbell for their criticisms of the manuscript.

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