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Interaction of bretylium and guanethidine on the relaxations of the rat isolated fundal strip preparation, evoked by indirect stimulation

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Summary

1. Isolated rat stomach fundal strip bathed in Krebs solution containing atropine (1 μg/ml), responded to indirect stimulation by a relaxation which was frequency dependent. These responses were blocked by phenoxybenzamine (6 μg/ml) or phentolamine (8 μg/ml).

2. Strips obtained from rats previously treated with reserpine did not show relaxation to indirect stimulation. These responses were therefore adrenergic in nature.

3. Bretylium (0·1–100 μg/ml) failed to block the relaxations produced by indirect stimulation, in fact relaxations were potentiated by the drug.

4. Guanethidine (10 μg/ml) blocked the relaxations induced by indirect stimulation.

5. Guanethidine may be taken up by adrenergic nerves actively since its action is not seen at 12° C.

6. Bretylium (10 μg/ml) prevented the actions of guanethidine at 37° C.

Introduction

Both bretylium and guanethidine block adrenergic transmission. Bretylium prevents release of transmitter from nerve endings (Boura & Green, 1959), while guanethidine depletes the transmitter from adrenergic neurones (Cass, Kuntzman & Brodie, 1960). In our experiments on isolated fundal strips of the rat, guanethidine blocked the effects of electrical stimulation while bretylium did not. In view of this we have analysed the actions and interactions of bretylium and guanethidine on this preparation.

Methods

Isolated fundal strips of rats were mounted in Krebs solution at 37° C gassed with 5% carbon dioxide in oxygen (Vane, 1957). Responses were recorded by means of a frontal writing lever with a tenfold magnification and a resting tension on the tissue of 1 g. The strip was excited electrically by field stimulation through two ring electrodes made of platinum wire. Stimulation was by rectangular pulses of 1 ms duration, at a frequency of 1–10 Hz, with supramaximal voltage (15 V) applied for 10 s every two minutes. Concentrations of drugs are expressed in terms of their salts. Drugs used were atropine sulphate (Merck), noradrenaline

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bitartrate (Unichem), guanethidine sulphate (Ismelin, Ciba), bretylium tosylate (Wellcome Research Lab.), phenoxybenzamine hydrochloride (Smith, Kline & French), phentolamine (Ciba) and reserpine acid phosphate (Serpasil, Ciba). The Krebs solution always contained atropine (1 μg/ml) and all drugs except noradrenaline were in contact with the strip for two minutes or more. Noradrenaline was in contact with the strip for 30 s only.

Results

Strips subjected to field stimulation in ten experiments produced relaxations which were frequency dependent and resembled those produced by noradrenaline in concentrations of 0·1–1·0 μg/ml.

Relaxations induced by field stimulation (up to 10 Hz) as well as by noradrenaline were completely blocked by α-receptor blocking drugs such as phenoxybenzamine (6 μg/ml ; 6 experiments) or phentolamine (8 μg/ml ; 6 experiments). Strips obtained from rats which were previously treated with reserpine (1·5 mg/kg, i.p. for two consecutive days) did not relax in response to indirect stimulation (1–10 Hz) under identical conditions although the strips relaxed to noradrenaline (0·01–1·0 μg/ml) and contracted to acetylcholine (0·1–4 μg/ml) (n=6) when atropine was not present in the Krebs solution.

The presence of bretylium (0·1–100 μg/ml) for periods from two minutes to two hours, failed to block the relaxations of the strips evoked by indirect stimulation in six experiments. Indeed, the drug potentiated these responses and the relaxations induced by exogenous noradrenaline (Fig. 1).

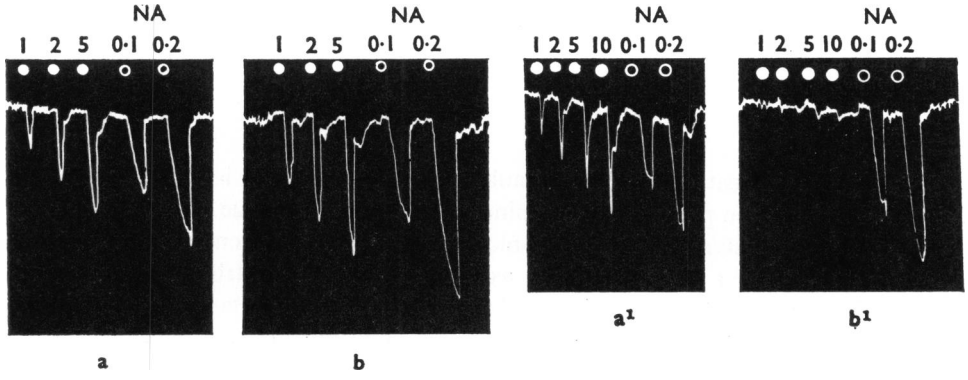

FIG. 1. Panels a and a¹ show relaxations of rat fundal strip, induced by indirect stimulation at frequencies 1, 2, and 5 Hz in panel a and 1, 2, 5 and 10 Hz in panel a¹, each for 10 s (closed circles), and by exogenous noradrenaline (NA, 0·1 μg and 0·2 μg/ml, open circles). Panel b shows the responses in presence of bretylium (10 μg/ml for 30 min at 37° C). Note the potentiation of the effects. Panel b¹ shows the responses in presence of guanethidine (10 μg/ml for 30 min at 37° C). Note the blockade of the responses to indirect stimulation while the responses to exogenous noradrenaline are potentiated.

Guanethidine (10 μg/ml ; 6 experiments) treatment for 30 min at 37° C, blocked the relaxations elicited by indirect stimulation but potentiated those produced by exogenous noradrenaline (Fig. 1). The guanethidine block persisted for 20–50 min after guanethidine was washed out. However, the responses induced by indirect stimulation were not blocked when the strip was exposed to guanethidine (10 μg/ml) for 30 min at 12° C prior to stimulation at 37° C.

In six experiments the presence of guanethidine (10 μg/ml) for 30 min at 37° C, blocked the responses to indirect stimulation. The tissue was then allowed to recover from the effects of guanethidine. When the relaxation induced by field stimulation had returned, the strip was treated with bretylium (10 μg/ml) for 10 minutes. Relaxation induced by field stimulation was now resistant to the effects of guanethidine (10 μg/ml) (Fig. 2).

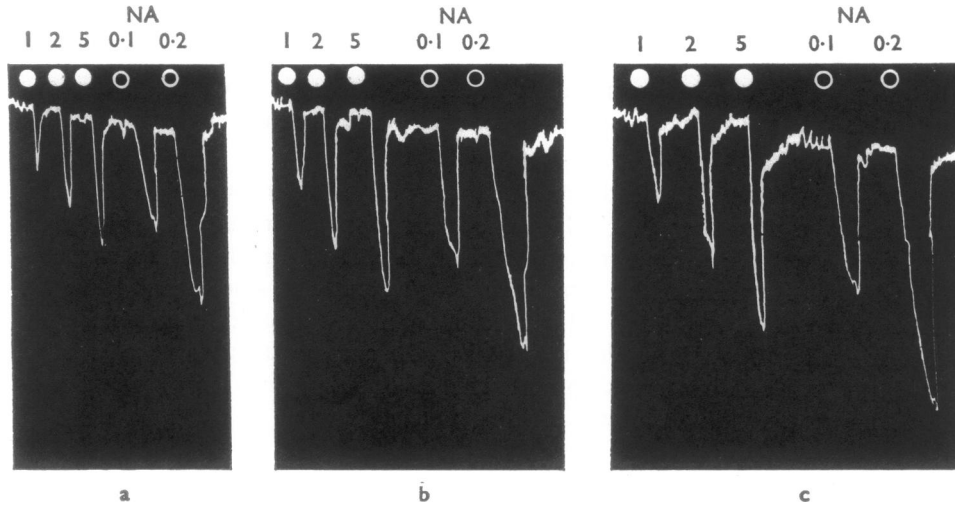

FIG. 2. Panels a, b and c show relaxations of rat fundal strip induced by indirect stimulation at frequencies 1, 2 and 5 Hz, each applied for 10 s (closed circles) and by noradrenaline (NA, 0·1 and 0·2 μg/ml) applied for 30 s, open circles. Between panels a and b the strip was exposed to bretylium (10 μg/ml for 10 min) and the responses in panel b were obtained in the presence of bretylium. Between panels b and c the strip was exposed to guanethidine (10 μg/ml for 30 min at 37° C) in the presence of bretylium and the responses obtained are seen in panel c.

Discussion

Our findings indicate that field stimulation of the isolated longitudinal fundal strip of the rat, in the presence of atropine, excites nervous tissue that is adrenergic in nature. For instance α-receptor blocking drugs like phenoxybenzamine or phentolamine blocked the relaxations, as did guanethidine; strips from rats pretreated with reserpine did not exhibit relaxation. Similar results were obtained by Paton & Vane (1963) in guinea-pig stomach although their experimental technique was different.

In our experiments the action of guanethidine was not seen when the strip was exposed to the drug at 12° C. This suggests that guanethidine is taken up actively, perhaps by adrenergic nerves.

Failure of bretylium to block the relaxations does not mean that electrical stimulation excites a non-adrenergic pathway, for all our other results point to an adrenergic mechanism. Besides, bretylium has many and varied actions including ganglion blockade (Boura & Green, 1959), prevention of uptake of adrenergic transmitter (Hertting, Axelrod & Patric, 1962) and monoamine oxidase inhibition (Furchgott, 1964; Kuntzman & Jacobson, 1963).

Bretylium not only failed to block the effects of indirect stimulation but also potentiated the effects of noradrenaline. This indicates that bretylium may interfere

with the uptake of noradrenaline. Further, bretylium prevents the action of guanethidine suggesting that active uptake of guanethidine is also prevented.

We, therefore, postulate that guanethidine and noradrenaline may share the same mechanism of uptake by the adrenergic postganglionic nerves in the rat stomach strip preparation.

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