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Central hypotensive effect of L-3,4-dihydroxyphenylalanine in the rat

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Mean arterial blood pressure was recorded through in-dwelling arterial catheters in conscious normotensive Sprague-Dawley rats. L-3,4-Dihydroxyphenylalanine (L-dopa) was given in various doses intraperitoneally, alone and after pretreatment with an inhibitor of dopa decarboxylase, α-hydrazino-α-methyl-β-(3,4-dihydroxyphenyl) propionic acid (MK 485) or seryl-2,3,4-trihydroxybenzylhydrazine (Ro 4–4602). L-Dopa (50 mg/kg) produced a hypertensive response which was abolished by MK 485 (100 mg/kg). A larger dose of L-dopa (200 mg/kg) after MK 485 caused a significant lowering of blood pressure after 15–20 min. After Ro 4–4602 (400 + 200 mg/kg), injection of L-dopa (200 mg/kg) had no significant effect on blood pressure. The hypotensive response to L-dopa (200 mg/kg) after MK 485 was not influenced by the central dopamine receptor blocking agent, spiroperidol (0·1 mg/kg), but could be completely inhibited by the dopamine β-hydroxylase inhibitor, bis-(4-methyl-1-homopiperazinyl-thiocarbonyl)disulphide (FLA 63) (40 mg/kg). Pretreatment with protriptyline (10 mg/kg) completely blocked the hypotensive effect of L-dopa after MK 485. In correlative biochemical experiments, levels of noradrenaline and dopamine were determined in brain, heart and femoral muscle. L-Dopa (200 mg/kg) alone caused a significant increase of dopamine levels in all tissues. After MK 485 and Ro 4–4602 L-dopa did not significantly increase the levels of dopamine in heart or femoral muscle; however, brain dopamine levels were increased more than after L-dopa alone, but brain dopamine levels after Ro 4–4602 were significantly lower than after MK 485, indicating some central decarboxylase inhibition by Ro 4–4602. L-Dopa alone reduced the noradrenaline content of the heart and this effect was prevented by MK 485 and Ro 4–4602. The results show that decarboxylation of L-dopa in both the central and the peripheral nervous system leads to an increase in blood pressure. Decarboxylation of L-dopa in the central nervous system only results in a hypotensive response, provided that high amounts of dopamine are formed in the brain. This effect was prevented by an inhibitor of dopamine β-hydroxylase but not by a dopamine receptor blocker. Therefore, a central noradrenaline mechanism seems to be involved. The presence of an intact membrane pump in noradrenaline neurons may be essential since protriptyline also blocked the hypotensive action.

Administration of the catecholamine precursor L-3,4-dihydroxyphenylalanine (L-dopa) to experimental animals produces a syndrome which involves effects elicited both from the central and the peripheral nervous system (see e.g. Butcher & Engel, 1969a, b; Carlsson, 1969). Since L-dopa seems to be pharmacologically inert (Blaschko & Chruschiel, 1960; Carlsson, 1964), it may be assumed that its actions are mediated via its metabolites. Of particular interest are the catecholamines, dopamine and noradrenaline which may act centrally as well as peripherally.

Potent inhibitors of dopa decarboxylase in peripheral tissues, but with little effect

in the central nervous system, have previously been used to dissociate central and peripheral effects of the dopa analogue L-α-methyldopa (Henning 1968, 1969a) as well as L-dopa itself (Butcher & Engel, 1969a, b). We have examined the influence of L-dopa on blood pressure in conscious rats before and after pretreatment with α-hydrazino-α-methyl-β-(3,4-dihydroxyphenyl)propionic acid (MK 485), a decarboxylase inhibitor with minimimal central actions (Porter, Watson & others, 1962; Bartholini & Pletscher, 1969). The influence of another decarboxylase inhibitor, seryl-2,3,4-trihydroxybenzylhydrazine (Ro 4–4602) was also studied. This inhibitor, when given in sufficiently large doses, acts on both central and peripheral decarboxylase (see e.g. Henning, 1969a; Bartholini & Pletscher, 1969). Injection of L-dopa alone gave an increase in blood pressure which could be converted to a hypotensive response by pretreatment with MK 485; after pretreatment with Ro 4–4602 L-dopa had no effects on blood pressure. The hypotensive action was analysed further by studying the influence of an inhibitor of dopamine-β-hydroxylase, bis-(4-methyl-1-homopiperazinyl-thiocarbonyl)disulphide (FLA 63; Carlsson, Corrodi, Florvall, Ross & Sjöberg, unpublished data; cf. Svensson & Waldeck, 1969), a central dopamine receptor blocking agent, spiroperidol (Andén, Butcher, & others, 1970) and protriptyline. Part of the results have been presented in a preliminary report (Henning & Rubenson, 1970).

EXPERIMENTAL

The mean arterial blood pressure was recorded in conscious unrestrained male Sprague-Dawley rats, 250–350 g, through in-dwelling arterial catheters connected to Statham pressure transducers writing on a Grass Polygraph (Popovic & Popovic, 1960). For technical details see Henning (1969b). The blood pressure values represent averages of the recordings for the 10 min periods immediately before the administration of the drugs except the values after L-dopa which are averages of the pressure for 15–20 min after the injection.

In the biochemical studies, rats of corresponding body weight were used. Noradrenaline was determined by the method of Bertler, Carlsson & Rosengren (1958), dopamine as described by Carlsson & Lindqvist (1962). Each analysis was made on pooled organs from two animals.

The drugs used were L-3,4-dihydroxyphenylalanine (L-dopa), α-hydrazino-α-methyl-β-(3,4-dihydroxyphenyl)-propionic acid (MK 485), bis-(4-methyl-1-homopiperazinyl-thiocarbonyl) disulphide (FLA 63), spiroperidol, seryl-2,3,4-trihydroxybenzylhydrazine (Ro 4–4602). All drugs were administered intraperitonally. Solutions were prepared immediately before injection. MK 485 and Ro 4–4602 were dissolved in 0·9% saline. L-Dopa was dissolved in slightly warmed 0·9% saline (pH 5–6). The spiroperidol was dissolved in a few drops of acetic acid and then diluted in 5·5% glucose solution to a concentration of 0·02 mg/ml. FLA 63 was dissolved in 0·9% saline (pH 6·7). Doses and time intervals are given with the results. Tests of significance were by Student's *t*-test and analysis of variance with one or two independent criteria of classification. *P* values less than 0·05 were regarded as significant.

RESULTS

Blood pressure experiments

L-dopa. A significant ($P < 0{\cdot}005$) rise in mean arterial blood pressure was observed 20 min after injection of L-dopa, 50 mg/kg, the maximum being reached after about

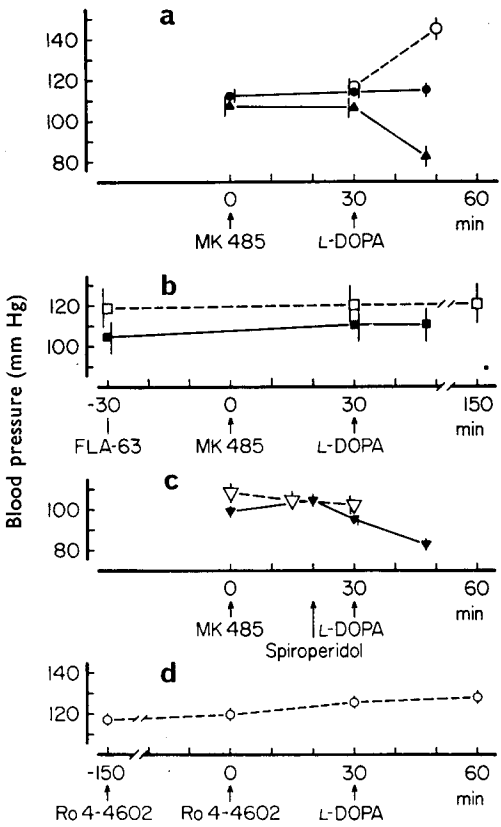

FIG. 1. Changes in mean arterial blood pressure of conscious normotensive rats after i.p. injection of various drugs. The blood pressure values represent averages of the recordings during the 10 min period immediately preceding the drug indicated except the values after L-dopa which are averages of the pressure for 15–20 min after the injection. (a) L-Dopa, 50 mg/kg ○ (9 exp.); L-dopa, 50 mg/kg, 30 min after MK 485, 100 mg/kg ● (5 exp.); L-Dopa, 200 mg/kg, 30 min after MK 485, 100 mg/kg ▲ (13 exp.). (b) L-Dopa, 200 mg/kg, 30 min after MK 485, 100 mg/kg and 60 min after FLA 63, 40 mg/kg ■ (8 exp.); FLA 63, 40 mg/kg alone □ (4 exp.). (c) L-Dopa, 200 mg/kg, 10 min after spiroperidol, 0·1 mg/kg, and 30 min after MK 485, 100 mg/kg ▼ (4 exp.); spiroperidol, 0·1 mg/kg ▽ (5 exp.). (d) Two doses of Ro 4–4602, 400 and 200 mg/kg, 3 and 0·5 h respectively, before L-dopa, 200 mg/kg (4 exp.).

20 min (Fig. 1a). The duration of the increase in blood pressure was over 60 min in all experiments. The animals showed piloerection and exophthalmus 15–30 min after the 50 mg/kg injection.

L-Dopa was also tested in 10, 20, 100 and 200 mg/kg doses; the lower doses either did not affect blood pressure or produced small increases. The higher doses consistently produced hypertensive responses.

MK 485 + L-*dopa.* Injection of MK 485, 100 mg/kg did not influence the mean arterial blood pressure significantly in 30 min ($P > 0{\cdot}1$) (Fig. 1a). Thirty min after MK 485, injection of L-dopa (50 mg/kg) did not produce any significant change in blood pressure. When the dose of L-dopa was increased to 200 mg/kg after MK 485, a significant fall in blood pressure was observed in 15–20 min ($P<0{\cdot}001$). Shortly after injection of L-dopa the animals had slight piloerection and exophthalmus and were aggressive and had an increased spontaneous motility. These behavioural changes reached a maximum 30 min after the L-dopa.

Ro 4–4602 + L-*dopa.* Four rats were given two doses of Ro 4–4602, 400 and 200 mg/kg, at 3 and 0·5 h, respectively before L-dopa (200 mg/kg). Mean arterial blood pressure was measured before the two doses of Ro 4–4602, before and 15–20 min after L-dopa. The results are shown in Fig. 1d. There were no significant changes in blood pressures ($P > 0{\cdot}1$). In these experiments, the animals showed no clear behavioural changes after the injection of L-dopa.

FLA 63 + *MK* 485 + L-*dopa.* FLA 63 (40 mg/kg) did not have any significant effect on the mean arterial blood pressure in the 1–3 h after the injection (4 experiments; Fig. 1b).

In another series of experiments FLA 63 (40 mg/kg) was given 30 min before MK 485 (100 mg/kg). When L-dopa (200 mg/kg) was injected 30 min later there was no significant change in blood pressure ($P > 0{\cdot}1$; Fig. 1b) and there were no signs of aggressiveness, but there was increased spontaneous motility with a predominance of stereotyped movements.

MK 485 + *spiroperidol* + L-*dopa.* Injection of spiroperidol (0·1 mg/kg) had no effect on mean arterial blood pressure ($P > 0{\cdot}10$, 5 experiments; Fig. 1c), but the animals were sedated. After a pretreatment with MK 485 (100 mg/kg, 30 min before) and spiroperidol (0·1 mg/kg, 10 min before), L-dopa (200 mg/kg) caused a significant drop in mean arterial blood pressure ($P < 0{\cdot}025$); there was very slight aggressiveness but a clear increase in spontaneous motility.

MK 485 + *protriptyline* + L-*dopa.* Four rats were given MK 485 (100 mg/kg) and protriptyline (10 mg/kg) 30 and 15 min, respectively, before L-dopa (200 mg/kg). The mean arterial blood pressure levels were as follows: before MK 485, 115 mm Hg (s.e. = 2·9); before protriptyline, 114 mm Hg (s.e. = 2·9); before L-dopa, 112 mm Hg (s.e. = 2·9); and 15–20 min after L-dopa, 116 mm Hg (s.e. = 2·9). The values are not significantly different from each other ($P > 0{\cdot}1$).

Biochemical experiments

Tissue concentrations of dopamine and noradrenaline were determined after L-dopa alone and after pretreatment with MK 485 or Ro 4–4602. The animals were killed 1 h after the injection of L-dopa. The results are in Table 1.

L-Dopa alone (200 mg/kg) caused a pronounced increase in dopamine concentrations in heart and femoral muscle. There was also a marked increase in the levels of brain dopamine. The noradrenaline content of the heart, but not femoral muscle or brain, was significantly ($P < 0{\cdot}001$) decreased.

Pretreatment with MK 485 30 min before L-dopa largely prevented the increase of dopamine in the heart and femoral muscle, but the increase in brain dopamine was more pronounced than after injection of L-dopa alone ($P < 0{\cdot}001$). There were also slightly increased noradrenaline concentrations in the heart and femoral muscle ($P < 0{\cdot}05$ and $P < 0{\cdot}005$, respectively) but not in brain.

As with MK 485, Ro 4–4602 before L-dopa (doses and time intervals as in the blood pressure experiments) prevented the accumulation of dopamine significantly in peripheral tissues (heart, femoral muscle). The increase in brain dopamine was not significantly different from after L-dopa alone. However, the increase in brain dopamine after Ro 4–4602 was significantly ($P < 0{\cdot}001$) less than after MK 485 plus L-dopa.

Table 1. *Levels of dopamine and noradrenaline in various tissues of normal rats and rats treated with* L-*dopa* (200 mg/kg i.p.) *or the same dose of* L-*dopa* 30 *min after MK* 485 (100 mg/kg, i.p.). The values are means with standard errors in μg/g and the number of analyses of 2 pooled organs are given in parentheses. *P* values were calculated by analysis of variance.

	Control	L-Dopa	MK 485+ L-dopa	Ro 4-4602 +L-dopa
Dopamine				
Brain	0·61 0·158 (5)	1·93 0·204 (3)	3·71 0·158 (5)	2·44 0·158 (3)
	$<0·001$	$<0·001$	$<0·001$	
	$<0·001$			
		$>0·10$		
	$<0·001$			
Hear	0·07 0·287 (5)	2·67 0·262 (6)	0·36 0·287 (5)	0·12 0·287 (5)
	$<0·001$	$<0·001$	$>0·10$	
	$>0·10$			
		$<0·001$		
	$>0·10$			
Femoral muscle	0·01 0·051 (5)	0·71 0·047 (6)	0·07 0·051 (5)	0·04 0·051 (5)
	$<0·001$	$<0·001$	$>0·10$	
	$>0·10$			
		$<0·001$		
	$>0·10$			
Noradrenaline				
Brain	0·38 0·029 (5)	0·38 0·038 (3)	0·40 0·029 (5)	0·38 0·029 (5)
	$>0·10$	$>0·10$	$>0·10$	
	$>0·10$			
		$>0·10$		
	$>0·10$			
Heart	0·96 0·047 (6)	0·53 0·047 (6)	1·13 0·057 (4)	0·83 0·051 (5)
	$<0·001$	$<0·001$	$<0·005$	
	$<0·05$			
		$<0·001$		
	$>0·10$			
Femoral muscle	0·10 0·006 (5)	0·09 0·005 (6)	0·12 0·006 (5)	0·07 0·006 (5)
	$>0·10$	$<0·001$	$<0·001$	
	$<0·005$			
		$<0·025$		
	$<0·005$			

Effects of dopamine on blood pressure

To examine the influence of dopamine on blood pressure, the amine was infused intravenously in graded doses in 4 conscious rats. At rates of 0·004 to 4 μg/min, there were no effects on blood pressure but at rates of 4 to 20 μg/min there were pressure responses.

DISCUSSION

Administration of L-dopa resulted in a pronounced increase in dopamine in sympathetically innervated tissues and a marked increase in brain concentrations. At the same time, L-dopa significantly reduced the noradrenaline content of the heart but not of the brain. Essentially similar findings have been reported previously (Bartholini, Burkard & others, 1967; Bartholini, da Prada & Pletscher, 1968; Bartholini & Pletscher, 1968; Butcher & Engel, 1969a, b). The blood pressure experiments revealed that L-dopa regularly elicited a hypertensive response in conscious rats. Previous studies have mostly been made in anaesthetized animals; the results are complex and indicate a wide species variation (Holtz & Credner, 1942; Dengler & Reichel, 1958; Gaillard, Schaeppi & Tissot, 1969; Pruss & McGill, 1969).

In animals pretreated with an inhibitor of peripheral decarboxylase (MK 485), the effect of L-dopa in increasing dopamine in peripheral tissues was almost completely prevented. Further, the effect of L-dopa in lowering heart noradrenaline was blocked, significantly indicating that this effect is due to a displacement of noradrenaline by dopamine formed from L-dopa. This mechanism is the same as that proposed for the noradrenaline-lowering effects of α-methyldopa and α-methyl-*m*-tyrosine which is also markedly reduced after decarboxylase inhibition (Carlsson & Lindqvist, 1962; Udenfriend & Zaltzman-Nirenberg, 1962; Gessa, Costa & others, 1962; Levine & Sjoerdsma, 1964; Henning, 1969a). In our experiments L-dopa increased brain dopamine levels significantly more after inhibition of peripheral decarboxylase, which confirms the results of other investigators (Bartholini & Pletscher, 1968; Butcher & Engel, 1969b) and is believed to result from an increased availability of L-dopa to the brain and, hence, an increased formation of dopamine.

The increase in blood pressure produced by L-dopa (50 mg/kg) was completely blocked by the peripheral decarboxylase inhibitor (MK 485). When the dose of L-dopa was increased, there was a significant fall in blood pressure. In contrast, the same dose of L-dopa had no effect on blood pressure after pretreatment with Ro 4–4602. Previous studies indicate that this compound is an effective inhibitor of both central and peripheral decarboxylase (see p. 554) although in our experiments Ro 4–4602 did not prevent the increase of brain dopamine produced by L-dopa. However, significantly more dopamine was formed in the brain after pretreatment with MK 485, indicating a significant degree of decarboxylase inhibition by Ro 4–4602 in the brain. Neither of the two decarboxylase inhibitors alone had any effect on blood pressure, as shown previously for corresponding time intervals (Henning, 1969a).

The interaction of the two decarboxylase inhibitors with the effects of L-dopa on blood pressure may be interpreted as follows. The hypertensive response seen after L-dopa alone is probably mediated through peripheral adrenergic mechanisms. Possibly, dopamine is involved, acting either directly by activation of the adrenergic receptors or indirectly by displacement of endogenous noradrenaline. The hypotensive response which is unmasked after inhibition of the peripheral decarboxylation of a

large dose of L-dopa is probably not due to peripheral actions of dopamine or noradrenaline though dopamine may have a dilator action in certain vascular beds in some species (see e.g. Holtz & Palm, 1966). The formation of dopamine in peripheral tissues was almost completely prevented by the decarboxylase inhibitor and in experiments with intravenous dopamine infusions in rats over a wide dose range, only hypertensive responses were encountered. Therefore, it seems most likely that an action of L-dopa metabolites in the central nervous system would explain the hypotensive effect of L-dopa after peripheral decarboxylase inhibition. Apparently, very high levels of such metabolites are required since a hypotensive response was associated only with the high dopamine levels produced after MK 485 pretreatment.

After pretreatment of the animal with an inhibitor of dopamine β-hydroxylase both the hypotensive response and the symptoms of aggressiveness produced by L-dopa were absent. In the dose used, FLA 63 markedly inhibited the β-hydroxylase (Svensson & Waldeck, 1969) and therefore the formation of noradrenaline from L-dopa was presumably prevented to a large extent. If this is so, an activation of noradrenergic mechanisms in the brain may account for the hypotension after L-dopa plus peripheral decarboxylase inhibition. A central noradrenaline receptor blocking action of FLA 63 is unlikely since this drug does not influence the noradrenaline receptors in the spinal cord in the rat (Andén, unpublished experiments). The lack of effect of spiroperidol, which in the dose used appears to block only central dopamine receptors in the rat (Andén, Butcher & others, 1970), could also be explained by a central noradrenergic activation by L-dopa metabolites. The antihypertensive drug clonidine (St 155) has recently been shown to have a central noradrenaline receptor stimulating action (Andén, Corrodi & others, 1970).

Pretreatment with protriptyline, which prevents the uptake of catecholamines in noradrenergic neurons (Carlsson, Fuxe & others, 1966; Jonason, 1969), blocked the hypotensive effect of L-dopa after peripheral decarboxylase inhibition. Assuming that the action of L-dopa in these experiments is mediated through central noradrenergic activation, the effect of protriptyline may be explained by a reduction in the neuronal uptake of dopamine. This could lead to a decreased availability of substrate for noradrenaline formation in these neurons, or to a diminished release of this amine, or both mechanisms in combination. It appears unlikely that the slight anticholinergic effect of protriptyline is of any relevance since unpublished experiments have shown that pretreatment with atropine sulphate (10 mg/kg) did not influence the hypotension after L-dopa plus MK 485. Another possible explanation is that protriptyline may exert a central noradrenaline receptor blocking effect.

The demonstration of a centrally mediated hypotensive response to L-dopa may have several important implications. As mentioned previously, L-dopa alone may cause lowering of blood pressure. During long-term oral L-dopa treatment of patients with Parkinson's disease, several investigators have noted episodes of hypotension, sometimes mainly of the postural type (Calne, Stern & others, 1969; Cotzias, Papavasiliou & Gellene, 1969; Yahr, Duvoisin & others, 1969). This may be related to an action of the drug of a similar nature to that found in rats pretreated with a decarboxylase inhibitor. Another indication is that similar effects could account for the effects of structural analogues of L-dopa. In preliminary experiments we have found that *m*-tyrosine behaves like L-dopa. However, this is not so with α-methyl-*m*-tyrosine which lacks hypotensive effects in the rat (Henning, 1967). α-Methyldopa,

which under certain conditions may produce hypertensive reactions, has previously been shown to retain its hypotensive properties after peripheral decarboxylase inhibition (Henning, 1969a). Further, preliminary experiments have shown that the hypotensive action of α-methyldopa is blocked after pretreatment with an inhibitor of dopamine β-hydroxylase. Taken together, these findings may indicate that L-dopa as well as its α-methylated derivative both act by activating central noradrenergic mechanisms.

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