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The Effects on Central Dopamine Function of Chronic L-DOPA (Methyl Ester Hydrochloride) Treatment of Mice

JOHN TABAR,* MAYKO HASHIZUME,* CATHY J. COOK,†
PHILIP M. BEART† AND DAVID M. JACKSON*[1]

**Department of Pharmacology, The University of Sydney, New South Wales 2006*
and †University of Melbourne, Clinical Pharmacology and Therapeutics Unit
Austin Hospital, Victoria 3084, Australia

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TABAR, J., M. HASHIZUME, C. J. COOK, P. M. BEART AND D. M. JACKSON. *The effects on central dopamine function of chronic L-DOPA (methyl ester hydrochloride) treatment of mice.* PHARMACOL BIOCHEM BEHAV **33**(1) 139–146, 1989.—Mice were treated for 28 days with drinking water containing L-DOPA methyl ester hydrochloride (DME) plus carbidopa, carbidopa alone, or with the vehicle. All mice were then given the vehicle for 1 day and behavioural and biochemical assessments made on the 29th day. On average, mice consumed between 181 and 302 mg/kg of DME (expressed as the base) each day. In behavioural experiments DME- and carbidopa-treated mice were subsensitive to the locomotor stimulating effects of apomorphine, after their pretreatment with reserpine plus α-methyl-p-tyrosine to remove endogenous stores of dopamine and to stop its synthesis. Even mice pretreated for only one day with chronic DME or carbidopa displayed some subsensitivity to apomorphine challenge, but the effect was more marked the longer the chronic treatment. Other mice were chronically treated for 28 days with α-methylDOPA or vehicle, and these mice when challenged with apomorphine after dopamine depletion (as described above), were also markedly subsensitive to the locomotor activating effects of apomorphine. There were no changes in sensitivity of drug-treated mice to the hypothermic effects of apomorphine, to the stereotypy-inducing effects of apomorphine or d-amphetamine, or to the locomotor activating effects of L-DOPA itself or to bromocriptine. There were, however, some changes in the basal grooming behaviour of both DME- and carbidopa-treated mice, and in their response to SKF38393 challenge. Striatal binding studies with [^{3}H]-spiperone and [^{3}H]-SCH23390 indicated that there were no marked changes in K_d or B_{max} of either D-1 or D-2 receptors. The data indicate that chronic treatment of mice with L-DOPA (as the methyl ester hydrochloride) produces a subsensitivity to one particular behavioural effect of apomorphine, in the absence of any change in D-1 and D-2 receptors. Furthermore, the behavioural subsensitivity to apomorphine would appear to be a result of the chronic treatment with carbidopa. Carbidopa, while not crossing the blood-brain barrier, can be metabolized in part to α-methylDOPA, which can cross the blood-brain barrier.

Chronic L-DOPA Supersensitivity Locomotor activity Stereotypy d-Amphetamine Apomorphine Mice
Receptor binding D-1 dopamine receptors D-2 dopamine receptors Carbidopa Temperature
α-MethylDOPA Autoreceptors SKF38393

THE hypothesis that the side effects of L-DOPA (L-3,4 dihydroxyphenylalanine) therapy may be caused by changes in dopamine (DA) receptor sensitivity (24–26) has generated considerable interest. In our previous studies, we showed that repeated injections of L-DOPA produced a behavioural subsensitivity to its own locomotor stimulant effects and that this change was dependent upon pharmacokinetic factors, and not upon changes in postsynaptic DA receptor sensitivity (1). However, despite the absence of postsynaptic receptor sensitivity changes, the DA autoreceptors were rendered subsensitive (1, 20, 21).

The literature, however, is not in any agreement about the effects of chronic L-DOPA. For example, a number of authors have reported a down-regulation of already supersensitive D-2 receptors (8, 11, 27, 32, 38), without altering the sensitivity of nonsensitized receptors (37,38). In agreement with this, it has been reported that chronic L-DOPA medication of patients with Parkinson's disease caused the down-regulation of the supersensitive D-2 receptors, without changing their sensitivity to below control levels (16). Other workers, however, have failed to detect any down-regulation of already supersensitive DA receptors (10, 13, 35). Furthermore, few studies have specifically examined the effects of chronic L-DOPA on D-1 receptor sensitivity. One group, using rats with unilateral lesions of the nigro-striatal DA pathway, has suggested that chronic L-DOPA enhances the

[1]Requests for reprints should be addressed to David M. Jackson, Ph.D., Department of Pharmacology, The University of Sydney, N.S.W., 2006, Australia.

supersensitivity of the DA-stimulated adenyl cyclase evident after such a lesion, while concomitantly down-regulating the supersensitive D-2 receptors (33).

There are a number of possible explanations for these inconsistencies. Firstly, the various models used by different workers may not measure the same thing. Thus, most of the binding studies cited above [see, e.g., (27)] used ligand displaying at least some selectivity for D-2 receptors, while most of the behavioural studies used DA agonists, such as apomorphine, d-amphetamine and L-DOPA, which have effects at both D-1 and D-2 receptors. This is a particularly important difference, given the complex interdependence of D-1 and D-2 receptors in motor function (5, 19, 29, 30). Secondly, there are major differences evident in experimental design. Several workers, for example, failed to control for the effect of the DOPA decarboxylase inhibitor included with the L-DOPA treatment (4, 8, 14, 27, 33, 35, 36), while others did not apparently include an inhibitor (11, 13, 17, 38). Thirdly, the effect of the chronic L-DOPA treatment is sometimes tested in animals with normosensitive DA receptors (1, 20, 21, 46) or in animals whose DA receptors are supersensitive (see above). Fourthly, the route of administration has varied, with some groups giving the L-DOPA once or twice a day orally by gavage (PO) (1, 4, 35, 45), intramuscularly (11), intraperitoneally (IP) (17, 20, 21, 38) or by putting the L-DOPA in the food (46). Finally, there are considerable differences between the various studies in the time of behavioural and biochemical assessment, with testing being conducted either during treatment or during withdrawal.

Most of our earlier work utilized 1 or 2 daily injections (IP or PO) of high doses of L-DOPA as the base or as the soluble methyl ester hydrochloride form. Such a schedule meant that the animals were effectively drug free for about 15–20 hr each day and offered the possibility that the high doses might have produced transient toxic levels in the plasma and brain. In order to overcome these criticisms, we began a series of experiments using the methyl ester hydrochloride form of L-DOPA, called DME in the present paper, dissolved in the animal's drinking water. In addition, because we have previously noted (unpublished data) that chronic treatment with peripheral decarboxylase inhibitors produces behavioural effects, we have included the appropriate control groups (1).

METHOD

General and Chronic Treatments

QS strain mice (20–25 g at the beginning of experimentation) were housed in groups of 15 to 20, under a 12 hr light, 12 hr dark cycle (light phase was 0630 to 1830 hr), at a temperature of $21 \pm 2°C$, with free access to food and drinking fluid. The chronic drug treatments were administered via the drinking fluid, which provided the only source of water. In pilot experiments, we varied the treatment time (14 or 28 days) and the chronic DME dose (2 or 4 mg/ml of drinking fluid). The higher dose produced an unacceptable morbidity and the following schedule was decided upon for the results presented in the present study. The first treatment group (the vehicle group) had access to a solution containing 0.3% ascorbic acid and 5.5% glucose in distilled water. The second treatment group (the carbidopa group) received the vehicle in which was dissolved carbidopa (0.2 mg/ml). The third treatment group received the vehicle in which was dissolved DME (2 mg/ml) plus carbidopa (0.2 mg/ml). This group is throughout this paper called the DME-treated group. Drug solutions were prepared freshly at least once every 2 days. In representative experiments, mice were weighed daily throughout the chronic treatment and the drug intake calculated for each 24 hr. The drug was withdrawn after 28 days and animals in all 3 treatment groups then received the vehicle only. All behavioural and biochemical observations were made 20 to 28 hr after withdrawal. In one additional chronic experiment, α-methyldopa (αMDOPA) was dissolved in the vehicle (0.2 mg/ml) and administered for 28 days and tests on these animals conducted the following day.

Measurement of Locomotor Activity

The locomotor activity of groups of 3 mice was measured in 4 matched rectangular activity cages intersected by 2 light beams on the shorter axis (2).

Measurement of Stereotyped Behaviour

After apomorphine or d-amphetamine challenge, mice were placed into small perspex boxes (95 mm wide × 73 mm high × 70 mm deep) which were screened visually from one another, and the degree of stereotypy was assessed each 5 min for 80 (apomorphine) or each 10 min for 120 (d-amphetamine) min according to the following schedule: 0, asleep or not moving; 1, alert and some movement; 2, some sniffing; 3, constant sniffing; 4, constant sniffing and intermittent rearing; 5. Constant sniffing and constant rearing. These scores were summed over the observation period to provide a single index of the degree of stereotypy. To assess the behavioural effects of the selective D-1 agonist SKF38393, mice were put into the small perspex boxes and challenged, 1 hr later, with SKF38393 or vehicle. The presence or absence of the following behaviours was assessed each 15 min for 3 hr, whether the animal was asleep, awake but still grooming sniffing or rearing. The animal was scored as grooming, sniffing or rearing if that behaviour was performed in a repetitive way during a 15-sec observation period. In some cases, an animal might display two of the behaviours at the same time (e.g., sniffing and rearing) and in such a case each behaviour was scored as being present. The most interesting data were the scores of grooming, and the total number of grooming incidences for each animal over the 3-hr observation was summed with the maximum for each animal being 12. All subjectively-assessed behaviour was scored by an experienced observer who was "blind" both to the chronic treatment schedule and to the challenge drug.

Measurement of Temperature

The rectal temperature was measured between 1300 and 1500 hr by inserting a Yellow Springs probe lubricated with warmed liquid paraffin 2 cm into the rectum. Readings were made 30 and 15 min before apomorphine challenge, and thereafter each 10 min for 90 min.

Drugs

d-Amphetamine sulphate (F. H. Faulding, Ltd.) was dissolved in saline. α-Methyl-p-tyrosine methyl ester hydrochloride (αMPT, Sigma) was dissolved in distilled water. αMDOPA (Merck, Sharp and Dohme*) was dissolved in the chronic vehicle as described. Reserpine (Sigma) was dissolved in a minimum of glacial acetic acid and diluted to volume with water. Apomorphine hydrochloride (Sigma and Sandoz*), SKF38393 hydrochloride (2,3,4,5-tetrahydro-7,8-dihydroxy-1-phenyl-1H-3-benzazepine HCl, Research Biochemicals Inc., Wayland, MA) and, when used acutely as a challenge (Table 1), carbidopa (Merck, Sharp and Dohme*) and DME, were dissolved in 0.01% ascorbic acid solution. The DME was prepared from L-DOPA base (Sigma). Bromocriptine mesylate (Sandoz*) was dissolved in 0.05% tartaric acid solution. In the stereotypy and temperature experiments, apomorphine was administered subcutaneously. Otherwise all drugs were administered IP

TABLE 1

THE SENSITIVITY OF MICE TREATED CHRONICALLY WITH VEHICLE, CARBIDOPA OR DME, TO AN ACUTE CHALLENGE WITH VEHICLE, CARBIDOPA (25 mg/kg) OR DME (PLUS CARBIDOPA)

	Challenge			
Chronic Treatment*	Vehicle	Carbidopa	200 mg/kg DME	400 mg/kg DME
Vehicle	595 ± 130 (9)	866 ± 268 (9)	1839 ± 370 (9)	4570 ± 540 (9)
Carbidopa	573 ± 98(10)	710 ± 170(10)	1854 ± 326(10)	5557 ± 536(10)
DME	962 ± 275(10)	1259 ± 224(10)	1279 ± 224(10)	4870 ± 606(10)

*Although DME produced significant stimulation in each of the 3 treatment groups when simple effects were analyzed (all $p<0.001$), there was no significant effect of chronic treatment, $F(2,103)=0.3266$, $p>0.05$.

The locomotor activity of groups of 3 mice was measured for 4 hr beginning immediately after challenge, and that occurring in the last 3 hr (the stimulant phase) is given above. The data represent the mean total activities ± SEM of (n) replicates.

in a dose volume to weight of 10 ml/kg.

Binding Assays

One day after 28 days of chronic treatment, mice were decapitated and their neostriata removed and frozen to −70°C. Binding assays were performed on crude membrane preparations of the neostriata of 3 or 4 brains using (^{3}H)-spiperone as a D-2 specific ligand in concentrations between 0.002 and 2.5 nM (15). Membrane preparations were washed extensively and an incubation (10 min, 25°C) included to eliminate endogenous catecholamines. Nonspecific binding was defined with an excess of dl-sulpiride (5 μM). Binding data were analyzed by iterative curve fitting procedures to yield the dissociation constant and receptor density (B_{max}) (28,31). Protein content was not altered by the chronic treatments (data not shown).

Statistics

The complete time-course of action of each challenge drug was monitored in all locomotor activity and stereotypy experiments. Experimental treatments were randomized to ensure that all sources of variation were distributed equally across all treatment groups. Locomotor activity scores were analyzed by appropriate analyses of variance (ANOVAR) (47) and, if significant differences were detected, further analyses were performed by post hoc F tests as described by Winer (47) to determine which of the groups differed from the control. Subjectively assessed behavioural changes induced by apomorphine, d-amphetamine and SKF38393 were analyzed by Kruskal-Wallis' ANOVARs, as supplied on the Macintosh computer statistical package Statview 512®. Binding data were analyzed as described in the text. We have used the behavioural pharmacology convention of presenting locomotor activity data as means (with standard errors as appropriate) and stereotypy data as medians (with ranges as appropriate).

RESULTS

Drug Intake and Body Weight

Animals consumed between 181 and 302 mg/kg of DME (in terms of the base) per day. There was no significant treatment effect on mouse body weight (data not shown).

Locomotor Response to L-DOPA Challenge (Table 1)

The chronic treatment did not significantly alter the animals' locomotor response to DME challenge, although as expected (41), the DME produced locomotor activation.

Locomotor Stimulant Response to Apomorphine (Fig. 1)

After the 28-day chronic treatment, mice were premedicated with reserpine and αMPT and challenged 4 hr later with various doses of apomorphine or vehicle. Apomorphine produced dose-dependent locomotor stimulation in all treatment groups (Fig. 1C). Carbidopa- and DME-treated mice were, however, subsensitive to apomorphine. In another experiment, mice were given the chronic treatments for 14 days followed by vehicle for 1 day, and then challenged, after reserpine plus αMPT pretreatment, with apomorphine. Once again, both carbidopa- and DME-treated mice were subsensitive to apomorphine (Fig. 1B). Other mice were given vehicle, carbidopa or DME for exactly 24 hr followed by vehicle for the next 20 to 24 hr, and then challenged acutely with apomorphine as described. Carbidopa- and DME-treated mice were less sensitive to apomorphine (Fig. 1A), but this was not as marked as with the longer treatments. Finally, mice were administered vehicle or αMDOPA for 28 days and then the active drug replaced by vehicle. Mice consumed between 15 and 31 mg/kg of αMDOPA per day, a dose similar to the daily intake of carbidopa. On the 29th day after pretreatment with reserpine and αMPT, mice were challenged with apomorphine. αMDOPA-pretreated mice were markedly subsensitive to apomorphine (Fig. 1D), and examination of the time-course curves (not shown) indicated that this was due to a slower onset, a lower peak activity and a shorter duration of action of apomorphine in the drug-treated animals.

Stereotypy Response to Apomorphine (Table 2)

There was a slight but nonsignificant increase in apomorphine-induced stereotypy in animals treated with carbidopa or DME, and this was due to a marginally increased response to both doses of apomorphine.

APO-Induced Hypothermia

Apomorphine produced hypothermia in all treatment groups, with no significant treatment effect being evident (data not shown).

APO-Induced Behavioural Depression (Table 3)

Low doses of apomorphine produce behavioural depression, an

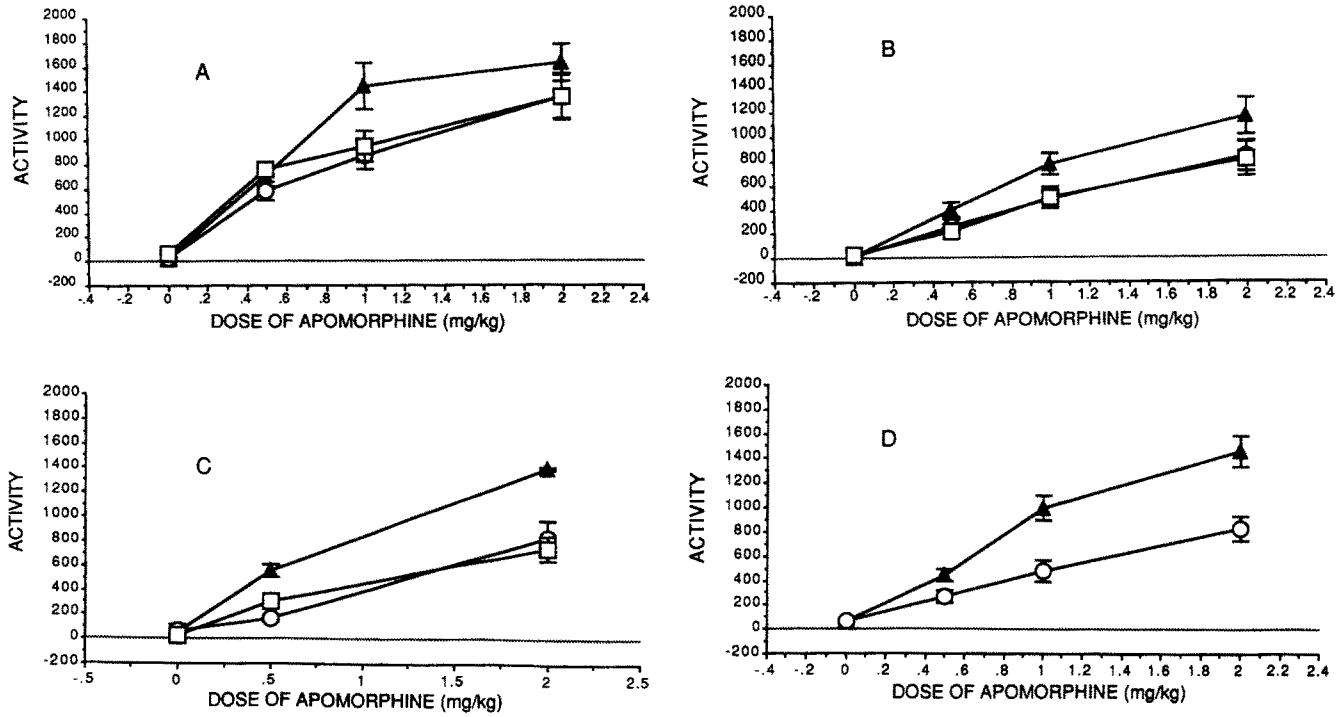

FIG. 1. Mice were treated for 1 (panel A), 14 (panel B) or 28 (panel C) days with DME (□), carbidopa (○) or vehicle (▲). One day after the withdrawal of the chronic treatments, mice were premedicated with reserpine (10 mg/kg) plus αMPT (250 mg/kg) and 4 hr later challenged with apomorphine or vehicle. The animals' total locomotor activity over the next 90 min was measured and the data represent the mean activity ± SEM of between 5 and 12 replicates. (A) Overall effect of the chronic treatment, $F(2,94)=2.90$, $p=0.06$. This marginal effect was primarily due to a difference in response of the 3 treatment groups to 1 mg/kg apomorphine, $F(2,94)=4.40$, $p=0.015$, with the response of each of the treatment groups being significantly less than the vehicle group ($p<0.05$). (B) Overall effect of the chronic treatment, $F(2,132)=7.14$, $p<0.001$. This difference was primarily due to less stimulation after apomorphine (1 and 2 mg/kg) in the DME and carbidopa groups than in the vehicle group ($p<0.05$ in both cases by post hoc F-tests). (C) Overall effect of the chronic treatment, $F(2,68)=10.00$, $p<0.001$, due to a markedly reduced stimulation after apomorphine in the DME and carbidopa groups. The response of each of the last 2 groups of 0.5 and 2 mg/kg was significantly less than the response of the vehicle group ($p<0.05$ in all cases, by post hoc F tests). (D) Displays the response of mice treated chronically with vehicle (▲) or αMDOPA (○) for 28 days, premedicated with reserpine plus αMPT as described above, and then challenged with apomorphine. Overall effect of chronic treatment, $F(1,87)=34.29$, $p<0.001$. Further analysis indicated that the αMDOPA animals were significantly less responsive to 0.5, 1 and 2 mg/kg of apomorphine (all $p<0.001$, by post hoc F tests).

effect mediated by DA autoreceptors (42). In the present experiment, apomorphine produced dose-dependent locomotor depression in all the treatment groups, but none of the chronic treatments significantly altered the magnitude of this response.

Locomotor Stimulant Response to d-Amphetamine

d-Amphetamine produced locomotor stimulation in all treatment groups. There was a marginal effect of the chronic treatment, and this was due to more locomotion in the DME group, compared to the vehicle and carbidopa groups. There was not, however, any particular change in response to d-amphetamine challenge (data not shown).

Stereotypy Response to d-Amphetamine (Table 2)

There was no significant effect of the chronic treatment on d-amphetamine-induced stereotypy.

Locomotor Stimulant Response to Bromocriptine (Table 4)

Chronically-treated mice were challenged with bromocriptine and the total activity during the stimulant phase [75 to 480 min after IP injection, (22)] is displayed in Table 4. Although there was no overall effect of treatment ($p>0.05$), there was a strong trend to an effect of treatment in response to 10 mg/kg, and this was due primarily to a lower response in the carbidopa group, $F(2,93)=3.8$, $p=0.026$.

Behavioural Response to SKF38393 (Fig. 2)

SKF38393 produced a significant dose-dependent increase in grooming behaviour in the vehicle-pretreated animals. In contrast, no significant increase was observed in either of the drug-treated groups. In these 2 treatment groups, there was an increase in the occurrence of grooming behaviours compared to that seen in the vehicle-treated group, even without SKF38393 challenge. This increase in basal activity may have been responsible for the higher frequency of grooming in the 2 drug treatment groups after challenge with 3 and 6 mg/kg SKF38393. After challenge with 12 mg/kg of SKF38393, the two drug treatment groups displayed significantly less grooming than that seen in the vehicle-treated animals.

Receptor Binding (Table 5)

Chronic carbidopa or DME did not significantly alter the

TABLE 2

THE EFFECT OF CHRONIC TREATMENT OF MICE WITH VEHICLE, CARBIDOPA OR DME ON THEIR SENSITIVITY TO THE STEREOTYPY PRODUCING EFFECTS OF d-AMPHETAMINE OR APOMORPHINE

Chronic Treatment	Challenge and Dose (mg/kg)					
	d-Amphetamine*			Apomorphine*		
	0	1.5	3.0	0	0.5	1.0
Vehicle	5(3–11,10)	21(13–33,10)	30(15–50,10)	15(12–20,8)	22(15–41,8)	34(26–63,8)
Carbidopa	5(1–19,10)	22(16–27, 9)	33(12–43,10)	16(14–21,8)	29(12–51,8)	42(30–52,8)
DME	6(2–19,10)	23(14–30,10)	31(22–46,10)	17 (5–23,8)	31(13–44,8)	41(25–60,8)

*Since the experiments with d-amphetamine and apomorphine were conducted at separate times using independent groups of mice, separate Kruskal-Wallis ANOVARs were calculated for these two groups of data. In neither experiment did the chronic treatment exert a significant effect on the response to d-amphetamine or apomorphine.

Stereotypy was assessed by an observer blind to the treatment schedule each 10 min for 120 min (d-amphetamine) or each 5 min for 80 min (apomorphine) and the data given here are the median total scores with the range and number of replicates in brackets for the whole observation period.

affinity (K_d) of the D-2 receptors, as defined with sulpiride ($p>0.05$). The B_{max}, however, appeared to be altered by the chronic treatment, $F(2,9)=4.97$, $p<0.05$, and post hoc F tests indicated that this was due to a marginally higher B_{max} in the carbidopa group than in the vehicle group, $F(2,9)=4.1$, $p=0.05$. Chronic αMDOPA treatment was without effect on either B_{max} or K_d (Table 5).

DISCUSSION

In our previous studies, we used oral or IP injections once or twice a day to produce high but short-lived plasma and brain levels of L-DOPA (1, 20, 21). In the present study, by administering the DME in the animals' drinking water, we were able to administer between 181–302 mg/kg/day, a dose, when expressed as the base, comparable to that utilized previously (200 or 400 mg/kg/day). However, the different treatment regimes produced different effects.

The present results provided no clear evidence for a change in the response of the animals to DME challenge, in contrast to our previous study in which we found that L-DOPA-treated mice were subsensitive to the stimulant effects of L-DOPA; an effect due to changes in the pharmacokinetics of the L-DOPA (1). The use of the methyl ester rather than the base itself would not seem to be a major factor because comparative studies indicate that they produce, dose for dose, similar behavioural and biochemical changes (6). In the present study, we also found that the sensitivity of the mice to d-amphetamine challenge was unchanged, in contrast to the subsensitivity that we showed behaviourally (1) and electrophysiologically (21). Since the dose of L-DOPA delivered as the base was similar through all our studies, it seems as though the route of administration may have caused the difference, with our previous studies using bolus injections and the present study using a schedule that would tend to distribute the intake throughout the day.

The most striking effect of the chronic treatments used in the present study was the marked subsensitivity of carbidopa and DME-treated mice to the locomotor stimulant effects of apomorphine. This subsensitivity was evident after a 1 day treatment with

TABLE 3

THE LOCOMOTOR DEPRESSANT EFFECTS OF APOMORPHINE IN MICE TREATED CHRONICALLY WITH VEHICLE, CARBIDOPA OR DME

Chronic Treatment*	Challenge Dose of Apomorphine (mg/kg)		
	0	0.075	0.15
Vehicle	863 ± 68(12)	481 ± 47(12)	324 ± 38(12)
Carbidopa	1027 ± 127(12)	513 ± 44(12)	354 ± 34(12)
DME	1098 ± 100(12)	502 ± 76(12)	343 ± 40(12)

*ANOVAR indicated that the chronic treatment exerted no significant effect on the degree of depression induced by apomorphine [overall effect of chronic treatment, $F(2,99)=1.42$, $p>0.05$].

Immediately after apomorphine challenge, mice were placed in groups of 3 into activity cages and their locomotor activity measured for 30 min. The data represent the mean total activity ± SEM of (n) replicates.

TABLE 4

THE LOCOMOTOR RESPONSE OF MICE TREATED CHRONICALLY WITH DME, CARBIDOPA OR VEHICLE TO A CHALLENGE WITH BROMOCRIPTINE

Treatment	Dose of Bromocriptine (mg/kg)		
	0	5	10
Vehicle	1441 ± 408(11)	2339 ± 770(11)	6834 ± 1970(12)
Carbidopa	1676 ± 227(11)	2198 ± 672(11)	4482 ± 1280(10)
DME	1781 ± 325(12)	2951 ± 881(12)	8382 ± 1070(12)

The data represent the mean total number of counts ± SEM of (n) replicates during the stimulant phase, 75 to 480 min after injection. Overall, there was no significant effect of the chronic treatment, $F(2,93)=1.86$, $p>0.1$. Interestingly, while bromocriptine produced significant stimulation in both the vehicle and DME groups (in both cases, analysis of simple effects indicated $p<0.001$), no significant stimulation was evident in the carbidopa group [analysis of simple effects, $F(2,93)=2.195$, $p>0.05$]. This latter effect was due to a significantly lower stimulant response to 10 mg/kg bromocriptine in the carbidopa group than in the other two groups (in both cases, post hoc F tests were $p<0.05$).

TABLE 5

[^{3}H]-SPIPERONE BINDING IN STRIATA FROM MICE TREATED WITH CHRONIC DME OR αMDOPA

Treatment Group	K_d (pM)	B_{max} (pmoles/g protein)
Vehicle	242 ± 71(4)	44 ± 4(4)
Carbidopa	405 ± 19(4)	72 ± 4(4)
DME	730 ± 439(4)	71 ± 11(4)
Vehicle	280 ± 30(3)	43 ± 4(3)
αMDOPA	200 ± 30(3)	44 ± 5(3)

The data represent the means ± SEM of (n) replicates.

DME, but was more marked after the longer treatments. Since the subsensitivity was present in both carbidopa and DME-treated animals, it is likely that the subsensitivity was due to the carbidopa component.

This subsensitivity raises several questions. Firstly, why was the subsensitivity to apomorphine seen only in the locomotor activity experiments and not in the stereotypy and temperature studies? One explanation may be that the DA receptors in various brain regions were differentially affected by whatever was causing the subsensitivity. A selectivity of this kind would be noteworthy since stereotyped behaviour, for example, is mainly a measure of neostriatal DA receptor function, while coordinated forwardly directed locomotion derives from the stimulation of DA receptors in the nucleus accumbens (18). Another possibility is that the reserpine and αMPT treatment used in the locomotor activity model (but not in the other models) may have altered the response to apomorphine. Alternatively, since apomorphine is a mixed D-1/D-2 agonist, it is feasible that the differential response to apomorphine in the various models is a reflection of the relative roles of D-1 and D-2 receptors. For example, it seems that D-1 receptors are not involved in any major way in the hypothermic effects produced by DA agonists (9), in contrast to an important involvement in the motor-activating effects of DA agonists (12, 19, 29, 30). Interestingly, we reported recently that mice, after long-term apomorphine treatment, were subsensitive to the hypothermic effects of a subsequent apomorphine challenge, unchanged in their sensitivity to the locomotor depressant effects ofapomorphine and supersensitive to the locomotor stimulant effects of apomorphine (43) in the absence of any change in D-1 or D-2 binding in the striatum (44). Thus, regardless of the cause, it seems clear that the effects of chronic drug treatments, including DME, can be manifested as variable responses to the one agonist. In any event, pharmacokinetic differences in the handling of apomorphine cannot explain the result as the treatments did not affect the locomotor depressant or hypothermic effects of apomorphine. Note also that Hall *et al.* (17) were unable to demonstrate any effect of chronic L-DOPA on apomorphine pharmacokinetics. The behavioural subsensitivity was not due to D-2 receptor subsensitivity, since there was no significant decrease in the number of D-2 receptors, as defined by [^{3}H]-spiperone and there was no significant alteration in the locomotor stimulant effects of the relatively selective D-2 agonist, bromocriptine. Indeed, the stimulation produced by bromocriptine in the carbidopa-treated animals was less than that seen in the vehicle- and DME-treated animals, to the extent where bromocriptine did not produce significant stimulation in these animals. Rather than a decrease, the D-2 receptor binding indicated that there may have been an increase in B_{max} of these receptors, with no change in the affinity, after both DME and carbidopa treatments. In this context, it was

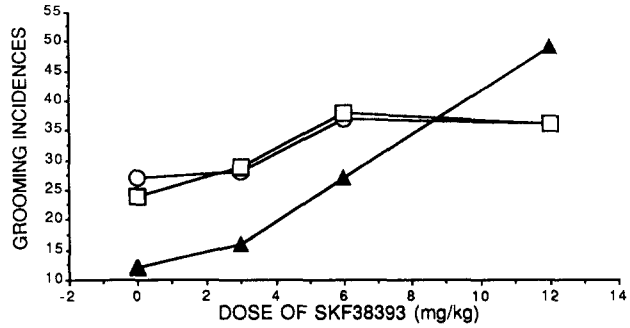

FIG. 2. Mice were treated for 28 days with DME (□), carbidopa (○) or vehicle (▲). One day after the withdrawal of the chronic treatments, mice were placed individually into experimental boxes for 1 hr, and then injected with SKF38393, or vehicle, and their grooming behaviour assessed as described in the Method section. For illustrative purposes, the data are expressed as the median total number of grooming incidences of each group for the whole total observation period. Kruskal-Wallis analysis of variance indicated that SKF38393 produced a highly significant change in grooming behaviour in the vehicle-treated animals, $H(3)=23.94$, $p<0.001$, but not in the DME- or carbidopa-treated animals (both $p>0.1$). Further analysis indicated that there was significantly more grooming in the vehicle-treated group than in either of the drug treatment groups after 12 mg/kg SKF38393 ($p<0.01$), and that both the carbidopa and DME groups exhibited more, but not significantly more, grooming behaviour after the vehicle challenge (i.e., SKF38393 0 mg/kg).

reported that L-DOPA treatment of rats produced an increase in the density of [^{3}H]-spiperone binding in the striatum (46), although other workers have reported no such change (17). The second aspect of the subsensitivity that needs to be considered is its cause. It seems clear that the carbidopa component was responsible. While this decarboxylase inhibitor does not readily cross the blood-brain barrier, its metabolite, αMDOPA, crosses readily (3,38). We administered αMDOPA to mice and, as predicted, demonstrated a subsensitivity to the locomotor stimulant effects of apomorphine, suggesting that the formation of αMDOPA from carbidopa may have been responsible for the behavioural effects observed. At the very least, these data indicate that studies which used carbidopa as a peripheral decarboxylase inhibitor may require reinterpretation [see, e.g., (8, 14, 27, 33, 35, 45)], especially where no appropriate control group was included (8, 14, 27, 33, 35, 45, 46). One item for future investigation will be to determine the precise neurochemical substrate of the subsensitivity.

Our results with apomorphine are also of interest because they indicate that the results obtained behaviorally with this drug may not agree with those from binding data. It is pertinent that we, and others, have previously noted an incongruity between D-2 binding data and behavioural assessments in a variety of models using apomorphine (7, 10, 17, 40, 43, 44). As mentioned above, part of this discrepancy may depend upon the fact that apomorphine is a mixed D-1/D-2 agonist.

There are few data reporting the effects of chronic L-DOPA on D-1 receptor function. One study (33) noted that apomorphine-induced contralateral rotation in the Ungerstedt rat was enhanced by chronic L-DOPA treatment, an effect antagonized by pretreatment with a selective D-1 antagonist. In another report, these workers found that SCH23390 binding to rat striatal membranes was unaltered by the chronic L-DOPA treatment, while the effect of the enkephalin D-Ala-2-Met-enkephalinamide on striatal adenyl cyclase was decreased in 6-hydroxydopamine-lesioned rats (34). In the present study, we used the ability of SKF38393 to produce an increase in grooming behaviour as a functional measure of D-1 receptor function. As expected, SKF38393 induced a rise in the

incidence of grooming behaviour in the control animals (5,29) and this was dose-dependent. No such significant increase was observed in the DME- and carbidopa-treated groups, and this seemed largely to be due to an increase in the incidence of basal grooming in these two groups. The highest dose of SKF38393, but not the lower doses, produced significantly more grooming in the vehicle than in the carbidopa and DME groups. This may indicate a reduced sensitivity to the effects of SKF38393, but this remains to be tested using ligand binding techniques and adenyl cyclase assays.

One of the hypotheses that initiated the present study was our finding that chronic L-DOPA can produce behavioural (1), electrophysiological (21) and biochemical (1) signs of DA autoreceptor subsensitivity. Using the depression of locomotor activity in mice as a behavioural index of DA autoreceptor function, we were unable to show any indication of a treatment effect, in agreement with biochemical data (35). Since DA autoreceptors are probably only of the D-2 subtype, this lack of behavioural evidence for a change in autoreceptor sensitivity is consistent with the lack of evidence for a change in post synaptic receptor sensitivity, as discussed above.

In comparing the present data with our previous results, it seems that the mode of drug administration is probably the cause for the marked differences in results. Similar differences are also probably partly responsible for the contradictions in the literature. The present treatment regime [and similar ones utilized in (46)] will (presumably) produce low plasma levels of L-DOPA which will be temporally related to food and/or fluid intake, whereas forced administration by injection or gavage [see, e.g., (1, 11, 35, 45)] will produce short-lived but high plasma levels. It seems likely that agonist-induced changes to normosensitive DA receptors may require a degree of DA receptor stimulation that cannot easily be achieved by the methods used in the present study. This is not, however, a unique observation as earlier workers have also observed that the mode of administration of a chronic drug treatment will influence the changes produced. For example, the effects of chronically administered amphetamines are to some extent determined by the way in which they are administered (32).

In conclusion, we have demonstrated that a 1-month treatment of mice with a moderate dose of L-DOPA produces complex alterations in the locomotor response to apomorphine that were not due to any change in the sensitivity of D-2 receptors, as assessed behaviourally and biochemically. Furthermore, the data provide strong evidence that carbidopa is pharmacologically active after chronic administration and will confuse the effects produced by L-DOPA alone.

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