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MK801 influences L-DOPA-induced dopamine release in intact and hemi-parkinson rats

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Abstract

In vivo microdialysis was used to investigate the influence of dizocilpine (MK801) on basal and levodopa (L-DOPA)-induced extracellular dopamine levels in striatum and substantia nigra of intact and 6-hydroxydopamine-lesioned rats. In lesioned rats, extracellular dopamine was decreased in striatum but not in substantia nigra. L-DOPA (25 mg/kg i.p. after benserazide 10 mg/kg i.p.) increased the dopamine levels in striatum and substantia nigra of intact and dopamine-depleted rats. This increase was significantly higher in dopamine-depleted compared to intact striatum. Pretreatment with MK801 (0.1 and 1.0 mg/kg i.p.) dose-dependently attenuated the L-DOPA-induced dopamine release in substantia nigra of intact rats. In dopamine-depleted striatum, MK801 enhanced L-DOPA-induced dopamine release. The present results indicate that the influence of MK801 on L-DOPA-induced dopamine release in striatum and substantia nigra depends on the integrity of the nigrostriatal pathway. In Parkinson's disease, NMDA receptor antagonists could be beneficial by enhancing the therapeutic efficacy of L-DOPA at the level of the striatum.

Keywords: Dizocilpine (MK801); Levodopa; 6-Hydroxydopamine; Microdialysis

1. Introduction

Until now, levodopa (L-DOPA) remains the most established anti-parkinsonian drug. Due to its conversion to dopamine by aromatic amino acid decarboxylase, it restores the dopamine depletion in the brain of patients suffering from Parkinson's disease.

The long-term use of L-DOPA is associated with several side effects, such as abnormal movements, which can become quite disabling (Lesser et al., 1979). Therefore, amelioration of long-term treatment of patients with Parkinson's disease is needed. Excessive glutamate input into several brain areas of the basal ganglia is a known physiopathological feature of Parkinson's disease (Bergman et al., 1990). Pharmacological intervention with glutamate receptor antagonists could therefore contribute to amelioration of the current L-DOPA therapy. Behavioral effects of the combined use of glutamate receptor antagonists and L-DOPA have been studied, both in intact and dopamine-depleted animals (for review, Starr, 1995). Dizocilpine (MK801), an antagonist of the NMDA receptor, induces motor stimulation by itself in intact rats, but these properties are lost after dopamine depletion. Lower doses of MK801 improve the efficacy of threshold doses of L-DOPA on behavioral activation in dopamine-depleted animals. However, only few studies exist on the neurochemical changes induced by the concomitant administration of MK801 and L-DOPA (Vila et al., 1999; Engber et al., 1993; Miller and Abercrombie, 1999; Biggs et al., 1996).

The striatum is probably a major site for pharmacological interactions between NMDA receptor antagonists and dopamine, as suggested by several findings: the greater striatal [^{3}H]MK801 binding compared to other basal ganglia nuclei (Ball et al., 1994), the upregulation of NMDA receptors in dopamine-depleted striatum (Tremblay et al., 1995; Wüllner et al., 1994; Samuel et al., 1990), the convergence of glutamatergic and dopaminergic inputs on

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individual medium spiny neurons (Cepeda et al., 1993; Kötter, 1994), and the potentiation of dopamine D1 receptor agonist-induced c-*fos* expression in the dorsolateral striatum with NMDA receptor blockade (Morelli et al., 1992). On the other hand, the substantia nigra is also regarded as a possible target area for the action of NMDA receptor blockers because of the presence of NMDA receptors in the substantia nigra (Christoffersen and Meltzer, 1995), which are substantially decreased in 6-hydroxydopamine-lesioned rats (Wüllner et al., 1994), the effect observed of MK801 on nigral aromatic amino acid decarboxylase activity (Fisher et al., 1998) and the interaction between MK801 and L-DOPA at this level (Biggs et al., 1996; Vila et al., 1999). Both brain areas have been shown to be target regions of L-DOPA decarboxylation, since in both intact and dopamine-depleted rats, systemic administration of L-DOPA increases extracellular levels of dopamine in striatum (Abercrombie et al., 1990; Miller and Abercrombie, 1999; Sarre et al., 1992; Orosz and Bennett, 1992) and substantia nigra (Sarre et al., 1992; Orosz and Bennett, 1992).

In this study, we used the microdialysis technique in freely moving rats to investigate the effect of the systemic administration of MK801 on L-DOPA-induced dopamine release. We used both intact rats and rats with a 6-hydroxydopamine lesion in the medial forebrain bundle to compare the neurochemical changes induced by both drugs. Since the striatum, as well as the substantia nigra, are important in L-DOPA pharmacology, extracellular dopamine levels were determined concomitantly in these two brain areas.

2. Materials and methods

2.1. Chemicals

Sigma, St. Louis, MO, USA: dopamine, L-DOPA methylester HCl, benserazide HCl, decane sulfonic acid, 6-hydroxydopamine. Merck, Darmstadt, Germany: sodium chloride, potassium chloride, calcium chloride hexahydrate, acetonitrile, orthophosphoric acid, sodium acetate trihydrate, disodium EDTA, sodium disulphite, hydrochloric acid. Janssen Chimica, Geel, Belgium: 3,4-dihydroxybenzylamine HBr. RBI, Natick, MA, USA: dizocilpine maleate. Roche: ascorbic acid.

All aqueous solutions were prepared in fresh water purified by a Seralpur Pro 90 CN system (Merck Belgolabo, Overijse, Belgium) and filtered through a membrane filter with pore size 0.2 μm.

2.2. Animals and surgery

Animal experiments were carried out according to the national guidelines on animal experimentation and were approved by the Ethical Committee for Animal Experiments of the Faculty of Medicine of the Vrije Universiteit Brussel.

2.2.1. 6-Hydroxydopamine lesions

Male albino Wistar rats weighing 180–200 g were anaesthetised with a mixture of ketamine/diazepam (50:5 mg/kg i.p.) and placed on a stereotaxic frame. The skull was exposed and a burr hole was drilled to introduce a syringe for injecting the 6-hydroxydopamine solution (containing 4 μg 6-hydroxydopamine per μl in 0.01% ascorbic acid, pH 5.00). The solution was injected into the left medial forebrain bundle, according to the atlas of König and Klippel (1963), for rats weighing 150–200 g. Coordinates relative to bregma were L −1.4, A −3.5 and V +7.8. A total volume of 4 μl was injected at a flow rate of 1 μl/min, introducing 16 μg 6-hydroxydopamine into the medial forebrain bundle. The syringe was left in place for 2 min and was then slowly removed over a 1–2-min time period. The skin was sutured, the animals received 4 mg/kg ketoprofen i.p. as analgesic, and were allowed to recover before returning to the animal housing facilities.

2.2.2. Microdialysis experiments

Microdialysis experiments were performed either on intact rats or on 6-hydroxydopamine-lesioned rats 18–21 days after the 6-hydroxydopamine lesion. The rats, weighing ±250 g, were anaesthetised with a mixture of ketamine/diazepam (50:5 mg/kg i.p.) and placed on a stereotaxic frame, with bregma placed 1.0 mm higher than lambda. The skull was exposed and burr holes were drilled to implant two guide cannulas (CMA Microdialysis, Stockholm, Sweden). Guide cannulas were positioned 3 mm above the left striatum and 2 mm above the ipsilateral substantia nigra, according to the atlas of Paxinos and Watson (1986), for rats weighing 250–320 g. Coordinates relative to bregma were L −2.4, A +1.2, V +2.8, and L −1.4, A −5.0, V +6.5, respectively. After surgery, the rats received 4 mg/kg ketoprofen i.p. as analgesic.

2.3. Brain microdialysis

After surgery, probes were introduced into the cannulae after removal of the guide (CMA 10 microdialysis probes). The membrane length was 3 mm for striatum and 2 mm for substantia nigra. The probes were perfused with modified Ringer's solution containing 147 mM NaCl, 1.1 mM $CaCl_2 \cdot 6H_2O$, and 4 mM KCl at a constant flow rate of 2 μl/min using a CMA 100 microdialysis pump (CMA Microdialysis). Animals were allowed to recover from surgery overnight; dialysate collection was started the day after the surgery. Samples were collected every 20 min in 10 μl of an antioxidant mixture (0.02 M HCl, 0.2% $Na_2S_2O_5$, 0.02% Na_2EDTA) to protect dopamine from

degradation. Dopamine levels were assayed on the day of the experiment.

2.4. Experimental setup

Before any pharmacological manipulation was performed, six dialysate samples were collected. The mean of these neurotransmitter dialysate concentrations is taken as basal value at time zero. Drugs were administered from collection seven onwards. For i.p. administration, the water-soluble salts of the drugs were dissolved in saline at a dose of 1 ml/kg. The animals received 25 mg/kg L-DOPA i.p. at time 60 min; 30 min after the blockage of peripheral aromatic amino acid decarboxylase by 10 mg/kg benserazide i.p., MK801 (0.1 or 1.0 mg/kg) was given immediately after the basal samples; at time 20 min, either alone or followed by the administration of L-DOPA/benserazide. The behavior of the animals was observed but not quantified. To determine the extent of dopaminergic denervation, the 6-hydroxydopamine-lesioned rat was killed with an overdose of pentobarbital immediately after the experiment; the brain was removed and both striata were dissected and frozen at −80°C until the determination of tissue levels of dopamine.

2.5. Determination of extracellular levels of dopamine

Determination of extracellular dopamine levels was performed by microbore liquid chromatography (column i.d. 1.0 mm) with automatic injection (10 μl) of the samples, and has been described in detail previously (Smolders et al., 1996). In summary, the assay of dopamine was based on ion pair reversed phase (C8) chromatography, coupled to electrochemical detection (Decade, Antec, Leiden, The Netherlands). The mobile phase consisted of 28 ml acetonitrile and 200 ml of the following buffer: 0.1 M sodium acetate trihydrate, 20 mM citric acid monohydrate, 2 mM decane sulfonic acid and 0.5 mM sodium EDTA adjusted to pH 5.5. The oxidation potential was set at +450 mV. The detection limit of the assay was 0.04 nM.

2.6. Determination of tissue levels of dopamine

To establish the extent of the dopamine depletion in striatum, the method described by Izurieta-Sanchez et al. (1998) was used. In brief, the striata were weighed, antioxidant and internal standard (dihydroxybenzylamine 100 ng/100 μl) were added. The tissue was homogenized, then centrifuged; the supernatant was diluted in acetic acid and analysed directly for dopamine content by means of ion pair reversed phase chromatography (C18) with a 20-μl injection loop. The mobile phase consisted of 0.1 M sodium acetate, 20 mM citric acid, 1 mM 1-octane sulfonic acid, 0.1 mM Na_2EDTA and 1 mM dibutylamine, adjusted to pH 3.8. Isopropanol 1.5% (v/v) was added as an organic modifier. The flow rate was set at 1 ml/min. Dopamine was detected electrochemically and the detector potential was +700 mV vs. the reference electrode (Ag/AgCl). Sensitivity was set at 2 nA full scale. Tissue dopamine content was expressed as nanogram dopamine/gram wet tissue; the detection limit of the system was 2.5 ng/g dopamine. The amount of dopamine on the intact side (contralateral) was equaled to 100%, and the dopamine content on the denervated side was expressed as percent depletion compared to this intact side.

2.7. Statistical analysis

Extracellular dopamine levels were expressed in nM. No corrections were made for probe recovery across the dialysis membrane. For statistical significance of differences in dopamine levels after administration of the drugs compared to baseline values, a one-way analysis of variance (ANOVA) for repeated measures and Fisher's protected least significant difference (Fisher's PLSD) test ($\alpha = 0.05$) were used. Two-tailed Mann–Whitney *U*-test was used to compare the concentrations for different treatments at the same time point.

3. Results

3.1. Influence of the 6-hydroxydopamine injection into the medial forebrain bundle on tissue levels of dopamine in striatum and substantia nigra

The rats included in this study had no detectable striatal tissue dopamine under the analytical conditions described. This corresponds to a residual tissue dopamine content of

Table 1
Extracellular basal values of dopamine in striatum and substantia nigra of intact and 6-OHDA-lesioned rats

	n	Mean (nM)	S.E.M.
Striatum			
Intact	35	1.86	0.25
6-OHDA	23	0.21	0.05
Substantia nigra			
Intact	34	0.25	0.03
6-OHDA	22	0.28	0.03

Extracellular basal values of dopamine, expressed in nM (mean ± S.E.M.), in striatum and in substantia nigra of intact rats and rats with a 6-hydroxydopamine lesion of the medial forebrain bundle.

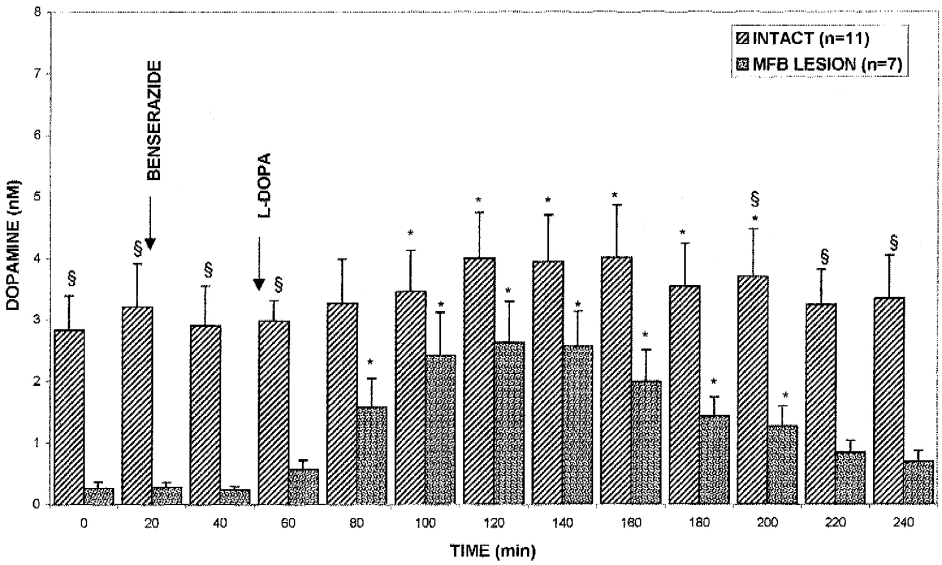

Fig. 1. The effect of 25 mg/kg levodopa (with 10 mg/kg benserazide as a blocker of peripheral dopa decarboxylase) on extracellular levels of dopamine in striatum of intact (hatched bars, $n = 11$) and 6-OHDA-lesioned rats (grey bars, $n = 7$). Data are expressed in nM (mean ± S.E.M.). The baseline value at time 0 is the mean of six baseline values. Significance of differences from the baseline value is determined by one-way ANOVA for repeated measures followed by Fisher PLSD test: $^{*}P < 0.05$. Differences between both groups were determined by Mann–Whitney: §$P < 0.05$.

less than 2.5 ng/g. In intact striatum, tissue dopamine content is about 18,000 ng/g, so this represents > 99% depletion of dopamine compared to control values. In a separate set of experiments, we determined the tissue dopamine content in the ipsilateral substantia nigra of rats with a > 99% depletion of tissue dopamine content in the striatum. Compared to the intact substantia nigra, tissue levels of nigral dopamine were depleted for 80 ± 6% (mean ± S.E.M., $n = 8$).

3.2. Extracellular basal values of dopamine in striatum and substantia nigra of intact and 6-hydroxydopamine-lesioned rats

The extracellular basal values of striatal and nigral dopamine levels (nM, mean ± S.E.M.) are shown in Table 1.

Comparing the data of intact and dopamine-depleted animals reveals that destruction of the nigrostriatal path-

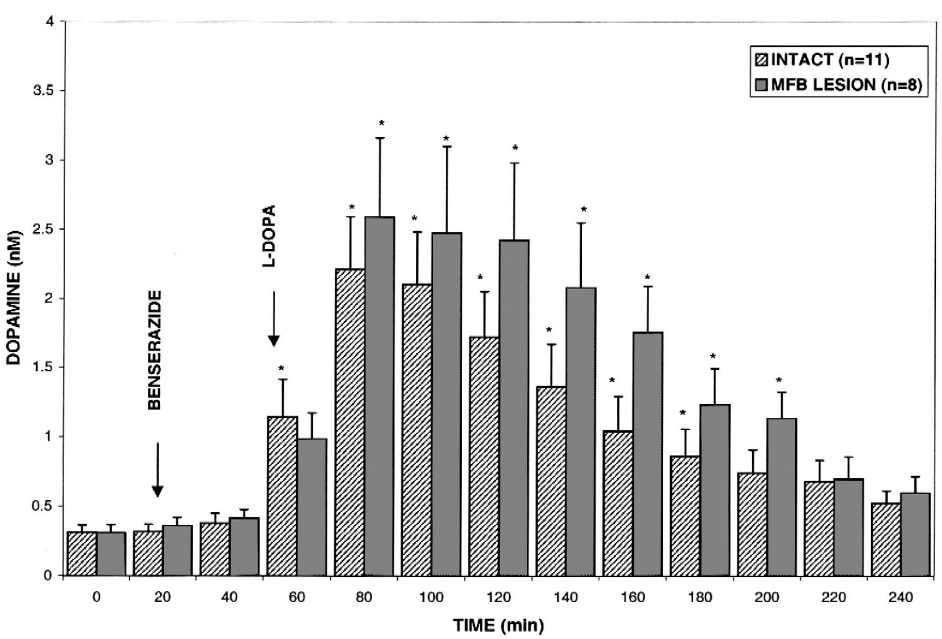

Fig. 2. The effect of 25 mg/kg levodopa (with 10 mg/kg benserazide as a blocker of peripheral dopa decarboxylase) on extracellular levels of dopamine in substantia nigra of intact (hatched bars, $n = 11$) and 6-OHDA-lesioned rats (grey bars, $n = 8$). Data are expressed in nM (mean ± S.E.M.). The baseline value at time 0 is the mean of six baseline values. Significance of differences from the baseline value is determined by one-way ANOVA for repeated measures followed by Fisher PLSD test: $^{*}P < 0.05$.

Table 2
Effect of MK801 on extracellular baseline values of dopamine in striatum and substantia nigra of intact and 6-OHDA-lesioned rats

	MK801 (mg/kg)	*n*	Mean (nM)	S.E.M.	*F*-test	*P* value
Striatum						
Intact	0.1	6	1.83	0.74	$F(12,60) = 1.251$	0.2716
	1.0	7	1.08	0.32	$F(12,72) = 1.344$	0.2137
6-OHDA	0.1	6	0.17	0.09	$F(12,60) = 1.073$	0.3986
Substantia nigra						
Intact	0.1	4	0.18	0.04	$F(12,36) = 0.768$	0.6783
	1.0	4	0.14	0.01	$F(12,36) = 1.639$	0.1241
6-OHDA	0.1	5	0.24	0.05	$F(12,48) = 1.280$	0.2608

Effect of MK801 on extracellular baseline values of dopamine, expressed in nM (mean ± S.E.M.), in striatum and in substantia nigra of intact rats and rats with a 6-hydroxydopamine lesion of the medial forebrain bundle.

way by 6-hydroxydopamine, significantly reduced extracellular levels of striatal dopamine (1.93 ± 0.25 nM, $n = 36$ vs. 0.18 ± 0.04 nM, $n = 22$ (mean ± S.E.M.), ($P \leq 0.001$) but nigral levels of dopamine were not influenced by the nigrostriatal lesion.

3.3. Effect of L-DOPA 25 mg / kg i.p. (after benserazide 10 mg / kg i.p.) on basal extracellular levels of dopamine in striatum and substantia nigra of intact and 6-hydroxydopamine-lesioned rats

Fig. 1 shows the influence of systemically administered L-DOPA on dopamine levels in the striatum. In intact striatum, dopamine increased from 2.82 ± 0.56 to 4.00 ± 0.85 nM (mean ± S.E.M.), which is 140% of its baseline value [$F(12,120) = 3.64$, $P < 0.0001$, $n = 11$]. In dopamine-depleted animals, striatal dopamine levels increased from 0.26 ± 0.10 to 2.63 ± 0.66 nM (mean ± S.E.M.), which is a 1000% increase [$F(12,72) = 8.90$, $P < 0.0001$, $n = 7$]. Although the baseline levels of striatal dopamine were significantly lower in dopamine-depleted rats, the amount of extracellular dopamine formed out of L-DOPA was not different compared to intact rats.

In intact substantia nigra (Fig. 2), the same dose of L-DOPA induced an increase of dopamine from 0.32 ± 0.05 to 2.20 ± 0.38 nM (mean ± S.E.M.), this is about 700% of its baseline value [$F(12,120) = 18.41$, $P < 0.0001$, $n = 11$]. In substantia nigra of 6-hydroxydopamine-lesioned animals, the increase in extracellular dopamine was about 800% of its baseline value, from 0.31 ± 0.06 to 2.59 ± 0.57 nM (mean ± S.E.M.) [$F(12,84) = 9.73$, $P < 0.0001$, $n = 8$]. Thus, the increase in dopamine levels after L-DOPA administration was not influenced by degeneration of the nigrostriatal bundle.

3.4. Influence of 0.1 and 1.0 mg / kg MK801 i.p. on basal extracellular levels of dopamine in striatum and substantia nigra of intact and 6-hydroxydopamine-lesioned rats

In intact rats, neither the low (0.1 mg/kg) nor the high (1.0 mg/kg) dose of MK801 influenced the basal extracellular levels of dopamine measured in both brain areas (Table 2). Only 1.0 mg/kg had a severe effect on the behavior of the animal, which was characterized by an overall activation, including sniffing, chewing and head weaving. These effects were not seen when 0.1 mg/kg MK801 was administered. In lesioned rats, extracellular

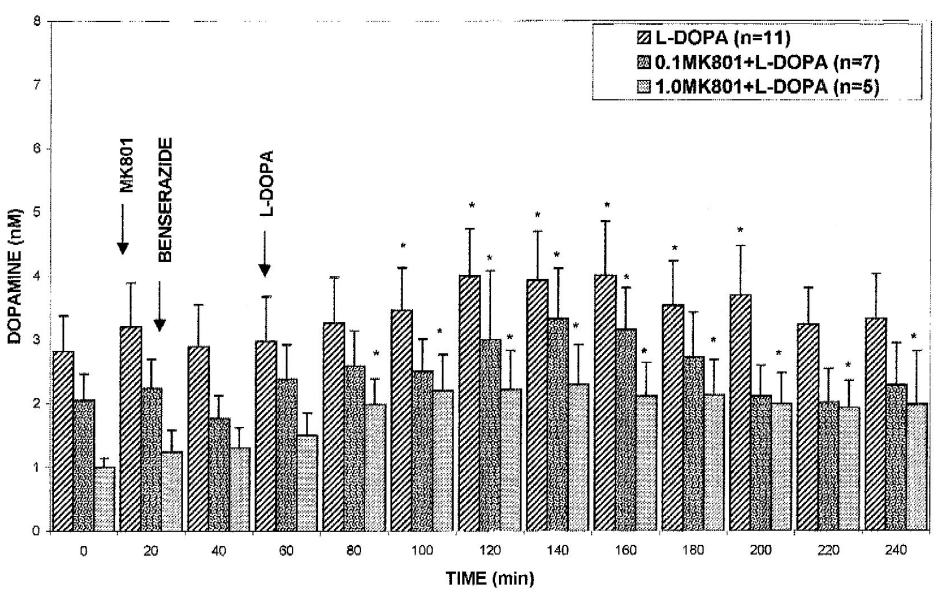

Fig. 3. The effect of 25 mg/kg levodopa (with 10 mg/kg benserazide as a blocker of peripheral dopa decarboxylase) alone (hatched bars, $n = 11$) and together with 0.1 mg/kg MK801 (grey bars, $n = 7$) or 1.0 mg/kg MK801 (dotted bars, $n = 5$) on extracellular levels of dopamine in striatum of intact rats. Data are expressed in nM (mean ± S.E.M.). The baseline value at time 0 is the mean of six baseline values. Significance of differences from the baseline value is determined by one-way ANOVA for repeated measures followed by Fisher PLSD test: $^{*}P < 0.05$.

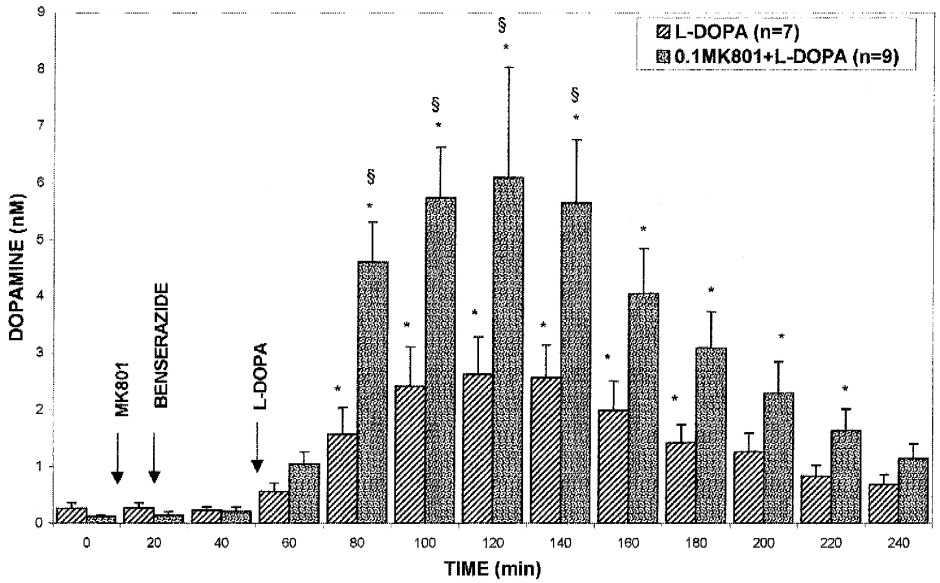

Fig. 4. The effect of 25 mg/kg levodopa (with 10 mg/kg benserazide as a blocker of peripheral dopa decarboxylase) alone (hatched bars, $n = 7$) and together with 0.1 mg/kg MK801 (grey bars, $n = 9$) on extracellular levels of dopamine in striatum of 6-OHDA-lesioned rats. Data are expressed in nM (mean ± S.E.M.). The baseline value at time 0 is the mean of six baseline values. Significance of differences from the baseline value is determined by one-way ANOVA for repeated measures followed by Fisher PLSD test: $^{*}P < 0.05$. Differences between both groups were determined by Mann–Whitney: $\S P < 0.05$.

dopamine levels in the striatum and the substantia nigra were not changed by 0.1 mg/kg MK801 either. Whereas, in intact rats, this dose never induced behavioral activation. In some of the lesioned animals, enhancement of motor activity was apparent. The effects were not seen in all the animals studied, though.

3.5. Effect of MK801 on L-DOPA-induced dopamine release in striatum of intact and 6-hydroxydopamine-lesioned rats

The pretreatment with MK801 did not alter the effect of L-DOPA on striatal dopamine levels of intact rats (Fig. 3).

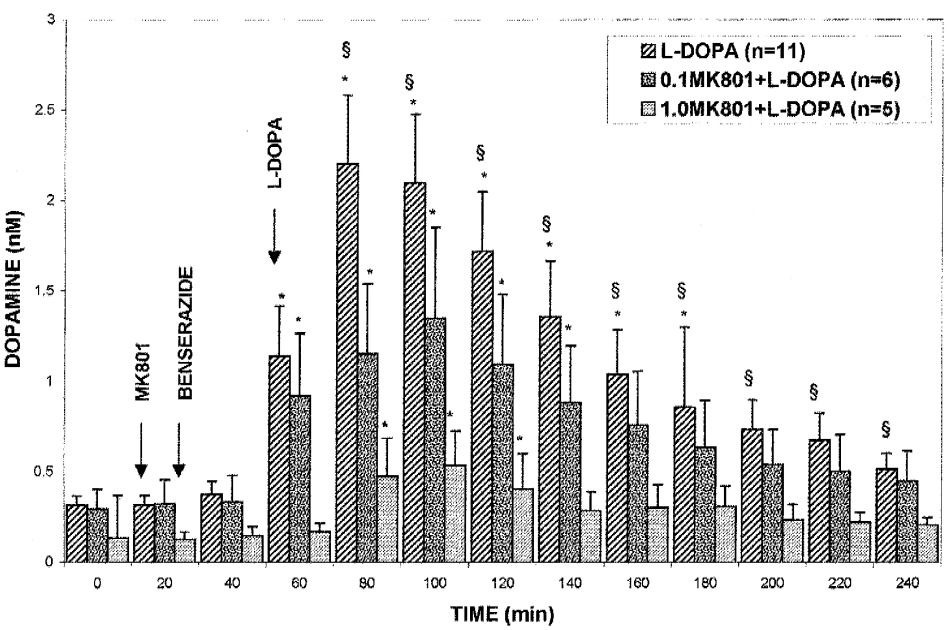

Fig. 5. The effect of 25 mg/kg levodopa (with 10 mg/kg benserazide as a blocker of peripheral dopa decarboxylase) alone (hatched bars, $n = 11$) and together with 0.1 mg/kg MK801 (grey bars, $n = 6$) or 1.0 mg/kg MK801 (dotted bars, $n = 5$) on extracellular levels of dopamine in substantia nigra of intact rats. Data are expressed in nM (mean ± S.E.M.). The baseline value at time 0 is the mean of six baseline values. Significance of differences from the baseline value is determined by one-way ANOVA for repeated measures followed by Fisher PLSD test: $^{*}P < 0.05$. Differences between both groups were determined by Mann–Whitney: $\S P < 0.05$.

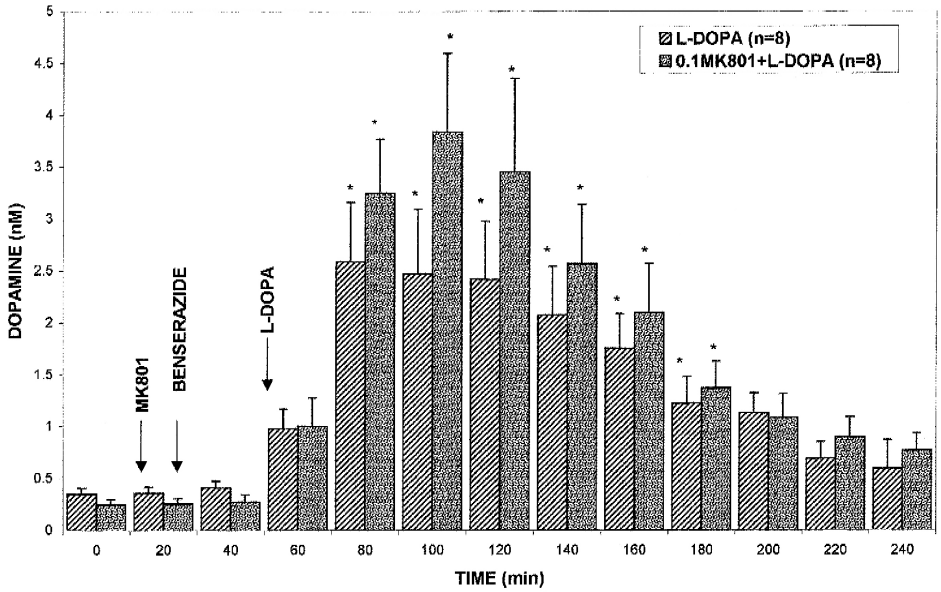

Fig. 6. The effect of 25 mg/kg levodopa (with 10 mg/kg benserazide as a blocker of peripheral dopa decarboxylase) alone (hatched bars, $n = 8$) and together with 0.1 mg/kg MK801 (grey bars, $n = 8$) on extracellular levels of dopamine in substantia nigra of 6-OHDA-lesioned rats. Data are expressed in nM (mean ± S.E.M.). The baseline value at time 0 is the mean of six baseline values. Significance of differences from the baseline value is determined by one-way ANOVA for repeated measures followed by Fisher PLSD test: $^{*}P < 0.05$.

The extracellular levels of dopamine increased from 2.08 ± 0.42 to 3.33 ± 0.79 nM (mean ± S.E.M., $n = 7$) when the rat was pretreated with 0.1 mg/kg MK801. Pretreatment with 1.0 mg/kg induced an increase in extracellular dopamine levels from 1.08 ± 0.14 to 2.29 ± 0.63 nM (mean ± S.E.M., $n = 5$). Although the overall increase was significantly different from baseline values, both for 0.1 mg/kg [$F(12,72) = 2.12$, $P < 0.05$, $n = 7$] and 1.0 mg/kg [$F(12,48) = 2.54$, $P < 0.05$, $n = 5$] MK801, it did not differ from the control group that received L-DOPA alone. Experiments were performed at random in time. By coincidence, the group of rats that received 1.0 mg/kg MK801, together with L-DOPA, had quite low baseline values of dopamine. Comparison of the baseline levels of the three groups did not show a significant difference.

When 6-hydroxydopamine-lesioned rats were pretreated with 0.1 mg/kg MK801, the extracellular levels of dopamine in the striatum increased from 0.12 ± 0.03 to 6.09 ± 1.05 nM (mean ± S.E.M.), [$F(12,96) = 19.59$, $P < 0.0001$, $n = 9$] (Fig. 4). This increase is significantly higher than the increase induced by L-DOPA alone (from 80 to 140 min, $P < 0.05$).

3.6. Effect of MK801 on L-DOPA-induced dopamine release in substantia nigra of intact and 6-hydroxydopamine-lesioned rats

In intact rats, the extracellular levels of dopamine induced by L-DOPA administration were attenuated by pretreatment with MK801 (Fig. 5). At a dose of 0.1 mg/kg MK801 i.p., extracellular dopamine levels increased significantly from 0.30 ± 0.11 to 1.16 ± 0.39 nM (mean ± S.E.M.) [$F(12,60) = 4.03$, $P < 0.0001$, $n = 6$]. When 1.0 mg/kg of MK801 was administered before L-DOPA, nigral dopamine levels increased significantly from 0.14 ± 0.03 to 0.48 ± 0.21 nM (mean ± S.E.M.) [$F(12,48) = 2.87$, $P < 0.05$, $n = 5$]. This increase was significantly smaller than the one induced by L-DOPA alone (from 60 to 240 min, $P < 0.05$).

In contrast, nigral dopamine levels of 6-hydroxydopamine-lesioned rats increased significantly after the combined administration of 0.1 mg/kg MK801 and L-DOPA from 0.25 ± 0.05 to 3.84 ± 0.76 nM (mean ± S.E.M.) [$F(12,84) = 13.16$, $P < 0.0001$, $n = 8$] but there was no difference with the group that received L-DOPA alone (Fig. 6).

4. Discussion

4.1. Effect of nigrostriatal denervation on basal extracellular dopamine levels in striatum and substantia nigra

The destruction of the medial forebrain bundle induces a > 99% depletion of tissue dopamine levels in the striatum, thereby decreasing extracellular levels of striatal dopamine significantly.

The injection of 6-hydroxydopamine into the medial forebrain bundle did not influence extracellular levels of dopamine in substantia nigra. To our knowledge, the maintenance of extracellular dopamine levels in substantia nigra

after degeneration of dopaminergic neurons has not been mentioned before. Although no dopaminergic input is left in the striatum, it is possible that 6-hydroxydopamine injection into the medial forebrain bundle affects cell bodies to a lesser extent. Dissection of both striatum and substantia nigra in the third week (18–21 days) after 6-hydroxydopamine lesion of the medial forebrain bundle has demonstrated that a $>99\%$ depletion of dopamine tissue levels in striatum corresponds to a depletion of 80% of dopamine tissue levels in substantia nigra. It is known that cell bodies are less sensitive to 6-hydroxydopamine (Robertson and Robertson, 1989). Furthermore, 6-hydroxydopamine was injected in the medial forebrain bundle and it is known that degeneration in the retrograde direction occurs more progressively than in the anterograde direction (Sauer and Oertel, 1994), so a longer time period would be necessary to obtain complete nigral denervation. On the other hand, injection of 6-hydroxydopamine into the substantia nigra has also shown to induce an 80% depletion of tissue dopamine (Robertson and Robertson, 1989). Extracellular dopamine levels in striatum are not influenced until the loss of dopaminergic neurons exceeds 80% (Altar and Marien, 1989; Robinson and Whishaw, 1988; Zhang et al., 1988). In substantia nigra, comparable compensatory mechanisms, as in striatum, might exist. Furthermore, the increased activity of the glutamate projection to the substantia nigra can contribute to maintain dopamine levels to normal values after a partial lesion. This can be achieved by stimulating release from dendrites as well as by promoting burst firing (Emmi et al., 1996).

4.2. Effect of nigrostriatal denervation on L-DOPA-induced dopamine release in striatum and substantia nigra

Both in intact and dopamine-depleted striatum, the administration of L-DOPA causes extracellular dopamine levels to increase significantly. Although the absolute amount of extracellular dopamine formed out of L-DOPA does not differ in intact and denervated striatum, the relative increase in denervated striatum greatly exceeds that in intact striatum, which is in agreement with previous studies (Sarre et al., 1992; Wachtel and Abercrombie, 1994; Miller and Abercrombie, 1999). The loss of presynaptic dopamine D2 receptor inhibition, reuptake sites and subsequent catabolic activity due to the nigrostriatal denervation causes extracellular dopamine to increase to higher extracellular levels after L-DOPA administration, compared to intact striatum. The dopamine is formed by other cells containing aromatic amino acid decarboxylase, which can cause neuronal release (e.g. serotonergic cells), (Wachtel and Abercrombie, 1994; Miller and Abercrombie, 1999) or non-neuronal release (e.g. glial cells, endothelial cells) (Sarre et al., 1994).

Also, in normal and dopamine-depleted substantia nigra, extracellular dopamine levels increased significantly following the administration of L-DOPA. In contrast to the striatum, the amount of dopamine formed did not differ between intact and denervated substantia nigra. The importance of the substantia nigra in L-DOPA-induced dopamine release after systemic administration has been emphasized, both in intact and in dopamine-depleted rats (Robertson and Robertson, 1989; Orosz and Bennett, 1992; Sarre et al., 1992). Only one study found approximately the same increase in dopamine levels in intact and dopamine-depleted substantia nigra after L-DOPA administration, but these authors measured tissue dopamine levels (Robertson and Robertson, 1989). All microdialysis studies performed on this issue found that in dopamine-depleted substantia nigra, the amount of dopamine formed out of L-DOPA was higher than in intact substantia nigra. Although the experimental setup was comparable to ours, both other studies used anaesthetised rats. Furthermore, the lesion was induced by 6-hydroxydopamine injection into the substantia nigra. This induced lower baseline levels of dopamine, so when the increases are expressed as a percentage of these baseline values, the relative increase is higher (Orosz and Bennett, 1992; Sarre et al., 1992).

The increases in striatal and nigral dopamine after L-DOPA administration are not associated with clearly enhanced motor behavior. This lack of correlation between dopaminergic increases in striatum and substantia nigra and the motor behavior has been discussed recently (Fisher et al., 2000) and is confirmed by our experiments.

4.3. Effect of MK801 on basal extracellular dopamine levels in striatum and substantia nigra of intact and denervated rats

Systemic administration of MK801 did not change extracellular dopamine levels in striatum and substantia nigra, neither in intact nor in dopamine-depleted rats.

These results indicate that in intact rats, extracellular dopamine levels in striatum and substantia nigra are not under tonic influence of glutamate via the NMDA receptor.

In striatum of extensively lesioned rats, a significant augmentation of NMDA receptor binding sites (Samuel et al., 1990; Wüllner et al., 1994), an increase in the expression of mRNAs encoding for NR1 and NR2A subunits of NMDA receptors as demonstrated by in situ hybridization studies (Andrés et al., 1998; Tremblay et al., 1995; Ulas and Cothman, 1996) and increased tyrosine phosphorylation of NR2B NMDA receptor subunit (Menegoz et al., 1995) were demonstrated. On the contrary, in substantia nigra, NMDA receptors decreased (Wüllner et al., 1994). Despite these changes in NMDA receptor density, extracellular dopamine levels were not influenced by pharmacological blockage of this receptor. Although MK801 did not directly influence extracellular dopamine levels in basal conditions, the NMDA receptors were effectively blocked

at the doses used since pretreatment with MK801 altered the L-DOPA-induced dopamine release.

4.4. Effect of MK801 on L-DOPA-induced dopamine release in intact and denervated rats

To our knowledge, the effect of MK801 on L-DOPA-induced extracellular dopamine levels in intact striatum have not yet been studied. The results presented here show that in intact striatum, the increase in striatal dopamine levels induced by L-DOPA was not influenced by pretreatment with MK801. In dopamine-depleted striatum, pretreatment with MK801 significantly enhanced the formation of dopamine out of L-DOPA. This is not a direct effect of MK801 on dopamine release since MK801 alone did not influence extracellular dopamine levels. Glutamate suppresses dopamine synthesis in striatal synaptosomes (Chowdhury and Fillenz, 1991) and MK801 enhances aromatic amino acid decarboxylase activity, both in striatum and substantia nigra (Fisher et al., 1998; Hadjiconstantinou et al., 1995). Since the glutamatergic tone is raised in dopamine-depleted animals (Albin et al., 1989), it is possible that blocking the hyperglutamatergic input by MK801 relieves the excessive aromatic amino acid decarboxylase suppression by glutamate and enables more dopamine to be formed out of L-DOPA. Since MK801 is a use-dependent NMDA antagonist, the results presented here indicate that in intact striatum, the glutamatergic pathway is not tonically active and that loss of striatal dopamine due to nigrostriatal denervation induces activation of the glutamatergic input into the striatum. This results in increased basal levels of glutamate in the striatum, as has been observed by other authors (Lindefors and Ungerstedt, 1990; Calabresi et al., 1993; Meshul et al., 1999).

In substantia nigra, the effect of MK801 on L-DOPA-induced dopamine release differs from the effects seen in the striatum. Pretreatment with MK801 dose-dependently attenuated L-DOPA-induced dopamine release in intact substantia nigra, whereas, it did not exert an effect in denervated substantia nigra. Rosales et al. (1997) showed that activation of the glutamatergic subthalamonigral pathway induced an increase in nigral dopamine levels and that subsequent D1 receptor stimulation increased glutamate levels. They proposed that the dopamine released after subthalamonigral stimulation affected both subthalamonigral and striatonigral projections via D1 receptors. This would implicate that there exists a positive feedback mechanism between glutamate and dopamine in substantia nigra. In our experimental setup, not the release of glutamate by subthalamonigral stimulation could have started the reciprocal interaction, but the dopamine formed out of L-DOPA. Dopamine could stimulate D1 receptors on subthalamonigral terminals, thereby inducing release of glutamate. Glutamate could further enhance the release of dopamine via the NMDA-receptors on dopaminergic cell bodies. Blocking the NMDA receptor beforehand would prevent the positive feedback and would prevent the enhancement of nigral dopamine release by D1 receptor stimulated glutamate release, which might explain the attenuation of the peak value of extracellular dopamine. However, our results are in contrast with recent findings on the increase of nigral aromatic amino acid decarboxylase activity by MK801, which would induce an increase of L-DOPA-induced dopamine release rather than a decrease (Fisher et al., 1998). Although these authors propose that the glutamatergic pathways in the basal ganglia are tonically active, thereby allowing MK801 to exert its effect, our results do not support this. Rather, the results presented here demonstrate that the glutamatergic pathway is not tonically active, since MK801 administration by itself did not influence extracellular dopamine. The subthalamonigral pathway is activated by the dopamine formed out of L-DOPA, thereby activating the NMDA receptor and making blockage by MK801 possible. Why the same effect is not seen in the striatum is not clear yet. It is possible that the relatively low L-DOPA-induced dopamine release and subsequent D1 receptor stimulation was too small to influence the massive glutamatergic input into the striatum.

In dopamine-depleted substantia nigra, the formation of dopamine out of L-DOPA is not influenced by pretreatment with MK801. This is not in accordance with earlier findings where concomitant perfusion of MK801 severely enhanced L-DOPA-induced dopamine release (Biggs et al., 1996). Two major differences in experimental setup exist. First, the drugs were perfused locally. Second, dopamine depletion was attained by reserpine administration, an effect which is different from a 6-hydroxydopamine-induced dopamine loss (Kannari et al., 2000). Several explanations are possible for the lack of effect of MK801 on L-DOPA-induced dopamine release. First, in the present study, extracellular dopamine levels in substantia nigra were not depleted by 6-hydroxydopamine. If the same compensation mechanisms exist in substantia nigra as are described in the striatum, the pharmacological profile of drug treatment is not altered because the substantia nigra is only partially denervated. Second, NMDA receptors in the substantia nigra are known to be localized at the dopaminergic cell bodies. Degeneration of these cell bodies includes loss of the NMDA receptors present on these cell bodies. It is not known if the NMDA receptors present on the 20% remaining cell bodies are sufficient to induce pharmacological effects. Lastly, NMDA receptors are located preferentially in the striatum, whereas, the subthalamic nucleus and its target areas, such as the substantia nigra, mainly express α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid (AMPA) receptors (Rouzaire-Dubois and Scarnati, 1987; Young et al., 1990; Albin et al., 1989; Tallaksen-Greene et al., 1992). This could indicate that AMPA receptors play a more important role in motor-related synaptic transmission in substantia nigra than do NMDA receptors (Albin et al., 1989). It would be

interesting to investigate whether an AMPA receptor antagonist would influence L-DOPA-induced dopamine formation in substantia nigra in vivo, as has been demonstrated in vitro.

In summary, the results presented here demonstrate that pretreatment with the NMDA receptor antagonist MK801, which by itself did not influence basal extracellular dopamine levels, differentially influenced L-DOPA-induced dopamine release in striatum and substantia nigra of intact and 6-hydroxydopamine lesioned rats.

In intact rats, MK801 altered L-DOPA-induced dopamine release only at the level of the substantia nigra. Blockage of subthalamonigral glutamate input, which is increased by local D1 receptor stimulation following the increase in nigral dopamine, could be responsible for the dose-dependent attenuation of L-DOPA-induced dopamine release. In dopamine-depleted rats, L-DOPA-induced dopamine release was influenced only at the level of the striatum. Local disinhibition of aromatic amino acid decarboxylase activity could account for this result.

It has been hypothesized that the striatum is the primary site for the therapeutic action of L-DOPA and that the neurochemical events in the substantia nigra are responsible for specific side effects of long-term L-DOPA therapy in Parkinson's disease, such as dyskinesias and on–off phenomena (Orosz and Bennett, 1992). Our results suggest that NMDA receptor antagonists could indeed be beneficial by enhancing the therapeutic efficacy of L-DOPA at the level of the striatum.

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