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ADRENERGIC TRANSMITTER CHANGES AND RESPONSE TO SYMPATHETIC NERVE STIMULATION AFTER DIFFERING PRETREATMENT WITH α-METHYLDOPA

BY

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α-Methyldopa is known to be metabolized to α-methylnoradrenaline, which replaces the physiological transmitter noradrenaline in sympathetic nerves. The formation of a less potent false transmitter in vasomotor fibres has been postulated to be the cause of the antihypertensive action of α-methyldopa in man (Day & Rand, 1964 ; see also Haefely, Hürlimann & Thoenen, 1966 ; and Muscholl, 1966). Despite the wide therapeutic use of this drug, no data have appeared either in man or in animals which would prove this hypothesis by showing a causal and quantitative relationship between impaired function of peripheral adrenergic neurones and the replacement of noradrenaline by α-methylnoradrenaline.

The nictitating membrane of the cat is a model extensively used for the study of drugs acting at the adrenergic neurone. It may be expected to be suitable also for the study of the action of α-methyldopa, since on this organ α-methylnoradrenaline is about three times less potent than noradrenaline (Haefely *et al.*, 1966). In a previous investigation (Haefely *et al.*, 1966) pretreatment of cats with α-methyldopa 100 mg/kg intraperitoneally or subcutaneously twice daily for 3 days did not depress neurally evoked contractions of the nictitating membrane. This lack of effect of α-methyldopa could be explained by the increased sensitivity to noradrenaline occurring after this pretreatment. It was realized, however, that further elucidation of the obviously complex action of α-methyldopa necessitated variations in the pretreatment schedule and the estimation of the replacement of noradrenaline by α-methylnoradrenaline. This has been undertaken in the present investigation. The results obtained make it unlikely that the formation of the false transmitter as such is causally related to impaired function of peripheral adrenergic neurones after α-methyldopa. A preliminary report of part of this work has appeared (Haefely, Thoenen & Hürlimann, 1967).

METHODS

Healthy cats of either sex weighing 2–4 kg were used.

Six treatment schedules with α-methyldopa (α-methyl-3,4-dihydroxy-DL-phenylalanine) were used. *Schedule A :* 100 mg/kg intraperitoneally twice daily for 3 days. Experiment on the spinal animal

on the fourth day—that is, 16 hr after application of the last dose. *Schedule B:* as in schedule A, but 200 mg/kg intraperitoneally. *Schedule C:* 100 mg/kg intraperitoneally 40, 24 and 16 hr before set-up of the spinal preparation. *Schedule D:* same timing as in schedule C, but 200 mg/kg intraperitoneally. *Schedule E:* 200 mg/kg intraperitoneally twice daily for 3 days, last dose 4 hr before the experiment. *Schedule F:* 200 mg/kg intraperitoneally 28, 20 and 4 hr before spinalization or killing for amine determination. *Controls:* cats housed as the drug-treated animals and injected with saline served as controls.

Experiments on spinal cats

The experiments on spinal cats were carried out as described previously (Haefely *et al.*, 1966). Briefly, the cats were spinalized at C_1 level under sodium pentobarbitone anaesthesia. The movements of both nictitating membranes were recorded isotonically under a tension of 4 g and magnified seven times. The cervical sympathetic trunk was stimulated with bipolar platinum electrodes using supramaximal pulses of 1 msec duration delivered by a Grass S 4 stimulator. "Stimulus number-response curves" were obtained by recording nictitating membrane contractions to 1, 3, 9 and 27 volleys at a stimulation frequency of 1.6/sec. For "frequency-response curves" repetitive stimulation at the rates of 0.2, 0.4, 0.8, 1.6, 3.2, 6.4, 12.8 and 25/sec was used in a cumulative manner, whereby the frequency was increased stepwise after a maximal response to the preceding one had been obtained. Partial dose-response curves for noradrenaline, DMPP (dimethyl-phenylpiperazinium iodide) and tyramine injected intravenously were constructed for the response of the nictitating membrane, the mean arterial blood pressure in the femoral artery and the heart rate.

Experiments on the isolated perfused spleen

The spleens were isolated as described previously (Thoenen, Hürlimann & Haefely, 1963) and perfused at a constant rate of 10 ml./min with a modified Krebs-Henseleit solution (Thoenen, Haefely, Gey & Hürlimann, 1965).

In a first series of experiments, designed to study the effect of pretreatment with α-methyldopa on the output of normal plus false transmitter as well as on the contractile response following splenic nerve stimulation, spleens of untreated cats and cats pretreated with 200 mg/kg 28, 20 and 4 hr before the experiment were used. The splenic nerves were stimulated with supramaximal rectangular monophasic pulses of 1 msec duration for 10 sec at a rate of 6 or 10/sec. The interval between these standard bursts of repetitive stimulation was 8 min. Changes in splenic volume were recorded with a plethysmograph and piston recorder. From the beginning of each stimulation period the venous effluent was collected for 90 sec, centrifuged and its pressor activity assayed on the blood pressure of the pithed rat. The pressor activity, expressed in terms of noradrenaline (base), includes both noradrenaline and α-methylnoradraneline present in the perfusates. Both amines have identical pressor potencies in the pithed rat (Muscholl & Maître, 1963).

A second series of experiments was designed to study the quantitative correlation between amines liberated by nerve stimulation and amines present in the spleen after pretreatment according to schedule B and E. The splenic nerves were stimulated repetitively for 2 min at a rate of 10/sec during continuous infusion of phenoxybenzamine (1 μg/ml.) into the arterial inflow which started 15 to 20 min before. Phenoxybenzamine prevented excessive contraction of the spleens and inhibited re-uptake of liberated noradrenaline and α-methylnoradrenaline into sympathetic nerve-endings, thus enabling quantitative differential estimation of the two amines. Intrasplenic removal and its inhibition by phenoxybenzamine is identical for noradrenaline and α-methylnoradrenaline added to the perfusion fluid of the spleen (unpublished). The splenic perfusate was collected during the repetitive stimulation and up to 30 sec after its termination. At the end of the collecting period the spleen was homogenized as described below.

Estimation of noradrenaline and α-methylnoradrenaline

The tissues (spleen, heart, iris and nictitating membrane) were frozen in petroleum ether on dry ice, weighed and homogenized in ice-cold 0.4 n $HClO_4$. Perfusates were deproteinized by adding 0.2 ml.

concentrated $HClO_4$ to 10 ml. perfusion fluid. The amines were isolated on Dowex-50 columns and eluted with 0.4 n HCl. Noradrenaline and α-methylnoradrenaline were determined by the fluorimetric-biological differential method described by Muscholl & Maître (1963). This method takes advantage of the fact that the pressor activity of equimolar quantities of noradrenaline and α-methylnoradrenaline is identical in the pithed rat, whereas the fluorescence of α-methylnoradrenaline is only very weak (<5%) under conditions used for fluorimetric estimation of noradrenaline. Noradrenaline was determined according to the method of Bertler, Carlsson & Rosengren (1958). The values of noradrenaline and α-methylnoradrenaline are corrected for recoveries determined in each experiment and found to vary between 70 and 90%.

In a few experiments the eluates of the Dowex-50 columns were evaporated to dryness *in vacuo* and the amines separated by paper chromatography (n-butanol/1 n HCl or phenol/0.1 n HCl descending for 20 to 26 hr; spray: 0.1% $K_3[Fe(CN)_6]$ in 5% aqueous ethylendiamine).

The substances used were: α-methyl-3,4-dihydroxy-DL-phenylalanine (α-methyldopa), tyramine hydrochloride, 1,1-dimethyl-4-phenylpiperazinium iodide (DMPP), (−)noradrenaline hydrochloride.

RESULTS

1. *Experiments on spinal cats*

Nictitating membrane. Figure 1 shows the effect of sympathetic nerve stimulation on the nictitating membrane in untreated controls and in cats pretreated with α-methyldopa

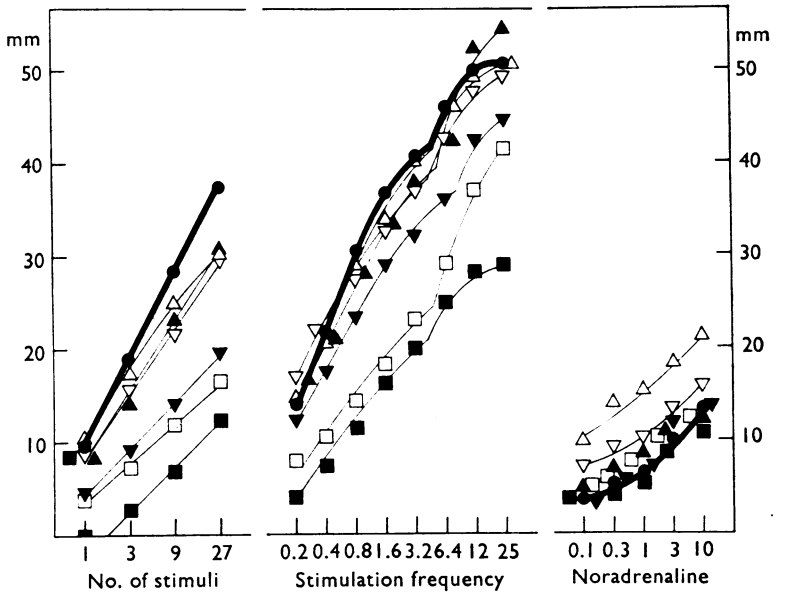

Fig. 1. The effect of sympathetic nerve stimulation (stimulus number-response curves and frequency-response curves) and noradrenaline intravenously (μg/kg) on the nictitating membrane after various pretreatments with α-methyldopa. Contraction height of the nictitating membrane is given on the ordinate as movement of the lever in mm on the kymograph. The following symbols are used: ● untreated controls (n=14), △ schedule A (n=8; figures taken from Haefely *et al.*, 1966), ▽ schedule B (n=4), ▲ schedule C (n=4), ▼ schedule D (n=4), □ schedule E (n=4), ■ schedule F (n=4). The points represent mean values. Standard errors are omitted for sake of clarity; they were (on the average) 20% of the mean value.

according to the six different treatment schedules. These schedules differed as to individual doses, total number of doses applied (which went parallel to the duration of pretreatment) and, most importantly, as to interval between the last application of drug and the experiment (post-drug interval). They will therefore be referred to as pretreatment with the lower or higher dose, the shorter or longer duration and the shorter or longer post-drug interval. As reported previously (Haefely *et al.*, 1966) pretreatment with 100 mg/kg twice daily for 3 days with the longer post-drug interval had no effect on the " frequency-response curve " and produced a questionable depression of the maximum of the stimulus number-response curve." This unexpected ineffectiveness of α-methyldopa was tentatively explained by the occurring supersensitivity of the nictitating membrane to noradrenaline. The first question was therefore whether or not this dosage was inadequate. Doubling the individual dose, but keeping the other variables of the treatment schedule constant, had effects on the responses to cervical sympathetic stimulation which were not significantly different from those obtained with the original schedule. The sensitivity to noradrenaline, however, increased much less after the higher dosage than after the lower. To test the possible importance of duration of pretreatment on the development of increased sensitivity to noradrenaline, cats were pretreated with the lower and higher dosage, but for a shorter time, keeping the longer post-drug interval unaltered. With the lower dosage (schedule C), the sensitivity to noradrenaline was not different from that of controls and the effect of sympathetic nerve stimulation unaltered except for a slight depression of the " stimulus number-response curve." With the higher dosage (schedule D) sensitivity to noradrenaline was unaltered, there was a clear depression of the whole " stimulus number-response curve," but only a slight depression of the uppermost part of the " frequency-response curve." The third variable in the schedule thought to be possibly of importance is the post-drug interval. An interval of 4 hr was chosen and the higher dose of 200 mg/kg given either six times within 3 days (schedule E) or three times within half that time (schedule F). In both groups sensitivity to noradrenaline was unchanged as compared with control animals, but the neurally evoked nictitating membrane contractions were drastically depressed, which can be seen both on " stimulus number-response curves " and on " frequency-response curves." Depression was more marked with the shorter treatment.

A clearer impression of the effects depicted in Fig. 1 is gained when one compares the responses of the nictitating membrane to 3 volleys at 1.6/sec, to continuous stimulation at 0.2/sec, and to noradrenaline 10 γ/kg intravenously in controls and after the various pretreatments (Fig. 2).

Only pretreatment with short post-drug interval markedly depressed neurally evoked contractions both under conditions of short train stimulation at a low frequency and of continuous stimulation at different frequencies. A moderate depression occurred after a short pretreatment with the higher dose and longer post-drug interval. Pretreatment for 3 days and with the longer post-drug interval had no effect on the " frequency-response curve " and only slightly depressed the upper part of the " stimulus number-response curve." Sensitivity to noradrenaline was increased only after the longer treatment and the longer post-drug interval, but supersensitivity was much more marked after the lower dosage than after the higher one. These two pretreatments (schedule A and B) also slightly increased the effect of tyramine and DMPP (Fig. 3).

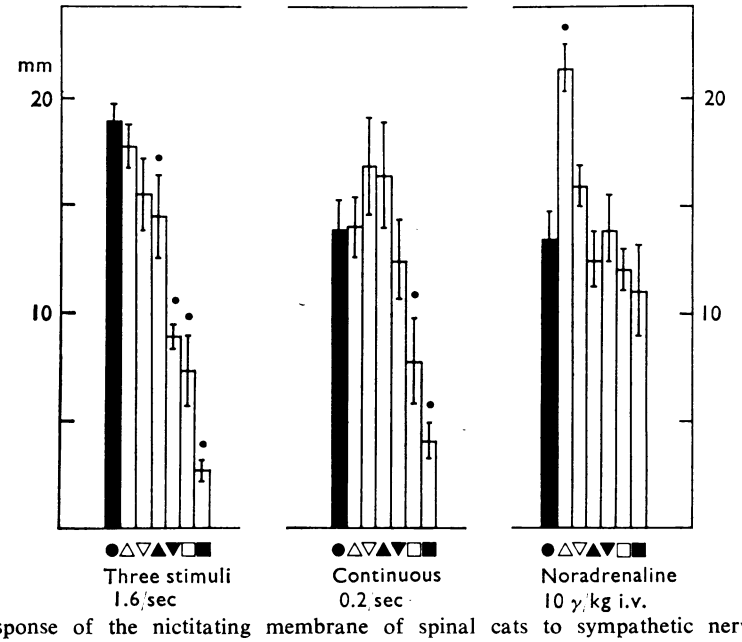

Fig. 2. Response of the nictitating membrane of spinal cats to sympathetic nerve stimulation (response to 3 volleys at a rate of 1.6/sec on the left-hand side; response to continuous stimulation at a frequency of 0.2/sec, middle) and to noradrenaline (10 μg/kg) intravenously (right-hand side) after various pretreatments. Symbols and calibration as in Fig. 1. Given are the mean values with standard errors. The points above the columns indicate statistical significance ($P<0.05$) against control values.

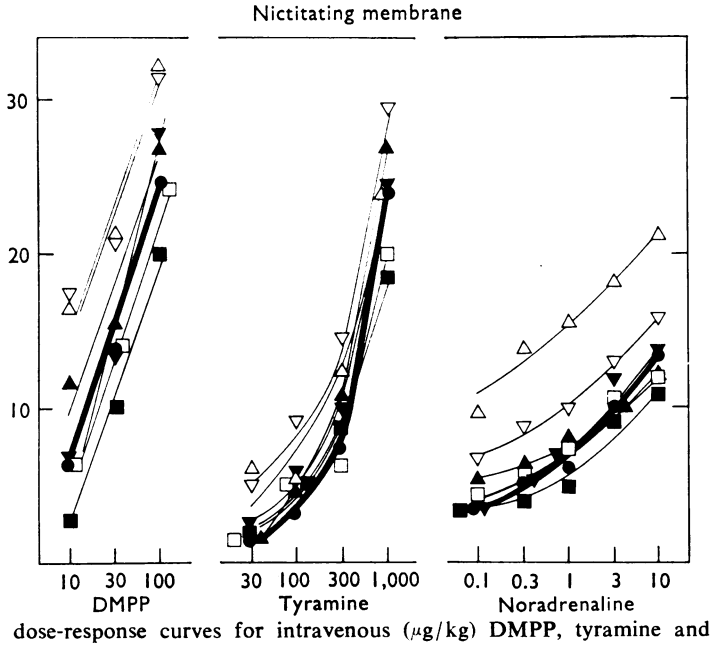

Fig. 3. Partial dose-response curves for intravenous (μg/kg) DMPP, tyramine and noradrenaline obtained on the nictitating membrane after various pretreatments with α-methyldopa (symbols and calibration as in Fig. 1).

Blood pressure. Blood pressure responses to noradrenaline, DMPP and tyramine were in the normal range after all pretreatment schedules (Fig. 4).

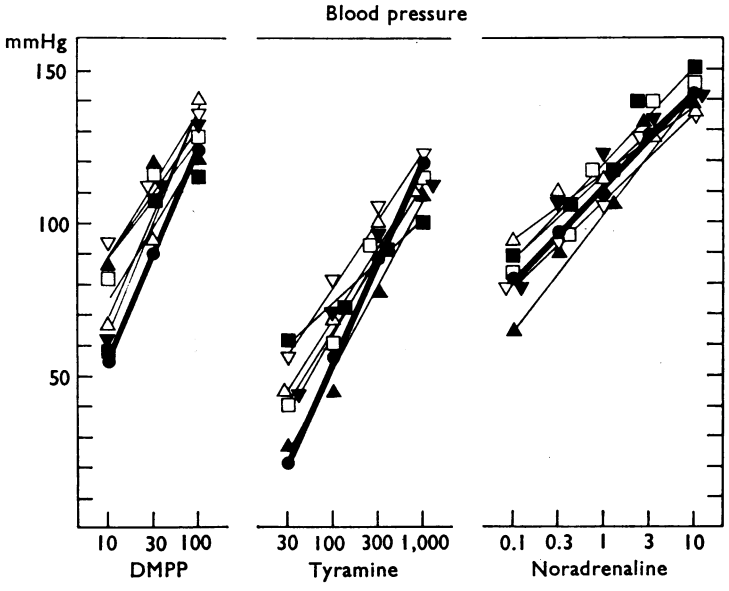

Fig. 4. Dose-response curves for the pressor effect of DMPP, tyramine and noradrenaline given intravenously (μg/kg) obtained after various pretreatments with α-methyldopa. The symbols are the same as in Fig. 1.

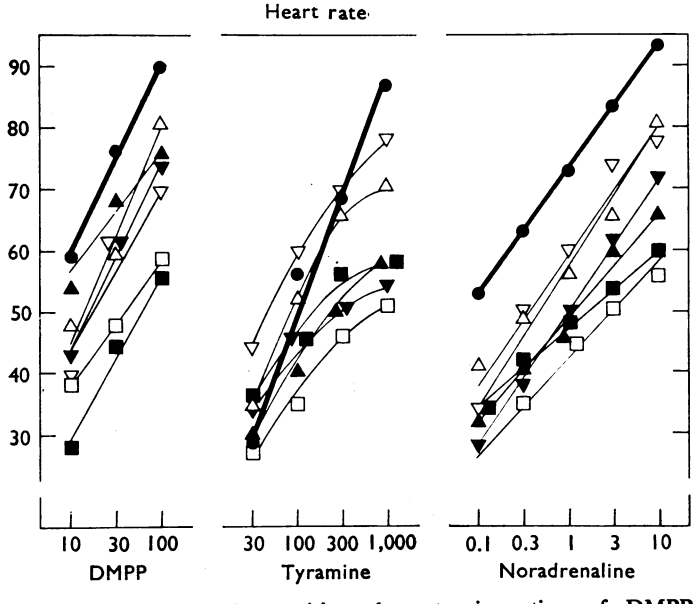

Fig. 5. Dose-response curves for the positive chronotropic action of DMPP, tyramine and noradrenaline in spinal cats after various pretreatments with α-methyldopa (symbols as in Fig. 1). Doses (μg/kg intravenously) are plotted against the maximum increase in heart rate (beats/min).

Heart rate. The sensitivity of the cardiac pacemaker to noradrenaline was decreased by all pretreatment schedules (Fig. 5). The subsensitivity was unrelated to the total dose of α-methyldopa applied. However, short pretreatment and short post-drug interval had the greatest depressant action on noradrenaline effects. The positive chronotropic effect of DMPP was decreased approximately to the same degree as that of noradrenaline. The effect of low doses of tyramine was unaltered or slightly increased, that of the highest dose diminished.

2. *Experiments on isolated perfused spleens*

In a first series of experiments the transmitter output and the contractile response resulting from sympathetic nerve stimulation with trains of stimuli at a rate of 6/sec and 10/sec were measured in spleens from cats pretreated with three doses of α-methyldopa 200 mg/kg intraperitoneally 28, 20 and 4 hr before the isolation. This pretreatment had been found to depress both " stimulus number-response curves " and " frequency-response curves " on the nictitating membrane. It was of particular interest to study the functional consequences of the partial replacement of noradrenaline by α-methylnoradrenaline in sympathetic nerves of an organ in which both amines had identical potency in producing contraction (Haefely, Hürlimann & Thoenen, 1964). The transmitter output was assayed

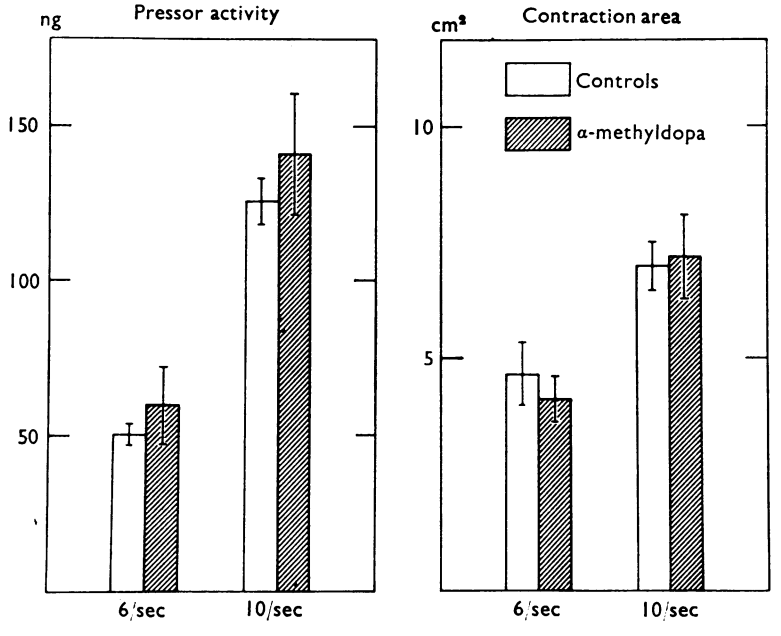

Fig. 6. Pressor activity (expressed in noradrenaline equivalents) of the venous effluent and contractile response of the spleen (" contraction area "—i.e., area on the kymograph enclosed by the lever's writing point from the beginning of stimulation to its return to the starting level) in response to a 10-sec period of repetitive stimulation of the splenic sympathetic nerves with stimulation frequencies of 6/sec and 10/sec. Results obtained on isolated perfused spleens from cats pretreated with 200 mg/kg α-methyldopa intraperitoneally 28, 20 and 4 hr before the experiment. Given are the mean values with standard errors.

in the pithed rat, where both amines have the same pressor activity. Figure 6 shows that the pressor activity of the venous effluent from spleens pretreated with α-methyldopa did not differ from that of untreated controls at either frequency of stimulation. Correspondingly the contractile response was the same in untreated and pretreated animals.

In a second series noradrenaline and α-methylnoradrenaline were estimated separately in the venous effluent after splenic nerve stimulation and in the spleen homogenized immediately after the collecting period. In order to eliminate effects on volume and vascular resistance of the spleen as well as on re-uptake of the amines into sympathetic nerve terminals, the spleens were perfused with Krebs-Henseleit solution containing phenoxybenzamine. The mean values of the α-methylnoradrenaline/noradrenaline ratios (Fig. 7) indicate that the false transmitter was present in approximately the same amounts as the physiological one in the spleen and in the effluent after the shorter pretreatment, but greatly exceeded noradrenaline after the longer treatment. Already from these values it seemed clear that both amines were liberated in the same proportion as they were present in the spleen. To settle this important point, the correlation between α-methyl-

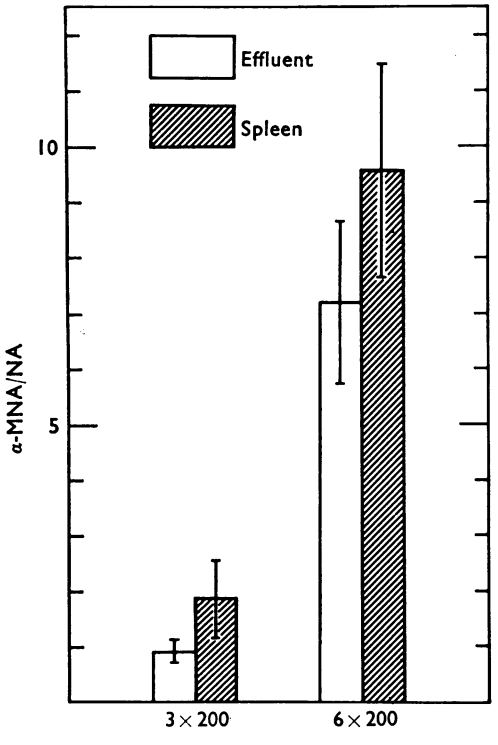

Fig. 7. Ratios of α-methylnoradrenaline/noradrenaline in the venous effluent following splenic nerve stimulation for 2 min at a rate of 10/sec and in the subsequently homogenized spleens. The preparations were made from cats pretreated according to schedule F (3×200 mg/kg) and schedule B (6×200 mg/kg). Phenoxybenzamine (1 μg/ml.) was added to the perfusion fluid. The spleens were homogenized immediately after stimulation.

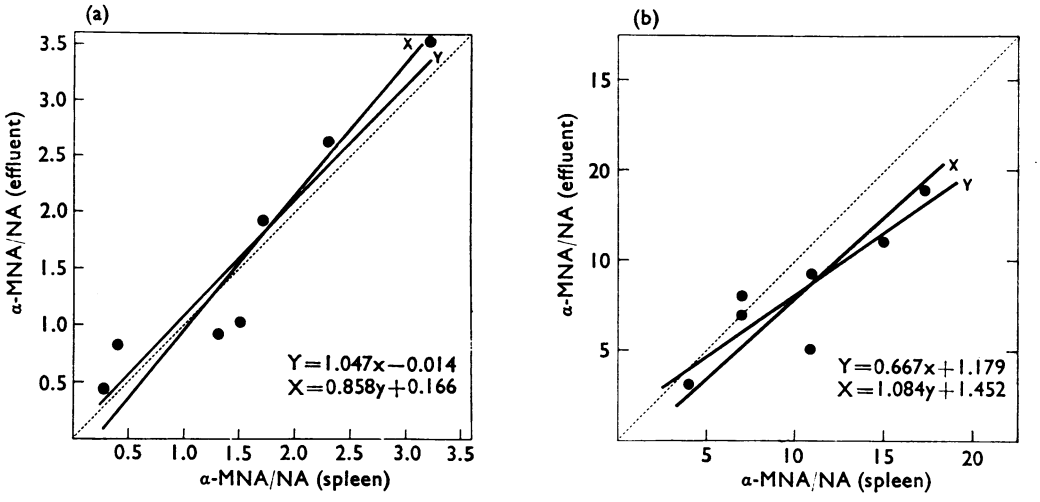

Fig. 8. Correlation between the ratio α-methylnoradrenaline/noradrenaline in the venous effluent and in the subsequently homogenized spleens of cats pretreated according to schedule F (a) and to schedule B (b). The points are the values of the individual experiments, the means of which were given in Fig. 7.

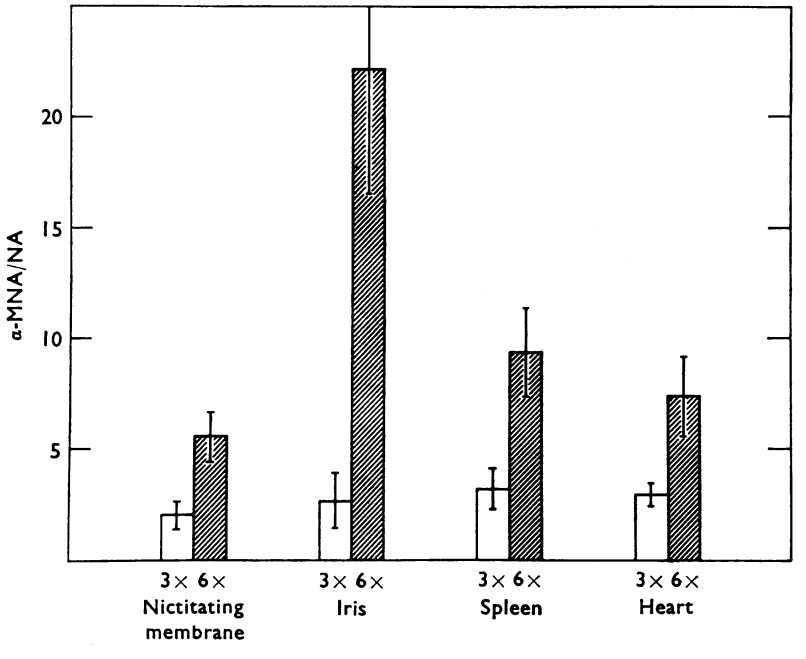

Fig. 9. Ratios of α-methylnoradrenaline/noradrenaline in nictitating membrane, iris, spleen and heart of cats pretreated with α-methyldopa according to schedule F (3×) and schedule B (6×). Given are the mean values with standard errors.

noradrenaline/noradrenaline ratios of the perfusion fluid and the splenic homogenates was calculated from the individual experiments. As shown in Fig. 8, a significant correlation was found between the ratios of both amines in the spleen and the perfusate (coefficient of correlation $r=0.94$, $P<0.01$ for schedule F, $r=0.85$, $P<0.05$ for schedule B). Coefficients of regression for both treatments did not differ significantly ($P<0.01$) from the ideal coefficient 1.0 for identical ratios of α-methylnoradrenaline/noradrenaline in effluent and homogenate.

3. *Estimation of noradrenaline and α-methylnoradrenaline in different organs*

The effect of α-methyldopa on neurally evoked contractions could not be correlated with the release of normal and false transmitter in the nictitating membrane. Therefore the results obtained in the isolated perfused spleen could be of value in interpreting the mode of action of α-methyldopa in the membrane only if the replacement of the physiological transmitter by α-methylnoradrenaline was similar in spleen and nictitating membrane. Figure 9 shows ratios of α-methylnoradrenaline/noradrenaline in nictitating membrane, iris, spleen and heart of cats pretreated with 200 mg/kg α-methyldopa either three times with short post-drug interval or six times with longer post-drug interval. The former schedule produced most marked depression of neurally evoked nictitating membrane contractions, whereas the latter was virtually without effect. The shorter pretreatment resulted in α-methylnoradrenaline/noradrenaline ratios which were not

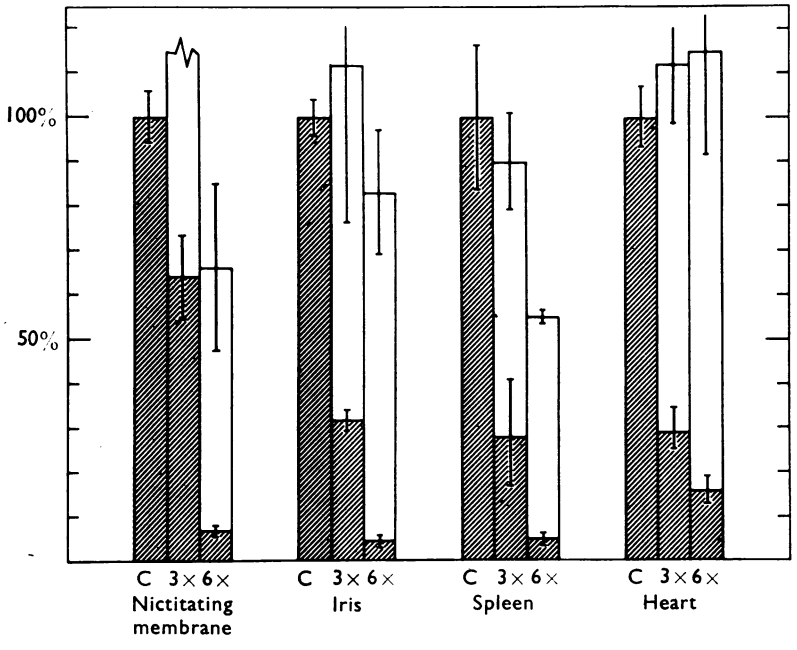

Fig. 10. Amounts of α-methylnoradrenaline (white columns) and noradrenaline (hatched columns) in nictitating membrane, iris, spleen and heart of cats pretreated according to schedule F (3×) and schedule B (6×). The amounts of both amines found in pretreated cats were expressed in per cent of the values of noradrenaline in untreated controls (C). Given are the means with standard errors.

statistically significant from one another in the four organs. The longer treatment resulted in an increase of the ratios similar in nictitating membrane, spleen and heart, where α-methylnoradrenaline represented 80 to 90% of the total amine present. In the iris the false transmitter almost completely replaced the noradrenaline. In Fig. 10 the absolute amounts of α-methylnoradrenaline and noradrenaline are given and compared with those of untreated controls which were taken as 100%. The total amount of noradrenaline plus α-methylnoradrenaline in the heart after both schedules was identical with the amount of noradrenaline present in untreated controls. In iris and spleen the total amount of amines tended to be less after the longer treatment than the noradrenaline content in controls. Unfortunately, our method of removing the nictitating membrane for the estimation was too crude. Because in the series reported varying amounts of orbital tissue and of the smooth muscles of the membrane were removed the absolute values of the amines should be disregarded and only amine ratios used.

4. *Acute effects of α-methyldopa infusions on nictitating membrane responses to nerve stimulation and noradrenaline*

In 3 spinal cats, α-methyldopa 200 mg/kg was injected intravenously (injection time 8 to 10 min) after several constant responses of the nictitating membrane to sympathetic nerve stimulation and to intravenous noradrenaline. In one cat α-methyldopa had no effect on blood pressure, heart rate and tone of the nictitating membrane. In the two other animals the injection was followed by an increase of the mean blood pressure of about 50 mm Hg lasting for 2 hr. There was a parallel increase of the heart rate by approximately 50 beats/min. The resting tone of the nictitating membrane augmented very slowly and was 3 to 4 g higher 2 hr after the injection than in the control period. The effect of noradrenaline on the blood pressure and the heart rate was unaltered after the injection of α-methyldopa ; the responses of the nictitating membrane to preganglionic volleys and to noradrenaline were usually slightly increased and prolonged, as is normal when they are superimposed on an increased tone.

DISCUSSION

In the present investigation we found no simple relationship between replacement of the physiological transmitter by α-methylnoradrenaline and the functional impairment of adrenergic neurones in the cat nictitating membrane after treatment with α-methyldopa. By varying the single doses, the total number of doses applied and the interval between the last application of the drug and the experiment (post-drug interval), several processes contributing to the final action of α-methyldopa could be differentiated.

First there is the metabolic fate of α-methyldopa resulting in a *decrease of noradrenaline and its replacement by α-methylnoradrenaline.* The mean ratios of α-methylnoradrenaline/noradrenaline were very similar (between 2 and 3) in the four organs studied (nictitating membrane, iris, spleen and heart) after pretreatment with three times 200 mg/kg intraperitoneally. Pretreatment with six times 200 mg/kg markedly increased the ratio in all organs studied. The mean ratios varied between 5.5 and 9.4 for nictitating membrane, spleen and heart and were not significantly different. In the iris,

however, this pretreatment increased the ratio much more. The absolute amounts of α-methylnoradrenaline and noradrenaline in all organs studied, except the nictitating membrane, indicate a stoichiometric replacement of the missing noradrenaline by α-methylnoradrenaline after three times 200 mg/kg α-methyldopa. Unfortunately, the removal of the whole nictitating membranes with adjacent tissues proved an unsatisfactory method ; therefore, in the nictitating membrane only the ratio between the two amines should be considered. Following the more prolonged treatment, there was still a stoichiometric replacement of noradrenaline by α-methylnoradrenaline.

If α-methyldopa had no other effects on the adrenergic neuromuscular junction in the nictitating membrane, one would expect in view of the three times weaker action of α-methylnoradrenaline as compared to noradrenaline and the dose-ratios found in the other three organs studied that the effect of sympathetic nerve stimulation would be reduced to about 70% after the shorter treatment and to less than 50% after the longer treatment. However, pretreatment with six times 200 mg/kg and the longer post-drug interval (α-methylnoradrenaline representing 85 to 90% of the total transmitter amines) had virtually no effect on neurally evoked contractions, whereas after only three times 200 mg/kg with the shorter post-drug interval (α-methylnoradrenaline representing 65 to 75% of the total transmitter amines) " stimulus number-response curves " and " frequency-response curves " were greatly depressed.

This complete discrepancy between replacement of noradrenaline by the weaker false transmitter and the functional consequences raises the *question whether the two transmitteer amines are liberated in the same proportion in which they are present in the organs.* This problem was studied on the isolated perfused spleen of cats pretreated according to schedule B and F. Pretreatment with six times 200 mg/kg increased the ratio α-methylnoradrenaline/noradrenaline much more than pretreatment with only three times 200 mg/kg. However, there was a significant correlation between the ratios in the perfusate and the ratios in the tissue. Therefore, the discrepancy between biochemical and functional findings cannot be due to a difference in the ratio found in the tissue and that in a small transmitter pool important for neural release. Furthermore, a difference in liberation or re-uptake of the physiological and false transmitter amine seems to be excluded.

An important finding for interpreting the action of α-methyldopa is the *development of increased sensitivity to noradrenaline by the drug.* This seems to depend on the duration of the pretreatment period, since it was observed only after a three-days treatment. Perhaps the increased sensitivity is related to the ratio α-methylnoradrenaline/noradrenaline in the nictitating membrane. Such an increased sensitivity to noradrenaline of the nictitating membrane has been found after other pretreatments, leading to the formation of an α-methylated amine such as after α-methyl-m-tyrosine (Haefely *et al.*, 1966) and after α-methyldopa plus disulfiram (Thoenen, Haefely, Gey & Hürlimann, 1966).

A mechanism counteracting the increased sensitivity to noradrenaline must be postulated from our results. After all treatment schedules with the shorter post-drug interval, sensitivity to noradrenaline of the nictitating membrane was found to be within the control range, irrespective of the amount of the single doses or the duration of pretreatment. This indicates that for several hours after the application of α-methyldopa the mechanism producing increased sensitivity to noradrenaline was suppressed. This is

most clearly demonstrated by comparing nictitating membrane contractions induced by nerve stimulation and intravenously injected noradrenaline in the groups treated with six times 200 mg/kg but differing by their post-drug interval. The time during which supersensitivity was depressed seemed to increase with the dose of α-methyldopa, as indicated by the fact that the sensitivity to noradrenaline was less 16 hr after the sixth 200 mg/kg dose than after the sixth dose of 100 mg/kg. The nature of this mechanism depressing increased sensitivity to noradrenaline is unknown. The simplest explanation, namely an α-adrenolytic action of α-methyldopa itself or one of its metabolites is difficult to accept in view of both the undiminshed sensitivity to noradrenaline of the nictitating membrane and the undiminished response of the spleen to sympathetic nerve stimulation after 3×200 mg/kg with short post-drug interval. In contrast to results obtained by Farmer (1965) in cats anaesthetized with chloralose, the intravenous infusion of α-methyldopa in our experiments did not diminish the response of the nictitating membrane to sympathetic nerve volleys and to exogenous noradrenaline. An effect of α-methyldopa on the adrenergic receptor level seems therefore improbable. The involvement of the intermediary metabolite α-methyldopamine is improbable because in the presence of this amine after combined pretreatment with α-methyldopa and disulfiram the sensitivity to noradrenaline was increased (Thoenen *et al.*, 1966). The question whether or not the intermediary metabolite α-methyldopamine plays a part as a false transmitter after α-methyldopa treatment can only be answered when methods for accurate estimation of this amine in the nictitating membrane are available.

If one accepts the adrenergic neuromuscular junction in the cat nictitating membrane as a model for human sympathetic vasomotor fibres, it becomes very unlikely that the formation of a less potent false transmitter in peripheral sympathetic neurones alone should be causally related to the antihypertensive action of α-methyldopa in man. Replacement of noradrenaline by α-methylnoradrenaline—at least in the nictitating membrane—is accompanied by increased sensitivity to noradrenaline, and only when this supersensitivity is depressed by some unknown action of α-methyldopa does the expected impairment of the effects of sympathetic nerve stimulation by the partial release of a less potent false transmitter become apparent. While to our knowledge the extent to which α-methylnoradrenaline replaces noradrenaline in human tissues has not been determined, Muscholl & Rahn (1966) found a decrease of the excretion of noradrenaline concomitant with an increase of α-methylnoradrenaline and α-methyldopamine in the urines of patients after single doses of α-methyldopa. But, as may be inferred from our experiments, even a temporal correlation between the peak of excretion of α-methylnoradrenaline and of blood-pressure fall does not permit to conclude a causal relationship. Furthermore, clinical experience shows that the hypotensive action of a single dose of α-methyldopa has a duration of not more than 16 hr even under chronic treatment (Holtmeier, v. Klein-Wisenberg & Marongiu, 1966). Although turnover rates for α-methylnoradrenaline formed by α-methyldopa are not known in man, it is questionable whether the ratio α-methylnoradrenaline/noradrenaline should be much less 16 hr after intake of the drug than at the peak of the hypotensive effect. In this context it may be mentioned that α-methyldopa has a definite hypotensive action in conscious dogs (Goldberg, Da Costa & Ozaki, 1960; Gerold, personal communication), although we found equal pressor potencies for α-methylnoradrenaline and noradrenaline in unanaesthetized dogs (unpublished results). The

same situation is found in the rat, where α-methyldopa has an antihypertensive action (Stanton & White, 1965; Gerold, Hürlimann & von Planta, 1966) despite identical pressor activities of noradrenaline and α-methylnoradrenaline (Maître & Staehelin, 1963). Recently, Varma (1967) found α-methyldopa still hypotensive in immunosympathectomized rats with experimental hypertension. The contribution of other effects of α-methyldopa to its antihypertensive action should therefore not be overlooked—such as central ones. It might also be worth while to look in man for the very marked subsensitivity to noradrenaline of the cardiac pacemaker present in cats pretreated with α-methyldopa and which has also been observed in the rabbit (Gillis, Shister & Melville, 1966).

SUMMARY

1. Cats were pretreated with α-methyldopa intraperitoneally, varying the individual doses, the duration of treatment and the interval between application of the last dose and the experiment. The functional consequences of treatment were studied on the nictitating membrane of spinal preparations. The amounts of noradrenaline and α-methylnoradrenaline were determined in several organs. The ratio α-methylnoradrenaline/noradrenaline in the effluent of the isolated perfused spleen during nervous stimulation was correlated with that in the whole organ.

2. There was a complete lack of correlation between replacement of the physiological transmitter by α-methylnoradrenaline and the impairment of the effects of sympathetic nerve stimulation after treatment with α-methyldopa under our experimental conditions.

3. After α-methyldopa noradrenaline is decreased and replaced by α-methylnoradrenaline in a dose-dependent manner in the nictitating membrane, iris, spleen and heart. The sensitivity to noradrenaline of the nictitating membrane increases with the duration of pretreatment. Reduction of the neurally evoked contractions of the membrane occurred only after a short post-drug interval, indicating that depression of the increased sensitivity to noradrenaline by a yet unknown mechanism is necessary in order to unmask the functional consequence of the release of a less potent false transmitter.

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