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COMPARISON OF THE EFFECTS OF BRETYLIUM, GUANETHIDINE AND BETHANIDINE ON SMOOTH MUSCLE RESPONSES TO DIFFERENT RATES OF SYMPATHETIC NERVE STIMULATION

BY

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The relative effects of bretylium, guanethidine and bethanidine on smooth muscle responses to different rates of sympathetic nerve stimulation have been compared. The responses studied were vasoconstriction in the femoral vascular bed and contraction of the spleen in anaesthetized cats, vasoconstriction in perfused ears of rabbits and inhibition of pendular movements in rabbit isolated ileum preparations. Except in the isolated ileum, the action of bretylium on curves relating the frequency of nerve stimulation and the effect on response was different from that of guanethidine. Whereas bretylium caused relatively greater inhibition of responses to high stimulus frequencies and depressed the slopes of the curves, guanethidine preferentially suppressed responses to low stimulus frequencies and caused roughly parallel shifts of the curves. In each situation tested bethanidine was the most potent of the three blocking agents and in general its effect on frequency/response curves was intermediate between those of bretylium and guanethidine.

Some differences between the effects of bretylium and guanethidine on the responses to sympathetic nerve stimulation have been described by Boura & Green (1962). Bretylium depressed the slope of regression lines relating frequency of sympathetic nerve stimulation to magnitude of contractions of the cat nictitating membrane, while guanethidine (and reserpine) preferentially abolished responses to low rates of nerve stimulation and caused a parallel shift of the regression lines. Some of the differences in clinical response to bretylium and to guanethidine may be related to the findings of Boura & Green (1962).

Bethanidine (BW467C60), a recently introduced potent adrenergic neurone blocking agent, has a hypotensive action in man (Montuschi & Pickens, 1962 ; Smirk, 1963). Its effect on the sympathetic nerve stimulus frequency/nictitating membrane response curves lies between those of bretylium and guanethidine (Boura & Green, 1963).

We report here the effects of bretylium, guanethidine and bethanidine on nerve stimulus frequency/response regression lines using other smooth muscle preparations innervated by the sympathetic nervous system.

METHODS

Perfused rabbit ears. These were perfused with Tyrode solution at 37° C, the outflow from the vein being measured with a Thorp impulse counter. The greater auricular nerve was stimulated with rectangular pulses of supramaximal voltage and 1 msec duration, at frequencies ranging from 1 to 20 shocks/sec, either for periods of 30 sec or until a maximal vasoconstriction had been obtained. Drugs were introduced into a cannula in the artery to the ear. The composition of the Tyrode solution was (g/l. of distilled water): NaCl 8.0, KCl 0.2, $MgCl_26H_2O$ 0.2, $NaH_2PO_42H_2O$ 0.05, $NaHCO_3$ 1.0, $CaCl_2$ 0.15 and dextrose 1.0.

Anaesthetized cats. Cats of either sex and weighing 2 to 4 kg were used. Anaesthesia was induced with ether and was maintained with chloralose (about 60 mg/kg, intravenously).

Spleen volume changes. These were recorded with a Perspex oncometer connected to a float recorder, usually of 30 ml. capacity. The splenic nerve was cut just below the coeliac ganglion and stimulated with rectangular pulses of supramaximal voltage and 1 msec duration, at frequencies of 0.5, 1, 2.5, 5, 10 and 20 shocks/sec. In some experiments, trains of stimuli lasting 30 sec at each frequency were applied in ascending and then in descending order leaving sufficient time between each train for full relaxation of the spleen. In other experiments, the nerve was stimulated continuously until the contraction was maximal with each frequency before passing to the next higher frequency without any rest period.

Peripheral vascular resistance. Studies of increases in this resistance caused by sympathetic nerve stimulation were carried out in anaesthetized cats which had received 2 mg/kg of atropine sulphate. Blood was driven at a constant rate of 10 to 20 ml./min from a cannulated carotid artery through a polyethylene tube into a femoral artery by a Sigmamotor pump. The pressure distal to the pump was recorded through a T-junction by a mercury manometer. This pressure was 140 to 170 mm Hg in the absence of nerve stimulation and increased in proportion to increased peripheral vascular resistance when the sympathetic chain was stimulated. The lumbar sympathetic chain on the same side as the cannulated femoral artery was sectioned at the level of L3 or L4 and the peripheral end was stimulated with rectangular pulses of supramaximal voltage and 1 msec duration applied at various frequencies until a maximal effect was obtained.

All drugs were injected into a femoral vein, except when otherwise specified. The doses refer to the sulphates of guanethidine and atropine, the *para*-toluene sulphonate of bretylium, and the hydrochloride of bethanidine.

Rabbit ileum preparation. The effects of the adrenergic neurone blocking agents on the inhibition of pendular movements of rabbit isolated ileum caused by stimulation of the periarterial mesenteric nerves were studied by the method of Finkleman (1930). The preparation was set up in a 100 ml. organ-bath containing oxygenated Tyrode solution at 37° C. The nerve was stimulated with shocks of supramaximal voltage at frequencies ranging in twofold increments from 3.125 to 50 shocks/sec, either for 20 sec or until a maximal inhibitory response was obtained. The frequencies were applied before and at intervals after adding bretylium or guanethidine to the bath fluid. In some experiments 0.1 μg/ml. of atropine was continually present in the organ-bath.

RESULTS

Perfused rabbit ear. Stimulation of the auricular nerve of perfused rabbit ears caused vasoconstriction to a degree dependent on the frequency of the applied stimulus within the range 1 to 20 shocks/sec (Fig. 1, *a* and *d*). Injections of 100 and 200 μg of bretylium (Fig. 1, *b* and *c*, respectively) preferentially inhibited the responses to the higher frequencies in each of three preparations and depressed the slope of the frequency/response curves constructed from these results. In contrast, submaximally effective concentrations of guanethidine preferentially suppressed the vasoconstrictor responses to the lowest rates of auricular nerve stimulation (Fig. 1, *e*).

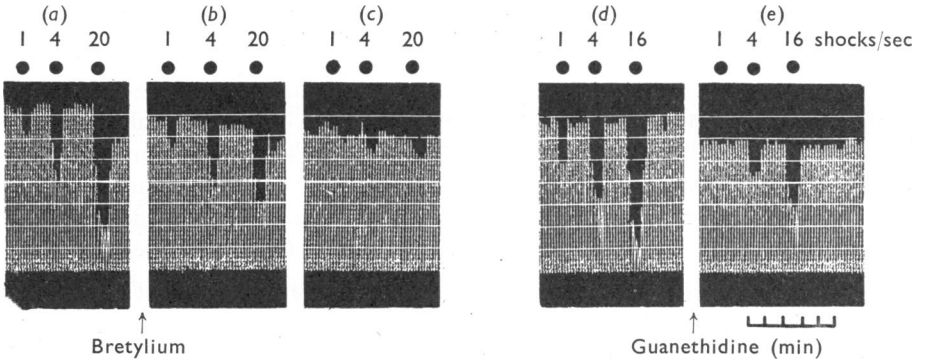

Fig. 1. Records of the venous outflow from a perfused rabbit ear. At the black dots vasoconstriction was produced by stimulation of the greater auricular nerve for 30 sec at the stimulus frequencies shown. (*a*), control responses; (*b*) and (*c*), responses after introduction into the arterial cannula of 100 and 200 μg of bretylium tosylate respectively, showing preferential inhibition of responses to the high stimulus frequencies; (*d*) and (*e*), before and after 50 μg of guanethidine sulphate respectively, showing preferential inhibition of the responses to low stimulus frequencies.

Bethanidine was tested in three preparations and showed effects on the frequency/response curves intermediate between those of bretylium and guanethidine.

Spleen volume changes. The results of experiments, in which the spleen volume changes produced by stimulation of the splenic nerve at various frequencies for 30 sec were measured, are summarized in Fig. 2. In these experiments sufficient time

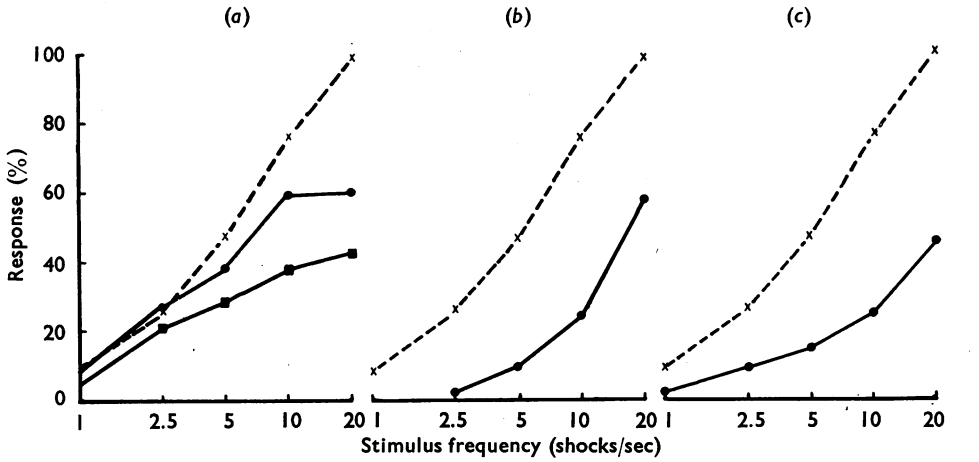

Fig. 2. The relationship of the frequency of splenic nerve stimulation (on log scale) to changes in spleen volume in anaesthetized cats. Each train of stimuli was applied for 30 sec, and adequate time was allowed for the spleen to relax fully between each train. All spleen volume changes have been expressed as a percentage of that produced by 20 shocks/sec in each animal before giving blocking agents. × —— ×, control responses (mean for twelve cats). (*a*), ● —— ● and ■ —— ■, after 1.0 and approximately 2.5 mg/kg of bretylium tosylate respectively, showing depression of slope; (*b*) ● —— ●, after 0.3 mg/kg of guanethidine sulphate showing a parallel shift of the regression line; (*c*) ● —— ●, after 0.3 mg/kg of bethanidine hydrochloride which produced an effect on the regression line intermediate between those of bretylium and guanethidine. Experimental means are for groups of three cats.

was allowed between trains of stimuli for the spleen to relax fully. The slope of the regression line relating the log of the stimulus frequency to volume changes was depressed by bretylium (Fig. 2, *a*). Guanethidine preferentially inhibited responses to low stimulus frequencies to produce a roughly parallel shift of the regression line to the right (Fig. 2, *b*). The effect of bethanidine on the frequency/response curve was intermediate between those of bretylium and guanethidine, the slope being reduced with the responses to low stimulus frequencies showing a greater percentage reduction than those to the higher stimulus frequencies (Fig. 2, *c*).

Fig. 3 summarizes experiments which were similar except that the nerve was stimulated by trains of stimuli at each frequency in ascending order until maximal contractions were obtained and no interval for relaxation was permitted. A similar

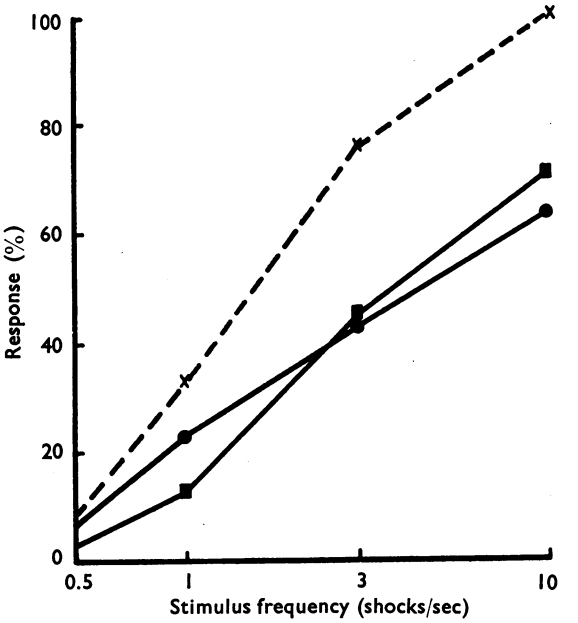

Fig. 3. The relation of frequency of splenic nerve stimulation (on log scale) to spleen volume change in anaesthetized cats, as in Fig. 2 except that the trains of stimuli were applied until contraction was maximal and no interval was allowed for relaxation. x - - - x, control responses (mean for nine cats); ● —— ●, after 2 mg/kg of bretylium tosylate (three cats); and ■ —— ■, after 0.3 mg/kg of guanethidine sulphate (three cats).

distinction between the acute effects of intravenous bretylium (Fig. 3, filled circles) and guanethidine (Fig. 3, squares) was again found.

Hind-limb perfusions. The maintenance of a constant blood flow through the femoral vascular bed with a Sigmamotor pump enabled changes in peripheral resistance caused by nerve stimulation to be recorded as changes in perfusion pressure. Resistance increased with the frequency of lumbar sympathetic stimulation and the blocking actions of bretylium and guanethidine on the responses to

the various frequencies differed in a characteristic manner (Fig. 4). Fig. 5 shows the effects of bretylium (empty circles), guanethidine (triangles) and bethanidine (squares) on the regression lines relating log frequency of nerve stimulation to increase in perfusion pressure. The regression line became shallower following

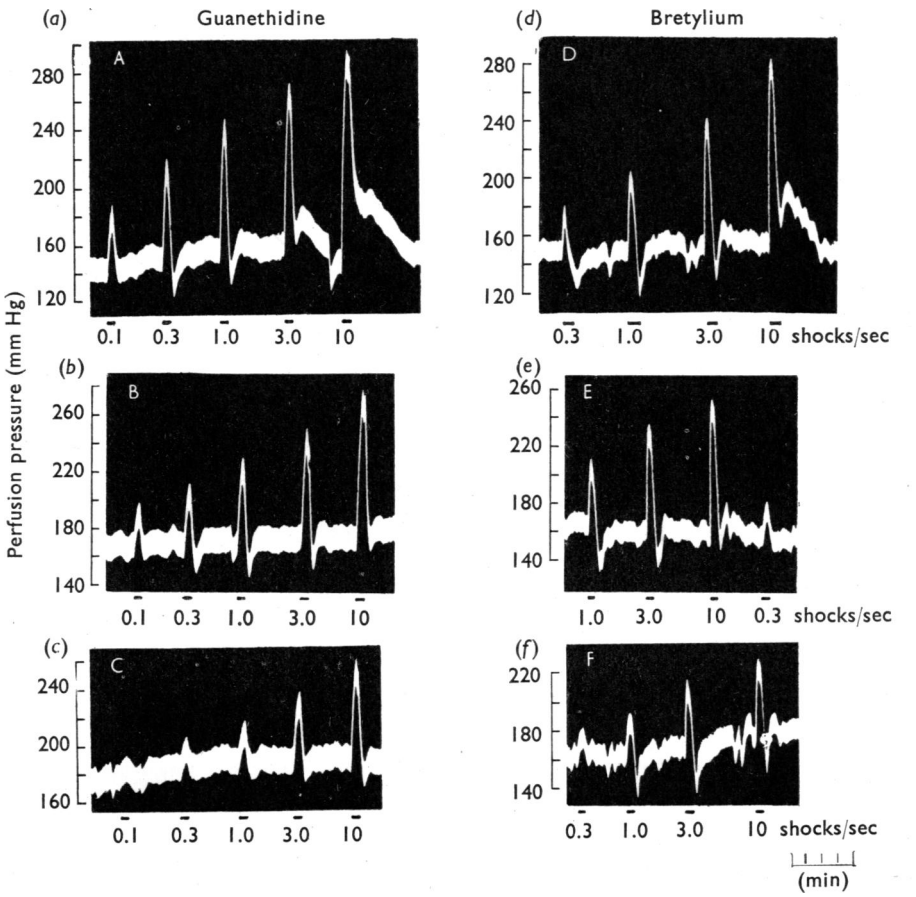

Fig. 4. Record of the pressure in the femoral arteries of two atropinized cats anaesthetized with chloralose when blood was driven into the artery at 20 ml./min. The lumbar sympathetic chain was stimulated for 30 sec for the duration of the horizontal lines at the stimulus frequencies shown. (*a*), before, and (*b*), after 0.1 mg/kg of guanethidine sulphate; (*c*), after a further 0.2 mg/kg of guanethidine; (*d*), before, and (*e*), after 1.0 mg/kg of bretylium tosylate; (*f*), after a further 0.5 mg/kg bretylium. Both drugs slightly increased peripheral vascular tone and reduced the vasoconstrictor responses to sympathetic stimulation.

the acute administration of bretylium (1 to 2 mg/kg), whereas after guanethidine (0.3 to 0.5 mg/kg) there was a roughly parallel shift of the curve to the right. The effect of 0.2 mg/kg of bethanidine was more like that of guanethidine than that of bretylium.

Rabbit ileum preparation. The inhibition of the pendular movement of isolated rabbit ileum caused by electrical stimulation of the sympathetic nerves was propor-

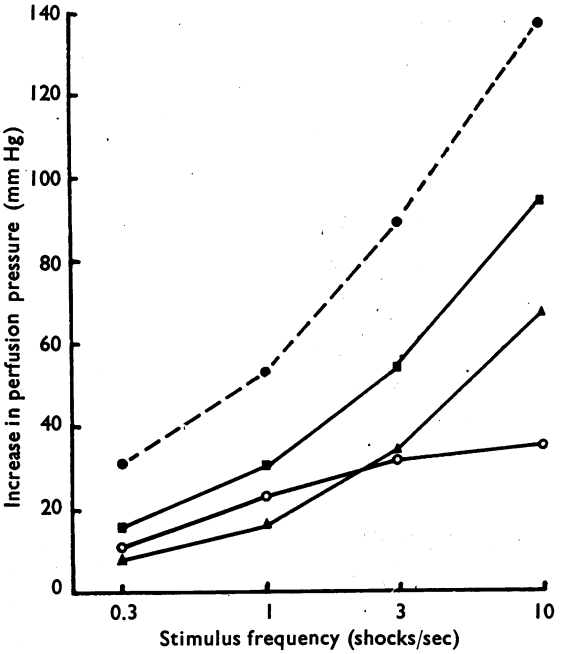

Fig. 5. The effects of adrenergic neurone blocking agents on regression lines relating frequency of lumbar sympathetic chain stimulation (on log scale) to increase in perfusion pressure in the femoral artery of atropinized cats. ●---●, control curves (means for ten cats); ○——○, after 1 to 2 mg/kg of bretylium tosylate (three cats) showing depression of slope; ▲——▲, after 0.3 to 0.5 mg/kg of guanethidine sulphate (three cats) showing parallel shift; ■——■, after 0.2 mg/kg of bethanidine hydrochloride showing another parallel shift.

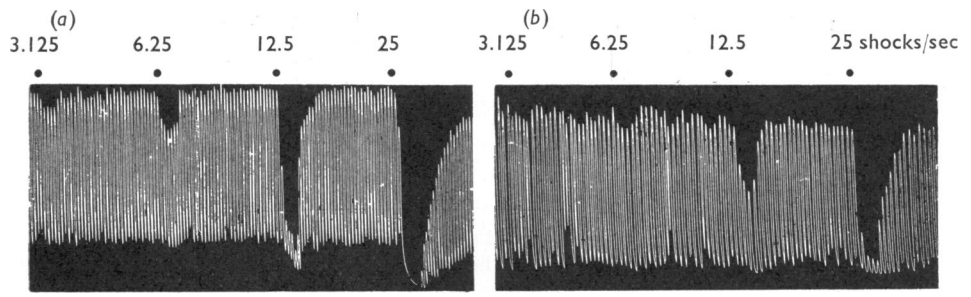

Fig. 6. Record of contractions of a rabbit isolated ileum preparation. At the dots, supramaximal shocks at the frequencies shown were applied to the periarterial mesenteric nerves for 20 sec. (*a*), before, and (*b*), 15 min after adding 1.5 μg/ml. of bretylium tosylate to the bath, showing reduction of the inhibitory response to nerve stimulation.

tional to the stimulus frequency (Fig. 6, *a*). Addition of bretylium (1.5 μg/ml.) to the bathing solution reduced the magnitude of the inhibitory action of all frequencies of nerve stimulation (Fig. 6, *b*). A similar effect was produced by guanethidine. The effects of each compound on curves relating stimulus frequency to the inhibitory response were also similar in experiments in which 0.1 μg/ml. of atropine had been added to the bathing solution.

DISCUSSION

Differences between the effects of bretylium and guanethidine on smooth muscle responses to various rates of sympathetic nerve stimulation, similar to those described for the nictitating membranes by Boura & Green (1962), have been found in studies of vasoconstriction in the hind limbs of anaesthetized cats and in perfused rabbit ears and of contraction of the spleen in anaesthetized cats. The findings in nictitating membrane experiments led Boura & Green (1962) to predict that the comparatively greater depressant action of guanethidine on low rates of sympathetic nerve stimulation would give rise to the following effects: (a) that tolerance would be less prominent with guanethidine than with bretylium ; (b) that, at doses matched to produce a similar lowering of blood pressure with the patient standing, guanethidine would produce the greater effect when the patient was supine and bretylium when the patient exercised ; (c) that guanethidine would be more inclined to induce bradycardia.

The relative effects of bethanidine on nictitating membrane responses to different frequencies of sympathetic nerve stimulation lay between those of bretylium and guanethidine (Boura & Green, 1963). The action of bethanidine on responses to different frequencies of nerve stimulation has now been found to lie between those of bretylium and guanethidine in some other test situations, but no major distinction between bethanidine and guanethidine was found in studies of the vasoconstrictor response to sympathetic nerve stimulation in the femoral vascular bed.

The only failure to distinguish between the effects of bretylium and guanethidine on different rates of sympathetic nerve stimulation has been in experiments using the isolated ileum preparation of rabbits, an artificial test situation remote from the mechanisms concerned in regulation of blood pressure.

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