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THE CARDIOVASCULAR EFFECTS OF L-DOPA IN THE PITHED RAT

E. EDEN & P.A. NASMYTH
Department of Pharmacology, St Mary's Hospital Medical School, Paddington, London W.2.

1 L-DOPA (1-2 mg i.v.) in the pithed rat reduced the arterial and increased the venous pressure responses to noradrenaline.

2 Infusions of dopamine (4-8 $\mu g\,kg^{-1}\,min^{-1}$) and noradrenaline (500 $ng\,kg^{-1}\,min^{-1}$) also reduced the pressor responses to noradrenaline. The pressor response did not recover after stopping dopamine infusions, but it usually did so after stopping noradrenaline infusions.

3 The effect of L-DOPA on the response to noradrenaline was prevented by the prior injection of the dopa decarboxylase inhibitor NSD 1024.

4 The prior injection of the dopamine-β-hydroxylase inhibitor diethyldithiocarbamate, only partially prevented the effect of L-DOPA on pressor responses to noradrenaline.

5 The perfusion of noradrenaline (400 $ng\,kg^{-1}\,min^{-1}$) together with Krebs solution (10 ml/min) through an organ bath containing an isolated aortic strip, depressed the response of the strip to doses of noradrenaline after the infusion was stopped. Infusions of dopamine (0.5-8.0 $\mu g\,kg^{-1}\,min^{-1}$) had a similar effect. Fifteen minutes after adding L-DOPA (0.5 mg) to the bath and 10 min after washing it out, the response to noradrenaline was depressed in three out of four experiments.

6 Infusions of noradrenaline (1 $\mu g\,kg^{-1}\,min^{-1}$) in an isolated heart perfused by Langendorf's method blocked the response to injected noradrenaline whilst perfusion was in progress. Infusions of dopamine (4-8 $\mu g\,kg^{-1}\,min^{-1}$) or of L-DOPA (200 $\mu g\,kg^{-1}\,min^{-1}$) did not have this effect.

7 It is concluded that the effect of L-DOPA on pressor responses to noradrenaline in the pithed rat are mediated by its conversion to dopamine and noradrenaline.

Introduction

L-DOPA produces hypotension in many patients with Parkinson's disease (Godwin-Austen, Tomlinson, Frears & Kok, 1969; Calne, Brennan, Spiers & Stern, 1970; Watanabe, Chase & Cardon, 1970). There is evidence in animal experiments that the hypotension is due to a central action of L-DOPA (Henning & Rubenson, 1970; Watanabe, Parks & Kopin, 1971). However, Dhasmana & Spilker (1973) concluded that it was possible to distinguish between the mechanisms by which supine and postural hypotension were produced by L-DOPA. They expressed the view that the hypotension induced by the drug in supine animals was centrally mediated, whereas the inhibition of the pressor response to tilting (postural hypotension) was probably due to dopamine acting as a false transmitter in the peripheral nervous system.

In 1960 Smith reported that the i.v. injection of L-DOPA in the pithed rat reduced pressor responses to noradrenaline and dopamine, which might indicate a different or additional mechanism of action to those so far considered. The i.v. infusion of noradrenaline produces a rise in blood pressure which is not sustained and which reduces the responses to the injection of pressor doses of the drug (Nasmyth, 1962; Gillespie & Muir, 1967). It is possible, therefore, that large doses of L-DOPA may be converted to noradrenaline and produce effects on the cardiovascular system in much the same way as infusions of noradrenaline. The present work examines this possibility and the effects of noradrenaline infusions on isolated preparations of the heart and thoracic aorta. A preliminary account of the work has been given to the Physiological Society (Eden & Nasmyth, 1973).

Methods

Blood pressure records

Male Wistar rats (250-400 g) were given atropine sulphate (1 mg kg^{-1} i.p.), anaesthetized with ether and pithed by the method of Shipley & Tilden (1947). After giving heparin (1000 i.u. i.v.), blood pressure was recorded from the carotid artery and drugs were injected into the femoral vein. When drugs were to be infused, both femoral veins were cannulated and infusions were given via one cannula and injections via the other. Infusions were made with a Palmer 12 speed continuous slow injector at rates not normally exceeding 0.025 ml/min and never exceeding 0.1 ml/minute.

In some experiments venous pressure was recorded from the jugular vein with a water manometer. In others, it was recorded with a pressure transducer (SEM 4-82) with a capacitance (250 uF) connected in parallel with the input to the recorder to damp the speed of the response.

Isolated thoracic aorta

Male Wistar rats (200-450 g) were killed by a blow on the head and exsanguinated. The aorta was removed from a point just above the emergence of the renal arteries and immediately immersed in Krebs solution of the following composition (mM): NaCl, 119; KCL, 4.7; $CaCl_2$, 2.7; $MgSO_4$ $7H_20$, 1.2; NaH_2PO4 $2H_20$ 1.2; $NaHCO_3$, 25; glucose, 0.2% w/v. The solution was bubbled with 95% O_2 and 5% CO_2 and a spiral strip about 2 mm wide was cut with fine curved scissors. The strip was mounted in an organ bath of 4 ml capacity and the Krebs solution was bubbled with 95% O_2 and 5% CO_2 via a sintered glass filter and kept at 37° C. The tissue was connected to a linear displacement transducer (SE 92/0125) in such a way that it was subjected to a tension of 2.0 grams. Isotonic contractions were recorded on a potentiometric recorder.

Experiments were begun 1 h after completing the preparation. Drugs were introduced in volumes of Krebs solution not exceeding 0.2 ml and were allowed to act for 90 s before being washed out of the bath. The interval between drug additions varied from 8-10 min in different preparations and was determined by the time required for the tissue to relax.

In experiments designed to determine the effects of infusion of noradrenaline or dopamine, Krebs solution was run through the bath at the rate of 10 ml/min and the drug was infused into the supply tubing (2 mm i.d.) at a point 20 cm from its entry into the organ bath. The noradrenaline was infused with a Palmer 12 speed continuous slow injector at rates not normally exceeding 0.025 ml/min and never exceeding 0.1 ml/minute.

Isolated heart perfusion

Male Wistar rats (200-400 g) were killed by a blow on the head and exsanguinated. Hearts were removed quickly, washed in cold Krebs solution and perfused by Langendorf's method using the apparatus described by Hancock & Nasmyth (1956). Injections and infusions were made into the rubber cap of the cannula. Drug infusions were made with a Palmer 12 speed continuous slow injector as described above.

Drugs used

Sodium diethyldithiocarbamate trihydrate pure A.R. (Koch-Light Laboratories); L-β-(3,4-dihydroxyphenyl)alanine (L-DOPA, Koch-Light Laboratories); 3,4-dihydroxyphenylethylamine hydrochloride (3-hydroxytyramine, dopamine hydrochloride, Sigma Chemical Company); (-)-noradrenaline (Levophed 1 : 1000, Winthrop Laboratories); 3-hydroxybenzyloxyamine dihydrogen phosphate (NSD 1024, Sandev Ltd).

The doses in the text represent bases or salts as described above.

Results

Blood pressure responses

Effects of L-DOPA. L-DOPA (1-2 mg) produced rises in blood pressure ranging from 20-100 mmHg. In each case, the peak of the rise was reached within 2 min and then returned to normal biphasically. The first phase was rapid, the pressure falling by 60-80% in the first 15 minutes. From then on the pressure fell very slowly, often not returning to the control level within one hour. Ten to fifteen minutes after injection of L-DOPA, pressor responses to i.v. doses of noradrenaline were maximally depressed. Usually, incomplete recovery of the responses occurred followed by a second decline until they stabilized about 1 h after giving the L-DOPA. At this time, the pressor responses to noradrenaline were reduced by 42 ± 7.6% (mean and s.e. mean n=7). Recovery of the responses to noradrenaline was seen in one experiment 90 min after giving L-DOPA. It did not occur in any of the other experiments during 2 h following L-DOPA. The inhibition of the response to noradrenaline did not appear to be related to either the dose of L-DOPA or to the rise in pressure produced by it. Indeed the largest

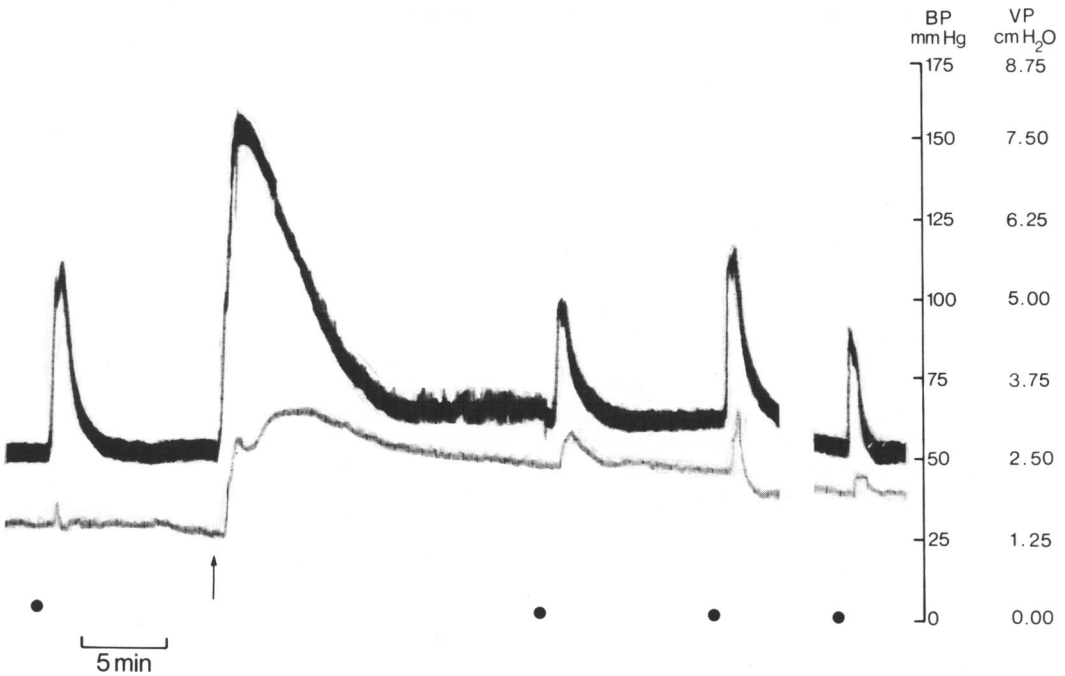

Fig. 1 The effect of L-DOPA (2 mg i.v. at the arrow) on arterial (upper tracing) and venous pressure (lower tracing) responses to noradrenaline (50 ng i.v.) at each dot. The response in the last panel was obtained 50 min after the injection of L-DOPA.

reduction in the response was produced by a dose of 1 mg of L-DOPA which caused a rise in pressure of only 20 mmHg.

L-DOPA caused rises in venous pressure ranging from 0.5 to 2.4 cmH_2O in the four experiments in which it was measured. The pattern of its return to normal was not identical with that of the blood pressure being monophasic and taking about one hour. The injection of noradrenaline before L-DOPA caused biphasic changes in venous pressure consisting of a small rise corresponding to the injection into the vein (volume 0.25 ml) followed by a small and transient fall coinciding with the peak of the pressor effect. Following L-DOPA, the venous pressure responses to noradrenaline were monophasic, transient and consisted of rises of the order of 0.4 cmH_2O. These effects are illustrated in Figure 1.

Infusions of dopamine. Infusions of dopamine of either 4 or 8 $\mu g\ kg^{-1}\ min^{-1}$ i.v. in three animals produced reductions in the pressor response to noradrenaline of 9, 37 and 75% respectively during the infusion and 15 min after its commencement. In the first animal (9% inhibition), the inhibition became progressively greater after the infusion was stopped, increasing to 48.5% in 30 minutes. In the other two, there was some recovery when the infusion was stopped, but it was incomplete in the hour for which it was followed.

The effect of dopamine infusions was determined in another two animals which had received (+)-tubocurarine (1 $\mu g/kg$) in preparation for stimulation of the sympathetic outflow (Gillespie, MacLaren & Pollock, 1969). These animals appeared to be insensitive to dopamine infusions of 4 or 8 $\mu g\ kg^{-1}\ min^{-1}$ which produced no rise in blood pressure and no inhibition of the response to noradrenaline. However, infusions of 32 and 64 $\mu g\ kg^{-1}\ min^{-1}$ in these animals did produce rises in pressure and did reduce the pressor response to noradrenaline by about 20% during the infusion, and accounted for the wide range of doses given in the preliminary report of this work (Eden & Nasmyth, 1973). Recovery was rapid, being complete within 20 min of stopping the infusion.

Infusions of noradrenaline. The lowest rate of infusion of noradrenaline previously shown to reduce pressor responses to bolus injections of noradrenaline was 2.8 $\mu g\ kg^{-1}\ min^{-1}$ (Nasmyth, 1962). No attempt to determine the lowest rate of infusion capable of producing the effect was made at that time.

In the present experiments, an infusion of 100 ng kg^{-1} min^{-1} produced a substantial rise in blood pressure. The pressor responses to noradrenaline were increased by 45% during the infusion and 28 min after it was started. They quickly returned to normal when the infusion was stopped. When the infusion rate was doubled in the same animal a rise in pressure occurred which was not sustained, but which fell and was maintained at a level 20 mmHg below the maximum 20 min after the infusion was started. Twenty-six minutes after starting the infusion, the responses to noradrenaline were potentiated by 60%, but 6 min after stopping it they were 50% less than the controls and there was no recovery 45 min later. In two more animals the same rate of infusion produced a similar pattern of blood pressure response, but venous pressure rose less than it did in animals given L-DOPA. In one of these animals, the responses were inhibited by 55% during the infusion and recovered as soon as it was stopped. In the other, the responses to noradrenaline were not affected either during the infusion or after it was stopped.

An infusion of 500 ng kg^{-1} min^{-1} in three animals caused a rise followed by a fall in blood pressure. The venous pressure (measured in two animals) increased by 0.5-1.0 cmH_2O which was commensurate with the effect seen in animals given L-DOPA. In all of them the responses to noradrenaline were reduced during the infusion and in two of them they recovered after the infusion was stopped. In the third animal however, the responses to noradrenaline continued to diminish after the infusion was stopped. Thirty-five minutes after stopping the infusion in this animal the blood pressure began to fall and venous pressure began to rise until after 3 h the blood pressure was nearly zero and the venous pressure had risen from 2.5 to 7.0 cmH_2O. At this point there was no response to injected noradrenaline.

The effect of metabolic inhibitors on the inhibition produced by L-DOPA

Having shown that the pressor responses to noradrenaline could be inhibited by infusions of dopamine or noradrenaline, it was important to determine whether or not the inhibition produced by L-DOPA was dependent upon its being metabolized to either of these substances.

When dopa decarboxylase was inhibited by NSD 1024 (20 mg/kg i.v.) given 30 min beforehand, the pressor response to L-DOPA was always of shorter duration than in untreated animals, the return to normal pressure being complete in 5-10 minutes. In two out of a total of three experiments in which L-DOPA (1 mg) was given, the magnitude of the response was also considerably less than in the untreated animals. In none of these experiments did L-DOPA inhibit pressor responses to noradrenaline at any time up to 88 min following the dose. In two experiments in which L-DOPA (2 mg) was given the responses to noradrenaline were depressed by 13% after 15 min in one experiment and after 45 min in the other.

In experiments in which diethyldithiocarbamate (250 mg kg^{-1} i.v.) was used to block dopamine β-hydroxylase, the inhibitor was given 50-60 min before the L-DOPA. Immediately following the injection of the inhibitor, responses to noradrenaline were reduced but they recovered after 20-30 minutes. The pressor response to L-DOPA was reduced and in two experiments it prevented the latter from inhibiting the pressor responses to noradrenaline. In four experiments, the maximum depression of the response to noradrenaline was 42, 13, 13 and 7.5% respectively. Thus, in only one case did it equal the mean inhibition produced by L-DOPA in untreated animals.

Isolated organs

Thoracic aorta. To determine the effect of exposing the aortic strip to noradrenaline for relatively long periods of time on responses to noradrenaline, two methods were tried. In the first, after establishing constant submaximal responses to noradrenaline (5 ng), a dose of 5 ng was added to the bath and allowed to remain there until, after 22 min without washing, the tissue recovered its normal tone. Now, when noradrenaline (5 ng) was added to the bath without washing, the response was unaltered compared with the controls. When noradrenaline (100 ng) was allowed to remain in contact with the tissue until its effect had disappeared (85 min) it too was without effect on the response to a 5 ng dose of the drug.

In the second method, noradrenaline was infused into the Krebs solution flowing through the bath as described in the methods section. The rate of infusion of noradrenaline was based on the weight of the animal from which the strip had been prepared. It was infused at the rate of 200 ng kg^{-1} min^{-1} in three experiments and was washed from the bath at the end of the infusion period. In one of these experiments, in which the duration of the infusion was 30 min, the responses to noradrenaline were not affected (Figure 2). In another, they were depressed and did not recover during 140 minutes. In the third experiment in which the infusion continued for 90 min, there was depression of the noradrenaline response followed by gradual recovery during the ensuing

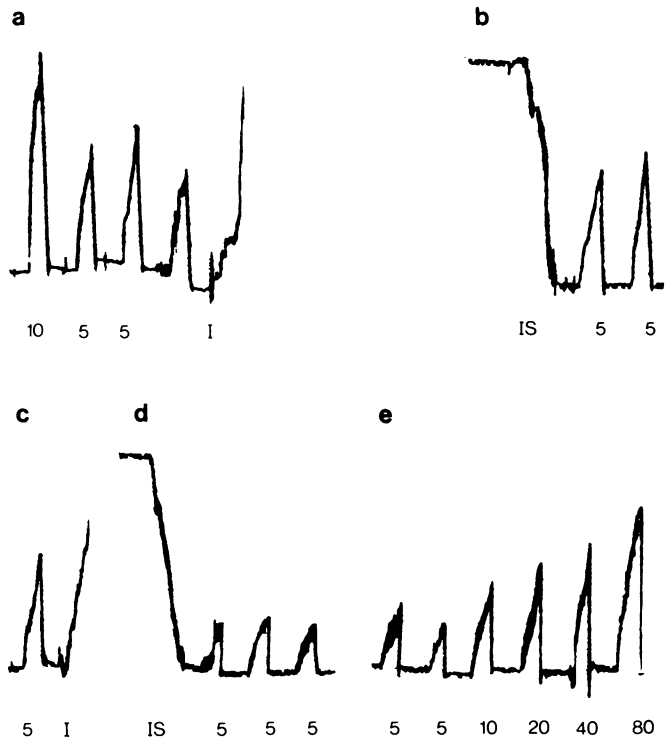

Fig. 2 Contractions of the rat isolated aortic strip. Figures refer to doses of noradrenaline in ng. (a) At I, noradrenaline (200 ng kg^{-1} min^{-1}, based on the weight of the rat) was infused into the Krebs solution which was flowing through the bath (4 ml) at 10 ml/minute. The infusion produced a sustained maximal contraction identical in height with that produced by the 10 ng dose of noradrenaline. Thirty minutes after starting it, the infusion was stopped at IS (b) and the responses to the 5 ng doses of noradrenaline were unaffected. Panels (b) and (c) are continuous. (c) At I an infusion of noradrenaline (400 ng kg^{-1} min^{-1}) was started together with Krebs solution at 10 ml/min and continued for 30 minutes. Again, a sustained maximal contraction was produced. (d) The infusion was stopped at IS. Responses to noradrenaline (5 ng) were now depressed and remained so for more than 88 min (the interval represents 56 minutes). Compare the maximal response to noradrenaline with responses to increasing doses in (e).

hour. In the experiment in which an infusion of 200 ng kg^{-1} min^{-1} was without effect, the infusion rate was doubled for 30 min and this did depress the responses to noradrenaline (Figure 2). The depression was confirmed in another experiment with 400 ng kg^{-1} min^{-1}. An infusion of 800 ng kg^{-1} min^{-1} produced the same effect in a fifth experiment.

In three experiments, dopamine (0.5, 2.0, and 8.0 μg kg^{-1} min^{-1}) infused in the same way as the noradrenaline, also reduced the responses to noradrenaline by 13, 33 and 58% respectively.

To determine the effect of L-DOPA on the response of the aortic strip to noradrenaline, repeated submaximal responses to two doses of noradrenaline were established. Then 15 min after introducing L-DOPA (0.5 mg) into the bath and 10 min after washing it out, the responses to the noradrenaline were re-established. In one experiment, the L-DOPA was without effect and in three others, all the responses to the two doses of noradrenaline were significantly depressed. In every case except one the value for P was <0.01. In the exceptional case P was 0.05.

Perfused isolated heart

Noradrenaline infusions. In three hearts good inotropic responses to noradrenaline (25 ng) were established. The infusion of noradrenaline (0.5 μg kg^{-1} min^{-1}) (based on the weight of the animal from which the heart had been removed) produced a very small increase in the amplitude of the contraction which returned to normal within 5 min despite the continued infusion of noradrenaline. Injections of noradrenaline (25 ng)

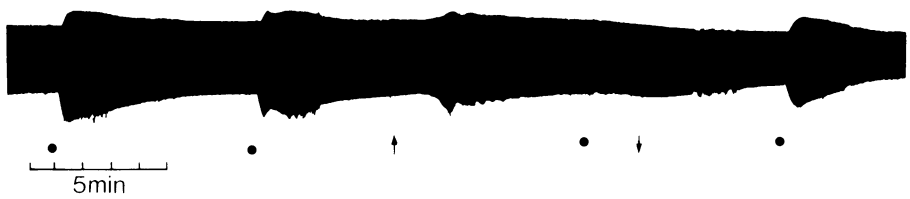

Fig. 3 Rat (215 g) isolated heart perfused by Langendorf's method. At each dot noradrenaline (25 ng) was injected into the perfusion fluid. At the first arrow, an infusion of noradrenaline (1 $\mu g\ kg^{-1}\ min^{-1}$) based on the weight of the animal was begun and it produced a transient increase in the amplitude of the beat. When the amplitude had returned to normal after 7 min, the response to noradrenaline (25 ng) was abolished. The infusion was stopped at the second arrow and the response to noradrenaline quickly returned.

given at 7 min intervals during the 30 min of the infusion continued to produce responses which were not significantly different from controls in two experiments. In the third experiment, the responses were halved in 30 min and completely recovered within 20 min of stopping the infusion. In five hearts in which noradrenaline 1 $\mu g\ kg^{-1}\ min^{-1}$ was infused, a small transient increase in amplitude occurred and in every case the response to injected noradrenaline was abolished within 7 minutes. The responses returned within 5 min of stopping the infusion (Figure 3). In one of these experiments, the inotropic responses to dopamine were also blocked and recovery occurred on stopping the infusion.

Dopamine infusion. An infusion of dopamine (4 $\mu g\ kg^{-1}\ min^{-1}$) produced small transient increases in the amplitude of the heart beat, but did not significantly affect the positive inotropic response to noradrenaline or dopamine in four experiments in which the infusion was continued for up to 16 minutes. In three experiments, dopamine (8 $\mu g\ kg^{-1}\ min^{-1}$) was infused for a similar time and it too failed to affect the responses to noradrenaline. In one experiment, dopamine (16 $\mu g\ kg^{-1}\ min^{-1}$) stopped the coronary flow, slowed the heart and reduced the amplitude of the beat so that it was not possible to make reliable observations of its effect on responses to noradrenaline.

L-DOPA infusions. In two experiments an infusion of L-DOPA (200 $\mu g\ kg^{-1}\ min^{-1}$) caused an increase in the amplitude of the heart beat that lasted for 13 min in one experiment and for 20 min in the other. Responses to noradrenaline were not significantly affected at times up to 18 min after commencing the infusion.

Discussion

In the preliminary account of this work (Eden & Nasmyth, 1973) we concluded that it was necessary for L-DOPA to be converted to noradrenaline to produce inhibition of the pressor responses to noradrenaline, believing that the small inhibition seen after the introduction of a β-hydroxylase inhibitor was due to incomplete inhibition of the enzyme. The present experiments have shown that both dopamine and L-DOPA are capable of inhibiting the responses of the isolated aortic strip to noradrenaline. Consequently, the inhibition of the pressor responses in the presence of the β-hydroxylase inhibitor could have been due to depression of the vascular responses to noradrenaline by dopamine formed from L-DOPA, if not to L-DOPA itself. The fact that the inhibition of the pressor response to noradrenaline by L-DOPA in the presence of a dopamine β-hydroxylase inhibitor was generally less than it was in experiments in which the enzyme inhibitor was absent, suggests that the formation of noradrenaline also contributed something to the phenomenon.

The present experiments have demonstrated that L-DOPA, dopamine and noradrenaline can all reduce the sensitivity of the aorta to noradrenaline, but that only noradrenaline affects the inotropic response of the heart. Gillespie & Muir (1967) concluded that the fall in blood pressure during the continuous infusion of noradrenaline is not due to desensitization of vascular smooth muscle but to diminished cardiac output. Our experiments on the isolated heart showing that the amine can abolish the response to itself confirm this conclusion. However, our experiments on the aortic strip do not agree with their conclusion regarding the vasculature. The reason for this disagreement is almost certainly due to the fact that the lowest rate of infusion that they used was 10 $\mu g\ kg^{-1}\ min^{-1}$ which would produce supramaximal responses in the blood vessels. Since the present experiments have shown that supramaximal responses in the isolated aortic strip are unaffected, the change in sensitivity of the vasculature would not have been apparent in their experiment.

The possibility that conversion to noradrenaline

contributes to the effect of L-DOPA on pressor responses to noradrenaline is supported not only by the observation that a dopamine β-hydroxylase inhibitor greatly reduces the effect, but also by the fact that pressor responses to noradrenaline after L-DOPA are accompanied by rises in venous pressure which do not occur before L-DOPA. This could well be caused by the failure of noradrenaline to increase cardiac output as efficiently as it did before L-DOPA.

The question arises whether or not the conversion of L-DOPA to dopamine and then to noradrenaline can take place extraneuronally and quickly enough to account for the activity of L-DOPA in terms of these metabolites. The liver contains substantial amounts of L-dopa decarboxylase which is believed to be extraneuronal (Iverson, Glowinski & Axelrod, 1966). Additionally, Tate, Sweet, McDowell & Meister (1971) reported dopa decarboxylase activity in human erythrocytes. This is now known not to be specific L-amino acid decarboxylase as it decarboxylates both the D and the L forms, nor is its activity stimulated by pyridoxal phosphate or inhibited by brocresine (Dairman, Christenson & Udenfriend, 1972). Nevertheless, they represent a possible site for the conversion of L-DOPA to dopamine. This, coupled with the observation here, that after giving the dopa decarboxylase inhibitor NSD 1024 the pressor response to L-DOPA was much reduced, suggests that conversion to dopamine, at least, is both possible and rapid. Calne, Karoum, Ruthven & Sandler (1969) observing the metabolism of orally administered L-DOPA in Parkinsonism noted that large quantities of the dopamine metabolite homovanillic acid but only small quantities of the noradrenaline metabolite 4-hydroxy-3-methoxymandelic acid were produced. This argued that the conversion of L-DOPA to dopamine was considerable, but that to noradrenaline was slight. However, our experiments demonstrate that very little noradrenaline is needed to produce profound effects on the heart and aortic strip. Dopamine β-hydroxylase is present in the serum (Goldstein, Freedman & Bonnay, 1971) and probably derives from the sympathetic nerve endings (Weinshilboum, Thoa, Johnson, Kopin & Axelrod, 1971). Goldstein *et al.* (1971) estimated human serum dopamine β-hydroxylase activity to be sufficient to form 50-90 nmol of product per ml of serum in 20 minutes. The figure for rat serum given by Goldstein Fuxe & Hökfelt (1972) is 0.87 nmol per ml of serum. Presuming this figure also to mean the amount of product formed in 20 min, the average 250 g rat might be expected to produce about 3 μg of noradrenaline in that time. The amount of noradrenaline infused in a 250 g rat at 500 ng kg^{-1} min^{-1} would have been 2.5 μg. It is clear then that the amount of noradrenaline which could have been formed from the L-DOPA given in these experiments is commensurate with what were effective rates of infusion of noradrenaline.

The fact that the introduction of a dopa decarboxylase inhibitor in the whole animal experiments prevented the depression of pressor responses to noradrenaline normally produced by L-DOPA, argues that none of the depression was due to the activity of L-DOPA itself. However, the amino acid was shown to depress the responses of the rat aortic strip to noradrenaline, but the concentration used was very high. This direct action of L-DOPA on the vasculature could have been a factor in the experiments of Henning, Rubenson & Trolin (1972) in which they observed a fall of blood pressure induced in rats given the peripheral decarboxylase inhibitor MK 486 when L-DOPA (200 mg kg^{-1} i.p.) was given.

It has been clearly demonstrated that L-DOPA in quite modest doses exercises a peripheral effect on the cardiovascular system of the rat reducing its responsiveness to noradrenaline. The effect is mediated by its conversion to dopamine and noradrenaline, the latter blocking its own effect on the heart and both metabolites depressing the response of the thoracic aortic strip to noradrenaline.

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