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The effects of blocking agents upon the isolated vas deferens of the guinea-pig stimulated by the hypogastric nerve

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When the guinea-pig isolated vas deferens preparation was stimulated by its hypogastric nerve, the responses obtained at different frequencies of stimulation were all reduced proportionately by guanethidine, bretylium, reserpine and pentamethonium. The difference between guanethidine and bretylium in affecting the responses to nervous stimulation at different frequencies of the cat nictitating membrane reported by Boura & Green was not observed with this preparation. Papaverine reduced responses to high frequencies of stimulation more than those to low frequencies. Amphetamine also reduced responses to all frequencies uniformly except in the smallest doses used, which potentiated the responses to high frequencies; these doses reversed the reduction in response by guanethidine, bretylium and reserpine. Preparations stimulated between parallel electrodes responded similarly to those stimulated by the hypogastric nerve.

THE isolated preparation of the guinea-pig vas deferens and hypogastric nerve as described by Hukovic (1961) responds to the adrenergic neurone blocking action of guanethidine and bretylium by a reduction in height of the contraction caused by electrical stimulation of the nerve (Boyd, Chang & Rand, 1961; Bentley, 1962). The technique of stimulating the nerve over a range of frequencies has been shown to distinguish between the blocking actions of guanethidine and bretylium when applied to the responses of the cat nictitating membrane to stimulation of the post-ganglionic sympathetic nerve of the superior cervical ganglion (Boura & Green, 1962). The study described here examined the results of applying this technique to the blocking action of these and other drugs on the vas deferens preparation.

Methods

The organ was prepared as described by Hukovic (1961) and set up in a 15 ml overflow-type gut bath. The preparation was bathed in Krebs solution (33 g NaCl; 17·5 ml 10% KCl; 14 ml 10% $CaCl_2$; 10·4 g glucose; 10·5 g $NaHCO_3$; 0·81 g KH_2PO_4; 1·47 g $MgSO_4 7H_2O$; in 5 litres of distilled water), which was bubbled with 95% O_2 and 5% CO_2 and maintained at 35°. Stimulation of the hypogastric nerve was effected by a shielded electrode around which the nerve was wound twice and the free end secured to the edge of the bath to prevent movement of the nerve on the electrode. The electrode was placed on the surface of the perfusion fluid. In some experiments the organ was stimulated between parallel electrodes completely immersed in the bath, following the method described as transmural by Birmingham & Wilson (1963). In these experiments, the vas deferens was cleaned of mesentery as closely as possible. With both methods of stimulation, the pulses were 2 msec wide and of

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supramaximal voltage. The nerve was stimulated for between 2 and 5 sec every minute, while stimulation between parallel electrodes was applied for 10 sec every 90 sec.

The frequency was varied within the range 4 to 32 pulses/sec; this usually elicited the complete range of response of the organ. It was found advantageous to have more observations at the lower than at the higher frequencies. If guanethidine, bretylium or reserpine were given in sufficient concentrations to produce a moderate reduction in the response, the response subsequently continued to fall progressively and did not level out. It was, therefore, necessary to make some allowance for this in the design of the experiment. In some cases it was possible to limit the fall by washing out the drug after a given interval, when a constant response was obtained.

In all these experiments, two series of frequency change were made for each standard and treatment, the frequency first being lowered progressively and then raised. This process was repeated after each addition of drug, time being allowed for equilibrium to occur. On changing the frequency, time was allowed for the response to settle to that determined by the new frequency. The frequency was not changed until at least three or four constant responses were obtained.

Results

The change in height of response of the preparation with change in frequency is shown in Table 1 and Fig. 1 *et seq.* The corresponding

TABLE 1. RESPONSE OF GUINEA-PIG ISOLATED VAS DEFERENS TO SUPRAMAXIMAL STIMULATION OF THE HYPOGASTRIC NERVE AT VARIOUS FREQUENCIES (Figures from typical experiment)

Frequency (shocks/sec)	Height of response (mm)*		
	On descending run	On ascending run	Mean
24	85	84	84·5
20	80	79	79·5
16	70	71	70·5
12	60	56	58
10	49	50	49·5
8	39	38	38·5
6	28	25	26·5
4	8	—	8

* Each figure quoted is the mean for the last three responses obtained at that frequency of stimulation.

change with frequency after addition of guanethidine to the bath, in amounts between 0·3 and 1·5 μg/ml is also shown in Fig. 1. The curve is typical of those obtained in seven such experiments and shows that a similar degree of depression is exerted over the whole frequency range. Similar relations between frequency and reduction of response were found for bretylium, in concentrations between 0·5 and 2·0 μg/ml (4 experiments; Fig. 2) and reserpine at 5 to 10 μg/ml (5 experiments; Fig. 3). The depression with reserpine was slow to develop and persistent; the

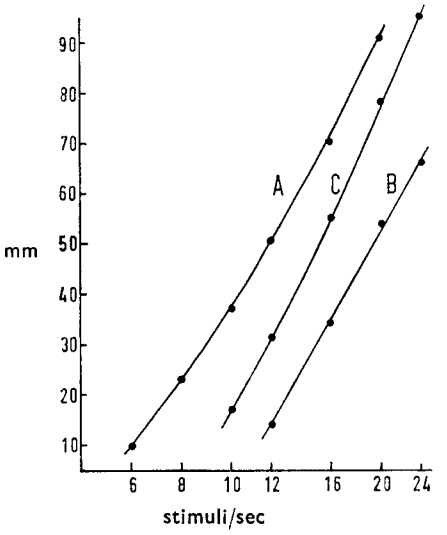

FIG. 1. Mean height of response, in mm of guinea-pig isolated vas deferens, stimulated by the hypogastric nerve with supramaximal shocks at various frequencies; from a single preparation. A. Before drug treatment. B. In the presence of guanethidine sulphate, 0·5 μg/ml. C. In the presence of amphetamine sulphate, 0·5 μg/ml following guanethidine sulphate, 0·5 μg/ml.

initial effect was to heighten the response, as also seen with lower, non-blocking doses (1 to 2 μg/ml). Papaverine however, affected the responses to high frequency stimulation more than those to lower frequency (Fig. 4).

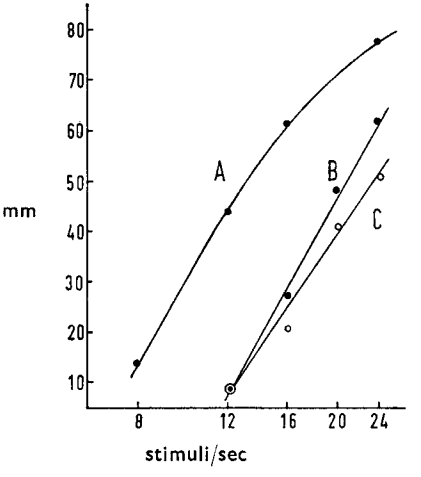

FIG. 2. Mean height of responses, in mm of guinea-pig isolated vas deferens, stimulated by the hypogastric nerve with supramaximal shocks at various frequencies; from a single preparation. A. Before drug treatment. B. In the presence of bretylium tosylate, 0·5 μg/ml. C. In the presence of pentamethonium iodide, 10 μg/ml.

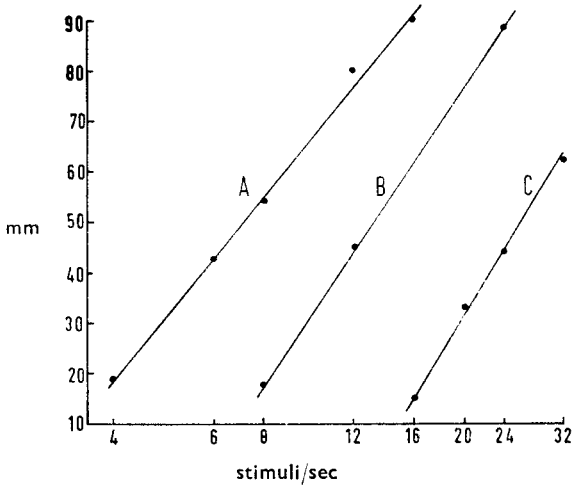

FIG. 3. Mean height of responses, in mm of guinea-pig isolated vas deferens, stimulated by the hypogastric nerve with supramaximal shocks at various frequencies; from a single preparation. A. Before drug treatment. B. 20 min after addition of reserpine, 5 μg/ml. C. 1 hr after washing out.

The ganglion-blocking agent, pentamethonium, at 10 μg/ml (Fig. 2) had a depressant action which was exerted similarly at all frequencies.

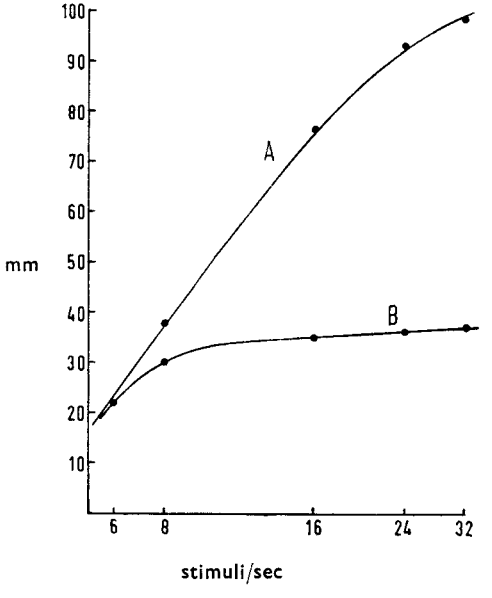

FIG. 4. Mean height of responses, in mm of guinea-pig isolated vas deferens, stimulated by the hypogastric nerve with supramaximal shocks at various frequencies; from a single preparation. A. Before drug treatment. B. In the presence of papaverine hydrochloride, 0·1 mg/ml.

In a few experiments in which the organ was stimulated between parallel electrodes alternately with stimulation of the hypogastric nerve, it was found that the reduction by hexamethonium of responses elicited by nerve stimulation, was greater than the reduction of those induced by stimulation between parallel electrodes, whilst both were equisensitive to guanethidine and bretylium (Fig. 5). Guanethidine and bretylium

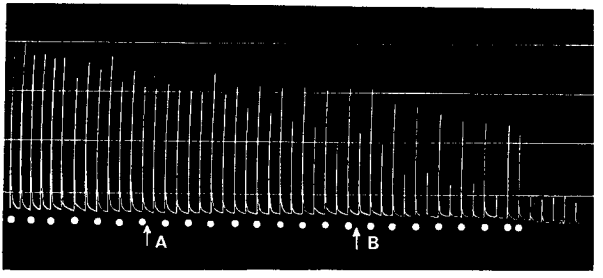

FIG. 5. Portion of kymograph record, guinea-pig isolated vas deferens, supramaximally stimulated by electrodes on the hypogastric nerve (unmarked) alternately with stimulation between parallel electrodes in the bath (marked). At A, addition of hexamethonium iodide to make a bath concentration of 5 μg/ml. At B, replacement with guanethidine sulphate to make a bath concentration of 0·6 μg/ml.

depressed the frequency-response curve in a parallel manner in preparations stimulated between electrodes as well as in the nerve-stimulated preparation, and similar doses of these drugs were required for reduction of the responses to both (Fig. 6; 4 experiments).

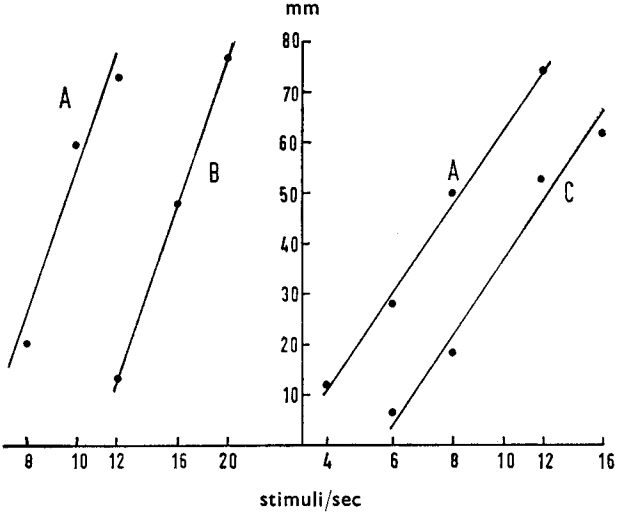

FIG. 6. Mean height of responses, in mm, of guinea-pig isolated vas deferens, stimulated between parallel electrodes with supramaximal shocks at various frequencies; each pair of curves from a single preparation. A. Before drug treatment. B. In the presence of guanethidine sulphate, 0·5 μg/ml. C. In the presence of bretylium tosylate, 1·5 μg/ml.

Amphetamine reduced the responses at concentrations above 10 μg/ml, but lower concentrations (0·5 to 5·0 μg/ml) potentiated the response to the higher frequencies of stimulation although reducing the response to lower frequencies (Fig. 7; 3 experiments). When added to the bath after

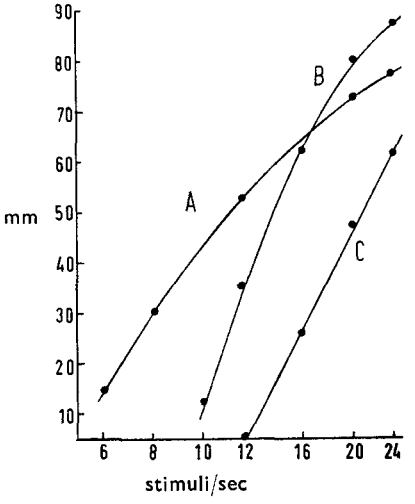

FIG. 7. Mean height of responses, in mm of guinea-pig isolated vas deferens, stimulated by the hypogastric nerve with supramaximal shocks at various frequencies; from a single preparation. A. Before drug treatment. B. In the presence of amphetamine sulphate, 0·5 μg/ml. C. In the presence of amphetamine sulphate, 20 μg/ml.

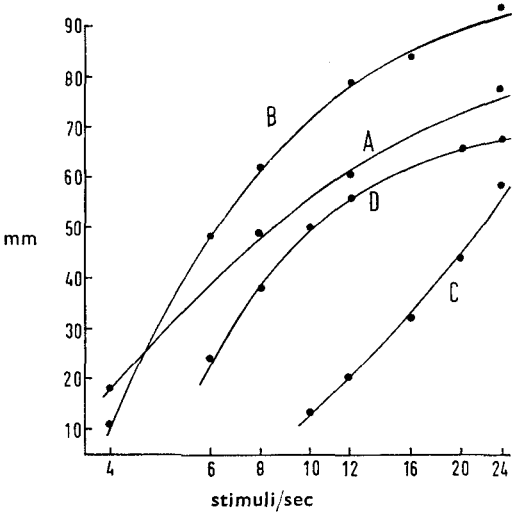

FIG. 8. Mean height of responses, in mm of guinea-pig isolated vas deferens, stimulated by the hypogastric nerve with supramaximal shocks at various frequencies; from a single preparation. A. Before drug treatment. B. In the presence of dibenamine hydrochloride, 10 μg/ml. C. In the presence of guanethidine sulphate, 0·33 μg/ml. D. In the presence of dibenamine hydrochloride, 20 μg/ml. following C.

depression of the response by guanethidine, amphetamine at 0·5 μg/ml restored the response at all frequencies (Fig. 1; 3 experiments). Amphetamine similarly reversed the block due to bretylium though the lower frequencies were less well restored than the higher ones. Amphetamine also reversed the block caused by reserpine; in this case, however, only the higher frequencies were restored, the lower ones being further reduced. This was very similar to the picture presented by amphetamine itself.

The adrenergic blocking agent, dibenamine, at 10 μg/ml, also potentiated the response to stimulation, predominantly at high frequencies (Fig. 8). Dibenamine also reversed the depression due to guanethidine (Fig. 8); the reversal was, however, of the same non-parallel form as the potentiation caused by dibenamine alone. It was noticed that there was a transient biphasic effect when dibenamine was added to the bath containing guanethidine, as there was initially a further depression before reversal of the guanethidine block.

Discussion

Investigation of effects upon the relation between response and frequency of stimulation may provide information on the mode of action of agents affecting the response. The approximately parallel degree of reduction over the range of frequencies found with guanethidine, bretylium and reserpine suggest uniform interference with the transmitter, either by competitive occupation of receptors or by reduction in the amount released. The gradual development and persistence of block due to reserpine, and the opposite action observed initially, recall the features of other actions of reserpine associated with amine depletion.

Boura & Green (1962) were able to distinguish between the action of bretylium and guanethidine in their effects on the cat nictitating membrane, stimulated by its sympathetic nerve, in that the frequency-response curve was depressed in a parallel manner by guanethidine, whereas bretylium had little effect on the response to low frequencies. From this they suggested that block due to guanethidine depended upon a competitive antagonism while the basis of the action of bretylium was non-competitive. In the results with the isolated vas deferens reported here, both guanethidine and bretylium appeared to depress the frequency-response curve in a parallel fashion, so that both would seem to exert a similarly competitive block.

A similar lack of differentiation between the blocking actions of guanethidine and bretylium has recently been found by Green & Robson (1964) using the isolated Finkleman preparation of the rabbit ileum, whereas the actions of these drugs on the stimulated responses of the vessels of the cat leg, the cat spleen and the vessels of the isolated rabbit ear resembled those on the nictitating membrane. A reason for the differences does not readily appear from consideration of the preparations concerned.

There is evidence that the sympathetic innervation of the vas deferens may not be entirely post-ganglionic (Sjöstrand, 1962a), supported by the

demonstration that ganglionic blocking agents reduce the response to stimulation of the hypogastric nerve (Sjöstrand, 1962b; Ohlin & Stromblad, 1963), a finding confirmed in this report.

Recent work has suggested that when the vas deferens is stimulated, either between parallel electrodes in the bath or transmurally (Birmingham & Wilson, 1963; Bentley & Sabine, 1963), this leads to stimulation of post-ganglionic fibres only, whereas hypogastric nerve stimulation involves preganglionic stimulation also. Results reported here confirm this evidence, in that while both means of stimulation were to some extent susceptible to ganglionic blockade, the responses due to hypogastric nerve stimulation were noticeably more reduced than those to stimulation between bath electrodes. Both, however, were equally sensitive to guanethidine. Bretylium and guanethidine affected the frequency-response curve in preparations stimulated between electrodes in the same manner as they did in those stimulated by the nerve, and thus the behaviour of the two drugs on the vas deferens does not seem to be related to the question of whether the stimulation is pre- or post-ganglionic.

The antagonism by amphetamine towards the block due to guanethidine reported by Day & Rand (1963) is confirmed in these experiments and its competitive basis is further suggested by frequency-response relations. Amphetamine given alone exerted a definite potentiating action in smaller doses. This might have contributed to reversal of the effects of guanethidine upon higher frequency responses but would not account for antagonism at lower frequencies, since in these circumstances amphetamine never potentiated responses.

References

Bentley, G. A. (1962). *Brit. J. Pharmacol.*, **19**, 85–98.
Bentley, G. A. & Sabine, J. R. (1963). *Ibid.*, **21**, 190–201.
Birmingham, A. T. & Wilson, A. B. (1963). *Ibid.*, **21**, 569–580.
Boura, A. L. A. & Green, A. F. (1962). *Ibid.*, **19**, 13–41.
Boyd, H., Chang, V. & Rand, M. J. (1961). *Arch. Int. Pharmacodyn.*, **131**, 10–23.
Day, M. D. & Rand, M. J. (1963). *Brit. J. Pharmacol.*, **20**, 17–28.
Green, A. F. & Robson, R. D. (1964). *Ibid.*, **22**, 349–355.
Hukovic, S. (1961). *Ibid.*, **16**, 188–194.
Ohlin, P. & Stromblad, B. C. R. (1963). *Ibid.*, **20**, 299–306.
Sjöstrand, N. O. (1962a). *Acta physiol. scand.*, **56**, 376–380.
Sjöstrand, N. O. (1962b). *Ibid.*, **54**, 306–315.