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Noradrenaline Synthesis from L-DOPA in Rodents and its Relationship to Motor Activity[1]

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DOLPHIN, A., P. JENNER AND C. D. MARSDEN. *Noradrenaline synthesis from L-DOPA in rodents and its relationship to motor activity.* PHARMAC. BIOCHEM. BEHAV. 5(4) 431–439, 1976. – Evidence has been obtained for an increase in noradrenaline (NA) turnover after administration of L-DOPA to rodents. Normal mice, and those pre-treated with either reserpine or α-methyl-p-tyrosine (AMPT) were given L-DOPA (200 mg/kg) plus MK 486 (α-methyldopahydrazine; 25 mg/kg). In all cases L-DOPA produced a rise in cerebral dopamine (DA) levels. Cerebral NA levels were increased by L-DOPA in reserpinised and AMPT-treated mice. The same dose of L-DOPA produced no change in NA in normal mice, although pre-treatment with the monoamine oxidase inhibitor pargyline (200 mg/kg) resulted in a greater rise in NA 1 hr after L-DOPA compared to animals receiving pargyline alone. This evidence suggests that NA is synthesized from L-DOPA in all these situations. But whole brain 3-methoxy-4-hydroxyphenylglcol sulphate (MOPEG-SO4), a major metabolite of NA, measured after administration of the same dose of L-DOPA plus MK 486, was unaltered in normal and AMPT-treated rats, and was significantly decreased in reserpinised rats. However, an elevation of whole brain MOPEG-SO4 was found in reserpinised and AMPT-treated rats after a lower dose of L-DOPA (50 mg/kg). This discrepancy may be explained by high doses of L-DOPA causing inhibition of catechol-O-methyl transferase (COMT), which is suggested by the observation that the forebrain homovanillic acid (HVA): 3,4-dihydroxyphenylacetic acid (DOPAC) ratio was significantly lower after the high dose of L-DOPA than in untreated mice. Such an inhibition would prevent formation of MOPEG-SO4. Pretreatment with the dopamine-β-hydroxylase inhibitor FLA (63(bis-(1-methyl-4-monopiperazinyl-thiocarbonyl)disulphide) prevented the increase in NA and MOPEG-SO4 formation observed following L-DOPA induced motor activity in these groups of animals suggesting the involvement of NA in the production of such behaviour.

L-DOPA Locomotion Noradrenaline MOPEG-SO4 FLA 63

IT is currently believed that dopaminergic neurotransmission is of basic importance in locomotor activity, but that noradrenaline (NA) plays a role in the facilitation and modification of this behaviour [2,38]. When L-DOPA is administered to patients with Parkinson's disease, the therapeutic benefit is attributed to the formation of dopamine (DA) in the brain [21]. Whether significant quantities of NA are also synthesised is uncertain, and the role of such NA as might be formed, in the therapeutic response in unknown.

In previous papers we have provided pharmacological evidence that in rodents NA receptor stimulation enhances the locomotor response attributable to DA formation from L-DOPA [16]. We have employed two animal models used to evaluate anti-parkinsonian drugs. The models involve pre-treatment of rodents with reserpine to prevent storage of monoamines [8] or with α-methyl-p-tyrosine to prevent catecholamine synthesis by inhibition of tyrosine hydroxylase [34]. Both produce a marked reduction in locomotor activity (akinesia) which may be reversed by specific DA receptor stimulants [9,13]. NA receptor stimulation alone has little effect on motor activity in the reserpinised animal, but is able to enhance the response to dopaminergic stimulation [2,27].

The use of dopamine-β-hydroxylase inhibitors (DBHI) to prevent the formation of NA from administered L-DOPA indicates that NA has a functional role in augmenting motor activity produced by DA stimulation [1, 26, 35]. Although several DBHI have been employed, FLA-63 (bis-[1-methyl-4-homopiperazinylthiocarbonyl] disulphide) appears to be the most convenient, as it can be administered in solution and produces an adequate blockade of DBH at a dose (25 mg/kg) which does not produce any marked toxicity [38].

In the present work we have attempted to clarify the extent of involvement of NA in the motor activity produced by L-DOPA in normal animals and those depleted of catecholamines. The time course of L-DOPA induced motor

[1] A preliminary report of this work was presented to the British Pharmacological Society, Cambridge, September 1975.
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activity has been compared with the levels of DA and NA after L-DOPA administration in the presence or absence of FLA-63. To determine the turnover of NA, changes in the level of the major metabolite of NA, 3-methoxy 4-hydroxyphenylglycol sulphate (MOPEG-SO4) [32], after L-DOPA administration has been investigated. It has been suggested that MOPEG-SO4 levels reflect the turnover of extraneuronally released and thus functionally active NA [6]. Our results indicate that NA synthesis occurs from administered L-DOPA in normal and amine-depleted rodents. This enhanced NA turnover is shown to be related in time to the locomotor response to L-DOPA.

METHOD

Animals

Male 'Swiss S' or 'P' strain mice (20–25 g) or Wistar rats (200–250 g) (Animal Suppliers Limited) were used in all experiments. Drugs were administered, locomotor activity monitored and animals sacrificed for biochemical determinations according to the schedule shown in Fig. 1.

Drugs

All drugs were administered intraperitoneally. In some experiments, L-DOPA was administered to normal animals. In other experiments mice and rats were pre-treated with either reserpine (10 mg/kg; Halewood Chemicals Limited) 18–24 hrs before administration of L-DOPA in behavioural experiments and 19 hr before L-DOPA in all biochemical experiments; or with AMPT (α-methyl-p-tyrosine methyl ester HCl) (200 mg/kg; Sigma) dissolved in physiological saline, 3 hr before L-DOPA in all experiments. FLA-63 (25 mg/kg; Labkemi, Sweden) dissolved in dilute HCl which was subsequently adjusted to pH7 with NaOH, was administered 1 hr prior to L-DOPA. Control animals received the same volume of vehicle. L-DOPA (200 mg/kg; Roche Products Limited) together with the peripheral decarboxylase inhibitor MK 486 (α-methyldopahydrazine) (25 mg/kg; MSD Limited) was administered in fine suspension in 1% methylcellulose in a volume of 0.5 ml. Control animals received the same volume of methylcellulose. In some experiments L-DOPA (50 mg/kg) alone was administered; in others pargyline (100 mg/kg; William Warner Limited) dissolved in saline was administered to normal animals 1 hour prior to L-DOPA.

Behavioural Pharmacology

Locomotor activity of mice was measured using two Animex activity meters (LKB Farad) [37]. The animals were housed in batches of 3 in clear plexiglass cages which enabled behavioural observations to be made at intervals during the experiments without disturbance. Experiments were performed between 9.00 a.m. and 9.00 p.m. under standard laboratory conditions of lighting and temperature. Food and water were withdrawn during the test period. Activity was recorded as total counts/10 min interval (± 1 S.E.M.), or as the total counts recorded for the duration of the experiment (± 1 S.E.M.). Animals were placed in the recording cages immediately after administration of AMPT or 1 hour before administration of FLA-63 in the case of untreated or reserpinised animals. Motor activity measurement was continued for 4 hr after administration of L-DOPA.

Biochemical Experiments

Mice were used for all determinations of cerebral NA, DA, homovanillic acid (HVA) and 3,4-dihydroxyphenylacetic acid (DOPAC). Rats were used for determination of MOPEG-SO4. Animals were killed by stunning and decapitation. For analysis of DA and NA, normal mice and AMPT-treated or reserpine-treated mice were sacrificed 1 hr prior to FLA-63 or saline treatment, 1 hr prior to L-DOPA or methylcellulose administration, and 1 hr and 4 hr after L-DOPA or methylcellulose administration. All experiments were performed on the same schedule, such that L-DOPA

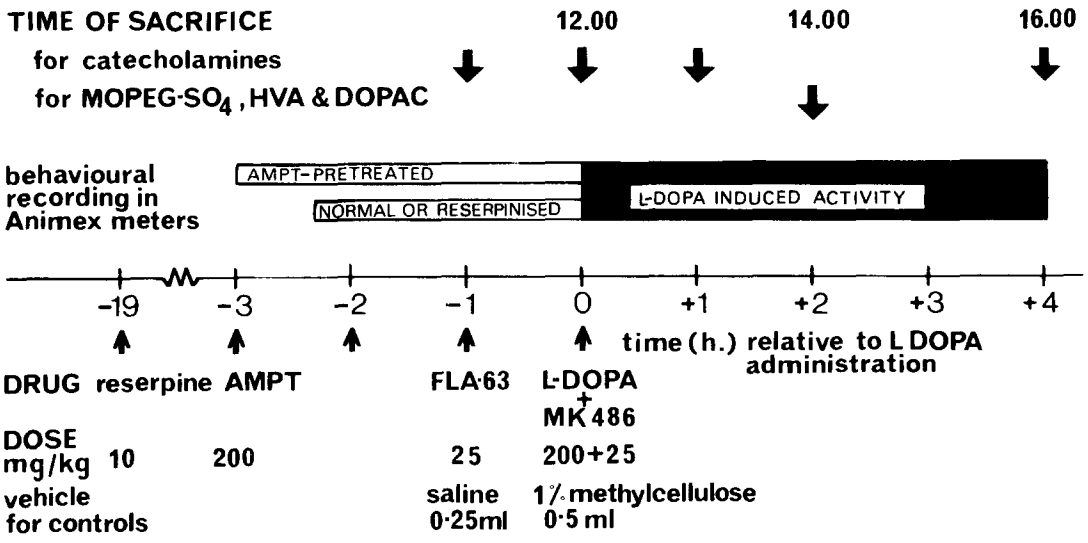

FIG. 1. Time schedule for drug administration, motor activity recording, and sacrifice for the determination of catecholamines and their metabolites.

was administered at 12.00 a.m. Two whole brains were pooled for each analysis of catecholamines. Separation was performed by the method of Atack [3]. DA was assayed according to this method, and NA by the method of Weil-Malherbe and Bigelow [40].

Analysis of forebrain HVA and DOPAC was performed in untreated mice and 2 hours after administration of L-DOPA to normal mice. The brain was dissected by removing a cortical slice and making a transverse cut immediately posterior to the striata. The anterior portion was taken as the forebrain sample. Three forebrains were pooled for each analysis of HVA and DOPAC. The analysis was carried out according to the method of Murphy *et al.* [30]. For determination of MOPEG-SO4, rats were sacrificed two hours after administration of L-DOPA. Analysis of MOPEG-SO4 was performed using single rat brains according to the method of Meek and Neff [29].

In all biochemical analyses, internal standards of DA (3-hydroxytyramine), NA (DL-arterenol), HVA, DOPAC (Calbiochem Limited), and MOPEG-SO4 (RO 4-2028; Roche Products Limited) were assayed simultaneously with the samples and tissue levels of the catecholamines and their metabolites were calculated from these without correction for recovery. Mean recoveries were for DA, 73%; for NA, 73%; for HVA, 70%; for DOPAC, 65% and for MOPEG-SO4, 85%.

RESULTS

Locomotor Activity

L-DOPA (200 mg/kg) plus MK 486 (25 mg/kg) induced a marked increase in locomotor activity in both normal mice and those pre-treated with AMPT (200 mg/kg) or reserpine (10 mg/kg) (Table 1). Peak motor activity was reached by the end of the first hour, and the response lasted for up to 4 hr. FLA-63 (25 mg/kg) attenuated the locomotor activity produced by the administration of L-DOPA to normal, AMPT-treated and reserpinised mice. The decrease in locomotor activity produced by FLA-63 occurred to the greatest extent in the 3rd and 4th hr after L-DOPA.

Effect of L-DOPA on brain catecholamine levels in normal mice

The results of measurements of whole brain catecholamines made one hour prior to, at the time of, and 1 and 4 hours after L-DOPA (200 mg/kg) + MK 486 (25 mg/kg) administration are shown in Fig. 2. The DA concentration was elevated 6-fold 1 hour after L-DOPA, compared to control animals given 0.5 ml methylcellulose ($p<0.01$). Four hours after L-DOPA, DA was still elevated 2.5-fold ($p<0.05$). No change in whole brain NA was found 1 hour after L-DOPA; 4 hours after L-DOPA, NA had fallen compared to methylcellulose-treated controls ($p<0.05$).

Pretreatment with FLA-63 (25 mg/kg) resulted in no change in DA levels either 1 hr or 4 hr after administration of L-DOPA compared to animals given L-DOPA alone. Pretreatment with FLA-63 resulted in a fall in NA 1 hour after the administration of L-DOPA alone ($p<0.01$). This fall was maintained 4 hours after L-DOPA ($p<0.01$). FLA-63 alone produced no change in DA levels compared to control animals not given L-DOPA. Two hr after administration, FLA-63 produced a 52% reduction in NA compared to normal animals ($p<0.01$) and an 83% reduction ($p<0.01$) 5 hr after administration. Compared to animals treated with FLA-63 alone, administration of L-DOPA plus FLA-63 caused a greater fall in NA 1 hour after L-DOPA ($p<0.01$) but an equal fall in NA 4 hours after L-DOPA.

Administration of the MAO inhibitor pargyline (100 mg/kg) 1 hour before L-DOPA (200 mg/kg) + MK 486 (25 mg/kg) resulted in an increase in whole brain NA 1 hour

TABLE 1

THE EFFECT OF FLA-63 (25 mg/kg) ON THE MOTOR ACTIVITY PRODUCED BY L-DOPA (200 mg/kg) PLUS MK 486 (25 mg/kg) IN NORMAL AMPT-TREATED AND RESERPINISED MICE

Pretreatment	FLA-63 25 mg/kg 1 hr prior to L-DOPA	Animex Counts						
		Hours Before L-DOPA		Hours After L-DOPA				Total Counts In 4 hr After L-DOPA
		−2	−1	+1	+2	+3	+4	
NONE	−	3800 ± 510	1377 ± 178	3872 ± 736	8547 ± 370	7305 ± 975	3769 ± 1000	23482 ± 1823
NONE	+	6023 ± 1265	1814 ± 181	4894 ± 652	7638 ± 1219	2597 ± 761†	1137 ± 227*	15355 ± 1274†
AMPT (200 mg/kg)	−	1111 ± 386	589 ± 75	2443 ± 712	8117 ± 828	7247 ± 1061	3289 ± 647	21157 ± 2976
AMPT (200 mg/kg)	+	428 ± 52	1277 ± 312*	2948 ± 438	5472 ± 879*	2744 ± 434†	518 ± 60†	11621 ± 1575*
RESERPINE (10 mg/kg)	−	−	45 ± 9	3688 ± 559	6950 ± 681	5318 ± 444	1944 ± 535	18230 ± 1423
RESERPINE (10 mg/kg)	+	−	298 ± 60‡	2864 ± 571	5628 ± 991	1554 ± 348‡	200 ± 177†	10073 ± 1333†

Male 'Swiss S' or 'P' mice (20-25g) were given reserpine (10 mg/kg) 18-24h prior to L-DOPA or AMPT (200 mg/kg) 3h prior to L-DOPA. Either FLA-63 (25 mg/kg) or 0.9% saline was administered 1h prior to L-DOPA (200 mg/kg) plus MK 486 (25 mg/kg). In the 2 hr before and the 4 hr after L-DOPA administration motor activity was measured in batches of 3 mice in Animex activity meters. The number of groups tested were 6 for the unpretreated groups, 5 for the AMPT-treated groups and 10 for the reserpine-treated groups. The results are expressed as total counts/hour and total counts/4 hr after L-DOPA ± 1 S.E.M. Significant differences between FLA-63 treated and saline treated groups are indicated by superscripts.

*$p<0.05$.
†$p<0.01$.
‡$p<0.001$.

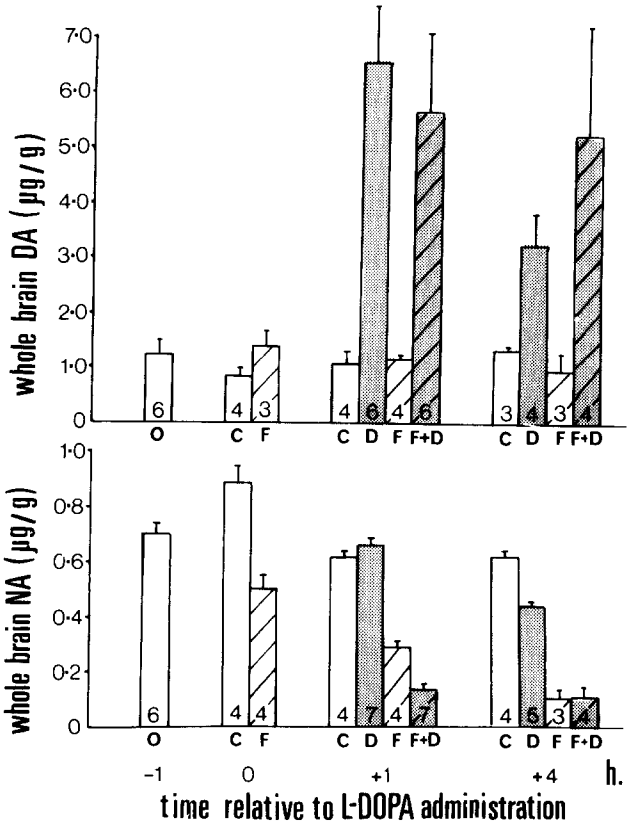

FIG. 2. The effect of pretreatment with FLA-63 (25 mg/kg) on whole brain NA and DA levels after administration of L-DOPA (200 mg/kg) plus MK 486 (25 mg/kg) to normal mice. FLA-63 (25 mg/kg) or 0.9% saline was administered to normal Swiss S or P strain male mice (20–25 g). One hour later the animals were treated with L-DOPA (200 mg/kg) plus MK 486 (25 mg/kg) suspended in 1% methlcellulose or with 1% methylcellulose alone. Whole brain NA and DA levels were measured at the time of FLA-63 administration, at the time of L-DOPA administration, and 1 and 4 hr after L-DOPA administration. Referring to the letters beneath the histogram, O indicates the whole brain NA or DA level at the time of FLA-63 administration, C indicates the level in saline plus methylcellulose treated mice, F the level in FLA-63 plus methylcellulose-treated mice, D the level in saline plus L-DOPA treated mice and F+D the level in FLA-63 plus L-DOPA treated mice. Each value is the mean of the number of determinations given at the base of each column, and the vertical bars represent ± 1S.E.M. The statistical significances of the differences between the results are calculated using Student's *t* test and quoted in the text.

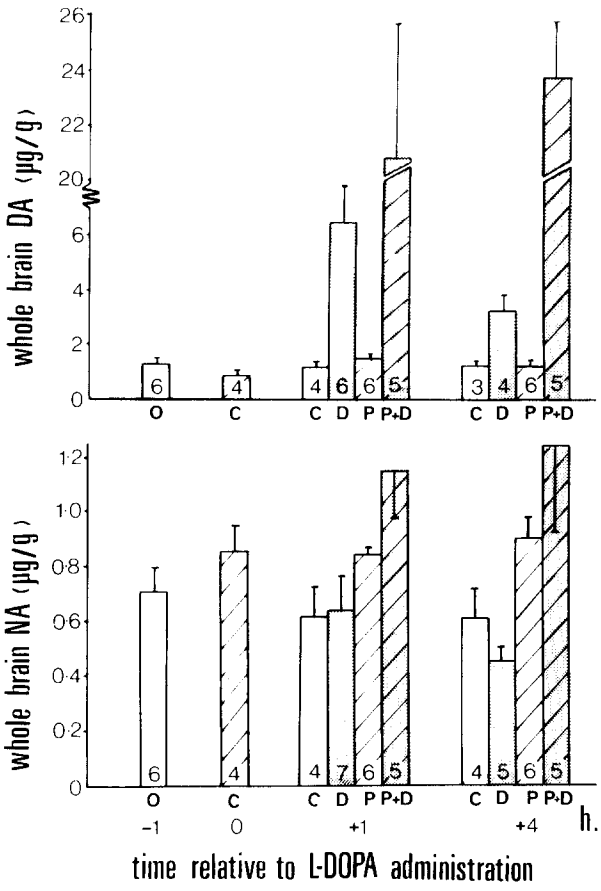

FIG. 3. The effect of pretreatment with pargyline (200 mg/kg) on the NA and DA levels after administration of L-DOPA (200 mg/kg) plus MK 486 (25 mg/kg) to normal mice. Pargyline (100 mg/kg) dissolved in 0.9% saline was administered to normal mice 1 hr before treatment with L-DOPA (200 mg/kg) plus MK 486 (25 mg/kg) or 1% methylcellulose. NA and DA levels were determined at the time of pargyline administration, at the time of administration of L-DOPA or its vehicle and 1 and 4 hr later. Referring to the letters below the histogram: O indicates the NA or DA level in control animals; C indicates the levels in animals treated with vehicle alone; P the level in animals treated with pargyline plus methylcellulose, D the level in animals treated with saline plus L-DOPA, and P+D the level in animals treated with pargyline plus L-DOPA. The vertical bars represent ± 1S.E.M. Each value is the mean of the number of determinations given at the base of each column. The statistical significances of the variations in catecholamines are quoted in the text.

after L-DOPA which was greater than that after pargyline given in combination with methylcellulose ($p<0.05$) (Fig. 3). The cerebral DA level in mice pretreated with pargyline, 1 hour after L-DOPA administration, was elevated 13-fold to 20,970 ± 5975 ng/g compared to animals given pargyline alone ($p<0.001$).

Effect of L-DOPA on brain catecholamine levels in AMPT-treated mice

The results of whole brain catecholamine measurements made prior to, at the time of, and 1 and 4 hours after L-DOPA (200 mg/kg) + MK 486 (25 mg/kg) administration are shown in Fig. 4. A rise in DA was found 1 hour after L-DOPA, compared to AMPT-treated animals given methylcellulose ($p<0.05$). Four hours after L-DOPA, the DA level was lower, but still elevated compared to controls ($p<0.05$). A rise in whole brain NA was found 1 hr after L-DOPA administration, compared to animals treated with methylcellulose ($p<0.05$). The level was restored to 90% of that found in normal untreated animals. Four hours after L-DOPA administration the NA level had decreased, but was still elevated compared to methylcellulose-treated animals ($p<0.05$). Administration of FLA-63 (25 mg/kg) to AMPT treated animals (1 hour prior to L-DOPA) resulted in no change in DA levels, 1 hour and 4 hours after L-DOPA administration. FLA-63 prevented the rise in NA found 1 hour after L-DOPA ($p<0.05$) and 4 hours after L-DOPA

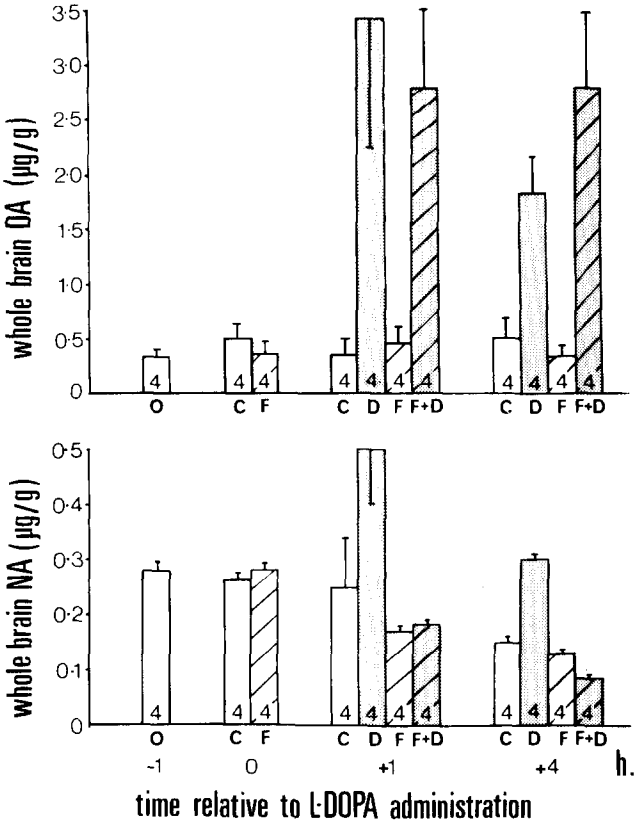

FIG. 4. The effect of pretreatment with FLA-63 (25 mg/kg) on whole brain NA and DA levels after administration of L-DOPA (200 mg/kg) plus MK 486 (25 mg/kg) to AMPT-treated mice. Drugs were administered and the histogram bars are identified as stated in the legend to Fig. 2 except that mice were pretreated with AMPT (200 mg/kg) dissolved in saline 2 hr before administration of FLA-63 or saline.

($p<0.01$). Administration of FLA-63 (25 mg/kg) alone did not alter the levels of DA or NA found after AMPT treatment.

Effect of L-DOPA on brain catecholamine levels in reserpinised mice

The results of whole brain catecholamine measurements made prior to, at the time of and 1 and 4 hr after L-DOPA (200 mg/kg) and MK 486 (25 mg/kg) administration are shown in Fig. 5. A rise in DA was found 1 hr after L-DOPA (200 mg/kg) + MK 486 (25 mg/kg) administration to reserpine-treated animals compared to controls given methylcellulose ($p<0.01$). Four hours after L-DOPA, the DA level was still elevated ($p<0.05$). A rise in whole brain NA was found 1 hr after L-DOPA compared to methylcellulose-treated animals ($p<0.05$), although no difference between NA levels in L-DOPA and methylcellulose treated animals was found 4 hr after L-DOPA. Administration of FLA-63 (25 mg/kg) to reserpine-treated animals prevented the rise in NA found 1 hour after L-DOPA but had no effect on the NA level found 4 hr after L-DOPA. FLA-63 produced an increase in DA levels 1 hr after L-DOPA ($p<0.05$) compared to untreated animals. Pretreatment with FLA-63 (25 mg/kg) 1 hour before L-DOPA (50 mg/kg) had no effect on after L-DOPA. FLA-63 (25 mg/kg) alone did not alter the

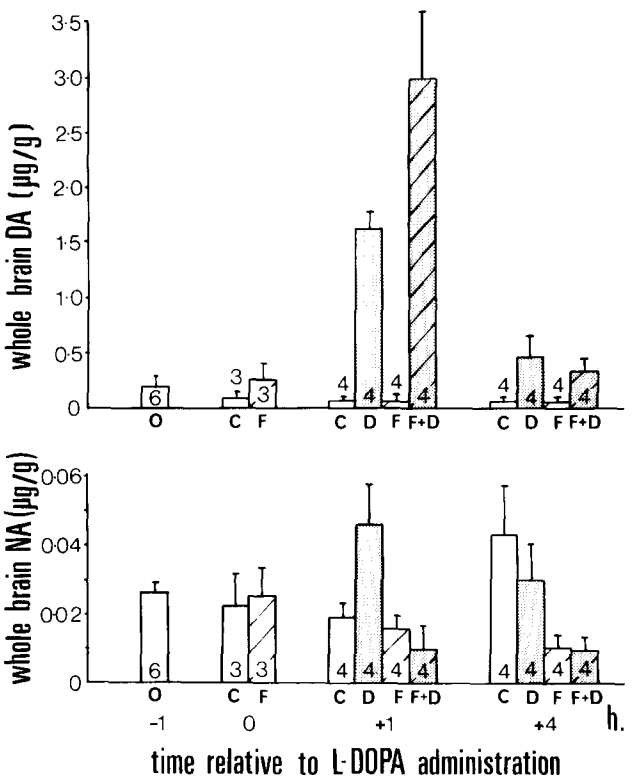

FIG. 5. The effect of pretreatment with FLA-63 (25 mg/kg) on whole brain NA and DA levels after administration of L-DOPA (200 mg/kg) plus MK 486 (25 mg/kg) to reserpinised mice. Drugs were administered and the histogram bars are identified as stated in the legend to Fig. 2 except that the mice were pretreated with reserpine (10 mg/kg) 18 hr before administration of FLA-63 or saline.

level of DA found after reserpine treatment 1, 2 or 5 hr after its administration, although it produced a fall in NA ($p<0.05$) 5 hr after its administration compared to animals given reserpine plus saline.

The effect of L-DOPA on MOPEG-SO4 levels in normal rats

Whole brain MOPEG-SO4 concentration was unaltered 2 hr after L-DOPA (200 mg/kg) + MK 486 (25 mg/kg) compared to untreated animals (Table 2). Treatment with FLA-63 (25 mg/kg) prior to L-DOPA + MK 486 resulted in a decrease in MOPEG-SO4 compared to the levels found in untreated animals ($p<0.001$) and when compared to animals treated with L-DOPA (200 mg/kg) + MK 486 (25 mg/kg) alone ($p<0.01$). Two hours after administration of a lower dose of L-DOPA (50 mg/kg) in the absence of the decarboxylase inhibitor, MOPEG-SO4 was unaltered compared to untreated animals. Pretreatment with FLA-63 (25 mg/kg) 1 hour before L-DOPA (50 mg/kg) alone, or animals MOPEG-SO4 compared to control untreated animals or those receiving L-DOPA (50 mg/kg) alone, or animals treated with FLA-63 alone. FLA-63 (25 mg/kg) alone caused a fall in MOPEG-SO4 compared to untreated animals ($p<0.01$).

The effect of L-DOPA on MOPEG-SO4 levels in AMPT-treated rats

Whole brain MOPEG-SO4 was reduced in AMPT-treated

TABLE 2

THE EFFECT OF FLA-63 ON MOPEG-SO4 LEVELS 2 HOURS AFTER L-DOPA (200 mg/kg) PLUS MK 486 (25 mg/kg) OR L-DOPA (50 mg/kg) IN NORMAL, AMPT-TREATED AND RESERPINISED RATS

Group	Treatment mg/kg L-DOPA	MK486	FLA-63	Normal	AMPT	Reserpine
1.	–	–	–	136 ± 3 (48)	75 ± 7 (16)	53 ± 4 (24)
2.	200	25	–	130 ± 7 (8)	89 ± 7 (16)	22 ± 3 (12)
3.	200	25	25	98 ± 5 (8)	60 ± 9 (8)	32 ± 3 (11)
4.	50	–	–	137 ± 9 (8)	125 ± 6 (16)	90 ± 12 (11)
5.	50	–	25	125 ± 9 (8)	101 ± 6 (7)	38 ± 4 (7)
6.	–	–	25	113 ± 9 (8)	78 ± 5 (8)	22 ± 2 (8)
Statistical Significances Between Groups						
	1 vs 2			N.S.	N.S.	$p<0.001$
	1 vs 3			$p<0.001$	N.S.	$p<0.01$
	1 vs 4			N.S.	$p<0.001$	$p<0.01$
	1 vs 5			N.S.	$p<0.05$	N.S.
	1 vs 6			$p<0.01$	N.S.	$p<0.001$
	2 vs 3			$p<0.01$	$p<0.01$	$p<0.05$
	2 vs 4			N.S.	$p<0.001$	$p<0.001$
	3 vs 6			N.S.	$p<0.05$	$p<0.01$
	4 vs 5			N.S.	$p<0.05$	$p<0.01$
	5 vs 6			N.S.	$p<0.01$	$p<0.001$

Untreated male Wistar rats (200-250g) or those pretreated with AMPT (200 mg/kg) 3 hr previously or reserpine (10 mg/kg) 19 hr previously were given L-DOPA (200 mg/kg) plus MK 486 (25 mg/kg or L-DOPA (50 mg/kg) alone. FLA-63 or 0.9% saline was administered 1 hr before L-DOPA. They wure sacrificed 2 hr after L-DOPA administration by stunning and decapitation. One whole brain was used in each determination of MOPEG-SO4 using the method of Meek and Neff [29]. The results are expressed as mean ± 1 S.E.M. The number of analyses performed for each group is given in parentheses. The statistical significances between the values were determined using Student's t test, and are given below the table.

controls compared to untreated animals ($p<0.001$) (Table 2). Two hr after L-DOPA (200 mg/kg) + MK 486 (25 mg/kg) no change in MOPEG-SO4 was found compared to AMPT-treated controls. Treatment with FLA-63 1 hr prior to L-DOPA + MK 486 caused a fall in MOPEG-SO4 compared to animals receiving either L-DOPA + MK 486 alone ($p<0.01$), or FLA-63 alone ($p<0.05$). Administration of the lower dose of L-DOPA (50 mg/kg) caused an increase in MOPEG-SO4 2 hours later, compared to animals given AMPT alone ($p<0.001$), and compared to those receiving the higher dose of L-DOPA + MK 486 ($p<0.01$). This increase was reduced by prior treatment with FLA-63 (25 mg/kg) ($p<0.05$).

The effect of L-DOPA on MOPEG-SO4 levels in reserpinised rats

Whole brain MOPEG-SO4 was reduced in reserpinised animals compared to untreated controls ($p<0.001$) (Table 2). Two hr after administration of L-DOPA (200 mg/kg) + MK 486 (25 mg/kg) a decrease in MOPEG-SO4 was found compared to animals treated with reserpine alone ($p<0.001$). Pretreatment with FLA-63 (25 mg/kg) 1 hr prior to L-DOPA + MK 486 resulted in an increase in MOPEG-SO4 2 hr after L-DOPA (200 mg/kg) + MK 486 (25 mg/kg) alone ($p<0.05$); but the MOPEG-SO4 level was still less than that found in animals given reserpine alone ($p<0.01$). Administration of the lower dose of L-DOPA (50 mg/kg) resulted in an increase in MOPEG-SO4 after 2 hr, compared to control reserpinised animals ($p<0.01$). This increase was prevented by pretreatment with FLA-63 (25 mg/kg) ($p<0.01$).

The effect of L-DOPA on HVA/DOPAC ratios in normal mice

The ratio of the two major DA metabolites HVA and DOPAC in the forebrain of normal mice was approximately unity. Two hr after administration of L-DOPA (50 mg/kg) there was no change in the HVA/DOPAC ratio. Two hr after administration of L-DOPA (200 mg/kg) + MK 486 (25 mg/kg) there was an 83% fall in the HVA/DOPAC ratio ($p<0.01$) (Table 3).

DISCUSSION

The administration of L-DOPA (200 mg/kg) plus the peripheral decarboxylase inhibitor MK 486 (25 mg/kg) resulted in a period of increased motor activity in both normal mice and those pretreated with AMPT or reserpine. The effect of the DBH inhibitor FLA-63 in decreasing total motor activity after L-DOPA was similar for the 3 classes of animal. This suggests, that if FLA-63 in the dose used is acting specifically as a DBH inhibitor [12,36], then NA is essential for maximal L-DOPA induced locomotor activity, in agreement with the work of others [1, 2, 35].

Interpretation of the biochemical experiments must take into account the several factors which are simultaneously influencing the levels of NA and its major metabolite MOPEG-SO4 after the administration of L-DOPA. In addition to providing the intraneuronal precursor for DA

TABLE 3

THE RATIO OF HOMOVANILLIC ACID TO DIHYDROXYPHENYLACETIC ACID IN THE FOREBRAIN OF NORMAL MICE AND 2 HOURS AFTER ADMINISTRATION OF TWO DOSES OF L-DOPA

Dose mg/kg L-DOPA	Dose mg/kg MK 486	Forebrain HVA/DOPAC
–	–	1.01 ± 0.19 (6)
50	–	0.87 ± 0.27 (6)
200	25	0.19 ± 0.02 (6)*

Male 'Swiss S' or 'P' mice (20-25g) were sacrificed 2 hr after administration of L-DOPA with or without MK 486 in suspension in 1% methylcellulose, or methylcellulose alone (0.5ml). The brains were removed rapidly and the forebrains from 3 mice were homogenised in 0.1 N HCl and assayed for HVA and DOPAC. The number of analyses performed per group is indicated in parentheses and a significant difference from the control group not receiving L-DOPA is indicated by an asterisk.

*$p<0.001$.

and NA synthesis, L-DOPA is also a substrate for COMT which is present extraneuronally, largely in the glia [5,33]. This enzyme is on the pathway of catabolism of NA to MOPEG-SO4 [19]. The rate of MOPEG-SO4 synthesis may be decreased after L-DOPA administration by two mechanisms; firstly L-DOPA and dopamine or their deaminated products may compete with NA as substrates for COMT; and secondly they also cause depeltion of its co-factor S-adenosylmethionine (SAM) [10,31]. It has been suggested that substrate competition is the most important mechanism by which L-DOPA inhibits COMT in doses greater than 150 mg/kg [39].

Another factor contibuting to the biochemical effects of L-DOPA is displacement by dopamine of NA from its storage granules [23]. These granules are intact in normal animals, present and functional but depleted in AMPT treated mice [13,14] and disrupted in reserpinised mice [8,15]. Such displacement releases NA into the cytoplasm where it is an immediate substrate for mitochondrial monoamine oxidase (MAO) [24]. The deaminated product DOPEG is then lost from the nerve terminals and becomes a substrate for COMT [24] unless it is protected by prior conjugation [6].

Treatment of normal mice with L-DOPA (200 mg/kg) plus MK 486 (25 mg/kg) caused a 6-fold increase in whole brain DA one hr later, suggesting that there is a large, normally unused, capacity for DA storage, where it is temporarily protected from MAO. By comparison DA levels at the same time after L-DOPA are increased 3-fold in AMPT-treated mice, and only 1.5-fold in reserpinised mice in whom granular storage for DA is diminished [11].

Four hr after the administration of L-DOPA (200 mg/kg) plus MK 486 (25 mg/kg) to normal mice, the NA level had fallen by 27% compared to methylcellulose treated controls. This is in agreement with the findings of Maj *et al.* [27]. As DBH is the rate-determining step in the biosynthesis of NA from L-DOPA, the failure of NA levels to increase after L-DOPA might indicate that there is only limited hydroxylation of DA [4]. However, if NA formation from L-DOPA does occur, either there is no additional storage capacity for NA in noradrenergic neurones, or alternatively, the increased synthesis and storage of NA after L-DOPA may be balanced by displacement of NA by DA from the storage granules, with resultant rapid cytoplasmic metabolism of displaced NA.

In order to determine whether increased NA synthesis was occurring in normal mice receiving this dose of L-DOPA, the MAO inhibitor pargyline was administered 1 hr before L-DOPA to block the other route of metabolism of NA by deamination. The results in Fig. 3 show that a higher level of NA was gained after the administration of pargyline plus L-DOPA than after pargyline alone. This suggests that increased NA synthesis does occur following L-DOPA administration, although in the absence of MAO inhibition the NA displaced from vesicular storage may result in increased formation of DOPEG and its sulphate conjugate [6]. this is in agreement with the results of Chalmers *et al.* [10] who found a transiently raised turnover of intracisternally injected [^{3}H]-NA after administration of L-DOPA.

In normal animals treated with FLA-63 alone, the NA level fell compared to saline treated controls, and pretreatment with FLA-63 1 hr before L-DOPA caused the NA levels to fall more rapidly than after FLA-63 alone, indicating that L-DOPA is increasing turnover of NA by the mechanism of granular displacement of NA by DA.

No change in MOPEG-SO4 was found 2 hr after administration of 200 mg/kg L-DOPA, or 50 mg/kg L-DOPA, although determination of the ratio of the 2 major metabolites of DA (HVA:DOPAC) (Table 3) suggested that COMT was inhibited at the higher but not the lower dose of L-DOPA, in agreement with Thoa *et al.* [39]. Because HVA is formed from DA by deamination and 0-methylation, whereas DOPAC requires only deamination, a lowering of the HVA/DOPAC ratio indicates a greater degree of inhibition of COMT [30].

FLA-63 reduced MOPEG-SO4 levels in normal rats and pretreatment with FLA-63 also reduced the MOPEG-SO4 level after L-DOPA (200 mg/kg) suggesting that some NA turnover was occurring to form MOPEG-SO4 and this was to some extent, inhibited by FLA-63.

The involvement of displacement in the action of 200 mg/kg L-DOPA is suggested by the greater fall in MOPEG-SO4 after treatment with FLA-63 plus L-DOPA (200 mg/kg) compared to treatment with FLA-63 plus L-DOPA (50 mg/kg).

The rise in NA 1 hr after the administration of L-DOPA to AMPT-treated animals (Fig. 4) to 90% of the level found in normal animals indicates that NA was being synthesized from L-DOPA, which by-passed the block in the synthetic pathway for catecholamines imposed by AMPT. This increased turnover of NA was not reflected by an increase in MOPEG-SO4 after the high dose of L-DOPA because of the demonstrated inhibition of COMT. Such an increase in MOPEG-SO4 was, however, observed 2 hr after the administration of the lower dose of L-DOPA, when COMT inhibition is not apparent. The fact that FLA-63 caused a fall in the MOPEG-SO4 level observed 2 hours after the administration of L-DOPA (200 mg/kg) suggests that L-DOPA in the absence of FLA-63 caused an increased turnover of NA.

That FLA-63 caused a greater fall in MOPEG-SO4 in the presence of L-DOPA (200 mg/kg) than in its absence, is further evidence for the involvement of COMT inhibition with this high dose of L-DOPA. It is probable that some displacement of NA was contributing to the observed turnover of NA.

The results in reserpinised animals show the same trend

as was observed in the AMPT-treated animals. L-DOPA increased NA levels, although to a much lesser absolute extent, and this increase was prevented by pretreatment with FLA-63. In correspondence with the result found in AMPT-treated animals, no increase in MOPEG-SO4 was observed 2 hr after administration of 200 mg/kg L-DOPA; indeed, the level fell compared to that in control reserpinised rats, probably because of COMT inhibition. However, a rise in MOPEG-SO4 was found 2 hr after the administration of 50 mg/kg L-DOPA, when COMT inhibition was not a contributing factor. This rise in MOPEG-SO4 was abolished by pretreatment with FLA-63. In reserpinised animals it is unlikely that displacement of NA by DA formed from L-DOPA can account for the increased NA turnover observed because of the low level of endogenous NA, and because intact granular storage for NA has been largely disrupted by reserpine [11,15]. The results indicate that NA synthesis can occur in reserpinised animals in agreement with the work of Glowinski et al [20].

The rise in DA levels observed 1 and 4 hr after administration of L-DOPA to normal or amine-depleted animals correlates well with the observed elevation of locomotor activity. The administration of the DBH inhibitor FLA-63 reverses to some extent this increased motor activity particularly in the 3rd and 4th hr after L-DOPA administration. However, the observed elevation of DA at 1 and 4 hr following L-DOPA was not prevented by pretreatment with FLA-63. On the other hand, the elevated NA levels observed at these time periods were prevented by FLA-63 administration. This would suggest that the inhibition of L-DOPA induced locomotor activity is due to the prevention of NA synthesis. This data suggests therefore that both DA and NA are important determinants of L-DOPA induced locomotor activity in rodents.

The mechanism of the NA effect is unknown. It is not even clear whether it is a cerebral mechanism or an action at the spinal cord level. The distribution of NA is so widespread that is might represent an action on the cortex, basal ganglia or cerebellum as well as on the spinal cord. Nor is it known whether the effect of NA is directly upon motor centres at one or more of these sites or alternatively whether it is acting indirectly via either the dopaminergic nigrostriatal system [17] or the 5HT pathways [25].

The conclusion from the behavioural and biochemical experiments presented here and from several other lines of research [2,28], is that effective noradrenergic neurotransmission is essential for maximal motor stimulation by L-DOPA in reserpine-treated, AMPT-treated and normal animals. The parallel conclusion drawn from our biochemical experiments is that, in all three classes of animal enhanced NA synthesis does occur after L-DOPA administration. That both locomotor activity and NA synthesis are similarly reduced by prior treatment with FLA-63, suggests that increased NA turnover may be, in part, responsible for the locomotor response to L-DOPA.

This study does, however, have obvious limitations. In particular the need to extrapolate from mice to rats to obtain an index of noradrenaline turnover is open to criticism. This is made necessary by the fact that the mouse, unlike the rat, does not conjugate MOPEG with sulphate groups [7,22]. The analysis of free MOPEG in mouse cannot, at present, be carried out by our fluorometric techniques. However, it would appear that the role of NA in motor activity is equally apparent in spontaneous activity and that induced by amphetamine and L-DOPA in rats [26]. Also, studies involving unilateral lesions of the ascending noradrenergic pathways in rats have been shown to induce circling behaviour *via* dopaminergic mechanisms [17]. Biochemically, the involvement of both dopamine and noradrenaline in L-DOPA induced locomotor activity in rats has also previously been demonstrated [27]. Likewise the proposed inhibition of COMT by L-DOPA in the rat has previously been observed in this species [31] and also shown to occur in the mouse by the present study. Thus, there are good grounds for assuming that the central mechanisms involved in the production of locomotor activity in the rat and mouse are not too dissimilar. Perhaps, the greatest limitation of this and other studies is the grossness of the analyses carried out when compared to the intricacy of the neuronal systems involved. Further study might be directed towards examination of the effects observed in discrete brain regions.

These conclusions may be of consequence in the L-DOPA therapy for Parkinson's disease. It has been shown that although the pathology of this disease is mainly associated with degeneration of the substantia nigra, other pigmented brain stem nuclei are also affected [18]. These include the nucleus locus coeruleus from which ascending noradrenergic fibres arise to innervate the cerebral cortex, cerebellum and midbrain. Thus, provided their degeneration is not too far advanced, the therapeutic effect of L-DOPA may be, in part, due to the formation of noradrenaline in these neurones, in addition to the well established replacement of dopamine in the nigro-striatal tract.

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