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The Relative Importance of Dopamine and Noradrenaline Receptor Stimulation for the Restoration of Motor Activity in Reserpine or α-Methyl-p-Tyrosine Pre-Treated Mice[1]

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A. C. DOLPHIN, P. JENNER AND C. D. MARSDEN. *The relative importance of dopamine and noradrenaline receptor stimulation for restoration of motor activity in reserpine or α-methyl-p-tyrosine pretreated mice.* PHARMAC. BIOCHEM. BEHAV. 4(6) 661–670, 1976 – Two animal models of Parkinsonism have been employed to investigate the role of noradrenaline in the motor effects of levodopa. Pretreatment with reserpine or α-methyl-p-tyrosine (AMPT) causes cerebral amine depletion and reduction of motor activity, which can be reversed by levodopa. The effect of inhibitors of noradrenaline (NA) synthesis and antagonists of NA and dopamine (DA) receptors on the action of levodopa have been studied For comparison, the effects of such treatments on apomorphine action has been investigated Reversal of reserpine (10 mg/kg) induced akinesia in mice by levodopa (200 mg/kg) plus the peripheral decarboxylase inhibitor MK 486 (L-α-methyl-dopahydrazine, 25 mg/kg) was inhibited by prior administration of phenoxybenzamine (20 mg/kg), haloperidol (1 mg/kg), pimozide (1 mg/kg) or the dopamine-β-hydroxylase inhibitor FLA-63 (bis [4-methyl-1-homopiperazinylthiocarbonyl] disulphide; 15 or 25 mg/kg). Apomorphine (2 mg/kg) reversal of reserpine akinesia was similarly inhibited by haloperidol (1 mg/kg) and pimozide (2 mg/kg) but not by phenoxybenzamine (20 mg/kg) or FLA-63 (25 mg/kg) Apomorphine (5 mg/kg) reversal of reserpine akinesia was enhanced by simultaneous administration of the noradrenergic agonist clonidine (1 mg/kg) and this effect was not significantly altered by prior administration of FLA-63. Clonidine, however, reversed the FLA-63 induced inhibition of the levodopa effect on reserpine akinesia. Levodopa reversal of akinesia induced by AMPT (200 mg/kg) was also inhibited by FLA-63, pimozide and haloperidol. Phenoxybenzamine, however, was without effect, but produced a different pattern of behaviour. Similarly, pimozide and haloperidol blocked apomorphine reversal of AMPT induced akinesia, FLA-63 was without effect but phenoxybenzamine produced marked inhibition. The results suggest that full restoration of motor activity in reserpine or AMPT pretreated animals requires stimulation of both DA and NA receptors.

Reserpine α-methyl-p-tyrosine Dopamine Noradrenaline Motor Activity

RESERPINE blocks the intraneuronal storage of noradrenaline, [NA] dopamine [DA] and serotonin thereby depleting the brain of these presumed neurotransmitters [7,17]. This depletion causes a parkinsonian syndrome in both animals and man. The reserpinised animal stops moving spontaneously (akinesia), adopts a flexed posture, and develops rigidity and tremor of the limbs and trunk, all of which are classical features of human Parkinsonism. The reserpine-induced akinetic-rigid state can be reversed dramatically by administration of levodopa [9], an observation that gave rise to the concepts DA deficiency and levodopa therapy in Parkinson's disease. The therapeutic response to levodopa in Parkinson's disease has been attributed to replenishment of depleted striatal DA stores [21]. Other evidence, however suggests that not only DA, but also NA, is involved in spontaneous locomotor activity in normal animals [34, 36, 40] and in that produced by levodopa in both normal and reserpinised animals [1, 14, 29, 30, 37]. In addition, the motor activity produced by direct cerebral DA receptor stimulation [15] is enhanced by simultaneous stimulation of NA receptors with clonidine although the latter had no effect when given alone [2, 3, 6, 27].

We have previously demonstrated in a preliminary communication, using a simple test of open field locomotor activity [26], that the reversal by levodopa of the akinetic-rigid syndrome produced by reserpine is modified by drugs affecting either DA or NA systems [30]. This study, however, is open to criticism on several counts.

The administration of reserpine to animals results in a

[1] A preliminary communication of this work was presented at the 6th International Pharmacology Meeting (Helsinki) July 1975
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complex syndrome of central and peripheral effects [17]. Depletion of DA, NA and serotonin occurs and the pattern of this depletion is itself complex [19]. Depletion of brain DA and NA can, however, be produced more specifically using an inhibitor of tyrosine hydroxylase (α-methyl-p-tyrosine; AMPT) the rate limiting step in amine synthesis [12,32]. We have therefore included this animal model of human Parkinsonism into the present study.

The involvement of a NA component in the levodopa induced restoration of locomotor activity hinges on the specificity of the NA antagonists used. In particular, the specificity of dopamine-β-hydroxylase inhibitors has been questioned in terms of a possible non-specific stressful effect mediated via peritoneal irritation. We have therefore tested the specificity of the NA antagonists employed in this study against locomotor activity induced by the DA agonist apomorphine [15]. Further, the involvement of a NA component can only be claimed if the effect of a dopamine-β-hydroxylase inhibitor can be overcome by the use of a NA agonist, such as clonidine

The open field test of locomotor activity previously employed was simple but limited as it involves an all or none response. It examines exploratory behaviour as well as spontaneous locomotor activity, for at each test the animal is introduced into a new environment. We have therefore carried out the present study using an automated technique for the measurement of motor activity of caged animals [35]. In this test, animals remain in a familiar environment throughout the period of observation, and the results emphasize spontaneous motor activity.

METHOD

Locomotor activity was measured using groups of 3 mice in 2 sets of Animex activity meters (LKB Farad Ltd) between 9 00 a.m. and 9 00 p.m., under conditions of standard laboratory lighting and temperature. Food and water were withdrawn during the test period. Animals were housed in clear Perspex cages with similar lids so that observations of behaviour could be made at intervals during the experiment. Activity was recorded as total counts per 10 min intervals (± 1 SEM) or as the total number of counts recorded during the period of activity (± 1 SEM) for all batches of animals. Between 3 to 8 batches of animals were used in each experiment.

Swiss S or P strain male mice (18–25 g) were used throughout this study (Animal Suppliers Ltd). Reserpine (10 mg/kg Halewood Chemicals Ltd) was administered IP 18–24 hr before recording commenced. Baseline activity was then recorded for 1 hr. At this point, 0.9% saline (0.1 ml), bis (4-methyl-l-homopiperazinylthiocarbonyl) disulphide (FLA-63, 25 mg/kg Labkemi AB), phenoxybenzamine (20 mg/kg, SKF Laboratories Ltd), haloperidol (1 mg/kg, Searle Ltd), or pimozide (1 or 2 mg/kg; Janssen Pharmaceuticals Ltd) was administered IP. After a further 1 hr recording (4 hr in the case of pimozide), levodopa (200 mg/kg, Roche Products Ltd) plus L-α-methyldopa hydrazine (MK 486), 25 mg/kg M S. D Ltd), or apomorphine (2 or 5 mg/kg, Evans Medical Ltd) with or without clonidine (1 mg/kg; Boehringer Ingelheim Ltd) was administered IP. Motor activity was then recorded for 4 hr in the case of L-DOPA and MK 486, or for 2 hr following apomorphine.

In some experiments FLA-63 (15 or 25 mg/kg) was administered orally by intubation 1 hr before the administration of levodopa plus MK 486 α-methyl-p-tyrosine methyl ester (H44/68, AMPT) (200 mg/kg; Labkemi AB) was administered IP to groups of animals whose activity was then recorded over a 2 hr period. Saline, FLA-63, phenoxybenzamine, haloperidol or pimozide was then administered followed 1 or 4 hr later by either apomorphine or levodopa plus MK 486 as described above.

All drugs were administered in aqueous solution (0.1–0.2 ml) except levodopa which was suspended in 1% methylcellulose (0.5 ml). Pimozide was dissolved in water by warming with a small quantity of tartaric acid. FLA-63 was dissolved in water (10 ml) containing a small quantity (0.05–0 10 ml) 6N hydrochloric acid. The pH of the resulting solution was brought towards neutrality using dilute ammonia solution (ca. 1 N) until a slight turbidity appeared. Reserpine (0.75 g) was dissolved in a mixture of benzyl alcohol (6.0 g) citric acid (0 81 g) and polyethylene glycol 400 (25.4 g) and then diluted with distilled water to 300 ml to give a 2.5 mg/ml solution which was stored under nitrogen in ampoules.

Comparison of behavioural data was carried out by Student's t test analysis. Results were considered significant when 2 $p<0.05$. Experiments were designed such that results for drug pretreated groups of animals were obtained in 1 experimental period and were compared with control groups of animals tested in another period in the same activity meter The results of critical experiments were further compared using alternate control and drug pretreated groups of animals and the results of such experiments are presented separately.

RESULTS

Levodopa Reversal of Reserpine Akinesia

The reserpinised animals were immobile and flexed, and exhibited rigidity, tremor and ptosis When placed in the activity meters the animals remained quiet and showed little or no spontaneous locomotion Administration of MK 486 (25 mg/kg) to these animals produced no alteration in the reserpine syndrome and no locomotor activity was observed. Administration of levodopa (200 mg/kg) plus MK 486 (25 mg/kg), however, rapidly and completely reversed the akinesia produced by prior reserpine (10 mg/kg) treatment. A reduction of the levodopa effect was observed following pretreatment IP with the dopamine β-hydroxylase inhibitor FLA-63, administered either IP (25 mg/kg) or PO (15 mg/kg). These doses produced approximately the same effect. The suppression by IP FLA-63 was partially reversed by the simultaneous administration of clonidine (1 mg/kg), a NA agonist, with levodopa, in particular activity in the second and third hr after levodopa was increased by clonidine, although peak activity was not restored in this experiment (but see below). Phenoxybenzamine (20 mg/kg) in a dose known to block spinal NA receptors [4,5], also reduced the reversal of reserpine induced akinesia by levodopa. Similarly, blockade of DA receptors by pimozide (1 mg/kg) or haloperidol (1 mg/kg) produced a marked reduction in the levodopa effect. None of the antagonists used above produced any significant additional behavioural changes when administered alone to reserpinised animals. (Table 1, Fig 1).

FLA-63 IP is claimed to produce non-specific inhibition of locomotor activity due to peritoneal irritation [38]. However, administration of FLA-63 orally at 15 mg/kg reduced Animex counts in the 4 hr period after levodopa administration to 9409 ± 881 (5) compared to 18,110 ± 2043 (5) in alternated groups of animals receiving levodopa

TABLE 1

THE EFFECT OF LEVODOPA PLUS MK 486 ALONE COMPARED WITH EFFECTS OBSERVED FOLLOWING PRETREATMENT WITH DRUGS INFLUENCING NORADRENERGIC AND DOPAMINERGIC NEURONAL SYSTEMS ON THE REVERSAL OF RESERPINE-INDUCED AKINESIA IN MICE AS MEASURED BY LOCOMOTOR ACTIVITY IN ANIMEX ACTIVITY METERS. RESERPINE (10 MG/KG IP) WAS ADMINISTERED 18-24H BEFORE ADMINISTRATION OF LEVODOPA (200 MG/KG IP) PLUS MK 486 (25 MG/KG IP). SALINE, FLA-63 (25 MG/KG IP OR 15 MG/KG PO), PHENOXYBENZAMINE (PBZ, 20 MG/KG IP) OR HALOPERIDOL (HPL, 1 MG/KG IP) WERE ADMINISTERED 1 HR BEFORE AND PIMOZIDE (PI, 1 MG/KG IP) 4 HR BEFORE LEVODOPA PLUS MK 486. CLONIDINE WAS ADMINISTERED SIMULTANEOUSLY WITH LEVODOPA. THE NUMBERS OF BATCHES OF 3 ANIMALS USED ARE SHOWN IN PARENTHESES

Drugs Administered	Dose (mg/kg)	Mean Animex Counts in 4 hr (± I S E M)	% Change from Levodopa +MK 486	Significance from Levodopa +MK 486
Saline + saline	—	342 ± 63 (6)	—	—
Levodopa + MK 486	200 + 25	13862 ± 2213 (5)	—	—
+ IP FLA63	25	5938 ± 1084 (5)	57 2	$p<0$ 01
+ PO FLA63	15	4230 ± 1026 (5)	69 5	$p<0$ 0025
+ IP FLA63 and clonidine	25 1	10026 ± 2309 (7)	27 7	$p>0$ 05
+ PBZ	20	8087 ± 1752 (5)	41 7	$p<0$ 05
+ HPL	1	9709 ± 1068 (8)	30 0	$p<0$ 05
+ PI	1	6252 ± 1495 (3)	54 9	$p<0$ 05

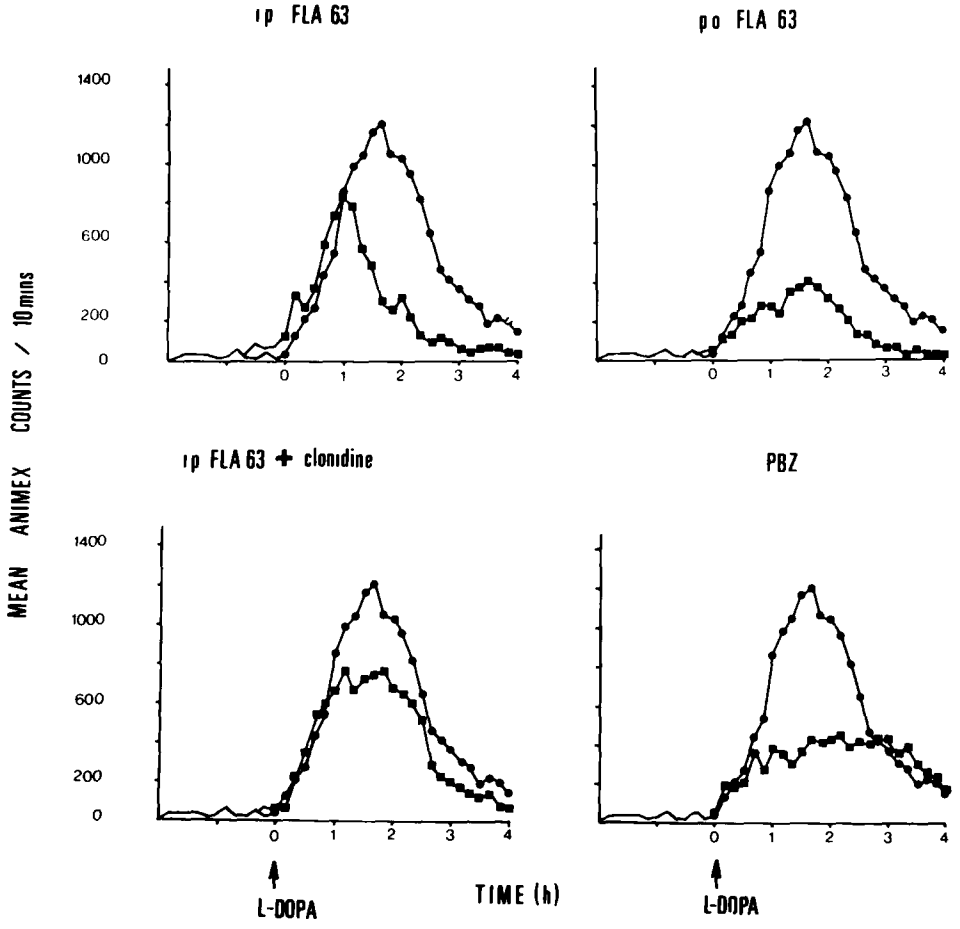

FIG 1. The effect of drugs influencing noradrenergic and dopaminergic neuronal systems on the reversal of reserpine-induced akinesia in mice by levodopa plus MK 486 as measured by locomotor activity in Animex activity meters. Reserpine (10 mg/kg IP) was administered 18–24 hr prior to levodopa (200 mg/kg IP) plus MK 486 (25 mg/kg IP). Animals were pretreated with FLA-63 (25 mg/kg IP or 15 mg/kg PO) or phenoxybenzamine (PBZ 20 mg/kg IP) 1 hr prior to levodopa plus MK 486 administration. Clonidine (1 mg/kg IP) was administered simultaneously with levodopa plus MK 486. The numbers of batches of 3 animals used are shown in Table 1.

TABLE 2

THE EFFECT OF LEVODOPA PLUS MK 486 ALONE COMPARED WITH THE EFFECTS OBSERVED FOLLOWING PRETREATMENT WITH DRUGS INFLUENCING NORADRENERGIC AND DOPAMINERGIC NEURONAL SYSTEMS ON THE REVERSAL OF AMPT-INDUCED AKINESIA IN MICE AS MEASURED BY LOCOMOTOR ACTIVITY IN ANIMEX ACTIVITY METERS AMPT (200 MG/KG IP) WAS ADMINISTERED 3 HR BEFORE ADMINISTRATION OF LEVODOPA (200 MG/KG IP) PLUS MK 486 (25 MG/KG IP) SALINE, FLA-63 (25 MG/KG IP), PHENOXYBENZAMINE (PBZ, 20 MG/KG IP) AND HALOPERIDOL (HPL, 1 MG/KG IP) WERE ADMINISTERED 1 HR BEFORE AND PIMOZIDE (PI, 1 MG/KG IP) 4 HR BEFORE LEVODOPA ADMINISTRATION THE NUMBER OF BATCHES OF 3 ANIMALS USED ARE SHOWN IN PARENTHESES

Drugs Administered	Dose mg/kg	Mean Animex Counts in 4 hr (± S.E M)	% Change from Levodopa +MK 486	Significance from Levodopa plus MK 486
Saline + saline	—	1381 ± 181 (4)	—	—
Levodopa + MK 486	200 + 25	21157 ± 1575 (5)	—	—
+FLA 63	25	11621 ± 1575 (5)	45 1	$p<0$ 0125
+PBZ	20	16189 ± 2306 (5)	23 5	$p>0$ 05
+HPL	1	1310 ± 117 (5)	93 8	$p<0$ 0005
+PI	1	10598 ± 4267 (4)	49 9	$p<0$ 05

alone ($p<0.005$). Similarly in animals receiving FLA-63 25 mg/kg PO Animex counts in the 4 hr period following levodopa administration were reduced to 9,133 ± 1073 (6) compared to 17,034 ± 1617 (4) in alternated groups of animals receiving levodopa alone ($p<0.005$). Administration of clonidine (1 mg/kg) plus levodopa to animals pretreated with FLA-63 (25 mg/kg IP) produced 14234 ± 2973 (6) Animex counts in the 4 hr period following levodopa administration in comparison to 13978 ± 2445 (6) in alternated groups of animals receiving levodopa alone ($p>0.05$)

This latter data suggests the actions of FLA-63 in these experiments to be brought about by a more specific mechanism than peritoneal irritation.

Levodopa Reversal of AMPT Induced Akinesia

Pretreatment of animals with AMPT in a dose known to cause inhibition of amine synthesis (200 mg/kg) produced a marked reduction in spontaneous locomotor activity during the first 2 hr after administration. After this time the animals remained quiet exhibiting only occasional locomotor activity. Rigidity and tremor were observed less frequently than in the reserpine model and ptosis was less marked. Administration of MK 486 (25 mg/kg) to these animals did not alter the behaviour pattern and no increase in locomotor activity was observed. However, administration of levodopa (200 mg/kg) plus MK 486 (25 mg/kg) to animals pretreated with AMPT (200 mg/kg) caused a marked increase in locomotor activity. The peak response was reached 1–1.5 hr after administration of levodopa and disappeared after about 4 hr. Pretreatment of animals IP with FLA-63 (25 mg/kg), haloperidol (1 mg/kg), or pimozide (1 mg/kg) significantly inhibited the reversal of AMPT induced akinesia by levodopa (Table 2, Fig 2).

Phenoxybenzamine (20 mg/kg) did not significantly decrease levodopa induced activity, although it did cause a reduction in peak motor activity. However, animals pretreated with phenoxybenzamine, in contrast to other drug combinations, showed a different behaviour pattern in response to levodopa. Running, pacing or walking activity was rarely evident. The animals remained prostrate but produced limb paddling movements and intermittently jumped or hopped. Stereotyped behaviour was also apparent in the form of gnawing, biting and the carrying of faeces and wood chippings by mouth. Each of these effects contributed to the activity measured by the Animex meters. Some evidence of this altered behaviour pattern was also observed in reserpinised animals receiving phenoxybenzamine and levodopa but was less marked than in AMPT pretreated animals.

None of the potential antagonists used above produced any significant behavioural change when administered alone to AMPT pretreated animals.

Apomorphine Reversal of Reserpine Akinesia

Apomorphine (2 mg/kg) caused a reversal of reserpine-induced akinesia which was maximal 10–20 min after administration and lasted approximately 1 hr. This reversal as judged by total Animex counts was unaffected by prior IP administration of phenoxybenzamine (20 mg/kg) or FLA-63 (25 mg/kg). It is apparent nevertheless from Fig. 3 that phenoxybenzamine causes a slight reduction in motor activity during the period of maximal response to apomorphine. However, a point for point comparison during this period also failed to show a significant effect The apomorphine effect, however, was significantly reduced by the prior administration of haloperidol (1 mg/kg) and pimozide (2 mg/kg) (Table 3, Fig. 3)

Apomorphine Reversal of AMPT Akinesia

Apomorphine (2 mg/kg) also increased motor activity in animals pretreated with AMPT (200 mg/kg). The effect was, however, of shorter duration lasting approximately 40 min. The effect was not significantly altered by prior IP administration of FLA-63 (25 mg/kg) but was markedly reduced by haloperidol (1 mg/kg) and pimozide (2 mg/kg).

Phenoxybenzamine (20 mg/kg) in contrast to its lack of effect in the reserpine model, produced a significant reduction of apomorphine-induced reversal of AMPT akinesia. (Table 4, Fig. 4).

The Effect of Clonidine on Apomorphine Reversal of Reserpine Akinesia

Apomorphine (5 mg/kg) induced reversal of reserpine

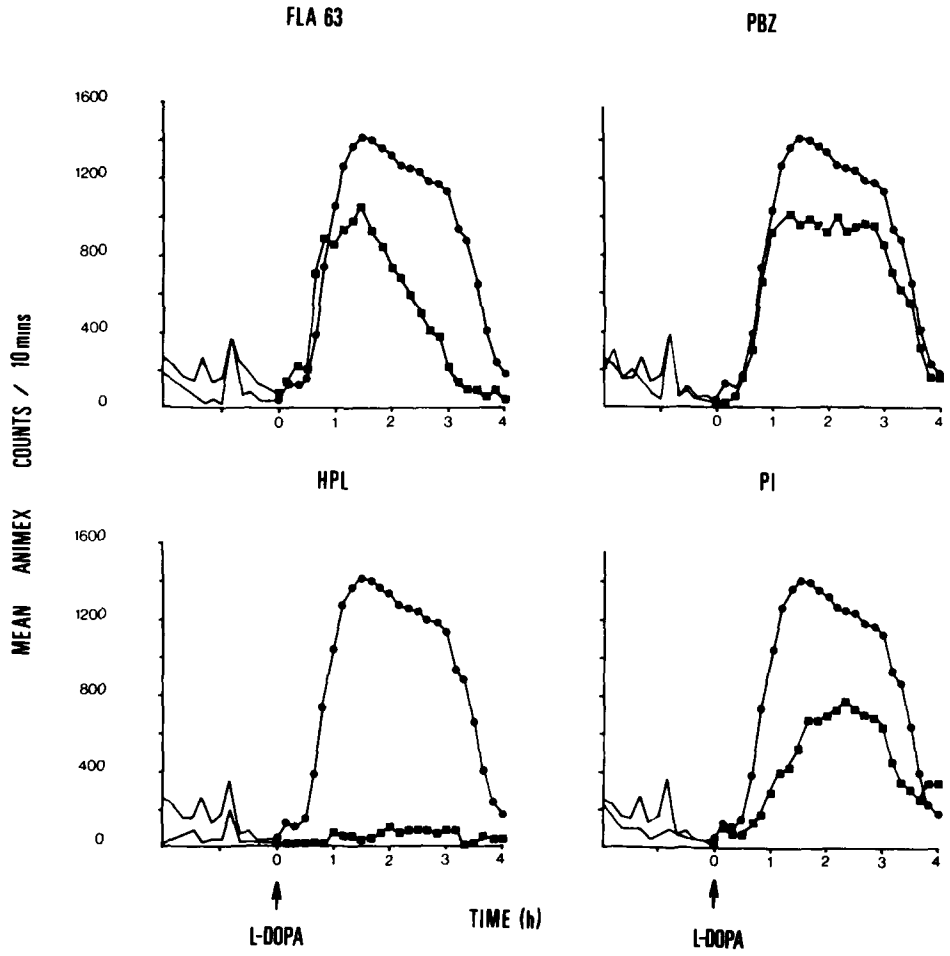

FIG. 2 The effect of drugs influencing noradrenergic and dopaminergic neuronal systems on the reversal of AMPT induced akinesia in mice by levodopa plus MK 486 as measured by locomotor activity in Animex activity meters AMPT (200 mg/kg IP) was administered 3 hr prior to levodopa (200 mg/kg IP) plus MK 486 (25 mg/kg IP) Animals were pretreated with FLA-63 (25 mg/kg IP), phenoxybenzamine (PBZ, 20 mg/kg IP) or haloperidol (HPL; 1 mg/kg IP) 1 hr prior to administration of levodopa plus MK 486 or with pimozide (PI, 1 mg/kg IP) 4 hr prior to administration. The numbers of batches of 3 animals used are shown in Table 2

TABLE 3

THE EFFECT OF APOMORPHINE ALONE COMPARED WITH THE EFFECTS OBSERVED FOLLOWING PRETREATMENT WITH DRUGS INFLUENCING NORADRENERGIC AND DOPAMINERGIC NEURONAL SYSTEMS ON THE REVERSAL OF RESERPINE-INDUCED AKINESIA IN MICE AS MEASURED BY LOCOMOTOR ACTIVITY IN ANIMEX ACTIVITY METERS DRUGS WERE ADMINISTERED AS INDICATED IN THE LEGEND TO TABLE 1 THE NUMBERS OF BATCHES OF 3 ANIMALS USED ARE SHOWN IN PARENTHESES

Drugs Administered	Dose mg/kg	Mean Animex Counts in 1 hr (± 1 S E.M)	% Change from Apomorphine Alone	Significance from Apomorphine Alone
Saline + saline	—	113 ± 25 (6)	—	—
Apomorphine	2	4341 ± 335 (5)	—	—
+ FLA 63	25	3643 ± 534 (5)	16 1	$p>0$ 05
+ PBZ	20	4193 ± 197 (4)	3.4	$p>0$ 05
+ HPL	1	1682 ± 464 (7)	61 3	$p<0$ 0025
+ PI	2	2139 ± 212 (5)	50 7	$p<0$ 0005

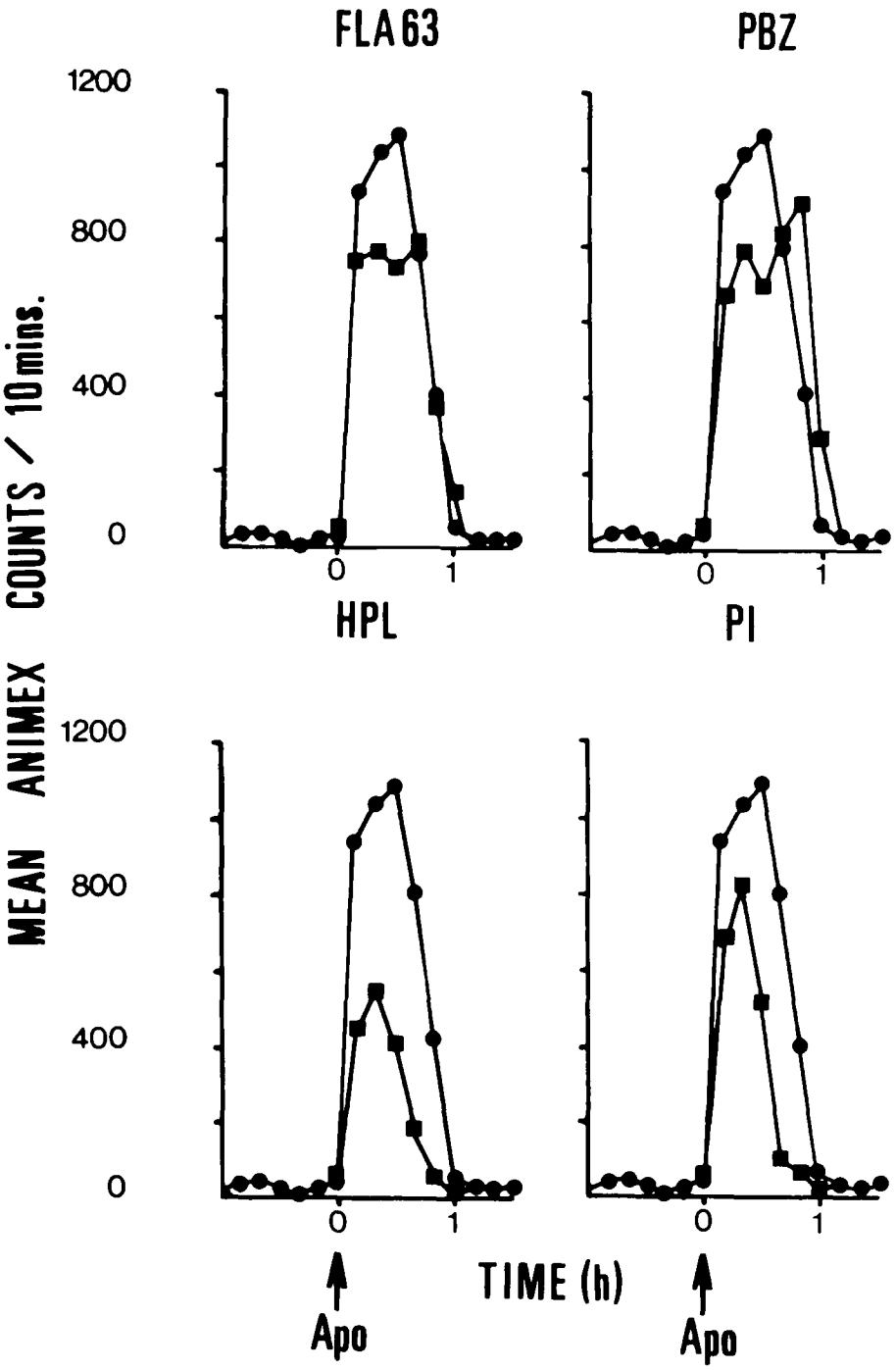

FIG 3. The effect of drugs influencing noradrenergic and dopaminergic neuronal systems on the reversal of reserpine-induced akinesia in mice by apomorphine as measured by locomotor activity in Animex activity meters. Reserpine (10 mg/kg IP), FLA-63 (25 mg/kg IP), phenoxybenzamine (PBZ, 20 mg/kg IP), haloperidol (HPL, 1 mg/kg IP) and pimozide (PI, 2 mg/kg IP) were administered prior to apomorphine (2 mg/kg IP) administration as indicated in the legends to Fig 1 and 2 The numbers of batches of 3 animals used are shown in Table 3.

akinesia was significantly enhanced by the simultaneous administration of clonidine (1 mg/kg) (Animex counts in the 2 hr after apomorphine 8082 ± 339 (3), apomorphine plus clonidine treated 15297 ± 525 (4), $p<0.001$). Prior administration of FLA-63 did not prevent the clonidine induced enhancement of apomorphine activity (Animex counts in 2 hr following administration of clonidine plus apomorphine after FLA-63 13531 ± 697 (3) $p<0.001$ compared to apomorphine controls; $p<0.05$ compared to apomorphine plus clonidine treated animals). (Fig. 5).

DISCUSSION

This data substantiates the notion that both DA and NA are of significance in locomotor activity. In both reserpine

TABLE 4

THE EFFECT OF APOMORPHINE ALONE COMPARED WITH THE EFFECTS OBSERVED FOLLOWING PRETREATMENT WITH DRUGS INFLUENCING NORADRENERGIC AND DOPAMINERGIC NEURONAL SYSTEMS ON THE REVERSAL OF AMPT-INDUCED AKINESIA IN MICE AS MEASURED BY LOCOMOTOR ACTIVITY IN ANIMEX ACTIVITY METERS DRUGS WERE ADMINISTERED AS INDICATED IN THE LEGEND TO TABLE 2 THE NUMBERS OF BATCHES OF 3 ANIMALS USED ARE SHOWN IN PARENTHESES

Drugs Administered	Dose mg/kg	Mean Animex Counts in 1 hr (± 1 S E M)	% Change from Apomorphine Alone	Significance from Apomorphine Alone
Saline + saline	—	711 ± 129 (4)	—	—
Apomorphine	2	2785 ± 278 (5)	—	—
+ FLA 63	25	2356 ± 325 (5)	15 4	$p>0$ 05
+ PBZ	20	1446 ± 142 (5)	48 1	$p<0$ 0025
+ HPL	1	225 ± 85 (5)	91 9	$p<0$ 0005
+ PI	2	388 ± 157 (4)	86 1	$p<0$ 0005

and AMPT treated animals, the effect of levodopa on motor activity was attenuated by FLA-63 administered orally or IP. It has been suggested that the actions of the dopamine-β-hydroxylase inhibitor FLA-63 on motor activity following IP administration are due to stressful effects of this compound mediated via corticosterone [38] However, since the administration of clonidine, a noradrenergic agonist, reversed the effect of FLA-63 on levodopa-induced activity, this would seem unlikely, although the antinociceptive action of clonidine might negate this finding. The probability of FLA-63 exerting a specific action on dopamine-β-hydroxylase is strengthened by the finding that FLA-63 was without effect on the motor activity induced by apomorphine in animals pretreated with either reserpine or AMPT, and by the observation that oral administration was as effective as intraperitoneal. Also, the administration of corticosteroids in high doses, or the IP administration of known irritants appears to be without effect on spontaneous or levodopa-induced motor activity (unpublished observations).

The effects of reserpine are not confined to catecholamine neurones, and it could be argued, for example, that changes in the storage of serotonin might influence the response to levodopa. Thus, there is evidence to suggest a functional link between the NA locus coeruleus and the serotonergic midbrain raphe nuclei [23,25].

Stimulation of noradrenergic receptors following levodopa administration might therefore affect 5-HT neuronal activity which, from other work, is known to influence locomotion. Alternatively, the effects of FLA-63 and other NA antagonists in reserpinised animals might be due to complex secondary effects on the altered activity of 5-HT neuronal systems caused by reserpine. However, the fact that similar results were obtained with FLA-63 in AMPT treated animals in whom 5-HT depletion does not occur, suggests that changes in cerebral NA are a direct cause of the altered motor activity that the drug produces.

The restoration of a normal levodopa response by the administration of the NA agonist clonidine [6] to animals previously treated with FLA-63 provides further evidence for the involvement of central NA receptors in the mediation of locomotor activity. The enhancement of DA-induced motor activity by the addition of a NA component was also seen when clonidine was combined with apomorphine, in agreement with earlier findings [3].

The reversal of reserpine akinesia by levodopa was also reduced by phenoxybenzamine, again suggesting the involvement of a NA component, since this compound was without effect on the action of apomorphine in reserpinised animals. These results are in agreement with previous findings in normal animals [29]. In AMPT treated animals, however, phenoxbenzamine had no effect on levodopa-induced overall locomotor activity, but caused a gross change in behaviour which may explain this apparent discrepancy. Stereotypy normally associated with DA activity was apparent in animals so treated, but the other movements observed were unusual, and walking or running did not occur. The total motor activity measured by the technique employed was not affected by phenoxybenzamine, but locomotion was abolished.

Phenoxybenzamine also significantly decreased the apomorphine reversal of AMPT akinesia in contrast to its lack of effect in the reserpine model. No obvious explanation for this observation is available, but it may be related to the effects of apomorphine on NA containing neuronal systems in the 2 models.

There is some evidence to suggest that DA agonists such as apomorphine [31] and piribedil [11,22] can exert a presynaptic effect on NA neurones. If such a presynaptic NA effect of apomorphine occurs it probably would not be operative in reserpinised animals for the effect of amphetamine on locomotor activity in reserpinised mice is not altered by pretreatment with phenoxybenzamine [22] although it is markedly inhibited in normal animals [28,29]. This might therefore explain why the effect of apomorphine on motor activity in reserpinised animals is not altered by phenoxybenzamine. In animals pretreated with AMPT, however, an effect on NA neuronal systems might contribute to motor effects of apomorphine since it has been shown that apomorphine enhances the disappearance of NA in this model [31]. The effect of phenoxybenzamine on apomorphine-induced locomotor activity in AMPT treated animals might therefore be not so surprising.

The involvement of a DA component in levodopa-induced motor activity in both reserpine and AMPT pretreated animals was confirmed using pimozide and haloperidol. In contrast to our previous communication [30] pimozide produced a significant effect on levodopa activity as expected. This discrepancy may be due to the

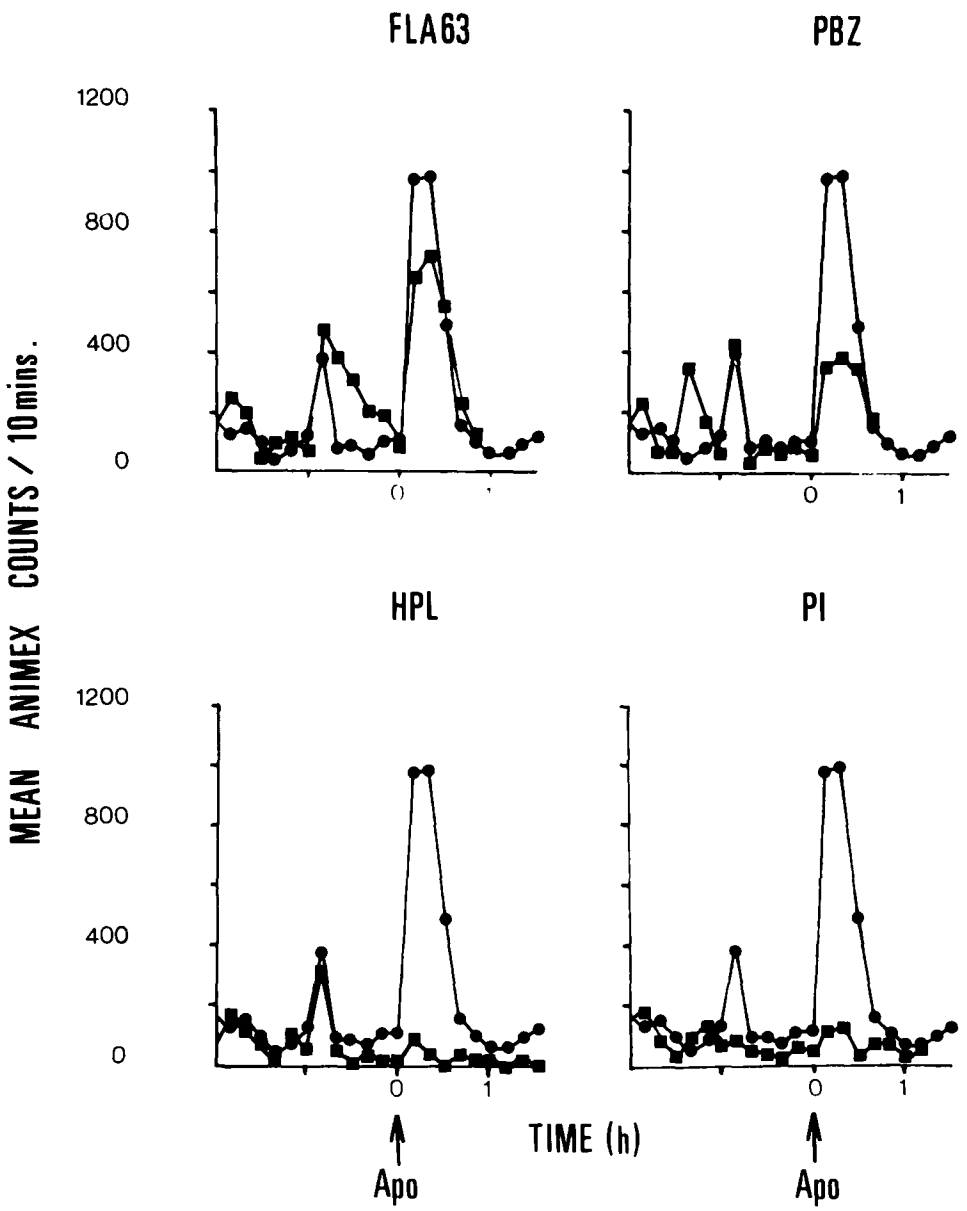

FIG. 4. The effect of drugs influencing noradrenergic and dopaminergic neuronal systems on the reversal of AMPT-induced akinesia in mice by apomorphine as measured by locomotor activity in Animex activity meters AMPT (200 mg/kg IP), FLA-63 (25 mg/kg IP) phenoxybenzamine (PBZ, 20 mg/kg IP), haloperidol (HPL; 1 mg/kg IP) and pimozide (PI, 2 mg/kg IP) were administered prior to apomorphine (2 mg/kg IP) as indicated in the legends to Fig 1 and 2 The numbers of batches of 3 animals used are shown in Table 4

different methods used to measure motor activity in our preliminary study, and highlights the difficulties involved in measuring behavioural changes in drug-induced activity

The failure of pimozide to antagonise locomotor activity provoked by levodopa in reserpinised animals as observed in open field conditions represented a failure of the drug to stop animals exploring their environment in a given time, i.e. they moved out of a given area despite pimozide. In contrast, pimozide reduced total locomotor activity in the same experiment when measured by activity meters, which recorded total activity whether exploratory or confined. Others [39] have hinted that exploratory activity may be related to a NA action, while overall locomotor activity including stereotypy is primed by DA stimulation. If this is true, pimozide's failure to antagonise open-field activity provoked by levodopa might represent residual NA exploratory activity despite a considerable reduction in overall motor activity.

The administration of levodopa to animals pretreated with AMPT and reserpine leads to a rapid increase in DA in the brain. In AMPT treated animals levodopa almost restores brain NA to normal (unpublished observation) but the increase in absolute levels of NA in reserpinised animals is slight [1]. However, synthesis of NA does occur in the reserpinised brain [18] but because its storage is disrupted it is rapidly broken down in the cytoplasm [17]. While it

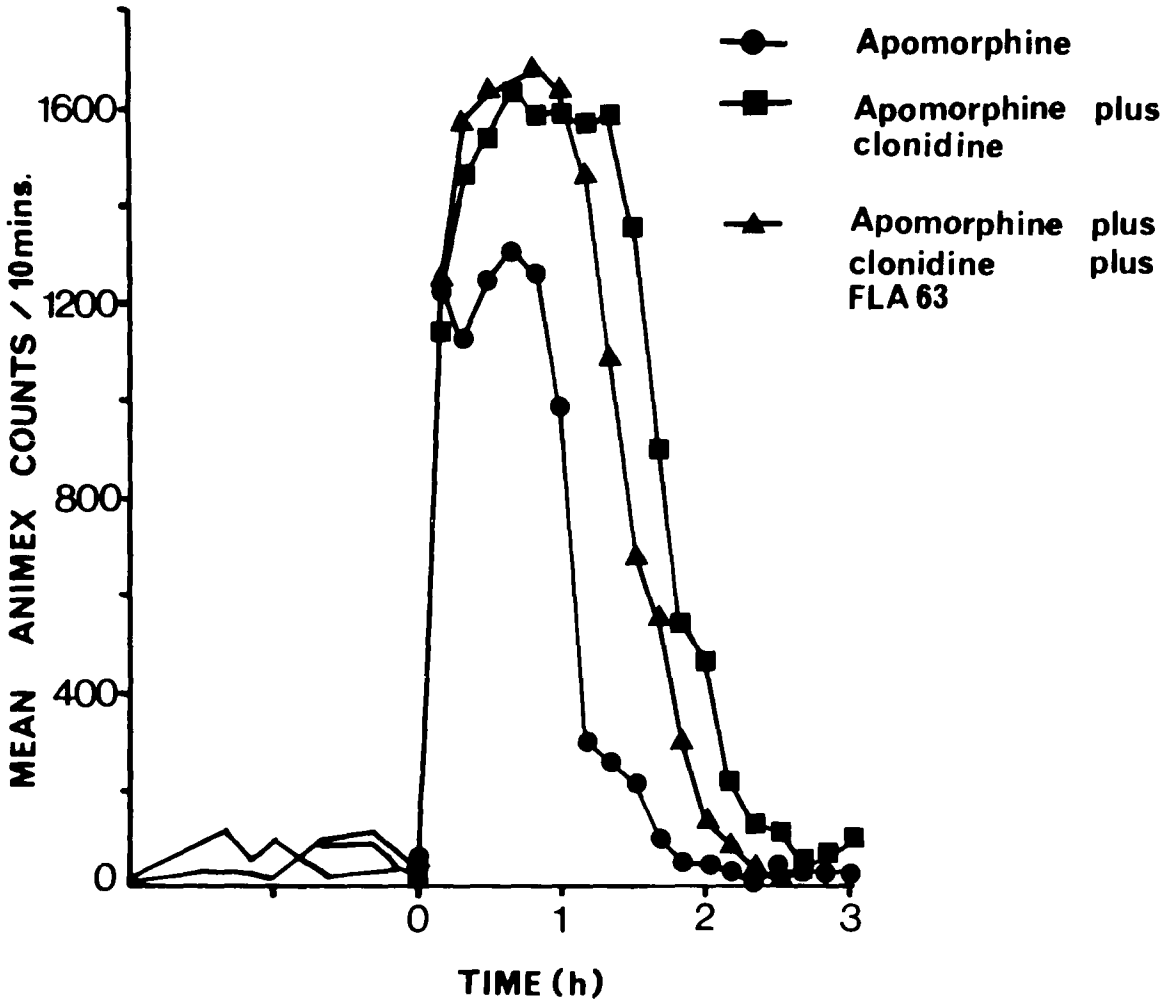

FIG. 5. The effect of noradrenaline receptor stimulation on motor activity produced by apomorphine alone or apomorphine plus FLA 63 in reserpinised mice as measured in Animex activity meters. Reserpine (10 mg/kg) was administered 18–24 hr prior to administration of apomorphine (2 mg/kg IP) FLA-63 (25 mg/kg IP) was administered 1 hr before and clonidine (1 mg/kg) simultaneously with apomorphine administration. The numbers of batches of 3 animals used are shown in the text

has been demonstrated that DA does not accumulate in NA neurones following levodopa treatment [10, 13, 14], there is recent evidence that the large quantities of DA formed enhance NA turnover by displacing it from neuronal stores [24]. The exogenous amino-acid is, however, the source of the newly synthesised NA and it is the release of newly synthesised catecholamines that is thought to be important in neural transmission [8, 18, 33].

Although changes in absolute levels of NA are small, compared to those in DA, our behavioural data would indicate that they are of significance in the restoration of normal motor function after disruption of catecholamine synthesis and storage. Such a finding must have relevance to Parkinson's disease. While the main pathological changes in this syndrome is degeneration of the substantia nigra, other pigmented brain stem nuclei are also affected including the locus coeruleus [20] which is a major source of ascending NA fibres to the cerebrum, diencephalon and cerebellum [16]. Loss of NA neurones in Parkinson's disease may exacerbate the effects of DA depletion, yet both would be compensated by levodopa therapy. However, selective DA agonists, such as piribedil or bromocriptine, might allow only partial restoration of function if they failed to cause concurrent stimulation of noradrenaline receptors. These drugs, recently introduced for clinical evaluation have not, in our hands been as effective as levodopa in many patients, perhaps because they lack adequate NA receptor stimulating activity. It may be that agonists that stimulate both NA and DA receptors should be the aim in developing new treatments for Parkinson's disease.

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