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ADRENERGIC NEURONE BLOCKING AGENTS: TOLERANCE AND HYPERSENSITIVITY TO ADRENALINE AND NORADRENALINE

BY

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Suppression of the release of adrenergic transmitter from stimulated postganglionic sympathetic nerves and increased responsiveness of end-organs to injected adrenaline and noradrenaline occurs after bretylium (Boura & Green, 1959), guanethidine (Maxwell, Plummer, Schneider, Povalski & Daniel, 1960) and bethanidine (Boura & Green, 1963; Fewings, Hodge, Scroop & Whelan, 1964). During daily injection of bretylium and guanethidine into cats, impairment of the response of the nictitating membrane to sympathetic nerve stimulation at first increases but later declines as the sensitivity to injected catechol amines rises (Green, 1960; Boura & Green, 1962). Tolerance to adrenergic neurone blockade in this system was therefore attributed to increased responsiveness to the small amounts of adrenergic transmitter released during incomplete adrenergic neurone blockade. In the above studies, and in similar experiments using bethanidine described in this paper, the peak increases in sensitivity occurred after 7 to 14 daily doses and were approximately thirtyfold for adrenaline and 100-fold for noradrenaline, matching those produced by postganglionic sympathetic nerve section (Boura & Green, 1962; Trendelenburg, 1963). In contrast, sensitivities to the pressor action of these amines seldom rose above threefold (Green, 1960; Boura & Green, 1962). This difference might be related to the relative complexity of the pressor responses, this depending on the balance of sympathetic α- and β-receptor activation and subject to various compensating reflexes. Consequently we have proceeded to similar studies of two other less-complex systems, choosing the smooth muscle responses of the femoral vascular bed and the spleen in cats. The results illustrate the difference in the increases of sensitivity of different smooth muscles to adrenaline and noradrenaline produced by adrenergic neurone blocking agents but fit the hypothesis that " tolerance " to adrenergic neurone blockade in any system is related to the rise in responsiveness to the adrenergic transmitter.

Some investigations of the changes in sensitivity to catechol amines of the perfused hind-quarter of guinea-pigs given adrenergic neurone blocking agents daily are also reported.

METHODS

Anaesthetized cats. Cats of either sex weighing 2 to 4 kg were used. Anaesthesia was induced with ether and maintained with chloralose (about 60 mg/kg, intravenously). Contractions of the nictitating membrane were recorded with an isotonic frontal-writing lever, with 7 g tension and $\times 15$ magnification.

Changes in spleen volume were recorded by enclosing the spleen in a Perspex oncometer connected to a float recorder. The hind-limb was perfused at constant flow with the cat's own blood via the femoral artery with the aid of a Sigmamotor pump; changes in perfusion pressure, indicative of altered peripheral vascular resistance, were recorded with a mercury manometer (Green & Robson, 1964). The preganglionic cervical sympathetic chain, the splenic nerve and the lumbar sympathetic chain were stimulated with trains of rectangular-wave shocks of supramaximal voltage and 1 msec duration at progressively increasing frequencies in the range of 0.1 to 10 shocks/sec; each frequency was applied for a sufficient period to produce a maximal response. Stimulation of the lumbar sympathetic chain was intermittent, recovery of femoral vascular resistance to prestimulation levels being allowed between each train of stimuli. Rest periods were not given between the ascending frequencies of cervical sympathetic or splenic nerve stimulation.

Sensitivities of the nictitating membrane and the spleen to intravenous (−)-adrenaline acid tartrate and (−)-noradrenaline bitartrate were determined by injection of doses in the ascending series: 0.4, 0.8, 1.6 μg, etc. The dose was always given in 1 ml. of 0.9% saline slowly over a period of 30 sec and the preparation was allowed to recover fully between each dose. The same procedure was used for the femoral vascular bed except that the amines were injected rapidly into the femoral artery in doses of 0.1, 0.3, 1, 3 and 10 μg contained in 0.2 ml. of saline. All studies of peripheral vascular resistance were carried out after injection of atropine sulphate (1 mg/kg, intravenously).

The spleens of two cats were denervated by sectioning the splenic nerve aseptically while the animals were anaesthetized. Sensitivity of the spleen to intravenous injections of adrenaline and noradrenaline was determined on the 5th day after operation in one cat and on the 14th day in the other.

Guinea-pig perfused hind-quarters. Guinea-pigs of either sex and weighing about 500 g were killed and their hind-quarters were perfused through the abdominal aorta with Ringer solution at 37° C at a pressure of approximately 1 m water. Outflow from the veins was measured with an Andrews recorder (C. F. Palmer). Vasoconstriction produced by noradrenaline and adrenaline, introduced into the arterial cannula

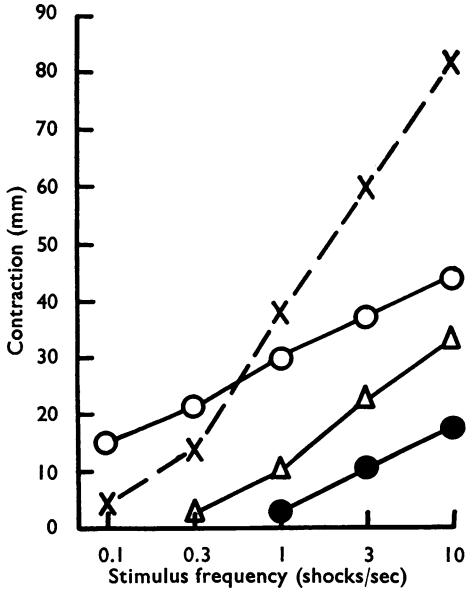

Fig. 1. Mean size of contractions of the nictitating membranes of anaesthetized cats caused by stimulation of the preganglionic cervical sympathetic nerve at various frequencies: ×---×, controls; Δ——Δ, 24 hr after a single dose of 1 mg/kg of bethanidine when responses were considerably depressed; ○——○, after 1 mg/kg daily for 14 days when inhibition was less. Depression of responses was great when 3 mg/kg of bethanidine had been given daily for 14 days (●——●). Group sizes: controls, eleven cats; treated groups, three cats.

in a volume of 0.1 ml., was assessed as the percentage reduction in flow rate. The composition of the Ringer solution was (g/l. of distilled water): NaCl 9.0, KCl 0.2, $MgCl_2$ $6H_2O$ 0.2, NaH_2PO_4 $2H_2O$ 0.05, $NaHCO_3$ 1.0, $CaCl_2$ 0.15 and dextrose 1.0.

Adrenergic neurone blocking agents were injected subcutaneously once only or daily; responses to nerve stimulation or injection of catechol amines were determined 18 to 24 hr after the last dose. The doses refer to the sulphate of guanethidine, the *para*-toluene sulphonate of bretylium and the hydrochloride of bethanidine.

RESULTS

Nictitating membrane and pressor responses. When 1 or 3 mg/kg of bethanidine was injected daily into unanaesthetized cats the nictitating membranes were relaxed for a few days but thereafter gradually retracted despite continued administration of the drug. Responses of the nictitating membrane to all frequencies of sympathetic nerve stimulation in the anaesthetized animal 24 hr after a single subcutaneous dose of 1 mg/kg of bethanidine were substantially less than those of controls but when this dose had been given daily for 14 days responses to low stimulus frequencies exceeded those in controls and the depression of responses to high frequencies was less marked (Fig. 1). At 20 hr after a single dose of 3 mg/kg of bethanidine, there was total block of responses to all frequencies studied, but after giving this amount daily for 14 days some recovery of responsiveness had occurred.

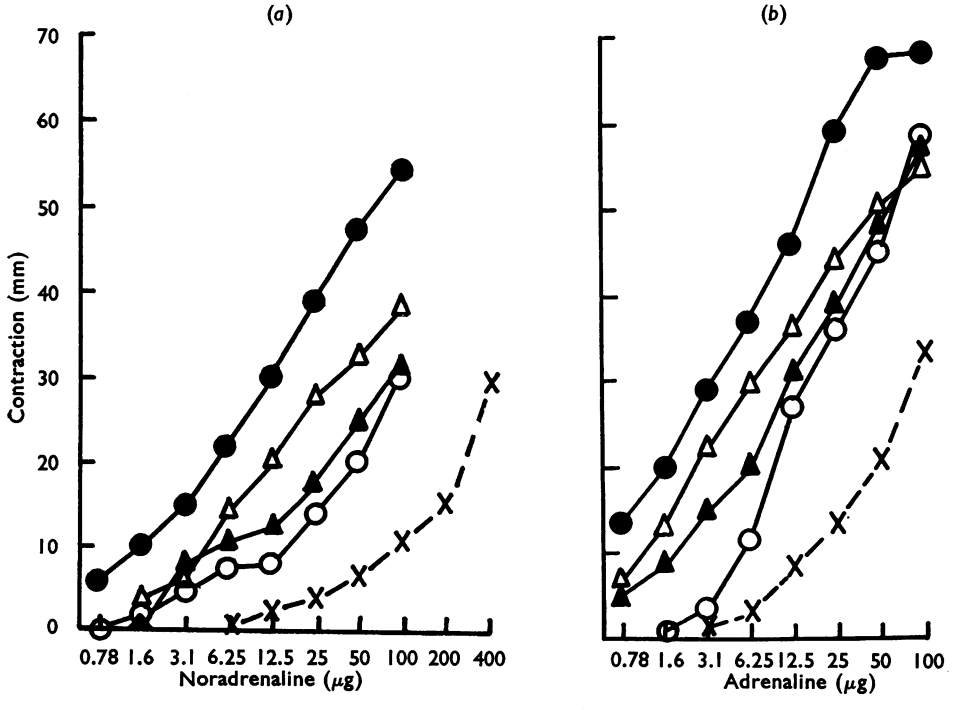

Fig. 2. Mean size of contractions of the nictitating membranes of anaesthetized cats caused by intravenous noradrenaline (*a*) and adrenaline (*b*): ×---×, controls; ▲——▲ and ○——○, after single doses of 1 mg/kg and 3 mg/kg of bethanidine respectively; Δ —— Δ and ● —— ●, after fourteen daily doses of 1 mg/kg and 3 mg/kg respectively. Hypersensitivity was greater with the multiple treatments. Group sizes: controls, twenty-three cats with doses up to 100 μg, two cats with higher doses of noradrenaline; treated groups, three cats.

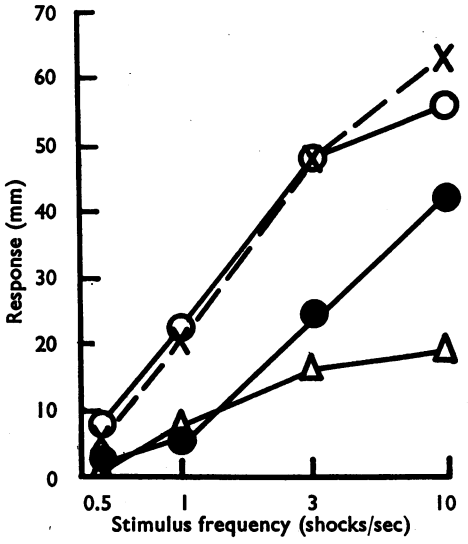

Fig. 3. Mean size of contractions of the spleen of anaesthetized cats caused by stimulation of the splenic nerve at various frequencies for sufficient time to allow a maximal response at each frequency: ×---×, controls (nine cats); ○——○, after fourteen daily doses of 3 mg/kg of bretylium (five cats); ●——●, after fourteen daily doses of 0.5 mg/kg of guanethidine (three cats); Δ——Δ, after fourteen daily doses of 2.5 mg/kg of guanethidine (two cats).

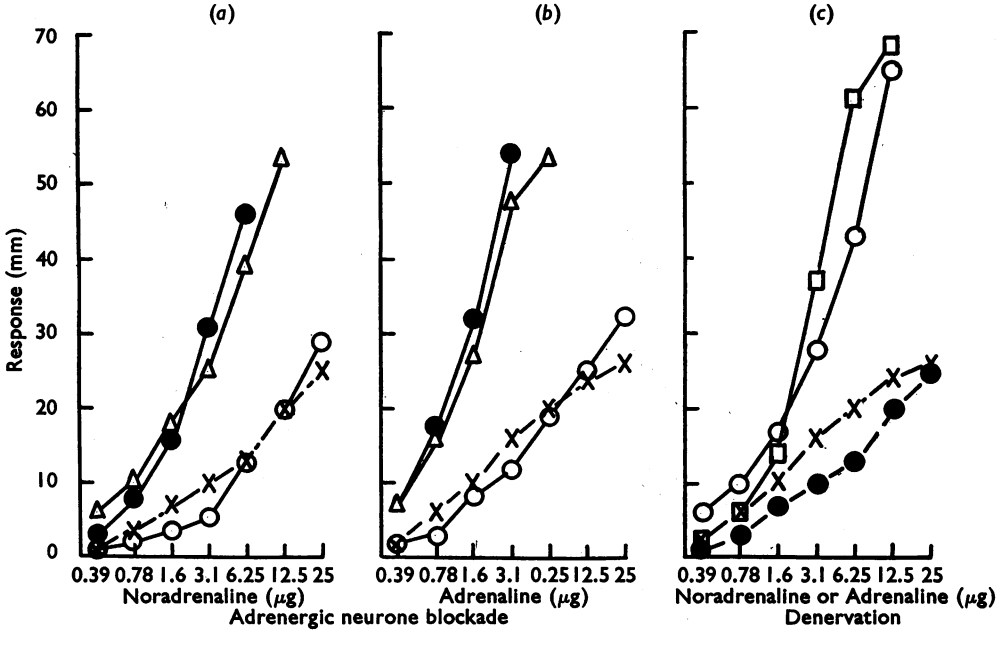

Fig. 4. Mean size of contractions of the spleen caused by noradrenaline and adrenaline in anesthetized cats. (*a*) and (*b*), sensitization by adrenergic neurone blocking agents: ×——×, controls (five cats) ○——○, after fourteen daily doses of 3 mg/kg of bretylium (two cats): ●--●, after fourteen daily doses of 3 mg/kg of bethanidine (three cats); Δ——Δ, after seven daily doses of 10 mg/kg of guanethidine (two cats). (*c*), Hypersensitivity found 5 to 14 days after cutting the splenic nerve: ●---● and ×---×, responses to noradrenaline and adrenaline respectively in controls (five cats); ○——○ and □——□, responses to noradrenaline and adrenaline respectively after denervation (two cats).

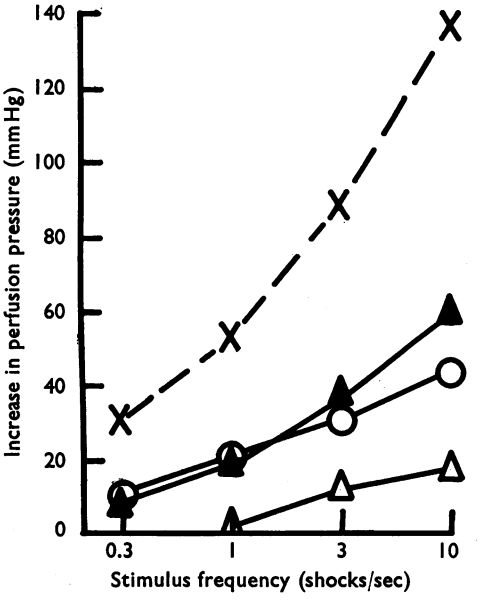

Fig. 5. The effects of adrenergic neurone blocking agents on regression lines relating frequency of lumbar sympathetic chain stimulation to increase in perfusion pressure in the femoral artery of anaesthetized cats given 1 mg/kg of atropine sulphate, intravenously: ×---×, control (mean of ten cats); ▲——▲, after fourteen daily doses of 1 mg/kg of bethanidine (three cats); ○——○, after fourteen daily doses of 3 mg/kg of bretylium (three cats); Δ —— Δ, after fourteen daily doses of 2.5 mg/kg of guanethidine (three cats).

The dose/response curves for noradrenaline (Fig. 2,*a*) and adrenaline (Fig. 2,*b*) showed a roughly parallel shift to the left after bethanidine, the increased sensitivity varying with the period of drug administration. The greatest hypersensitivity, approximately sixtyfold for noradrenaline and twentyfold for adrenaline, occurred when 3 mg/kg of bethanidine had been given daily for 14 days.

Pressor responses were recorded from a carotid artery in the same animals. Sensitivity to noradrenaline and adrenaline increased after administration of bethanidine but, even when 3 mg/kg had been given daily for 14 days, never by more than fourfold. These results are in keeping with those with bretylium and guanethidine (Boura & Green, 1962).

Spleen. At all frequencies of splenic nerve stimulation, contraction of the spleen was less than in controls when 0.5 or 2.5 mg/kg of guanethidine had been injected daily for 14 days, but little impairment was apparent after 3 mg/kg of bretylium daily for 14 days (Fig. 3).

The dose/response curves for noradrenaline (Fig. 4,*a*) and adrenaline (Fig. 4,*b*) were steeper after daily administration of the blocking agents. After daily administration of 10 mg/kg of guanethidine for 1 week, 2.5 mg/kg of guanethidine for 14 days (not in Fig. 4) or 3 mg/kg of bethanidine for 14 days the increases of responses to adrenaline and noradrenaline were large and comparable with those after splenic denervation (Fig. 4,*c*). When 3 mg/kg of bretylium had been given daily for 14 days there was no increase in

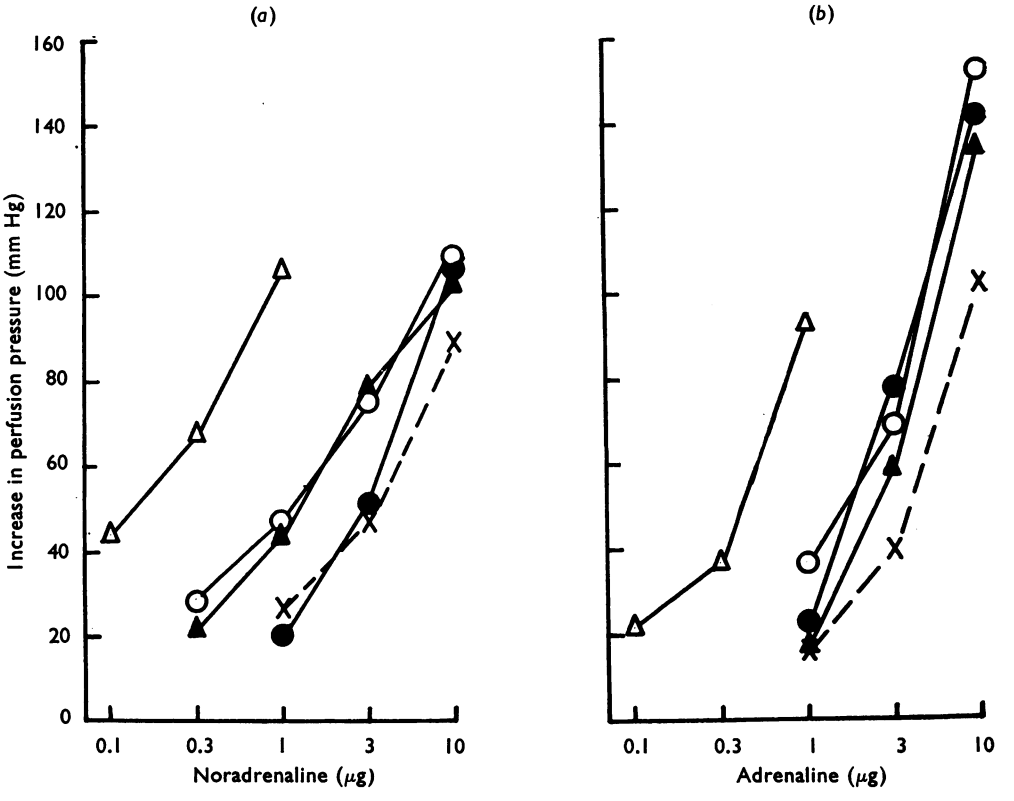

Fig. 6. Mean increase in perfusion pressure in the femoral vascular bed of anaesthetized cats given atropine caused by intra-arterial injection of noradrenaline (*a*) and adrenaline (*b*): ×---×, controls (five cats); ●——●, after fourteen daily doses of 1 mg/kg of bethanidine (four cats); ▲——▲, after fourteen daily doses of 2.5 mg/kg of guanethidine (four cats); Δ —— Δ, after seven daily doses of 10 mg/kg of guanethidine (two cats); ○——○, after fourteen daily doses of 3 mg/kg of bretylium (five cats). Sensitivity to the catechol amines was greatly increased only by the large dose of guanethidine.

responses to the catechol amines in keeping with the lack of the impairment of responses of the spleen to sympathetic nerve stimulation.

Femoral vascular bed. The rise in peripheral vascular resistance, caused in the hind-limbs of cats when the lumbar sympathetic chain was stimulated, increased with the frequency of nerve stimulation (Fig. 5). At all frequencies the responses were substantially less than in controls in cats given 14 daily subcutaneous doses of 3 mg/kg of bretylium, 2.5 mg/kg of guanethidine or 1 mg/kg of bethanidine. Responses were abolished when 10 mg/kg of guanethidine had been given daily for 7 days.

Following these treatments the slopes of the dose/response curves for noradrenaline (Fig. 6,*a*) and adrenaline (Fig. 6,*b*) generally showed no major change. Hypersensitivity did not reach significant levels after doses of the adrenergic neurone blocking agents causing partial suppression of nerve responses, but a ten- to twentyfold increase was produced by guanethidine in amounts causing abolition of nerve responses.

TABLE 1

AMOUNTS OF NORADRENALINE AND ADRENALINE REDUCING VENOUS OUTFLOW BY 30% IN PERFUSED GUINEA-PIG HIND-QUARTERS

Bethanidine treatment: 10 mg/kg daily for 14 to 21 days; guanethidine treatment: 5 or 10 mg/kg daily for 10 to 16 days. Values are means with ranges in parentheses

Treatment	No. of experiments	Noradrenaline (μg)	Adrenaline (μg)
Control	9	0·83 (0·2–6·4)	0·75 (0·3–1·6)
Bethanidine	5	0·57 (0·1–1·5)	0·81 (0·1–2·1)
Guanethidine	4	0·15 (0·02–0·8)	0·16 (0·03–1·5)

Guinea-pig perfused hind-quarters. Table 1 shows the mean doses of intra-arterial noradrenaline and adrenaline estimated from dose/response curves to cause a 30% reduction in flow. Although the means for the guanethidine-treated group were lower than for the controls and the bethanidine-treated group, because of the wide variation in the sensitivity of preparations these differences were not statistically significant.

DISCUSSION

Nictitating membrane and pressor responses produced by intravenous injections of adrenaline and noradrenaline into cats increased in magnitude after administration of bethanidine, in a similar manner to the increases found after bretylium or guanethidine (Green, 1960; Boura & Green, 1962). In particular, the sensitivity of the nictitating membranes increased with the duration of drug administration to a maximum sensitivity after fully blocking amounts had been given for 14 days, an increase similar to that produced after postganglionic nerve section. Similarly, when bethanidine or guanethidine had been administered daily in amounts sufficient to maintain substantial depression of the contractions of the spleen caused by splenic nerve stimulation, the increased sensitivity to the catechol amines again resembled that following sympathectomy; we tested only two cats after splenic nerve section but their sensitivity was similar to those tested by Burn & Rand (1959). These studies together with those concerned with the effects of bretylium on the salivary glands (Emmelin & Engström, 1961) strongly suggest a general similarity between the effects on sensitivity to catechol amines of adrenergic neurone blockade and chemical sympathectomy.

The increase in sensitivity to noradrenaline found in the systems studied in cats varied greatly. For example, whereas the greatest changes for intravenous noradrenaline in experiments using the nictitating membranes were approximately 100-fold, the sensitivity of the femoral vascular bed to intra-arterial noradrenaline never rose higher than ten- to twentyfold. Again, whereas the sensitivity of the nictitating membranes to noradrenaline increased twenty or more times after daily administration of 1 mg/kg of bethanidine, 3 mg/kg of bretylium or 2.5 mg/kg of guanethidine for 1 or 2 weeks, these treatments did not significantly increase the sensitivity of the vascular bed. That the sensitization of the femoral vascular bed to intra-arterial noradrenaline was less than the sensitization of the nictitating membrane to intravenous noradrenaline may be in part due to the different routes of administration. The inhibitory action of the adrenergic neurone blocking agents on noradrenaline and adrenaline uptake, which has been considered to be a major cause of hypersensitization (Vane, 1962), would presumably have greater opportunity of modifying the proportion of noradrenaline reaching effector muscle receptors when the amine is given

by the intravenous route than when given by close-arterial injection. In this connection Laurence & Nagle (1963) found that in man single doses of bretylium and guanethidine increased the pressor response to intravenous noradrenaline without increasing vasoconstrictor responses in the forearm produced by intra-arterial injection. However, Fewings *et al.* (1964) report an increased sensitivity to intra-arterial noradrenaline after intra-arterial infusion of bethanidine in hand blood flow experiments.

The change in responsiveness of the spleen was different again, especially in that the dose/response curves for intravenous noradrenaline and adrenaline became steeper after sustained adrenergic neurone blockade whereas in experiments using the other two systems they showed a roughly parallel shift; changes in noradrenaline threshold were relatively small.

In comparison with the variation in the levels of hypersensitivity to noradrenaline developed in the nictitating membrane, femoral vascular bed and spleen, the increases in susceptibility to adrenaline were relatively uniform. The hypersensitivity to noradrenaline greatly exceeded that to adrenaline only for the nictitating membranes; these are normally far more responsive to adrenaline than to noradrenaline but become about equally responsive to the two amines after sustained adrenergic neurone blockade.

Hypersensitivity to catechol amines develops also in other species following adrenergic neurone blockade (Boura & Green, 1965). Most of the studies have been concerned only with single doses, but Zimmerman & Harris (1963) reported that the mesoappendix vasculature of the rat became some three times more sensitive to topically applied adrenaline when 7.5 mg/kg of guanethidine had been given daily for up to 22 days. We found a similar trend using the perfused hind-quarters of guinea-pigs. In man, hypersensitivity to the amines occurs after single doses of the blocking agents (Laurence & Nagle, 1963) but it remains to be determined how far sensitivity increases with prolonged treatment.

After partial adrenergic neurone blockade the response of the effector organ to nerve stimulation must depend upon the amount of transmitter released and the sensitivity to it. Consequently, since bretylium, in contrast to guanethidine, depresses the slope of the curve relating frequency of sympathetic nerve stimulation to nictitating membrane contraction and produces minimal effects on transmitter released at low rates of nerve stimulation, responses to these become greater than in controls when bretylium has produced marked hypersensitivity to the transmitter (Boura & Green, 1962). The acute effect of bethanidine on the slope of such response curves is intermediate between those of bretylium and guanethidine (Boura & Green, 1963). In keeping with this we found a small enhancement of responses to low rates of nerve stimulation when administration of low doses of bethanidine daily had produced considerable hypersensitivity to adrenaline and noradrenaline, though higher doses suppressed these responses. Analogous differences between the effects of bretylium, bethanidine and guanethidine on the slopes of nerve stimulus frequency/response curves have been found in other systems (Green & Robson, 1964) and they can be related to the finding that, when these compounds are used to control hypertension in man, the need to increase dosage occurs most often with bretylium and least often with guanethidine (Montuschi & Pickens, 1962; Smirk, 1963). That tolerance to the hypotensive action of these agents in man has not been as precipitous as was expected from studies of the nictitating membrane responses in cats, even in the case of bretylium

(Boura, Green, McCoubrey, Laurence, Moulton & Rosenheim, 1959), suggests lesser sensitization to adrenergic transmitters. In cats, impairment of responses of the femoral vascular bed persisted with dosage schedules of the blocking agents that failed to maintain comparable suppression of nictitating membrane contractions caused by nerve stimulation in these experiments or in those reported by Boura & Green (1962). This can be at least partially attributed to the lesser susceptibility of the nictitating membrane response to adrenergic neurone blockade (Boura & Green, 1959, 1963; Green & Robson, 1964).

SUMMARY

1. Smooth muscle responses to nerve stimulation and intravenous injections of noradrenaline and adrenaline have been studied in anaesthetized cats given bretylium, bethanidine or guanethidine daily for up to 14 days.

2. The sensitivity of the nictitating membranes to intravenous noradrenaline and adrenaline increased during daily administration of bethanidine to reach a level similar to that known to follow postganglionic sympathetic nerve section or injection of equivalent doses of bretylium or guanethidine.

3. Like the other adrenergic neurone blocking agents, bethanidine caused a relatively small hypersensitivity to the pressor action of adrenaline and noradrenaline.

4. When bethanidine, bretylium or guanethidine had been given daily the responses of the femoral vascular bed to close arterial injection of noradrenaline or adrenaline showed a hypersensitivity less than that found in the nictitating membrane experiments; the hypersensitivity after large doses of guanethidine was greater than that revealed by the pressor responses to intravenous injections of the amines.

5. After daily administration of bethanidine or guanethidine in amounts that maintained substantial depression of the response of the spleen to nerve stimulation, responsiveness to noradrenaline and adrenaline reached the same level as after splenic nerve section.

6. Whereas the nictitating membranes showed a much greater rise in sensitivity to noradrenaline than to adrenaline, the other systems studied did not; after continued administration of the blocking agents all the systems examined showed approximately equal sensitivity to noradrenaline as to adrenaline.

7. Maintenance of suppression of the responses of the femoral vascular bed to sympathetic nerve stimulation required lower doses of the adrenergic neurone blocking agents than did maintenance of suppression of the nictitating membrane responses. These results are in keeping with the contention that " tolerance " is at least partially a sequel to the rise in the responsiveness of smooth muscle to the adrenergic transmitter.

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