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THE EFFECT OF STIMULATION OF SYMPATHETIC NERVES IN THE CAT TREATED WITH RESERPINE, α-METHYLDOPA AND α-METHYLMETATYROSINE

BY

W. HAEFELY, A. HÜRLIMANN AND H. THOENEN

From the Department of Experimental Medicine, F. Hoffmann-La Roche & Co., Basel, Switzerland

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Most drugs in use for treatment of arterial hypertension act by reducing rather specifically the effectiveness of sympathetic impulses on vascular smooth muscle and on myocardium. They differ, however, by the manner in which they affect the postganglionic adrenergic neurone.

One type of drug, represented by reserpine, acts by reducing the amount of transmitter stored in adrenergic nerve fibres (Muscholl & Vogt, 1958; Trendelenburg & Gravenstein, 1958; Chidsey, Braunwald & Morrow, 1962). Another class of agents, examples of which are bretylium and guanethidine, acts mainly by inhibiting the release of noradrenaline from adrenergic neurones (Hertting, Axelrod & Patrick, 1962).

α-Methyldopa [β-(3,4-dihydroxyphenyl)-α-methylalanine], a new antihypertensive drug, was originally introduced into therapy because of its inhibitory effect on aromatic amino-acid decarboxylase (Oates, Gillespie, Udenfriend & Sjoerdsma, 1960). It was, however, soon realized that its cardiovascular action could not be explained by this biochemical mechanism (Hess, Connamacher, Ozaki & Udenfriend, 1961; Maître & Staehelin, 1963; Levine & Sjoerdsma, 1964). Carlsson & Lindquist (1962) and Day & Rand (1963) advanced the hypothesis of a false transmitter mechanism at sympathetic nerve endings, at that time on the basis primarily of indirect evidence. The demonstration of the liberation of α-methyl-noradrenaline by sympathetic nerve stimulation from the heart of rabbits previously treated with α-methyldopa (Muscholl & Maître, 1963) and from the isolated perfused spleen of the cat (our unpublished results) has provided the direct proof. There is, however, still some uncertainty as to the functional consequences of replacing noradrenaline by α-methyl-noradrenaline on the effect of sympathetic neurochemical transmission in different organs and species (Goldberg, Da Costa & Ozaki, 1960; Stone, Porter, Watson & Ross, 1961; Stone, Ross, Wenger, Ludden, Blessing, Totaro & Porter, 1962; Gaffney, Rousseau, Woronkow & Peagler, 1963; Mason & Braunwald, 1964). Divergent values are also given in the literature for the relative potency of noradrenaline and α-methylnoradrenaline (Mueller & Horwitz, 1962; Pettinger, Horwitz, Spector & Sjoerdsma, 1963; Day & Rand, 1964; Lindmar & Muscholl, 1965).

The amino-acid α-methylmetatyrosine (β-(3-hydroxyphenyl)-α-methylalanine) is very closely related chemically to α-methyldopa. It undergoes a similar metabolic transforma-

tion, being decarboxylated to α-methylmetatyramine and further hydroxylated to metaraminol (3-hydroxynorephedrine) (Carlsson & Lindqvist, 1962). This latter amine is even more effective than α-methylnoradrenaline in displacing noradrenaline (for references see Haefely, Thoenen & Hürlimann, 1965) and can also be released by sympathetic nerve stimulation (Crout, Alpers, Tatum & Shore, 1964). α-Methylmetatyrosine has antihypertensive properties in man when given parenterally (Horwitz & Sjoerdsma, 1964), whereas no such effect could be observed in experimental animals (Stone *et al.*, 1961). In contrast to Andén (1964), we found that previous treatment with α-methylmetatyrosine markedly reduced the effect of sympathetic nerve stimulation on the cat nictitating membrane and isolated perfused spleen (Haefely *et al.*, 1965).

In the present study cats were treated for three days with α-methyldopa or with α-methylmetatyrosine in order to replace part of the noradrenaline in sympathetic nerves by α-methylnoradrenaline and metaraminol respectively. The effect of graded sympathetic nerve stimulation on the nictitating membrane was then studied quantitatively on spinal preparations. The sensitivity of the nictitating membrane and of the cardiovascular system to noradrenaline, 1,1-dimethyl-4-phenylpiperazinium iodide and tyramine was tested in the same animals. A group of cats was treated with low doses of reserpine in order to compare the effect of simple depletion of the normal transmitter with that of its partial replacement by a false transmitter.

METHODS

Four groups of cats were used. One group of twelve untreated cats served as control. Eight animals were treated with α-methyldopa given for 3 days twice daily in a dose of 100 mg/kg subcutaneously as an aqueous suspension. Four cats underwent the same treatment except that the drug was given intraperitoneally. This mode of application was used in order to test the absorption of subcutaneously injected α-methyldopa and because it was used by Day & Rand (1964). Since the results obtained were essentially the same after intraperitoneal and subcutaneous administration, only the latter will be reported. Four cats received α-methylmetatyrosine instead of α-methyldopa. The fourth group (five cats) was treated for 3 days with daily intraperitoneal injections of 0.1 mg/kg of reserpine.

At 16 to 20 hr after the last injection the animals were anaesthetized with sodium pentobarbitone (Nembutal; 30 mg/kg, intraperitoneally). The spinal cord was then cut at the level of the first cervical segment and the brain was destroyed. The preparations were maintained by positive-pressure artificial ventilation. Body temperature was held constant at 38° C. The distal end of the severed cervical sympathetic trunk was laid on platinum electrodes for stimulation. The contractions of both nictitating membranes were recorded isotonically with ink-writing frontal levers. The tension on the membrane was 4 g, the magnification of recording sevenfold. The stimuli were rectangular biphasic pulses of 1 msec duration and supramaximal voltage. The stimulus frequency was either kept constant at 1.6 shocks/sec for "stimulus number/response curves" (single shock and trains of 3, 9 and 27 stimuli) or progressively doubled, beginning with 0.2, up to 25 shocks/sec for cumulative "frequency/response curves." In the latter instance the nerve was stimulated at each rate until the contraction of the nictitating membrane had reached a plateau. Arterial blood pressure was recorded with a mercury manometer; the heart rate was continuously recorded by a collector driven by an electrocardiograph. Injections of increasing doses of the ganglionic stimulant dimethylphenylpiperazinium, tyramine and noradrenaline were made through a polyethylene catheter into a femoral vein. A typical experiment is presented in Fig. 1. From the results obtained in the individual animals of the four groups mean values and standard errors were calculated and submitted to the *t*-test for significance. Values of *P* below 0.05 were considered significant.

The drugs used were: reserpine (Serpasil; Ciba), DL-α-methyldopa (prepared by Dr Hegedüs in the Chemical Research Laboratories of Roche Basle), DL-α-methylmetatyrosine monohydrate (Regis Chemical Company), (−)-noradrenaline hydrochloride (Arterenol; Hoechst), dimethylphenylpiperazinium iodide (Parke Davis) and tyramine hydrochloride (Roche). The doses of the amines refer to the salts.

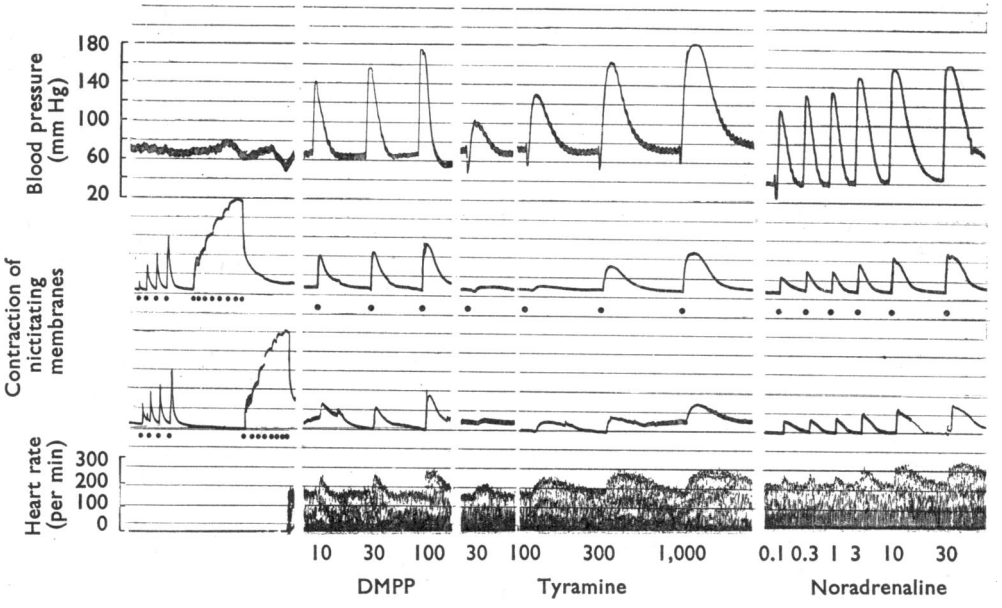

Fig. 1. Cat, 3.0 kg, treated with α-methyldopa (100 mg/kg, subcutaneously, twice daily for 3 days) and made spinal 16 hr after the last injection. Uppermost trace: arterial blood pressure; second and third traces: contractions of nictitating membranes; lowest trace: heart rate. The record shows the responses of the nictitating membranes to indirect stimulation, first to a single shock and to trains of 3, 9 and 27 stimuli at 1.6 shocks/sec (stimulus number/response curve), thereafter to continuous stimulation of the cervical sympathetic nerves at 0.2, 0.4, 0.8, 1.6, 3.2, 6.4, 12.8 and 25.6 shocks/sec (frequency/response curve). After the responses of the membranes to indirect stimulation the effects on the three parameters of intravenous injections of dimethylphenylpiperazinium (DMPP), tyramine and noradrenaline were tested. The doses are given as μg/kg, and injections were at the black dots.

RESULTS

General observations on pretreated animals

Cats treated with α-methyldopa did not differ from control animals except by a questionable decrease of spontaneous locomotor activity. Cats treated with α-methylmetatyrosine began to show definite signs of increased alertness about 30 min after the injections. Although the locomotor activity did not change, the cats continuously turned their heads from side to side, watching the room very attentively as if waiting for something. The animals appeared neither aggressive nor anxious. Various signs of moderately increased sympathetic activity such as mydriasis and bristling reaction of the hairs occurred. These phenomena disappeared gradually after about 1 hr. The cats receiving reserpine were not visibly disturbed after the first injection. Shortly after the second and third injection the animals began to whine. Most developed some diarrhoea. Ptosis and miosis were generally less pronounced than after the usual high doses of 2 mg/kg or more. Muscular co-ordination was not grossly affected. In no group did the animals refuse food. Since all were in a warm room, body temperature did not vary substantially.

The effect of sympathetic nerve stimulation

α-Methyldopa. Fig. 2 shows the results in the four groups. There was no significant difference between the frequency/response curves of controls and animals treated with α-methyldopa. This contrasts with the finding of Day & Rand (1964). In the stimulus number/response curves, however, the values of the two groups tended to diverge for the

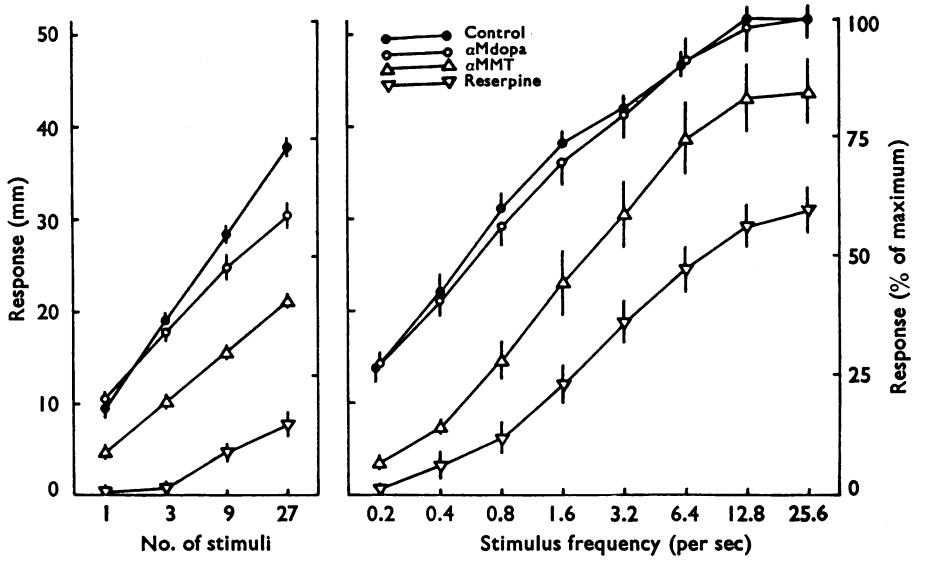

Fig. 2. Responses of the nictitating membranes to stimulation of the cervical sympathetic nerve. Left: stimulus number/response curves. Right: cumulative frequency/response curves. The vertical bars indicate standard errors of the means. αMdopa = α-methyldopa; αMMT= α-methylmetatyrosine.

upper three points; the difference was statistically significant only for the response to a train of twenty-seven shocks. This finding is surprising, since for controls the contraction height of the nictitating membrane was the same whether obtained as a response to an isolated train of twenty-seven shocks at 1.6 shocks/sec or to continuous stimulation at the same rate (cumulative frequency/response curve). Thus the only differences between these two stimulation parameters was continuity of stimulation and stimulation at lower frequencies which preceded that at 1.6 shocks/sec in the latter experiment. It is interesting to note that the difference between the response to a train of twenty-seven shocks at 1.6 shocks/sec and that to the same rate in the cumulative frequency/response curve was seen not only after α-methyldopa but also after α-methylmetatyrosine and after reserpine.

α-Methylmetatyrosine. In contrast to the response of the α-methyldopa group, treatment with α-methylmetatyrosine greatly reduced the effect of sympathetic nerve stimulation. The difference between the control and treated animals was statistically significant for all stimulation parameters. The frequency/response curve was shifted in a parallel manner to the right, the frequency-ratio being about 4.7, and the maximal obtainable contraction was reduced to 85%. The stimulus number/response curve was, however, not shifted in parallel, the effect of a train of three shocks after α-methylmetatyrosine being equivalent to that of

a single shock, and the response to a train of twenty-seven shocks after treatment equivalent to a train of six shocks without treatment.

Reserpine. Treatment with reserpine (0.1 mg/kg) on three consecutive days greatly reduced the effect of sympathetic nerve stimulation on the nictitating membrane. The shift of the frequency/response curve to the right was roughly parallel, the ratio being approximately 20. The maximum contraction obtainable was reduced to 60%. Fleming & Trendelenburg (1961), studying only the lower range of the frequency/response curve, had earlier found a similar reduction after the same dose schedule of treatment with reserpine. On the stimulus number/response curve the reduction caused by reserpine was greater with longer trains of stimulation. As after α-methyldopa and α-methylmetatyrosine stimulation at a frequency of 1.6 shocks/sec was more effective when determining cumulative frequency/response than with an isolated train of twenty-seven shocks.

The effect of treatment on the response of the nictitating membrane to humoral stimulation

Noradrenaline. Due to the very low sensitivity of the nictitating membrane to injected noradrenaline only the lower part of its dose/response curve could be studied. Treatment with α-methyldopa, α-methylmetatyrosine and reserpine resulted in a large, approximately equal increase in the sensitivity of the membrane to intravenously injected noradrenaline (Fig. 3). The increased sensitivity to these low doses of noradrenaline was high and—as far as reserpine is concerned—in sharp contrast to the results of Fleming & Trendelenburg (1961), who found no change of sensitivity of the nictitating membrane to noradrenaline after the same treatment.

Dimethylphenylpiperazinium. This is assumed to act as a ganglionic stimulant and as an indirect sympathomimetic agent, possibly at the same sites as tyramine (Lindmar, 1962).

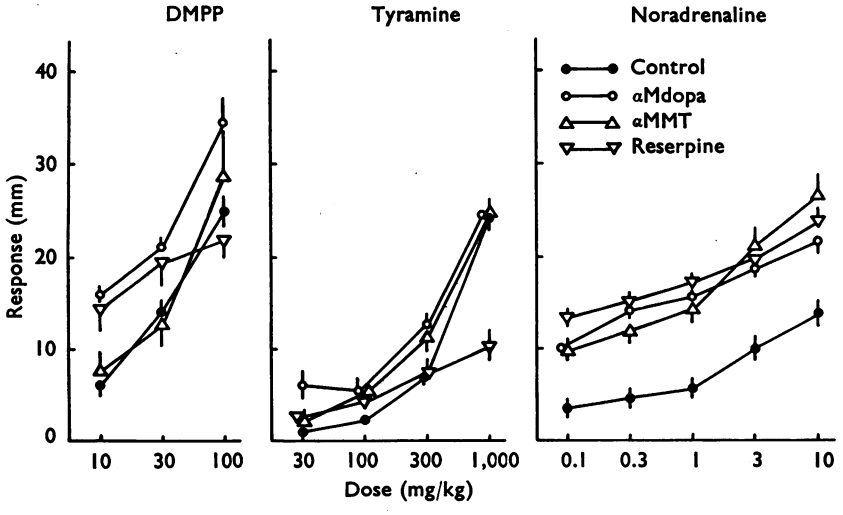

Fig. 3. Responses of the nictitating membranes to humoral stimulation by dimethylphenylpiperazinium (DMPP), tyramine and noradrenaline. The abscissa gives doses of these drugs in μg/kg. αMdopa = α-methyldopa; αMMT= α-methylmetatyrosine.

The results on the nictitating membrane in our study accord with this view. As can be seen from Fig. 1, the two lower doses of dimethylphenylpiperazinium produced a smooth contraction of the membrane much like that resulting from tyramine. With the higher dose this contraction was preceded by a sudden short-lived one similar to the response seen with nicotine or electrical stimulation of the cervical sympathetic nerve. The dose/response curve for dimethylphenylpiperazinium on the nictitating membrane was unchanged after α-methylmetatyrosine. In the α-methyldopa group all three doses of dimethylphenylpiperazinium produced significantly greater contractions. Treatment with reserpine increased the effect of the two lower doses of dimethylphenylpiperazinium and somewhat diminished that of the higher dose (although this difference was not statistically significant). Most strikingly, the sudden short-lived contraction was never seen in the reserpine group.

Tyramine. The effect of the three lower doses of tyramine was significantly increased in the α-methyldopa group, while that of 1 mg remained unchanged. After α-methylmetatyrosine only the response to the second dose of tyramine was significantly different from the control response. After treatment with reserpine the two lower doses of tyramine were slightly (although statistically not significantly) more effective than in the control group, whereas the third dose did not differ from the control and the response to 1 mg/kg was much reduced.

Basal blood pressure and heart rate in the four groups

As seen in Fig. 4, the basal values for blood pressure and heart rate were not significantly different in the four groups. The sensitivity to dimethylphenylpiperazinium, tyramine and noradrenaline could therefore be compared without interference from differences in initial values.

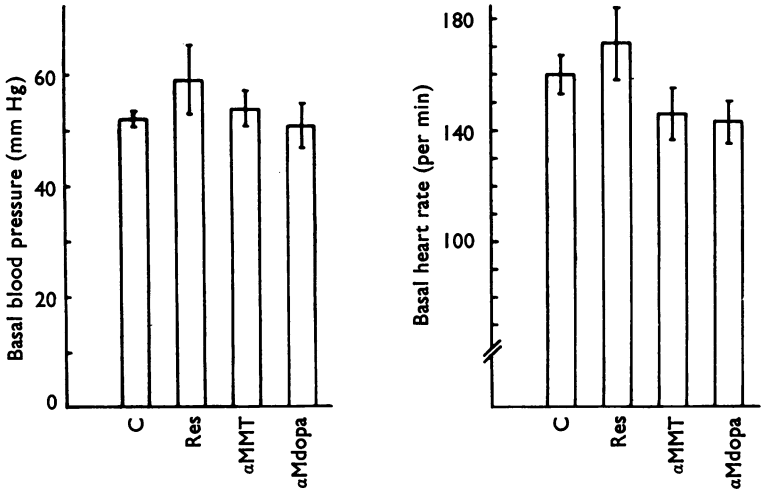

Fig. 4. Values of initial blood pressure and heart rate of control animals (C), of cats treated with reserpine (Res; 0.1 mg/kg/day for 3 days), with α-methyldopa (αMdopa; 100 mg/kg, subcutaneously, twice daily for 3 days) and with α-methylmetatyrosine (αMMT; 100 mg/kg, subcutaneously, twice daily for 3 days). Bars indicate standard errors. The differences are not statistically significant.

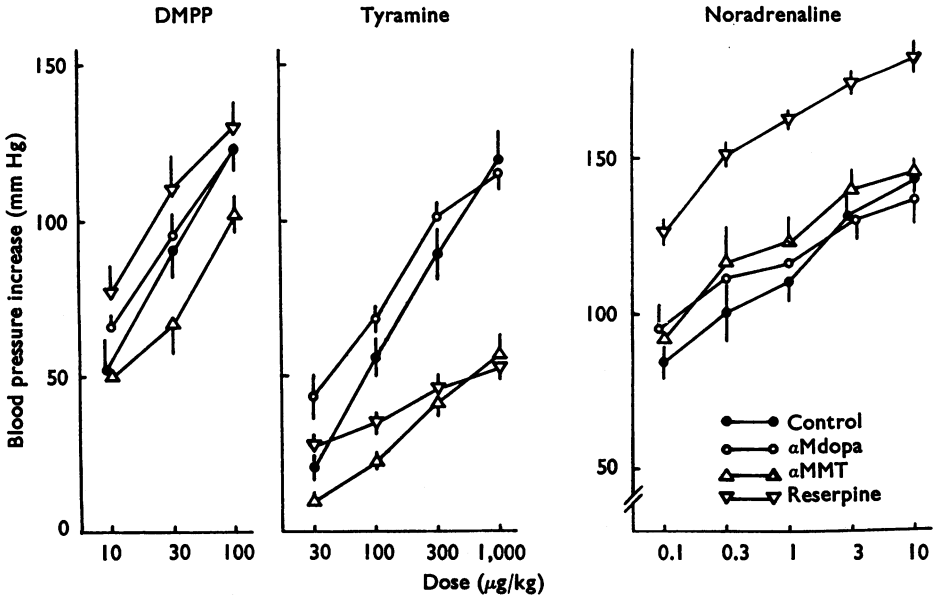

Fig. 5. Pressor responses to intravenous injections of dimethylphenylpiperazinium (DMPP), tyramine and noradrenaline. Abscissa gives doses of these drugs in μg/kg. αMdopa = α-methyldopa; αMMT= α-methylmetatyrosine.

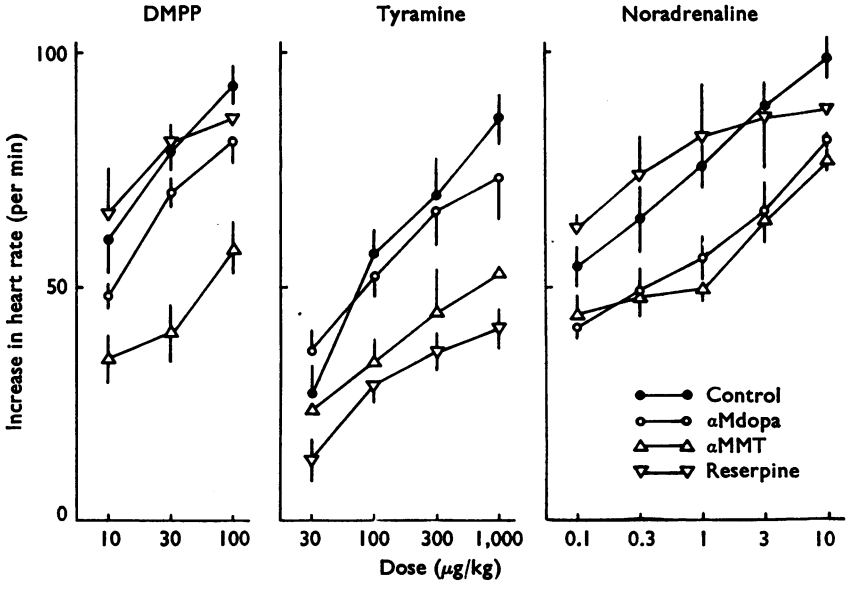

Fig. 6. Increase of heart rate in response to intravenous injections of dimethylphenylpiperazinium (DMPP), tyramine and noradrenaline. Abscissa gives doses of these drugs in μg/kg. αMdopa = α-methyldopa; αMMT= α-methylmetatyrosine.

The pressor responses to dimethylphenylpiperazinium, tyramine and noradrenaline

The dose/response curves for dimethylphenylpiperazinium were not significantly different from one another in the four groups (Fig. 5). Tyramine was slightly more effective in the lower dose-range after α-methyldopa. Treatment with α-methylmetatyrosine and reserpine greatly depressed the dose/response curves (statistically significant from the second dose upwards). The pressor effect of noradrenaline was not significantly altered after treatment with either amino-acid. Treatment with reserpine produced a parallel shift of the dose/response curve to the left, the sensitivity being about ten-times higher.

The effect of humoral stimulation of the pacemaker (Fig. 6)

The positive chronotropic effect of dimethylphenylpiperazinium was slightly depressed after treatment with α-methyldopa, but remained virtually unchanged after α-methylmetatyrosine. Treatment with reserpine markedly depressed the effect of dimethylphenylpiperazinium. No change occurred in the effect of tyramine after treatment with α-methyldopa, whereas the higher doses of tyramine were less effective after α-methylmetatyrosine. Treatment with reserpine depressed the dose/response curve. The pacemaker was approximately ten-times less sensitive to noradrenaline after α-methyldopa and α-methylmetatyrosine, whereas treatment with reserpine left the dose/response curve virtually unaltered.

Relative potencies of (−)-noradrenaline, (−)-α-methylnoradrenaline and (−)-metaraminol on nictitating membrane, blood pressure and heart rate

(−)-α-Methylnoradrenaline and (−)-metaraminol were compared with (−)-noradrenaline on spinal cats for their effects on blood pressure, heart rate and nictitating membrane contractions. The cats were either normal or previously treated with 2 mg/kg of reserpine, intraperitoneally 24 hr before, or had the right superior cervical ganglion removed 6 to 10 days before. The amines were injected in equimolar amounts intravenously except for comparisons on the chronically denervated nictitating membrane, when injections were made into a carotid artery by the cannulated lingual artery. On normal animals a complete dose/response curve was made first with noradrenaline and then with α-methylnoradrenaline or metaraminol. Reserpine-treated animals were used to determine the direct effect of the amines on catechol amine receptors. In these animals the administration of noradrenaline before one of the other amines might have refilled to some extent the depleted stores and thereby enabled the other amines to act indirectly on catechol amine receptors by liberation of noradrenaline. Therefore the order of testing was reversed and dose/response curves for α-methylnoradrenaline or metaraminol were made before that for noradrenaline. This order could, however, falsify the real potency ratio by the development of increased sensitivity to noradrenaline after metaraminol and less regularly and less clearly after α-methylnoradrenaline. This difficulty was overcome by testing the sensitivity to noradrenaline with a small dose of this amine before either α-methylnoradrenaline or metaraminol. The dose/response curve for noradrenaline was made only when the sensitivity was the same as at the beginning of the experiment. Average dose/response curves were constructed from the individual experiments and the potency ratio was determined. The values are given in Table 1. A single value for the relative potency appears when the dose/response curves were parallel. When the curves differed in their slope both

TABLE 1

RELATIVE POTENCIES OF (−)-NORADRENALINE, (−)-α-METHYLNORADRENALINE AND (−)-METARAMINOL IN SPINAL CATS

Potencies were calculated from average dose/response curves. Numbers of experiments are indicated in parentheses. When the average dose/response curves were not parallel both the highest and the lowest ratios are given

		Relative potency of		
Test organ	Condition	(−)-Noradrenaline	(−)-α-Methylnoradrenaline	(−)-Metaraminol
Blood pressure	Normal	1	1 (5)	0·03 (3)
	Reserpinized	1		0·03 (2)
Nictitating membrane	Normal	1	0·5–0·3 (5)	0·03–0·02 (2)
	Reserpinized	1		0·1 (1)
	Chronically denervated	1	0·3 (5)	0·03 (2)
Heart rate	Normal	1	1 (4)	0·003 (3)
	Reserpinized	1		0·02 (2)

the lowest and the highest value calculated are indicated. The effects of α-methylnoradrenaline were qualitatively indistinguishable from those of noradrenaline, whereas metaraminol differed greatly by the much longer duration of its effect, mainly on the heart rate and on the nictitating membrane, where its time course resembled very closely that of amphetamine. The duration of action was unchanged by previous treatment with reserpine. Of special relevance for our study were the relative potencies of the three amines on the chronically denervated membranes, since they represent the direct effectiveness on catechol amine receptors of the membrane: α-methylnoradrenaline was about one-third as potent as noradrenaline, and metaraminol one-tenth to one-thirtieth.

DISCUSSION

The effect common to the three drugs studied is a reduction of the amounts of noradrenaline stored in adrenergic neurones. In the case of reserpine this reduction is caused by an impairment in the capacity of storage organelles to retain the amine by an active process, whereas the amine seems to be displaced competitively by the decarboxylation products of α-methyldopa and α-methylmetatyrosine (for references see Introduction). The diminished effects of sympathetic nerve impulses after reserpine are easily explained by the reduced amounts of transmitter substance available at nerve terminals. After either amino acid the effectiveness of sympathetic nerve stimulation depends undoubtedly on the amounts of noradrenaline still present and available, and on the biological potency of the false transmitter substituting for the missing noradrenaline. But other factors must also play a role, as we shall note below.

The three drugs studied differ in their relative potency in decreasing the amounts of noradrenaline. In the present investigation no measurement of the noradrenaline content of the organ being studied was made and the three drugs may not have caused comparable reductions of noradrenaline stores. There is, however, a reasonable correlation between the doses used in this study and the clinical dosage. All three drugs were administered in a five- to six-fold higher dose per body weight than is customary in hypertensive patients.

Treatment with α-methyldopa

Treatment with α-methyldopa did not impair the effect of sympathetic nerve stimulation on the nictitating membrane as judged from the unaltered frequency/response curve. There was, however, some indication of a small impairment in the stimulus number/response curve. The response to an isolated train of twenty-seven shocks at 1.6 shocks/sec was smaller than to continuous stimulation at the same rate in the course of testing the cumulative frequency/response curve, although in control animals the contraction height was the same for both stimulation parameters. Continuous stimulation may overcome—perhaps by some facilitating mechanism—the slight impairment observed with short trains of stimuli. This same phenomenon was also observed after treatment with α-methyl-metatyrosine and reserpine. We cannot therefore exclude the possibility that treatment with α-methyldopa might reduce the effectiveness of sympathetic nerve activity occurring under physiological conditions in the cat. Day & Rand (1964) observed a shift to the right of the frequency/response curve after 3 days' treatment with α-methyldopa (100 mg/kg, intraperitoneally). In our four cats injected intraperitoneally for 3 days twice daily with 100 mg/kg the results were essentially the same as when the drug was administered subcutaneously (a slight shift of the frequency/response curve to the right was statistically not significant). The different results obtained by Day & Rand (1964) might be due to their use of chloralose as the anaesthetic for their cats.

The sensitivity of the nictitating membrane to noradrenaline was greatly increased after α-methyldopa. It is unlikely that this increased sensitivity is due to a nonspecific mechanism operating when sympathetic discharge to the effector organ is diminished for some time (Trendelenburg, 1963), since such a reduced sympathetic tone must have been very small if present at all. A mechanism involving altered inactivation of noradrenaline is more probable, in view of the inhibition of its uptake by α-methylnoradrenaline (Iversen, 1964; Dengler, 1964). Since after α-methyldopa sympathetic nerve stimulation must have liberated less noradrenaline than normally, and since the substituting α-methylnoradrenaline is roughly three-times less active on the denervated nictitating membrane, one would actually expect a clear-cut reduction of the effects of sympathetic nerve stimulation. That this did not occur might be due to the concomitant increase of sensitivity to noradrenaline. In contrast to the nictitating membrane, the increase of responsiveness of the blood pressure to noradrenaline was questionable, which corresponds to the finding in man (McCurdy, Prange, Lipton & Cochrane, 1964). This might be caused by the great reduction of sensitivity to noradrenaline of the cardiac pacemaker. We have no explanation for this rather selective reduction of sensitivity to noradrenaline of the heart, which also occurred after α-methylmetatyrosine.

The effects of dimethylphenylpiperazinium and tyramine on the nictitating membrane and on the blood pressure were slightly increased but their effect on the heart rate tended to be diminished. One could explain the greater effectiveness of tyramine by assuming that part of the noradrenaline released by tyramine is deaminated and thereby inactivated in untreated animals, whereas α-methylnoradrenaline, which is resistant to the monoamine oxidase, would reach the receptors in active form after release by tyramine. The diminished effect of tyramine on the heart and the increased effect on blood pressure and the nictitating membrane make it seem more probable that the responsiveness to tyramine simply reflects the sensitivity of the effector cells to noradrenaline.

Treatment with α-methylmetatyrosine

Treatment with α-methylmetatyrosine greatly reduced the effect of sympathetic nerve stimulation on the nictitating membrane. We know from experiments with the isolated spleen of cats previously treated with the same dose-schedule that sympathetic nerve stimulation liberates considerably less noradrenaline than in untreated animals. Furthermore, the replacing amine metaraminol is about thirty-times less active on the nictitating membrane. This may explain why the effect of sympathetic nerve stimulation is so clearly reduced despite a concomitant increase of the sensitivity to noradrenaline which was of the same order of magnitude as after α-methyldopa. This increased sensitivity is very probably due to inhibition of noradrenaline re-uptake, since it can also be seen after a single injection of metaraminol in acute experiments. The slight increase of sensitivity to noradrenaline of the blood pressure is not statistically significant and the lack of a greater increase may, as in the case of α-methyldopa, be explained by low sensitivity of the cardiac pacemaker. Accordingly the effects of tyramine and dimethylphenylpiperazinium are considerably reduced on the heart and somewhat less reduced on the blood pressure. The unaltered effect of dimethylphenylpiperazinium and the even slightly increased effect of tyramine on the nictitating membrane is difficult to understand.

Treatment with reserpine

The reduction of neurally mediated nictitating membrane contractions after three relatively small doses of reserpine confirms the findings of Fleming & Trendelenburg (1961). In contrast to these authors, we found a large increase of the sensitivity of this organ to exogenous noradrenaline which was of the same order of magnitude as after the two amino acids. The greater reduction of the effects of sympathetic nerve stimulation after reserpine than after the other two drugs could reflect a greater depletion of noradrenaline by the former agent, or a lack of replacement of the equally depleted normal transmitter by an artificial one. The very potent depleting action of α-methylmetatyrosine makes the latter possibility the more probable (Falck, 1962). The increased sensitivity to noradrenaline of the blood pressure was of the same order of magnitude as that of the nictitating membrane, whereas the cardiac pacemaker was only minimally more sensitive than in controls. In this respect the cat heart seems to behave like rat isolated atria (Bhagat, Booker & West, 1964) and differs from the dog heart (Bejrablaya, Burn & Walker, 1958). The reduction of the effects of tyramine on all three responses needs no comment. In contrast, the reduction of the effect of dimethylphenylpiperazinium was much smaller or even absent on the cardiovascular system, most likely demonstrating the different actions of dimethylphenylpiperazinium and tyramine on the adrenal medulla. In our experiments reserpine did not reduce the initial heart rate, as had been found by Fleming & Trendelenburg (1961) who used the same dose schedule. The different result may be explained by the use of ether as anaesthetic by these authors. Ether is known to increase sympathetic activity, and indeed the initial heart rate found in untreated animals by Fleming & Trendelenburg (1961) was higher by 30 beats/min than in our cats. It is evident that treatment with reserpine may lower a basal heart rate which would be higher without treatment due to some increased sympathetic activity outlasting the section of the spinal cord. We have also observed that cats made spinal during ether anaesthesia have a higher initial blood pressure than those made spinal during pentobarbitone anaesthesia.

From our results some general conclusions can be drawn concerning drugs which reduce the efficiency of sympathetic nerve activity by a false transmitter mechanism. Obviously the results with α-methyldopa on the nictitating membrane demonstrate that replacement of the normal transmitter by a substance capable of being stored and released at sympathetic nerve endings and having a weaker action on the specific receptors must not necessarily impair the effect of sympathetic nerve activity. A concomitant development of increased sensitivity to noradrenaline or a slower inactivation (uptake, metabolism) of the substituting false transmitter may compensate for the release of reduced amounts of the normal and of a less potent false transmitter substance. It also becomes clear that no conclusion as to the effectiveness of sympathetic nerve activity after substitution of the normal transmitter may be drawn from the measurement of the response to indirectly acting sympathomimetic amines. This demonstrates essential differences in the mode of physiological and pharmacological release. Furthermore, results obtained by studying the response to stimulation of the sympathetic nerve supply of a certain organ, such as the nictitating membrane, may not be representative of the adrenergic transmission in other effector organs such as the blood vessels and the heart. Different efficacy at the various receptors and perhaps organ differences in the inactivation of a false transmitter substance may be responsible for a lack of uniform reactivity of the sympathetic system. Species differences in the responsiveness of certain organs to substances capable of acting as false transmitters call for caution in extrapolating results from experimental animals to man. A typical example is α-methylnoradrenaline, which does not differ from noradrenaline in its pressor effect in cats and dogs (Conradi, 1964), whereas in man it seems to be three-times less potent (Mueller & Horwitz, 1962; Pettinger *et al.*, 1963).

SUMMARY

1. Groups of cats were treated for 3 days with either reserpine (0.1 mg/kg/day, intraperitoneally) or α-methyldopa or α-methylmetatyrosine (100 mg/kg, subcutaneously, twice daily). On the fourth day the animals were set up as spinal preparations. The following effects were measured and compared with those obtained in a control group of untreated animals: contraction of the nictitating membrane to graded stimulation of the cervical sympathetic nerves, and the effects of several doses of dimethylphenylpiperazinium, tyramine and noradrenaline on the nictitating membrane, blood pressure and heart rate.

2. Treatment with α-methyldopa did not substantially alter the effect of sympathetic nerve stimulation. It increased the sensitivity to noradrenaline of the nictitating membrane, left the effect of noradrenaline on the blood pressure unaltered and depressed it on the heart rate. The effect of dimethylphenylpiperazinium was increased on the nictitating membrane, and unaltered on the blood pressure and on the heart rate. The response to tyramine was increased on the nictitating membrane, slightly greater on the blood pressure and unaltered on the heart rate.

3. Treatment with α-methylmetatyrosine greatly reduced the effect of sympathetic nerve stimulation. There was a concomitant increase of the sensitivity to noradrenaline of the nictitating membrane, a decrease on the heart rate, but a questionable increase on the blood pressure. The action of dimethylphenylpiperazinium was unchanged on the nictitating membrane, tended to be diminished on the blood pressure and was greatly depressed on the heart rate. The effect of tyramine was slightly increased on the nictitating membrane and substantially depressed on the cardiovascular system.

4. Treatment with reserpine greatly depressed the effect of sympathetic nerve stimulation and greatly increased the sensitivity to noradrenaline of the nictitating membrane and the cardiovascular system. The influence on the sympathomimetic effects of dimethylphenylpiperazinium and tyramine was dependent on the dose of these two drugs; the effects of low doses were in general slightly increased or unchanged, whereas those of higher doses were depressed.

5. The results are discussed on the basis of the known facts that reserpine empties the noradrenaline stores in the peripheral sympathetic neurones, whereas α-methyldopa and α-methylmetatyrosine replace part of the normal transmitter by equal amounts of their decarboxylation products.

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