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Effect of L-dopa alone and with benserazide on the spontaneous activity of striatal neurones in normal and 6-hydroxydopamine-lesioned rats

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1 The effects of L-dopa methylester (LDME), an analogue of levodopa, on the spontaneous activity of dopamine sensitive neurones in the rat striatum, after 6-hydroxydopamine induced degeneration of the nigrostriatal tract were compared with those in unlesioned animals both in the absence and presence of benserazide, a peripheral DOPA decarboxylase inhibitor (PDI).

2 Studies were performed at 5–7 days post lesion (group 1 animals), at 21 days (group 2) when denervation supersensitivity was evident by contralateral turning to apomorphine and at the same time but following 7 days dosing with LDME plus benserazide (group 3).

3 In unlesioned animals, LDME alone inhibited spontaneous firing by some 45% over 60 min including a marked but transient early phase which was still present in all lesioned animals even though the later inhibition was significantly reduced in group 1 and 3 animals.

4 When given after benserazide in unlesioned animals LDME still produced a similar level of overall inhibition but without the early phase. The lesion reduced the overall inhibition, except in group 2 animals, and after chronic dosing (group 3) it was almost absent.

5 It is proposed that since the early inhibition with LDME alone is still seen after lesion of the nigrostriatal tract but not after the PDI benserazide, it is caused by peripherally formed dopamine and that as the delayed inhibition with LDME alone and after benserazide are all reduced by nigrostriatal lesions, as is its amphetamine like ipsilateral turning, that this depends on locally (striatal) synthesized dopamine.

6 This study also shows that chronic levodopa/PDI treatment reduces the compensating increased activity of surviving dopaminergic neurones and the functional supersensitivity to dopamine suggests that the long term administration of levodopa may reduce its own utilization and activity in the striatum and in the treatment of Parkinson's Disease.

Keywords: L-Dopa methylester; extracellular single unit recording; 6-hydroxydopamine lesion; striatal neurone; spontaneous activity

Introduction

There have been numerous behavioural and biochemical studies related to the actions of L-3,4-dihydroxyphenyl-alanine (L-dopa) on dopaminergic function in the central nervous system. However, little is known about the effect of systemic L-dopa on the activity of dopamine sensitive neurones in the striatum, which are the principal target of the dopaminergic input from substantia nigra pars compacta (SNc, A9 neurones) and which ultimately have a primary role in regulating the activity of neurones in the globus pallidus and substantia nigra pars reticulata (SNr) (Chevalier & Deniau, 1990; Twery *et al*., 1993). They consequently determine the outcome of some L-dopa-induced changes in dopamine neurotransmission and most probably its efficacy in the treatment of Parkinson's Disease (PD).

The activity of striatal neurones and their responsiveness to dopamine will also depend on their degree of innervation and it is generally believed that in man the degeneration of the dopaminergic nigrostriatal tract in PD gives striatal neurones a supersensitivity to dopamine that declines with chronic levodopa therapy (Hornykiewicz, 1963). Whether levodopa can only be converted to dopamine in the striatum is unlikely (Kang *et al*., 1992) since it can still produce behavioural responses in rats after near complete lesion of the nigrostriatal tract (Breese & Taylor, 1970; Ungerstedt, 1971). Thus whilst dopamine may be essential for the action of dopa, dopaminergic neurones may not be (Zigmond *et al*., 1990b).

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We have therefore compared the effect of systemic L-dopa on the spontaneous activity of striatal neurones in normal rats with its action in the following groups: (1) rats 5–7 days after denervation of striatal neurones by 6-hydroxydopamine (6-OHDA) induced degeneration of the nigrostriatal tract; (2) those allowed 14–21 days following denervation for the development of supersensitivity and (3) those kept for 21 days but given L-dopa daily from day 14.

Since in Parkinsonian patients L-dopa is invariably given with a peripheral decarboxylase inhibitor (PDI), we have also compared the effects of L-dopa alone with those seen after it was given with benserazide in the same three groups.

It was hoped that comparing the effects of dopa on striatal neurone activity in normal and lesioned animals with and without a PDI would provide evidence for the site of synthesis of dopamine from dopa after degeneration of the nigrostriatal tract, and its mode of action in PD. Due to the difficulty in dissolving L-dopa in aqueous solution for intravenous administration at the required concentration it was given as the methyl ester, L-dopa methylester, (LDME).

Methods

Degeneration of nigrostriatal tract

Male Sprague-Dawley rats (200–220 g, University College London) were anaesthetized with Hypnorm (0.3 ml kg^{-1}, i.m.) plus diazepam (2.5 mg kg^{-1}, i.p.) and positioned in a stereotaxic frame with the incision sites and pressure points in-

filtrated with local anaesthetic (lidocaine 1%). A 0.3 mm needle was lowered 8 mm into the left substantia nigra through a small burr hole (caudal 5.2 mm; lateral 1.9 mm, relative to bregma) (Paxinos & Watson, 1986) and 8 μg (free base) 6-hydroxydopamine (6-OHDA) injected in 4 μl sterile saline at a rate of 0.8 μl min^{-1}. The location of the 6-OHDA injection site was verified histologically at the end of the electrophysiological experiments.

Rotational responses

Lesioned animals were tested behaviourally for their rotational response to D-amphetamine (2.5 mg kg^{-1}, i.p.) or apomorphine (0.25 mg kg^{-1}, i.p.) in random order at least 24 h before the commencement of extracellular single unit recording. Only complete rotations (360°) were counted and rats which showed less than 60 turns per h were considered to be inadequately lesioned (Pan *et al*., 1990) and not included in electrophysiological studies. Animals with an effective acute lesion show ipsilateral rotation to D-amphetamine since dopamine can only be released on the intact side whilst contralateral turning to the dopamine agonist apomorphine indicates that supersensitivity has developed on the denervated side (van Horne *et al*., 1992).

Extracellular single unit recording

For electrophysiological experiments, anaesthesia was induced and maintained with halothane (4% and 0.8%, perspectively) carried in 95% O_2 and 5% CO_2 delivered at a flow rate of 400 ml min^{-1}. A femoral vein was cannulated for drug administrations. Body temperature was maintained at 37 ± 0.5°C with a heating blanket and the animal held in and grounded to a stereotaxic frame. A glass-coated tungsten microelectrode with an impedance of 5–10 MΩ (Merril & Ainsworth, 1972) was lowered through a small burr hole 0.7–1.0 mm anterior to bregma and 2.7 mm lateral to the midline suture (Paxinos & Watson, 1986) to depths between 3.5 and 6 mm to monitor the spontaneous activity of single neurones within the striatum. Signals were amplified and displayed on an oscilloscope and a cumulative spike count (reset every 10 s) recorded on an alpha-numeric printer and connected to a calibrated chart recorder.

Extracellular recording was made from one neurone per animal. A single unit was identified as dopamine-sensitive on the basis of its electrophysiological characteristics i.e. wave form, firing pattern (Bunney *et al*., 1973; Napier *et al*., 1991) and its inhibition by apomorphine (0.1 mg kg^{-1}, i.v.). The basal firing rate was monitored for at least 10 min before drug adminstration. In all experiments LDME (50 mg kg^{-1}, i.p.) was injected alone and then 2 h later but 20 min after benserazide (20 mg kg^{-1}, i.v.). There was no significant difference between the basal firing rate before LDME alone or with benserazide (see Figure 3). At the end of some experiments apomorphine was given to determine the responsiveness of the neurone to changes in dopamine activity. Some typical results are shown in Figure 2. Saline administration did not affect firing, which was maintained over periods equivalent in time to those of the drug studies.

Biochemical analysis

At the end of recording, the brains were removed immediately after exsanguination and the tissue levels of dopa, dopamine and their metabolites in both the intact and denervated striata determined by high performance liquid chromatography (h.p.l.c.) (Chang & Webster, 1995). Each striatum was homogenized in 1 ml 1 M $HClO_4$, centrifuged for 45 min ($12000 \times g$) at 4°C to remove precipitated proteins and the supernatant frozen at −70°C for later analysis.

Drugs

L-3,4-Dihydroxyphenylalanine methylester (LDME), 6-hydroxydopamine (6-OHDA), L-ascorbic acid, apomorphine hydrochloride, dopamine hydrochloride and haloperidol were purchased from Sigma (U.K.). All drugs were dissolved in normal saline. Although LDME presented as a distinct h.p.l.c. peak from levodopa in aqueous solution it was only ever present as dopa in plasma.

Statistics

The experimental data, expressed as mean ± s.e.mean, were analysed statistically by Student's t test for paired or grouped data and one way ANOVA of appropriate variables coupled with the Newman-Keuls test. A P value less than 0.05 was considered statistically significant.

Results

Rotational response after nigrostriatal lesions

The effectiveness of the lesion was shown by the fact that when tested after 5–7 days group 1 rats showed ipsilateral turning (5.6 ± 1.3 turns min^{-1}) to D-amphetamine (2.5 mg kg^{-1}, i.p.) (Figure 1). After 14–21 days (group 2) there was contralateral turning (8.9 ± 2.3) to apomorphine (0.25 mg kg^{-1}, i.p.), which is indicative of the development of denervation supersensitivity on the lesioned side. Animals given LDME plus benserazide (50 and 20 mg kg^{-1}, i.p., respectively) daily from 14 to 21 days (group 3) showed ipsilateral turning (7.1 ± 1.6) to an acute administration of the same drugs but no response to apomorphine.

The effect of nigrostriatal lesion on the spontaneous activity of striatal neurones

The mean spontaneous firing rate of 34 active striatal neurones recorded in normal rats was 10.7 ± 1.3 spikes per 10 s (range 3.2 to 29.4). Destruction of the nigrostriatal pathway significantly ($P < 0.01$) increased the spontaneous discharge in all three groups of animals (Table 1). This increase was even greater ($P < 0.05$) after 14–21 days in group 2 animals than that in the acutely lesioned (group 1) animals and partly (but not significantly) reversed after one-week subchronic administration of LDME with benserazide (group 3) (Figure 2).

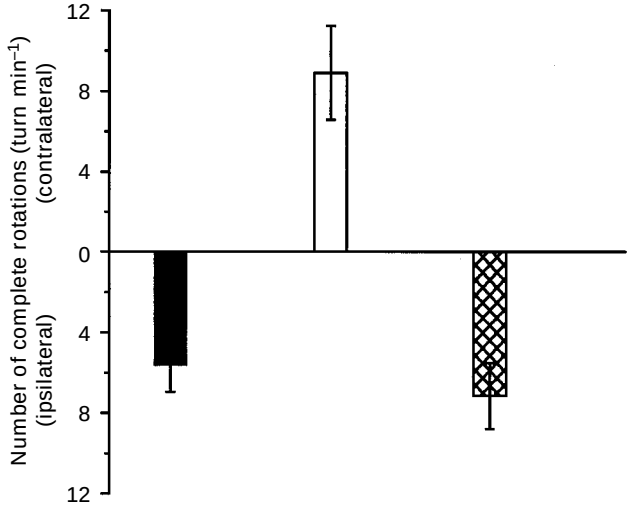

Figure 1 Rotational behaviour of nigro-striatal lesioned rats after administrations of D-amphetamine (2.5 mg kg^{-1}, i.p.; solid column), apomorphine (0.25 mg kg^{-1}, i.p.; open column) and LDME with benserazide (50 mg kg^{-1} and 20 mg kg^{-1}, i.p.; hatched column). For group details see text. Each value is the mean ± s.e.mean of 6 to 9 estimations.

Table 1 The effects of destruction of the nigrostriatal tract on the basal firing rate of rat striatal neurones

Group	Number of cells	Spikes 10 s^{-1} Mean	Min	Max	Significance§
Control	34	10.7±1.3	3.2	29.4	
1	7	28.9±6.8	5.5	58.5	$P<0.001$
2	7	49.0±9.4	12.1	90.0	$P<0.001$ ($P<0.05$†)
3	8	36.6±7.0	15.3	63.0	$P<0.01$

Values are expressed as mean±s.e.mean. Statistical analysis was done by one way ANOVA with Neuman-Keuls test. §Significantly different from control group, †Significantly different from Group 1.

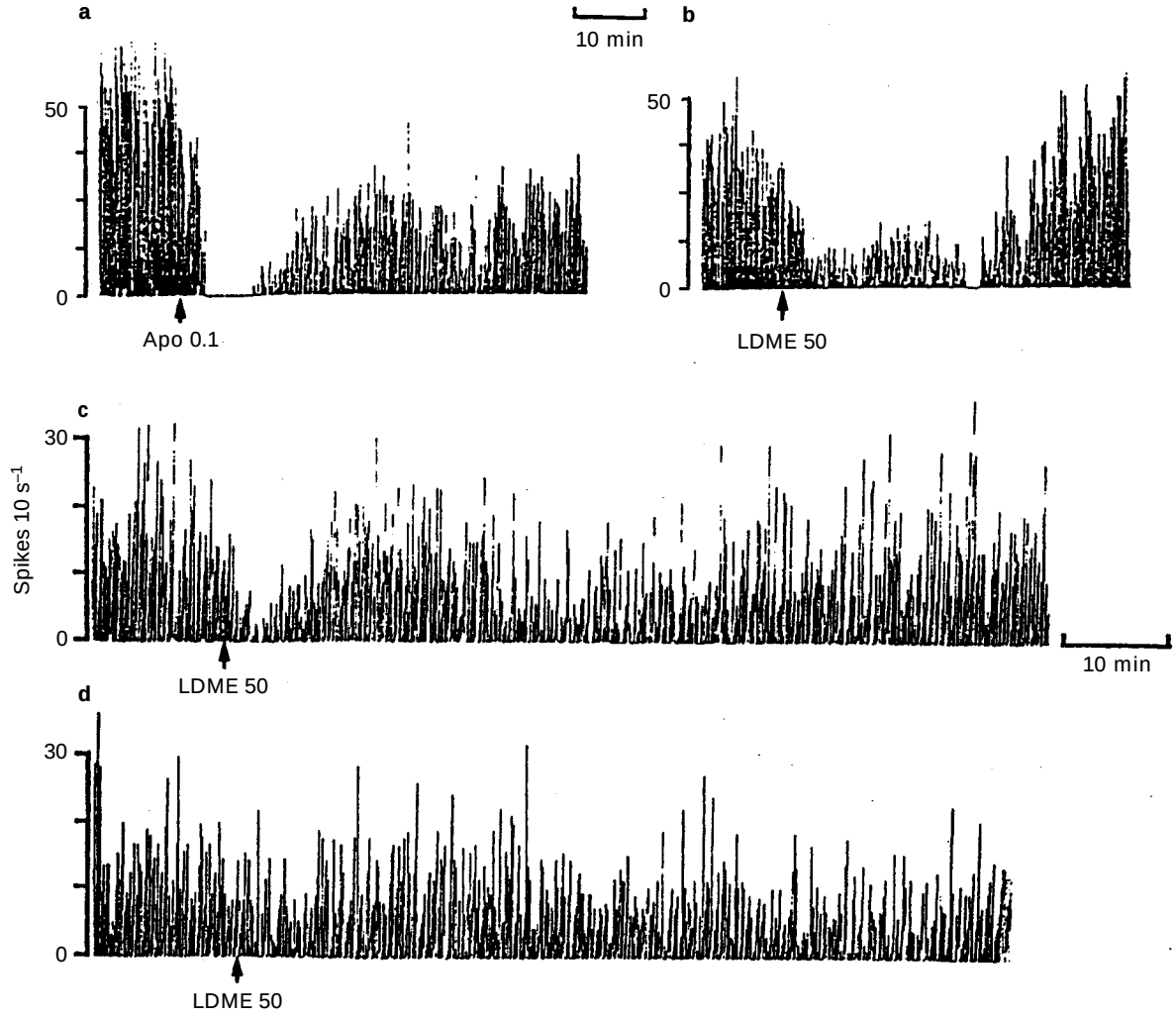

Figure 2 Representative rate histograms illustrating the inhibitory effects on the spontaneous discharge rate of a dopamine-sensitive neurone of apomorphine (0.1 mg kg^{-1}, i.v., a), and LDME (50 mg kg^{-1}, i.v., b) in 6-OHDA-lesioned rats (group 2) and the same dose of LDME alone (c) or given after benserazide (20 mg kg^{-1}, i.v., d) in group 1 animals.

Effect of LDME alone and after benserazide on striatal neurone firing

Unlesioned rats In animals with an intact nigrostriatal pathway LDME (50 mg kg^{-1}) produced a marked reduction in the spontaneous activity of striatal neurones. The inhibitory effect, which tended to be biphasic, was fairly rapid in onset to about 50% of basal firing rate within 5 min and then, after some transient recovery, showed a further reduction to 40% at 45 min. Thirty min after the same dose of LDME given after benserazide (20 mg kg^{-1}), striatal neurone activity was also inhibited but not to the same extent as with LDME alone but the early inhibition (within the first 20 min) was virtually absent. Nevertheless, the overall inhibitory effects for the two treatments were not statistically different (Figure 3).

Group 1 animals (acute lesion) After LDME alone there was a rapid, transient and significant inhibition of the spontaneous discharge of striatal neurones to about 70% (within 5 min) followed after some recovery by a secondary reduction to the same level at 40 min. This inhibitory effect was analogous in time course to, but significantly less ($F_{(1,112)}=16.511$, $P<0.001$) than, that produced by LDME in the intact animals (Figure 4a). When LDME was given after benserazide, there was little inhibition of neurone firing (Figure 5a) and the overall effect was significantly less ($F_{(1,112)}=7.20$, $P<0.01$) than that in intact rats with the same drug combination.

Group 2 animals (chronic lesion) In these animals, 14–21 days post lesion, the overall time courses of the inhibitory effects exerted by LDME alone and with benserazide on the

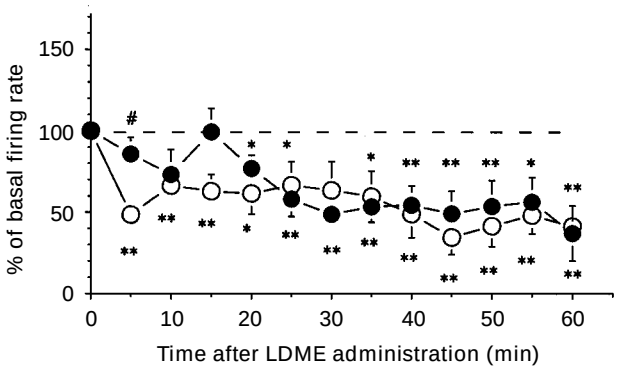

Figure 3 Time course of the effect of intravenous L-dopa methylester (LDME, 50 mg kg^{-1}) alone (○) or with benserazide (20 mg kg^{-1}) (●) on the spontaneous activity of neurones in the intact striatum. The mean basal discharge rate (100%) before LDME alone and after benserazide were 14.8 ± 4.2 and 10.8 ± 2.5 spikes $10\ s^{-1}$, respectively. Each value is the mean and vertical lines s.e.mean of 6 experiments. $^{*}P<0.05$ and $^{**}P<0.01$ significant difference from time 0 by paired t test; $^{\#}P<0.05$ significant difference from LDME alone by Student's t test.

spontaneous firing rate of striatal cells were almost identical with those in intact rats (Figure 4b and 5b) except for a significantly greater inhibition seen within 10 min after LDME alone (Figure 4b). In accord with the results obtained in the intact animals, the overall inhibitory effects of LDME alone and with benserazide did not differ significantly ($F_{(1,137)}=3.04$, $P=0.084$).

Group 3 animals (chronic lesion but subchronic administration of LDME with benserazide) In lesioned animals which had received LDME plus benserazide daily from days 14 to 21, LDME alone produced an equivalent inhibition of neurone firing to that seen in intact animals within the first 20 min post drug. However, this inhibition did not last and the overall inhibitory effect was significantly less ($F_{(1,112)}=5.82$, $P<0.02$) than in the intact group (Figure 4c). Unexpectedly, little inhibitory effect was observed within the one hour recording period after intravenous LDME in the presence of benserazide. The total effect of LDME with benserazide was not only significantly less ($F_{(1,112)}=13.81$, $P<0.001$) than that obtained in the intact animals (Figure 5c) but also significantly less than that of LDME alone in the same group ($F_{(1,112)}=9.26$, $P<0.01$)

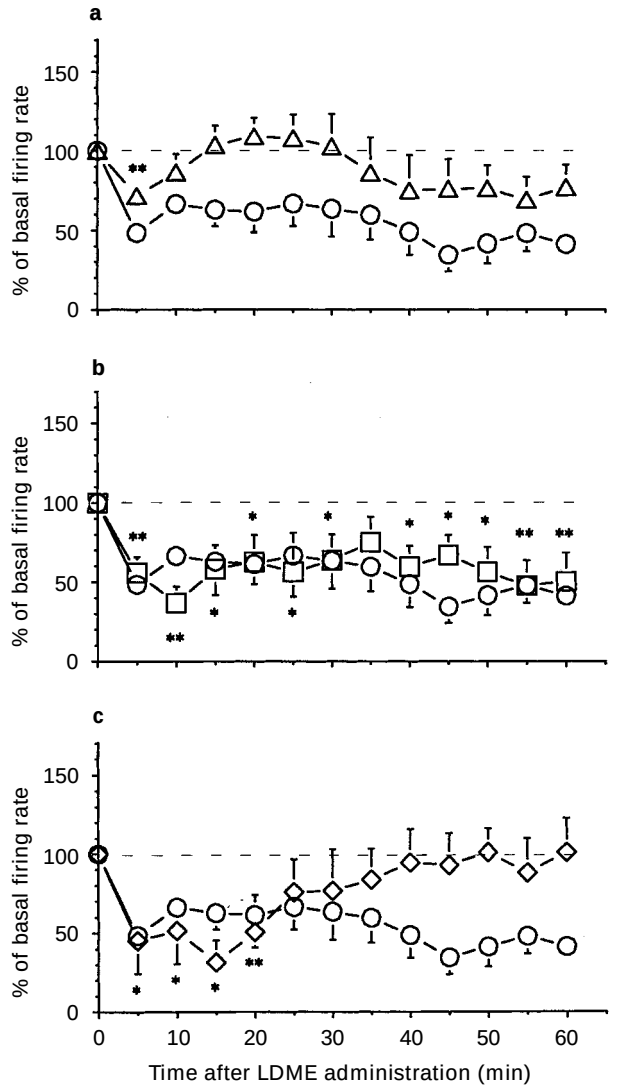

Figure 4 Time-course of the effect of LDME (50 mg kg^{-1}, i.v.) on the spontaneous activity of striatal neurones in 6-OHDA-lesioned rats compared with that in control animals (○) (see Figure 3). Results are shown (mean ± s.e.mean) for (a) group 1 animals with acute lesions (△, $n=5$), (b) group 2 animals 14–21 days post lesion (□, $n=7$) and (c) group 3 animals at 21 days (but treated with LDME 50 and benserazide 20 mg kg^{-1}, i.p.) (◇, $n=6$). $^{*}P<0.05$ and $^{**}P<0.01$ significant difference from time 0 by paired t test.

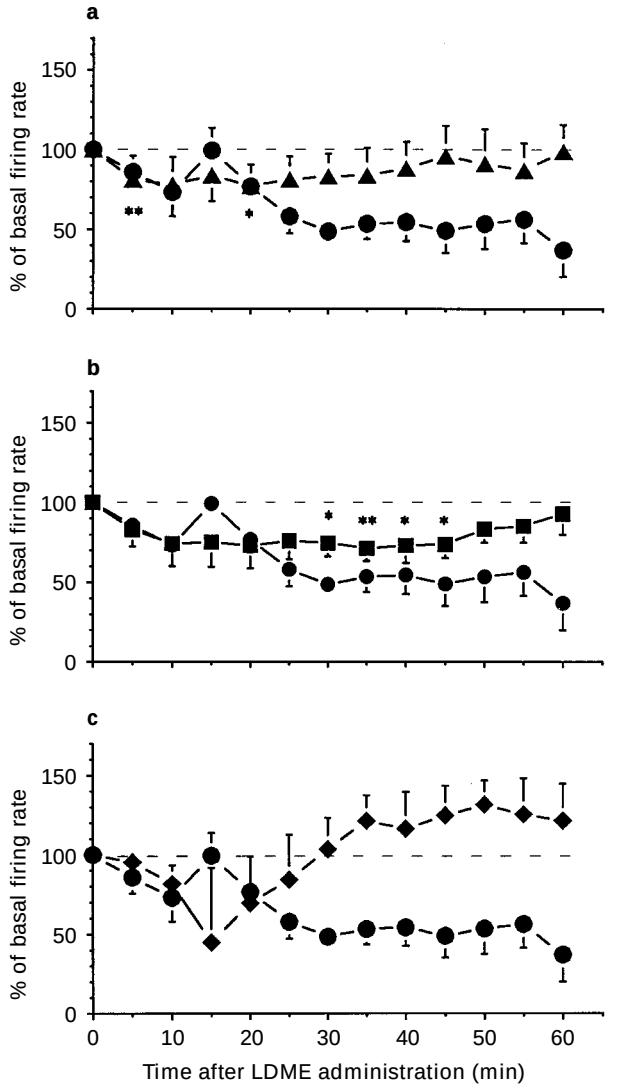

Figure 5 Time-course of the effect of LDME (50 mg kg^{-1}, i.v.) given 20 min after benserazide (20 mg kg^{-1}, i.v.) on the spontaneous activity of striatal neurones in 6-OHDA-lesioned rats compared with that in control animals (●) (see Figure 3). Results are shown (mean ± s.e.mean) for (a) group 1 animals with acute lesions (▲, $n=5$), (b) group 2 animals 14–21 days post lesion (■, $n=7$) and (c) group 3 animals (at 21 days but treated with LDME 50 and benserazide 20 mg kg^{-1}, i.p. for 14–21 days) (◆, $n=6$). $^{*}P<0.05$ and $^{**}P<0.01$ significant difference from time 0 by paired t test.

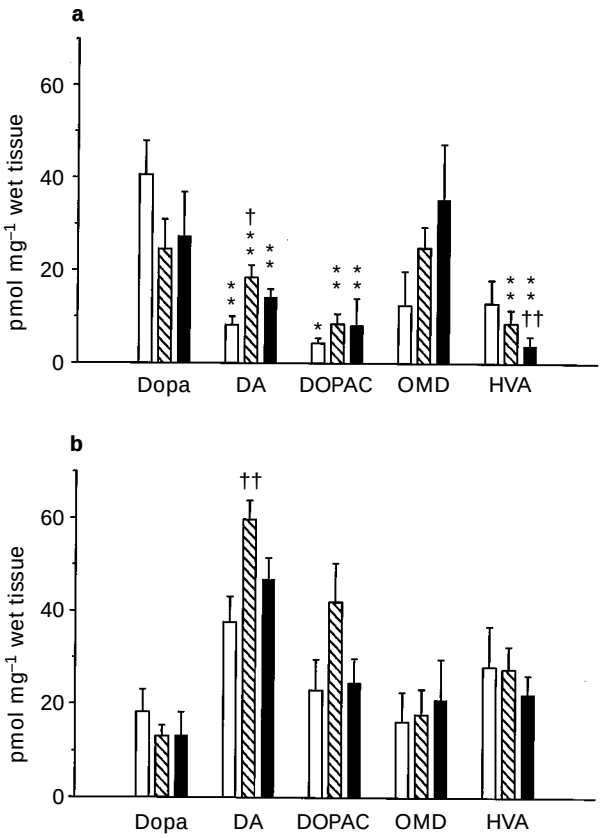

Figure 6 Levels of dopa, dopamine and their metabolites in denervated (a) and intact (b) striata of unilaterally 6-OHDA-lesioned rats. Animals with acute lesions (group 1, open columns), after 14–21 days (group 2, hatched columns) and after a similar period but with daily administrations of LDME plus benserazide from 14–21 days (group 3, solid columns). All animals were given LDME (50 mg kg^{-1}, i.v.) alone 3 h and then after 20 mg kg^{-1} benserazide 1 h before death. Values are expressed as pmol mg^{-1} wet tissue (mean ± s.e.mean of 6 or 7 rats). Statistically significant difference from the intact striatum are as indicated: $*P<0.05$; $**P<0.01$ (Student's paired t test). $\dagger P<0.05$; $\dagger\dagger P<0.01$ significantly different compared to rats with acute lesions.

as well as other lesioned groups. However, a profound inhibition on neurone firing with rapid onset could still be seen after the intravenous administration of apomorphine (0.1 mg kg^{-1}) at the end of the recording period.

Utilization of dopa after 6-OHDA lesions

As shown in Figure 6 and Table 2 the levels of dopamine and 3,4-dihydroxyphenylacetic acid (DOPAC) were significantly lower in the denervated compared with the intact striata of all three groups. HVA levels were also reduced but this only reached significance in group 3 animals. There was no significant difference in the levels of dopa and 3-O-methyldopa (OMD) between the sides, although dopa levels were higher in the denervated side. Surprisingly dopamine levels were significantly higher in both striata of group 2 animals than in those of the acutely lesioned animals (group 1).

Discussion

Before the effects of lesion of the nigrostriatal tract on the response of rat striatal neurones to levodopa, given in these experiments as its methyl ester (LDME), can be evaluated it is necessary to establish the existence of the lesion and the action of LDME in normal animals.

Apart from histological validation the lesions were confirmed by the ability of systemic amphetamine to induce ipsilateral turning (Ungerstedt, 1971; Sullivan *et al*., 1994) in rats 5–7 days (group 1 animals) after injecting 6-OHDA into one substantia nigra (Figure 1) and by the reduced levels of dopamine and its metabolites DOPAC and homovanillic acid (HVA) in the lesioned striata (Figure 6). Despite this the HVA/DA ratio (Table 2), an index of dopamine turnover in remaining terminals (Zigmond *et al*., 1990a, b) was still significantly elevated in the acutely lesioned (group 1) animals confirming the finding of Altar *et al*. (1987) that turnover is maximally elevated at the earliest post-operative time studied. The increased levels of DOPA in denervated striata also indicate a loss of terminal to synthesize dopamine although in their absence it is not clear where the dopa is retained. The fact that 21 days post lesion (group 2) the dopamine agonist apomorphine was able to induce contralateral turning not only

Table 2 Striatal tissue contents of dopa, dopamine, DOPAC, OMD and HVA in 3 groups of 6-OHDA-lesioned rats after LDME and benserazide

Group	n	*Tissue contents* (pmol mg^{-1}) *Lesioned*		*Intact*		*Percentage lesioned/intact*
Dopa						
1	6	40.6 ± 7.4		18.2 ± 4.9		313 ± 64
2	7	24.6 ± 6.4		13.1 ± 2.3		222 ± 46
3	8	27.4 ± 9.6		13.1 ± 5.0		249 ± 38
Dopamine						
1	5	8.2 ± 1.9**		37.6 ± 5.5		21.8 ± 3.3
2	7	18.4 ± 2.7**†		59.7 ± 4.1††		31.8 ± 4.6
3	7	14.2 ± 1.7**		46.8 ± 4.6		33.7 ± 6.7
DOPAC						
1	6	4.3 ± 1.1*		23.0 ± 6.6		15.8 ± 4.3
2	7	10.8 ± 2.1**		41.9 ± 8.3		18.2 ± 4.2
3	7	8.2 ± 5.7**		24.6 ± 5.2		16.4 ± 12.6
OMD						
1	5	12.4 ± 7.3		16.2 ± 6.3		152 ± 76
2	7	24.8 ± 4.5		17.8 ± 5.4		164 ± 50
3	7	35.4 ± 11.9		21.0 ± 8.6		162 ± 31
HVA						
1	6	12.9 ± 5.0	(1.9 ± 0.7)	28.0 ± 8.8	(0.7 ± 0.2)	47.7 ± 10.5
2	7	8.5 ± 2.9**	(0.7 ± 0.1)	27.5 ± 4.9	(0.4 ± 0.0)	29.5 ± 6.5
3	7	3.9 ± 1.9††	(0.1 ± 0.0)	22.2 ± 4.0	(0.4 ± 0.0)	9.1 ± 3.3††

Group 1, with acute lesions; Group 2, 3 weeks after the lesions; Group 3, as for Group 2 rats but treated every day for 1 week with two intraperitoneal injections of LDME (50 mg kg^{-1}) plus benserazide (20 mg kg^{-1}). Each value represents the mean ± s.e.mean of n determinations in each group. $*P<0.01$; $**P<0.01$ significantly different from the intact (right) side; †, $P<0.05$; ††, $P<0.01$ significantly different from the acute lesioned group (Group 1). All animals were given LDME (50 mg kg^{-1}, i.v.) alone 3 h and then after 20 mg kg^{-1} benserazide 1 h before death. Values in parentheses show HVA/dopamine ratio.

establishes the presence of denervation supersensitivity but, as this is thought to occur only when dopamine depletion exceeds 90% (Creese & Snyder, 1979), further establishes the effectiveness of the lesions.

The nigrostriatal lesions resulted in a significant increase in the basal firing of striatal neurones (Table 1). Although the nigrostriatal tract may provide both excitatory and inhibitory influences on striatal cells (Akaike *et al*., 1987; Ohno *et al*., 1987; Hu & Wang, 1988; Williams & Millar, 1990) the elevated discharge implies that inhibition dominates (Schultz & Ungerstedt, 1978; Florio *et al*., 1993) and indeed Orr *et al*. (1986) found the most marked increase in striatal cell firing to be associated with a near total depletion of dopamine after 7–9 days of lesion. This may explain why the firing was highest at 21 days (group 2, Table 1) and indicates that as dopamine is inhibitory its reduced release must be more important than the developed supersensitivity (and increased receptor number) confirmed to exist in this group by the contralateral turning to apomorphine (Figure 1). Surprisingly the levels of striatal dopamine were actually higher in this group but because the animals had received LDME previously this may not have been in a releasable pool. After dosing for several days with LDME plus benserazide (group 3) there was a partial reversal of the elevated firing. If this reflects a dopa-induced restoration of dopamine inhibition, it further supports the value of dopamine release over a change in postsynaptic responsiveness, since the loss of contralateral turning to apomorphine after LDME dosing would suggest a reduced receptor number and dopamine effect.

In control animals the overall inhibitory effect of LDME over the 60 min recording period was similar whether given alone or with benserazide (Figure 3) but there was a pronounced initial inhibition (to 30%) over the first 20 min with LDME alone that was not seen in the presence of benserazide. As the obvious difference between the two groups is the peripheral synthesis of dopamine without benserazide it is possible that such dopamine could be the cause of the early inhibition. The fact that it was still present after LDME alone in all three groups of lesioned animals (Figure 4), when little dopamine could have been formed in the striatum, supports this contention as does its absence in all lesioned animals when LDME was given with benserazide (Figure 5) and dopamine could not have been formed peripherally.

Although dopamine is not generally thought to cross the blood brain barrier (BBB), plasma and striatal dialysates from rats show similar early dopamine peaks after intravenous LDME alone (Chang & Webster, 1993). Such peaks are not seen after LDME with benserazide but match timewise the early inhibition of firing which could reflect the neuronal action of dopamine crossing into the striatum from the periphery. In fact intravenous dopamine (1–25 mg kg^{-1}) can cause a dose-related inhibition of cell firing and neither this, nor that achieved after levodopa administration, is associated with any change in blood pressure other than a small transient rise (Chang, 1995).

The later and slightly lower level of inhibition (42%) seen after LDME alone in control animals (Figure 3) is more likely to depend on dopamine formed from dopa within the striatum because it is also found, although surprisingly not to a greater extent, when dopa is given with benserazide and when it can only be decarboxylated centrally. This is supported by the reduction in this inhibition in all lesioned animals, except after the development of supersensitivity (Figure 5b), when there can be less local synthesis of dopamine. The ipsilateral, amphetamine-like, turning produced by LDME plus benserazide (Figure 1) in lesioned animals also indicates its reliance on the local striatal synthesis and release of dopamine.

Looking at the lesioned groups in more detail. The early inhibition with LDME alone was slightly reduced in acutely lesioned animals (group 1, Figure 4a) possibly due to the greater difficulty of overcoming the increased basal firing after lesion (Table 1), whereas in group 2 animals it was significantly enhanced, despite an even higher basal firing, possibly because of the increased number of receptors for the peripherally formed dopamine to act on and the loss of presynaptic terminal uptake (Snyder *et al*., 1990). The retention of overall inhibition, at a level similar to that in controls, after both treatments in group 2 animals is probably explained by and emphasizes the importance of, denervation supersensitivity in the response to exogenous dopamine. Similarly the significant loss of inhibition, apart from the early phase with LDME alone, in group 3 animals when they had been treated daily with LDME plus benserazide shows how the supersensitivity can be reduced by L-dopa therapy. This contention is supported by the failure of apomorphine to induce contralateral turning in those animals (group 3) and the reduction (non-significant) in basal firing rate compared with group 2 animals.

The fact that LDME with benserazide was no more effective than LDME alone in control animals and less effective in all lesioned animals (especially group 3) is difficult to reconcile with the clinical use of L-dopa plus benserazide or carbidopa. Possibly the effectiveness of L-dopa with a PDI in PD relies on the retention of some nigrostriatal innervation or at least striatal synthesis of dopamine, and may explain why such therapy loses its effectiveness in some patients with further degeneration of the dopaminergic pathway. The elevated level of OMD, even if not significant, on the lesioned side of group 3 animals (Figure 6), compared with that on the control side (or any side of group 1 and 2 animals) was also accompanied by lower levels of HVA ($P<0.01$) and since it has been shown that OMD significantly reduces dopamine efflux in striatal slices after superfusion with L-dopa (Chang & Webster, 1995) it is possible that OMD affects the turnover of dopamine after long term treatment with L-dopa plus a PDI. Certainly the cause of the ineffectiveness of this drug combination appears to be pre- rather than postsynaptic since the neuronal response to the directly acting dopamine agonist apomorphine was still retained.

These results provide experimental support for the contention that in PD degeneration of the nigrostriatal tract causes an initial supersensitivity to dopamine which may be reduced by subsequent dosing with L-dopa. They also suggest that compared with oral dosing there is no benefit in giving intravenous DOPA with a PDI rather than alone even though it increases the striatal levels of DOPA. Possibly the neurones we recorded from or their response to DOPA were not typical of those affected in PD (despite responding to apomorphine) or the technical difficulties of not being able to record for long periods missed a later effect of LDME plus benserazide. A 6-OHDA lesion may not, of course, be truly characteristic of that in PD but these results suggest that L-dopa, especially when given chronically with a PDI, may reduce its own utilization and activity in the striatum and so in the treatment of PD. Since the results also indicated that dopamine may cross the blood brain barrier, a study of intravenous L-dopa alone with a peripherally acting dopamine antagonist such as domperidone could be worthwhile.

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