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On the mechanism of L-DOPA-induced postural hypotension in the cat

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Summary

1. The effects of L-DOPA on postural hypotension and carotid occlusion pressor effect were studied, mainly in cats ; the recovery of the blood pressure upon tilting was used as a measure of postural hypotension.

2. L-DOPA (30 mg/kg) partially depressed the carotid occlusion pressor effect and caused some degree of postural hypotension, L-DOPA (100 mg/kg) had more marked effects ; the responses returned to control after 90 to 150 minutes. L-DOPA itself caused a pressor response in all cats.

3. The dopa decarboxylase inhibitor N^1-(DL-seryl)-N^2-(2,3,4-trihydroxybenzyl) hydrazine (RO4-4602, 50 and 10 mg/kg) had no effect itself on the tilt response but completely prevented the effects of L-DOPA on the carotid occlusion pressor effect and postural hypotension.

4. After RO4-4602 (3 and 1 mg/kg), L-DOPA (100 mg/kg) caused a brief rise of blood pressure followed by a longer lasting fall in horizontally-orientated cats (i.e. ' supine ' hypotension). No postural hypotension was observed after L-DOPA under these conditions.

5. Noradrenaline elicited only small and transient effects on postural hypotension, but dopamine's effects were more marked and longer lasting. Pressor dose-response relationships for noradrenaline were the same before and after L-DOPA, as well as in cats pretreated with L-DOPA for 4 days.

6. In cats with kidneys and intestines removed, the tilt reflex was still present. Dose-response curves to L-DOPA were the same as in normal animals. RO4-4602 (3 mg/kg) prevented postural hypotension and block of the carotid occlusion pressor effect ; supine hypotension was also observed after L-DOPA.

7. The recovery response to tilting in spinal cats was markedly depressed or absent unless the blood pressure was elevated by angiotensin, in which experiments L-DOPA depressed the recovery upon tilting (i.e. induced postural hypotension).

8. Blood pressure responses to tyramine were increased after 10 mg/kg of L-DOPA, but depressed after 100 mg/kg. The response to tyramine was not depressed, however, when RO4-4602 was given to block the dopa-dopamine conversion.

9. The response to sympathetic stimulation in pithed rats was depressed after L-DOPA and dopamine, but not after α-methyldopa.

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10. α-Methyldopa (300 mg/kg) given acutely caused a moderate degree of postural hypotension and a more marked postural hypotension if given for two days.

11. It is concluded that it is possible to differentiate between the supine and postural hypotension caused by L-DOPA and that supine hypotension is due to a central effect and postural hypotension to an extracerebral effect. Postural hypotension is discussed in relation to six hypotheses presented to explain its effect. Postural hypotension after L-DOPA is probably not due to α-adrenoceptor blockade, a central effect or any effect on the kidney. The most likely hypothesis is that L-DOPA forms dopamine which acts as a false transmitter in the peripheral sympathetic nervous system.

Introduction

A major side effect of L-DOPA in many Parkinsonian patients is hypotension. Both orthostatic (postural) and supine hypotension (usually referred to as hypotension) have been observed in man (Calne, Brennan, Spires & Stern, 1970; Watanabe, Chase & Cardon, 1970). There is substantial evidence from animal experiments that hypotension is due to a central mechanism whereby L-DOPA is effective after conversion to dopamine and/or noradrenaline in certain areas of the brain (Henning & Rubenson, 1970a; Robson, 1971; Minsker, Scriabine, Stokes, Stone & Torchiana, 1971). No common opinion exists about the mechanism of the postural hypotension observed after L-DOPA. Various hypotheses have been proposed as an explanation. The purpose of the present investigation was to study whether one of these hypotheses could be validated. It was deduced from our studies that the most likely hypothesis to account for postural hypotension after L-DOPA is that it is converted to dopamine which acts as a false transmitter peripherally. A preliminary account of this work has been presented (Spilker & Dhasmana, 1972).

Methods

Cats (2·3–3·5 kg) were anaesthetized with chloralose (70 mg/kg i.v.) after induction of anaesthesia with oxygen (2 l./min), nitrous oxide (4 l./min) (1:2) and halothane (2%) mixture. Systolic, diastolic and mean blood pressures were recorded from the permanently occluded carotid artery on a Philips Cardiopan 3R recorder and the femoral vein was cannulated for injections. For studies on postural hypotension, cats were firmly attached to a table with the fulcrum at the position of the heart. The table was tilted 75° for two minutes before it was returned to the horizontal position. Upon tilting, there was a sharp drop in blood pressure which was quickly followed by a partial recovery due to reflex mechanisms. This recovery effect has been used for studying postural hypotension (Constantine, McShane & Wang, 1971). The term postural hypotension will be used to denote a decreased recovery response to tilting. This procedure was followed within 15 min by a test of carotid occlusion whereby the common carotid artery was occluded for 45 seconds. The carotid occlusion reflex in cats anaesthetized with chloralose has been shown (Sattler & Van Zwieten, 1967) to be due to two components, withdrawal of baroreceptor stimulation and stimulation of chemoreceptors, of which the latter contributes about half to two-thirds of the total effect. Although only one carotid artery was occluded in the experiments described here, the rise in blood pressure

(40 ± 13 mmHg, ± S.D., $n=31$) was about the same as that elicited in other experiments on cats where both carotid arteries were occluded (44 ± 14 mmHg, ± S.D., $n=44$). In a limited number of experiments, dogs, anaesthetized with Na pentobarbitone (35 mg/kg i.v.) were also studied on the tilt table. The vagi were left intact in all experiments. Pithed male rats (200–275 g) were prepared as described by Gillespie, Maclaren & Pollock (1970). The stimulating electrode was inserted through the eye for 8 cm and a 1 cm segment of the spinal cord was stimulated at vertebral level T7–9. This level was chosen in order to obtain maximal blood pressure responses and minimal effects of other sympathetic responses. Pulses (frequency 10 Hz, duration 1·0 ms) were given for 30 s at five times threshold voltage. This procedure was repeated every 5 min ; recovery from each stimulation was complete within 4 minutes. Blood pressure was measured with a Statham P23Db transducer from the carotid artery. All rats were pretreated with (+)-tubocurarine (1 mg/kg i.v.) and maintained by artificial respiration.

Procedure 1. A dose-response relationship was determined in cats for the effects of L-DOPA on postural hypotension, the carotid occlusion test and blood pressure. The time-course of recovery following a single dose of L-DOPA (100 mg/kg i.v.) was also determined.

Procedure 2. A dose-response relationship was determined in cats for the preventative effects of the dopa-decarboxylase inhibitor RO4-4602 on the postural hypotension and carotid occlusion block caused by L-DOPA. Only one dose of RO4-4602 was studied per cat. Noradrenaline and dopamine were studied in other experiments on postural hypotension.

Procedure 3. Cats were pretreated with L-DOPA (50 mg/kg s.c.) twice on one day and once the following morning 45 min before anaesthesia. RO4-4602 was given after L-DOPA had elicited its effects. Cats were also pretreated with L-DOPA for four days but not on the morning of the experiment (50 mg/kg s.c., twice per day).

Procedure 4. In cats, the intestines and kidneys were removed (ligatures were placed around the renal and mesenteric arteries and 3 min later around the veins, before the organs were removed) and the effects of L-DOPA studied as well as its interactions with RO4-4602.

Procedure 5. L-DOPA and RO4-4602 were studied in spinal cats. The spinal cord was sectioned at C2 and the brain damaged. Blood pressure was maintained at normal levels in some experiments by a continuous infusion of angiotensin (0·54 to 0·94 (μg/kg)/min).

Procedure 6. The effect of L-DOPA in causing postural hypotension was studied in dogs.

Procedure 7. A dose-response relationship was determined for the effects of noradrenaline on blood pressure in normal cats before and after L-DOPA, as well as in cats pretreated with L-DOPA (50 (mg/kg)/day) for four days. Effects of tyramine and dimethylphenylpiperazinium (DMPP) on blood pressure were studied in other cats before and after L-DOPA. The effect of RO4-4602 on the tyramine response was also studied.

Procedure 8. The response of pithed rats to sympathetic stimulation was studied after L-DOPA, dopamine and α-methyldopa. Only one agent was studied per rat and effects were observed for 2 h after administration.

Procedure 9. A dose-response relationship was determined for effects of α-methyldopa on postural hypotension and the carotid occlusion pressor effect in normal cats as well as in cats pretreated with α-methyldopa (200 mg/kg s.c.) for two days plus 1 h before anaesthesia on the third day, when the tilt experiment was performed.

All errors are expressed as standard deviations (S.D.) and n refers to the number of cats used. Each cat was used for only one procedure or part thereof. Results are expressed in terms of a depression of the recovery response of the blood pressure observed upon tilting. This value was calculated by first determining the recovery as a fraction of the total fall in blood pressure upon tilting, and then by subtracting this value for the experimental response from that of the mean of the control responses and expressing this result as a percentage of the control response. The level of control blood pressure prior to tilting was found to have no influence upon the degree of recovery observed.

The drugs used were L-α-methyldopa (Merck, Sharp & Dohme), angiotensin (CIBA), *N,N*-dimethyl-*N'*-phenylpiperazinium iodide (DMPP, Aldrich), L-3,4-dihydroxyphenylalanine hydrochloride (L-DOPA) (Brocades), dopamine hydrochloride (Koch-Light), (−)-noradrenaline bitartrate, N^1-(DL-seryl)-N^2-(2,3,4-trihydroxybenzyl) hydrazine hydrochloride (RO4-4602, Roche) and tyramine hydrochloride (Koch-Light). All drugs were freshly prepared each day and weights are expressed in terms of the salts used.

Results

Studies on postural hypotension and the carotid occlusion pressor effect after L-DOPA (Procedure 1)

Normal cats responded to a two minute period of tilting with an almost immediate lowering of mean blood pressure ($\pm$S.D.) by 76 ± 19 mmHg ($n=24$) from a control of 152 ± 22 mmHg. The pressure recovered within 15 s usually by between 35–60% and in general remained at this level until the tilt period was completed and the table righted. Upon this manoeuvre the blood pressure rapidly rose above the original control level, but returned to control usually within five minutes (Fig. 1). Five to ten minutes after control blood pressure levels were re-established the carotid occlusion pressor effect was determined. This procedure elicited a mean blood pressure rise of 40 ± 13 mmHg ($n=31$).

After L-DOPA (10 mg/kg i.v.) neither the test for postural hypotension nor carotid occlusion was affected by more than 9% (Fig. 1A); but after L-DOPA (30 mg/kg), the partial return of blood pressure upon tilting was delayed and was 64 ± 30% less in magnitude than in control experiments ($n=6$). The carotid occlusion reflex was depressed 63 ± 13% by this dose of L-DOPA (Fig. 1A). L-DOPA (100 mg/kg) abolished the blood pressure return observed after tilting in all 11 experiments and this remained completely blocked for the duration of the tilt period (Fig. 1A). After a single intravenous dose of L-DOPA (100 mg/kg) the control response to tilting was not re-established until 90–150 min later (Fig. 1B; $n=3$). The carotid occlusion reflex was depressed 72 ± 14% by L-DOPA (100 mg/kg) and the time-course of its recovery was approximately the same as that observed for postural hypotension. Saline (0·9% w/v NaCl) had no effect on

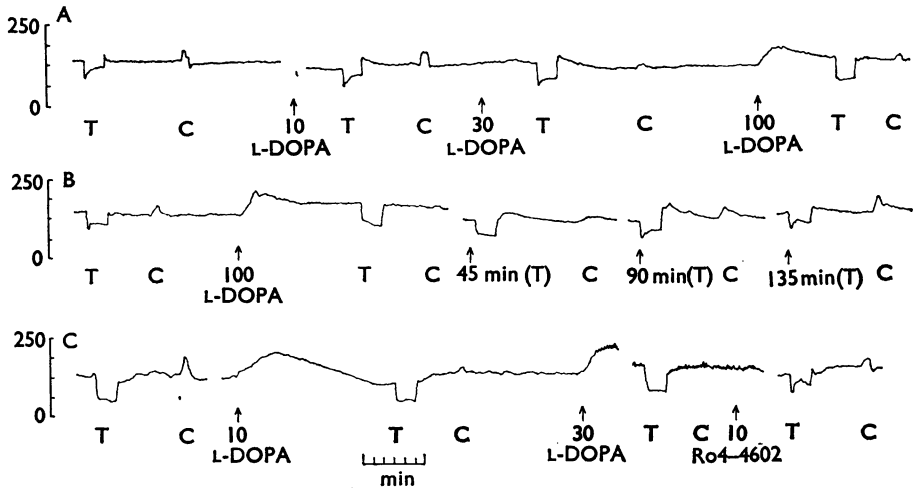

FIG. 1. Mean blood pressure responses in cats to a 2 min 75° tilt (T) followed by a test of carotid occlusion (C). Panel A: Dose-response relationship to L-DOPA. Panel B: Duration of postural hypotension and depression of the carotid occlusion after L-DOPA (100 mg/kg). Panel C: Effect of L-DOPA in a cat that was pretreated with L-DOPA (50 mg/kg, s.c.) twice on one day and once the next morning before the experiment. Note that although postural hypotension is present at the start of the experiment, the carotid occlusion pressor effect is normal.

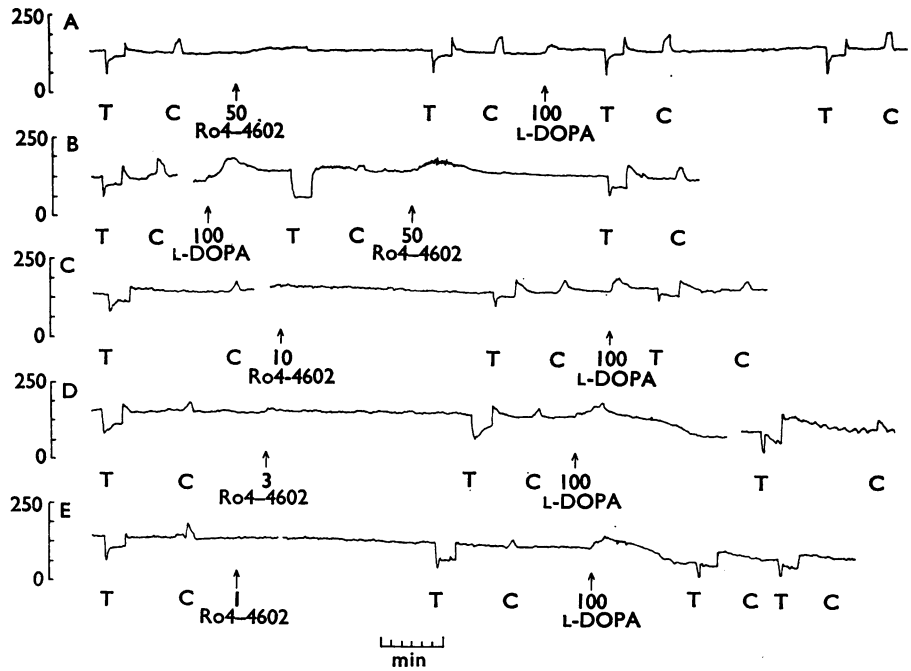

FIG. 2. Effects of various doses of RO4-4602 (50, 10, 3, 1 mg/kg) on preventing and reversing (Panel B) the postural hypotension and carotid occlusion depression due to L-DOPA (100 mg/kg). Note the hypotensive effect of L-DOPA in panels D and E. T=tilt reflex and C=test of the carotid occlusion.

either of these tests. Mean blood pressure was unchanged after 10 mg/kg of L-DOPA, rose 12±7 mmHg after 30 mg/kg and 61±20 mmHg after 100 mg/kg.

Effect of L-DOPA after RO4-4602 (Procedure 2)

The DOPA decarboxylase inhibitor RO4-4602 (50 mg/kg i.v.) sometimes caused a slight non-significant increase in blood pressure (Fig. 2), but this effect was not noted when lower doses of RO4-4602 were used. The mean blood pressure rise of 61±20 mmHg with L-DOPA (100 mg/kg) was reduced to 21±8 mmHg after RO4-4602 (50 mg/kg ; $P<0.01$). RO4-4602 had no effect itself on the tilt response. The carotid occlusion reflex was usually also unaffected, but was sometimes slightly altered in these experiments. RO4-4602 completely prevented the effects of L-DOPA (100 mg/kg) given 30–35 min later ($n=4$; Fig. 2A) on carotid occlusion reflex depression or postural hypotension during an observation period of 90 min after L-DOPA. A lower dose of RO4-4602 (10 mg/kg) was as effective as the higher dose (50 mg/kg) in this respect ($n=4$; Fig. 2C). When still lower doses of RO4-4602 (1 and 3 mg/kg) were given ($n=6$) L-DOPA (100 mg/kg) invariably caused supine hypotension (mean=57±13 mmHg below control) usually after a transient period of hypertension (Fig. 2D and 2E) that was not significantly different in magnitude from that observed after higher doses of RO4-4602. These low doses of RO4-4602 always prevented the occurrence of postural hypotension due to L-DOPA, although only the 3 mg/kg dose always prevented the block of the carotid occlusion reflex as well. In three experiments in which 1 mg/kg RO4-4602 was used, variable effects on the carotid occlusion reflex (but not on the tilt response) were observed, indicating that this dose is approximately the threshold dose necessary to prevent the carotid occlusion reflex depression caused by L-DOPA. RO4-4602 (50 mg/kg) was also given in five other experiments 20 min after L-DOPA (100 mg/kg) had elicited postural hypotension and depressed the carotid occlusion reflex. These reflexes were tested every 5–7 min and both effects of L-DOPA were found to be reversed 20 to 25 min later (Fig. 2B), which was 45 to 105 min faster than if the effect of L-DOPA had been allowed to wear off on its own. This effect of RO4-4602 remained for a further 90 min of testing.

Since RO4-4602 prevented postural hypotension after L-DOPA, it was of interest to determine whether two of the metabolites of L-DOPA, noradrenaline or dopamine could induce postural hypotension. Noradrenaline (2 to 10 μg/kg) elicited only a small depression of the recovery from tilt ranging from 0 to 21% (mean=7%, $n=8$). This effect persisted less than 30 minutes. Dopamine, however, decreased the recovery from tilt by a mean of 67±16% for a period in excess of 45 min ($n=8$), in a dose that gave an approximately equal pressor effect to that of noradrenaline (0·3 to 3·0 mg/kg).

Effect of L-DOPA after chronic pretreatment with L-DOPA (Procedure 3)

Four cats were pretreated with L-DOPA (50 mg/kg s.c.) twice on one day and once the following morning 45 min before anaesthesia. The control responses to tilting in these animals were markedly depressed or completely abolished (Fig. 1C). The carotid occlusion reflex was 40% depressed in only two cats and not depressed in the other two, as judged by values of the carotid occlusion reflex observed in

normal cats. Each of the cats pretreated with L-DOPA was more sensitive to subsequent L-DOPA injections (Fig. 1C). The mean blood pressure rise after L-DOPA 10 mg/kg was 45 ± 20 mmHg whereas in the control it was zero; and after 30 mg/kg it was 57 ± 17 mmHg compared with 12 mmHg in the control. The mean blood pressures before injection of L-DOPA did not differ between normal and L-DOPA pretreated cats. RO4-4602 (50 mg/kg) when given after L-DOPA, reversed the effects of L-DOPA on tilting and the carotid occlusion reflex in two experiments and RO4-4602, 10 mg/kg, was almost as effective as the larger dose in reversing the effects of L-DOPA in the other two experiments performed. Four cats were pretreated for four days with L-DOPA (50 mg/kg s.c.) but not on the morning of the experiment. In these cats the response to tilting was the same as in normal cats, but hypertensive pressor responses to injection of low doses of L-DOPA (1, 3, 10 and 30 mg/kg) were observed. Thus, the postural hypotension observed in cats pretreated on one day and once the following morning was due to the last dose given just before the experiment.

Effect of L-DOPA in cats with kidneys and intestines removed (Procedure 4)

In order to determine the importance of kidneys and intestines for the reflex governing postural changes and the effects of L-DOPA on this reflex, experiments were performed in cats where the intestines and kidneys were removed. At least 1 h was allowed before the experiments were continued. The reflex to tilt was still present in all four cats studied. In two it was the same as control and in the other two the recovery was more marked than in control (Fig. 3). Pressor responses to L-DOPA (10, 30 and 100 mg/kg) were comparable to those of normal animals. L-DOPA (100 mg/kg) caused postural hypotension (recovery abolished in each experiment) and decreased the carotid occlusion reflex by 53% (Fig. 3A). In two experiments, RO4-4602 (3 mg/kg) prevented the occurrence of postural hypotension and depression of the carotid occlusion reflex by L-DOPA (100 mg/kg; Fig. 3B). The supine hypotension (mean=48 mmHg below control) was also observed as in normal animals. Thus, responses to L-DOPA in animals with kidneys and intestines removed were approximately the same as in normal cats.

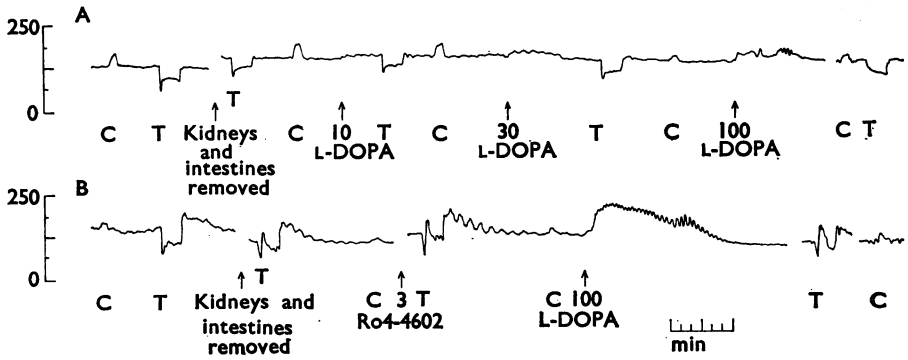

FIG. 3. Mean blood pressure effects of L-DOPA on the tilt reflex (T) and carotid occlusion (C) in cats with intestines and kidneys removed. Panel A: Dose-response to L-DOPA (10, 30 and 100 mg/kg). Panel B: Prevention of effects of L-DOPA by RO4-4602. The hypotension due to L-DOPA after a hypertensive effect is also evident.

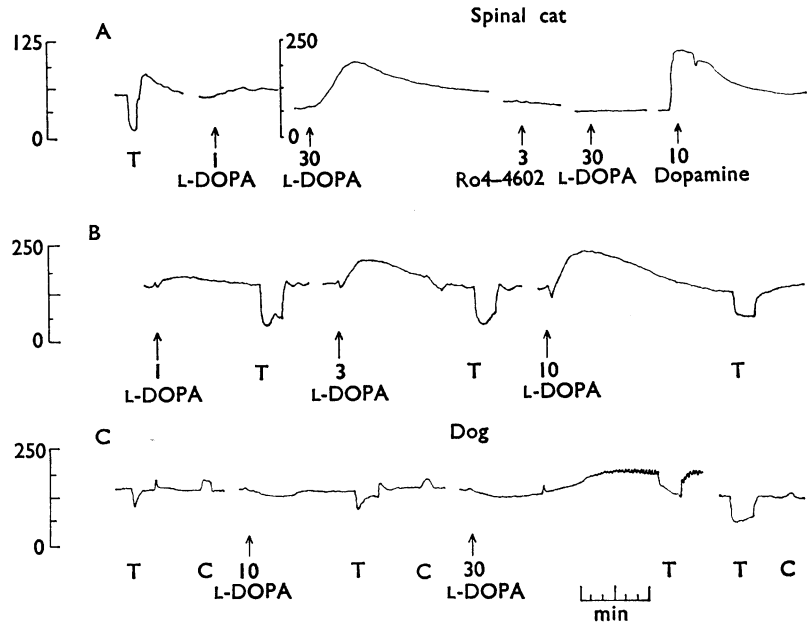

FIG. 4. Panel A: The absence of response to a 1 min tilt (T) in a spinal cat, plus mean blood pressure effects of L-DOPA, its block by RO4-4602 and effect of dopamine. Panel B: T and blood pressure response to L-DOPA in a spinal cat receiving a constant infusion of angiotensin. Panel C: Effects of L-DOPA on T and carotid occlusion (C) in a dog. (Doses in mg/kg.)

Effect of L-*DOPA in spinal cats (Procedure 5)*

Spinal cats were studied to evaluate further the central versus peripheral effects of L-DOPA. The recovery from tilt in spinal cats was completely absent (Fig. 4A) or present to only a limited extent. As blood pressure was rather low in these animals (mean=54 mmHg) experiments were also performed in spinal animals that were given a constant infusion of angiotensin to maintain the blood pressure at pre-spinal levels. Under these conditions (n=4) the fall in blood pressure on tilting was slower than in normal cats and there was a recovery which was also slower (100 to 200%). Nevertheless, the effect of L-DOPA could be studied on this reflex, elicited by tilting the cat. All spinal cats (n=6) were markedly sensitive to L-DOPA and the blood pressure increased in response to a minimum dose of 1 or 3 mg/kg L-DOPA (Fig. 4A). RO4-4602 (3 mg/kg) prevented this increase in blood pressure to L-DOPA but not to dopamine (Fig. 4A). Postural hypotension (recovery to tilt abolished) was observed after L-DOPA (10 mg/kg). A dose of 1 mg/kg of L-DOPA had no effect and 3 mg/kg had an intermediate effect (Fig. 4B).

Effect of L-*DOPA in dogs (Procedure 6)*

Because the recovery of the response to tilt is less in cats than in dogs or man, experiments with L-DOPA were also performed on dogs. The degree of recovery observed in our experiments was greater in dogs (75 to 97%) that in cats (Fig. 4C; n=3). Low doses of L-DOPA (10 mg/kg) depressed the degree of recovery from 11 to 20% but had no effect on the carotid occlusion reflex.

L-DOPA (30 mg/kg) caused postural hypotension (recovery to tilt abolished in all dogs) and depressed the carotid occlusion reflex by 62%.

Studies on possible α-adrenoceptor and ganglion blocking activity of L-DOPA (Procedure 7)

In experiments designed to test possible α-adrenoceptor blocking actions of L-DOPA, dose-response relationships for noradrenaline (0·01 μg/kg to 10·0 μg/kg) on blood pressure were determined. After two control series of noradrenaline injections, L-DOPA was injected and the series repeated. This was done with several doses of L-DOPA. At 10, 30 and 100 mg/kg L-DOPA had no effect on the dose-response relationship for noradrenaline ($n=6$). Four cats were pretreated for each of 4 days with L-DOPA (50 mg/kg s.c.) and the above tests performed on the fourth day, 1 h after the last L-DOPA injection. The control dose-response curve for noradrenaline in these cats was the same as in the animals not pretreated with L-DOPA and L-DOPA also had no effect on the curves obtained for noradrenaline.

The effects of tyramine and DMPP on blood pressure before and after L-DOPA were studied in other cats ($n=13$; both agents were studied in five of these cats). Standard doses were given every 15 min until the response after L-DOPA had returned to control values. The blood pressure responses to tyramine (200 μg/kg) and DMPP (50 μg/kg) were increased after an injection of 10 mg/kg of L-DOPA but after 100 mg/kg responses to tyramine were depressed (Table 1). The depressed

TABLE 1. *Effects of tyramine and DMPP on blood pressure in cats after L-DOPA*

	Dose (μg/kg)	n	Rise in blood pressure after: L-DOPA (10 mg/kg)*	L-DOPA (100 mg/kg)*
Tyramine	200	8	157±49	52±19
DMPP	50	7	139±27	—†

* Control response=100. All values are ± S.D. Mean rise in blood pressure upon tyramine injection was 45±19 mmHg ($n=44$) and for DMPP 65±15 mmHg ($n=29$). †Responses to DMPP after L-DOPA (100 mg/kg) were increased in three experiments and decreased in four.

responses to tyramine returned to control values after 90 minutes. When RO4-4602 (50 mg/kg) was given prior to L-DOPA (100 mg/kg) the responses to tyramine were not depressed ($n=3$). Responses to DMPP after L-DOPA (100 mg/kg) were variable. In three experiments they were increased by up to 33% of control and in four experiments they were decreased by up to 55% of control.

Studies in pithed rats (Procedure 8)

Control rats responded to sympathetic stimulation with an increase in mean blood pressure from 67±20 to 168±26 mmHg ($n=61$). The magnitude of this response was remarkably constant for up to 4 h in any one rat. Control injections of saline in the same volume as for drug additions had little effect upon the blood pressure or response to sympathetic stimulation. The effects of L-DOPA, dopamine and α-methyldopa on blood pressure and responses to sympathetic stimulation are summarized in Table 2. Each agent transiently increased the mean blood pressure and the order of potency was dopamine>L-DOPA>α-methyldopa. Dopamine and L-DOPA both markedly decreased the amplitude of the blood pressure response to sympathetic stimulation, but α-methyldopa had no effect upon this parameter.

TABLE 2. *Response of pithed rats to sympathetic stimulation after* DOPA, *dopamine and α-methyldopa*

	Dose (mg/kg)	*n*	Rise in blood pressure (average in mmHg)	Maximal decrease of the blood pressure response to sympathetic stimulation* (average in mmHg)
DOPA	100	5	32	65
Dopamine	10	3	80	75
α-Methyldopa	100	4	11	1
	200	3	8	9†

* These values were obtained after the blood pressure had returned to control levels. †This value was not observed until 2 h after α-methyldopa was given.

Studies on postural hypotension and the carotid occlusion reflex with α-methyldopa (Procedure 9)

The effects of α-methyldopa were studied on the tilt reflex and carotid occlusion reflex in cats. α-Methyldopa (10 and 30 mg/kg i.v.) had no effect on either response. At higher doses (100 and 300 mg/kg) some evidence of postural hypotension was observed (recovery from tilt depressed 32% after 300 mg/kg) which was never as marked as with L-DOPA (100 mg/kg), even up to 3 h after the 300 mg/kg dose was administered. The most marked effect was an increase in the time for recovery by 50 to 100%. The carotid occlusion reflex was depressed 65% by the highest dose of α-methyldopa. Two cats were pretreated with α-methyldopa (200 mg/kg) for two days and given a third injection on the third day one hour before anaesthesia. In these cats the time for recovery from tilt was increased by 100 to 400% and the carotid occlusion reflex was virtually absent.

Discussion

The recovery from tilt in cats has been shown to be mediated in part through aortic arch baroreceptors (Edholm, 1940). The value of tilt experiments in animals as a measure for predicting postural hypotension in humans was considered by Parra & Vidrio (1969) and by Constantine *et al.* (1971); both groups observed that drug-induced changes on the tilt response were similar to their effects on postural hypotension in humans and concluded that there *is* predictive value in such experiments. The recovery phase of the tilt reflex in cats is not as marked as in dogs or man but this was not considered to affect the interpretation of the present results, since L-DOPA elicited similar effects on the tilt response and carotid occlusion reflex in dogs.

Interference of the decarboxylase inhibitor RO4-4602 with the effects of L-DOPA on circulation is in general regarded as convincing evidence that L-DOPA effects are caused by dopamine and/or another metabolite of L-DOPA. Peripherally formed metabolites such as dopamine and noradrenaline increase blood pressure and could be responsible for the pressor effect observed upon L-DOPA administration, while these metabolites when formed centrally could induce a lowering of blood pressure. RO4-4602 does not readily penetrate into the brain. Rather high doses of the compound are therefore needed to block the central L-DOPA to

dopamine conversion in rats (Kuruma, Bartholini, Tissot & Pletscher, 1972), doses far in excess of those necessary for inhibition of decarboxylase activity in peripheral tissue. In our experiments in cats, RO4-4602 in doses of 50 and 10 mg/kg i.v. markedly reduced the pressor effect after L-DOPA without uncovering a supine hypotension. At doses of 3 and 1 mg/kg, however, a supine hypotension was observed. Bartholini, Blum & Pletscher (1969) reported that RO4-4602 in a dose of 500 mg/kg i.p. in rats inhibited central as well as peripheral dopa decarboxylase (using L-DOPA 200 mg/kg i.p.), whereas 50 mg/kg i.p. was said to probably cause a selective inhibition of extracerebral dopa decarboxylase. Further experiments (Pletscher & Bartholini, 1971) supported these results. Thus it seems warranted to conclude that doses of 1 and 3 mg/kg i.v. RO4-4602 produce inhibition of peripheral dopa-decarboxylase while doses of 10 and 50 mg/kg i.v. produce an additional inhibition of central dopa-decarboxylase and possibly block the central receptors responsible for causing hypotension. The fact that postural hypotension induced by L-DOPA is still blocked at doses of 1 and 3 mg/kg i.v. RO4-4602 can then be interpreted as a strong indication of an extracerebral origin of the postural hypotension caused by L-DOPA. Further support for this hypothesis comes from the experiments in spinal cats. Spinal cats normally elicited little or no recovery phase upon tilting, but when the blood pressure was maintained at normal levels by angiotensin infusion, a recovery phase was evident (the recovery was not as rapid as in controls but provides evidence that the tilt reflex can be maintained at least partially by mechanisms peripheral to the brain). L-DOPA depressed this reflex in spinal cats i.e. caused postural hypotension, which could have occurred only through an extracerebral mechanism. The conclusion reached is contrary to the suggestion of Gross, Bannister & Godwin-Austen (1972) that L-DOPA acts centrally above the level of the medulla to interfere with the reflex controlling the response to postural changes and thus causes postural hypotension. There are many studies in supine anaesthetized animals which provide strong evidence that *supine hypotension* after L-DOPA is due to a central effect of dopamine and/or noradrenaline (Henning & Rubenson, 1970a, b; Robson, 1971; Minsker *et al.*, 1971; Andén, Butcher & Engel, 1970). This evidence, however, does not support the possibility raised by Gross *et al.* (1972) that *postural hypotension* can be explained by a central mechanism. Calne *et al.* (1970) and Watanabe *et al.* (1970) both presented clinical evidence that patients treated with L-DOPA who had supine hypotension had a greater fall in blood pressure when erect (postural hypotension). These two groups (Watanabe *et al.*, 1970; Calne, Petrie, Rao, Reid & Vakil, 1972) reported that postural hypotension was not improved when lower doses of L-DOPA were combined with a peripheral dopa-decarboxylase inhibitor, although Calne *et al.* did observe a significant increase in the absolute level of both supine and erect systolic blood pressure in patients receiving L-DOPA plus MK-486. Such an improvement or even prevention of postural hypotension would have been expected on the basis of the present experiments. It is possible that this discrepancy reflects differences between MK-485 and MK-486 used in the clinical studies and RO4-4602 used in the present experiments, or that these differences reflect variations in experimental conditions such as species, anaesthesia, length of treatment, dose-ratio of the two agents used, etc. Contrary to these observations of Watanabe & Calne and their co-workers, Pletscher & Bartholini (1971) stated that some investigators found hypotensive effects in clinical trials of RO4-4602 plus L-DOPA to be less frequent and less severe.

The results of the present investigation as well as other studies have been examined in terms of hypotheses which may possibly explain the extracerebral mechanism of L-DOPA-induced postural hypotension.

Hypothesis 1

L-DOPA or one of its metabolites produces an α-adrenoceptor blockade (Godwin-Austen, Lind & Turner, 1969 ; Leon, Solomon, Ross, Golden & Abrams, 1970).

The main evidence in favour of this hypothesis is that in Parkinsonian patients receiving L-DOPA, mydriatic responses to phenylephrine were reduced (Godwin-Austen *et al.*, 1969) and that some patients had a decreased pressor sensitivity to noradrenaline (Leon *et al.*, 1970). Leon *et al.* (1970) also observed, however, that most patients had an increased response to tyramine. However, there are other possible explanations for the decreased noradrenaline pressor effects observed than α-adrenoceptor blockade, such as an increased MAO activity, which has been reported to occur in rats after chronic treatment with L-DOPA (Tarver, Berkowitz & Spector, 1971). Noradrenaline pressor responses were found to be the same in dogs before and after L-DOPA (Whitsett, Halushka & Goldberg, 1970 ; Whitnack, Leff, Mohammed & Gaffney, 1971). Our results in normal and L-DOPA pretreated cats also show no differences in the blood pressure effects of noradrenaline before and after L-DOPA. Responses to tyramine were increased after low doses of L-DOPA in dogs (Whitsett *et al.*, 1970) and in the present experiments in cats. Responses to tyramine were depressed, however, after a high dose of L-DOPA (100 mg/kg) in cats. This decreased response is probably due to replacement of noradrenaline stores by dopamine formed after L-DOPA injection, thus leaving less noradrenaline available for release by tyramine. This mechanism is supported by the finding that the response to tyramine was not depressed when RO4-4602 was given to block the dopa-dopamine conversion, and is more fully discussed under Hypothesis 5. Our results are not consistent with an α-adrenoceptor blockade by L-DOPA, since responses to noradrenaline were never depressed. Our results with DMPP indicate that L-DOPA does not possess ganglion blocking properties. This is in agreement with the conclusion reached by Whitsett *et al.* (1970) on the basis of electrophysiological experiments in cats. Interestingly, Whitsett *et al.* observed that the evoked action potential recorded from the postganglionic neurone of the superior cervical ganglion was increased in three experiments and depressed in four, and we observed that the blood pressure responses to DMPP after L-DOPA (100 mg/kg) were also increased in three experiments and depressed in four.

Hypothesis 2

L-DOPA forms dopamine which causes vasodilatation in the renal and mesenteric vascular beds, thus diminishing the reflex vasoconstriction caused by postural changes (Whitsett *et al.*, 1970).

In favour of this possibility is the observation that dopamine does cause this vasodilatation (Eble, 1964 ; McDonald, Goldberg, McNay & Tuttle, 1964 ; McNay, McDonald & Goldberg, 1965 ; McNay & Goldberg, 1966). However, our results in cats with kidneys and intestines removed showed that this procedure did not prevent the postural hypotension after L-DOPA nor did it inhibit the prevention of this effect by RO4-4602, suggesting that L-DOPA does not cause postural hypotension by this mechanism in the cat.

Hypothesis 3

L-DOPA markedly decreases the already low plasma renin levels in Parkinsonian patients which may account for the hypotension observed (Barbeau, Gillo-Joffroy, Boucher, Nowaczynski & Genest, 1969).

The authors themselves questioned this hypothesis in that decreased renin levels might occur 'secondary to a deficient sympathetic nervous system or related to a defect in dopamine metabolism'. Our results show that experimental postural hypotension after L-DOPA can be obtained in normal cats in acute experiments where marked changes in renin levels would not be expected to occur. In addition, removal of both kidneys did not affect the postural hypotension caused by L-DOPA. These results suggest that renin levels are not the central factor in L-DOPA-induced postural hypotension.

Hypothesis 4

L-DOPA forms dopamine which causes Na^+ and H_2O excretion which could decrease plasma volume and exacerbate the postural hypotension caused by one of the above mechanisms, or cause postural hypotension itself (Goldberg & Whitsett, 1971).

The evidence for this view comes from the finding that L-DOPA does cause Na^+ and H_2O excretion (McDonald *et al.*, 1964). In our experiments the postural hypotension occurred rapidly after L-DOPA administration, even in the absence of both kidneys so that it is unlikely that Na^+ and H_2O excretion is the major cause of postural hypotension, although it may well exacerbate the situation.

Hypothesis 5

L-DOPA forms dopamine which acts peripherally as a false transmitter (Yahr, Duvoisin, Schear, Barrett & Hoehn, 1969 ; Whitsett *et al.*, 1970 ; Calne, 1970).

In favour of this hypothesis are *in vitro* experiments showing that L-DOPA administration leads to a noradrenaline depletion in some tissues, possibly due to its replacement by dopamine (Harrison, Levitt & Udenfriend, 1963 ; Eränkö & Räisänen, 1966 ; Breese & Prange, 1971). The incubation of rabbit ileum with L-DOPA is followed by dopamine release upon stimulation of sympathetic nerves (Collins & West, 1968). Chronic injections of L-DOPA in cats for 21 days resulted in a lowered noradrenaline content of spleen and a lowered noradrenaline output upon postganglionic sympathetic stimulation of nerves to the spleen (Puig, Wakade & Kirpekar, 1972). The activity of the sympathetic nervous system was also reported to be attenuated by L-DOPA both in patients (Leon *et al.*, 1970) and in dogs (Whitsett *et al.*, 1970 ; Whitnack *et al.*, 1971). The depressed postganglionic sympathetic neural activity could account for the depressed carotid sinus reflex and postural hypotension upon L-DOPA administration.

Our results do not provide conclusive evidence in favour of this hypothesis, although the decreased blood pressure response to sympathetic stimulation in the pithed rat observed after L-DOPA or dopamine is in accordance with this hypothesis, as are the results obtained with tyramine. We consider dopamine, rather than noradrenaline, to be the metabolite of L-DOPA responsible for the postural hypotension since noradrenaline had much less effect on postural hypotension than

dopamine. Dopamine has been reported to depress bilateral carotid occlusion in cats (Whitsett *et al.*, 1970). The false transmitter hypothesis was considered the most likely cause of L-DOPA-induced postural hypotension in the cat. Despite evidence in favour of the false transmitter hypothesis, it is possible that the cause of postural hypotension induced by L-DOPA is more complex and that a mixture of various activities are involved.

Farmer (1965) observed a decreased response of the nictitating membrane in cats to post-ganglionic sympathetic nerve stimulation after α-methyldopa at a dose of 200 mg/kg, but not of 100 mg/kg. α-Methyldopa causes postural hypotension in some hypertensive patients (Dollery & Harington, 1962). Our results show that α-methyldopa also caused some degree of postural hypotension acutely and prolonged the recovery from tilt. These effects were more marked after chronic administration, although under other conditions α-methyldopa did not decrease the response to supramaximal preganglionic sympathetic stimulation in the pithed rat at a dose of 100 or 200 mg/kg.

We conclude that it is possible to differentiate between the supine and postural hypotension caused by L-DOPA and that supine hypotension is due to a central action of dopamine or possibly noradrenaline ; postural hypotension is probably due to dopamine acting as a false transmitter in the peripheral sympathetic nervous system.

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