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Modulatory effects of L-DOPA on D2 dopamine receptors in rat striatum, measured using in vivo microdialysis and PET

J. Opacka-Juffry, S. Ashworth*, R. G. Ahier*, and **S. P. Hume**

PET Methodology Group, MRC Cyclotron Unit, Hammersmith Hospital, London, United Kingdom

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Summary. Putative modulatory effects of L-3,4-dihydroxyphenylalanine (L-DOPA) on D2 dopamine receptor function in the striatum of anaesthetised rats were investigated using both in vivo microdialysis and positron emission tomography (PET) with carbon-11 labelled raclopride as a selective D2 receptor ligand. A single dose of L-DOPA (20 or 100 mg/kg i.p.) resulted in an increase in [^{11}C]raclopride binding potential which was also observed in the presence of the central aromatic decarboxylase inhibitor NSD 1015, confirming that the effect was independent of dopamine. This L-DOPA evoked D2 receptor sensitisation was abolished by a prior, long-term administration of L-DOPA in drinking water (5 weeks, 170 mg/kg/day). In the course of acute L-DOPA treatment (20 mg/kg), extracellular GABA levels were reduced by ~20% in the globus pallidus. It is likely that L-DOPA sensitising effect on striatal D2 receptors, as confirmed by PET, may implicate striato-pallidal neurones, hence a reduced GABA-ergic output in the projection area. Since the L-DOPA evoked striatal D2 receptor supersensitivity habituates during long-term treatment, the effects reported here may contribute to the fluctuations observed during chronic L-DOPA therapy in Parkinson's disease.

Keywords: GABA, glutamate, globus pallidus, chronic L-DOPA.

Introduction

L-Dihydroxyphenylalanine (L-DOPA) appears to be an endogenous neuroactive compound that is released from neurones and acts as a neurotransmit-

* Present addresses:
S. Ashworth, DMPK, SmithKline Beecham, The Frythe, Welwyn, Herts, United Kingdom
R. G. Ahier, Department of Psychology, Institute of Psychiatry, Denmark Hill, London, United Kingdom

ter or neuromodulator in the brain (Misu and Goshima, 1993; Tedroff et al., 1997). Acute or chronic administration of exogenous L-DOPA affects both dopamine (DA) receptor status and aromatic L-amino acid decarboxylase activity (AADC, L-DOPA decarboxylase), the type and severity of the effects depending on the duration of the treatment (reviewed by Opacka-Juffry and Brooks, 1995).

There is evidence of an increase in ligand binding to striatal D1 and D2 receptors, as confirmed ex vivo (Murata and Kanazawa, 1993), and to D2 receptors as shown in vivo by means of positron emission tomography (PET) (Hume et al., 1995), following acute L-DOPA administration in rats. At present, little is known about direct L-DOPA effects on D2 dopamine receptors although this issue is of interest in the context of Parkinson's disease (PD). It is known that PD management is often complicated by fluctuations in levodopa efficacy, the "on-off" responses and "wearing off" observed during chronic L-DOPA treatment (Marsden and Parkes, 1977).

The aim of the present study was to assess whether the reported in vivo D2 receptor sensitisation caused by acute L-DOPA administration in rat striatum is altered by long-term L-DOPA treatment and whether it has any functional implications within the basal ganglia. The in vivo effects of acute L-DOPA administration on rat striatal D2 receptors were confirmed using PET and extended to long-term L-DOPA treatment. In order to investigate whether L-DOPA induced D2 receptor sensitisation leads to altered neuronal activity in striatal afferents and efferents which are regulated by D2 receptors, extracellular amino acid neurotransmitters were monitored in the striatum and external globus pallidus during acute L-DOPA treatment, using in vivo microdialysis. Extracellular glutamate was measured in the striatum, to assess whether L-DOPA affects the D2 receptors which control cortico-striatal glutamatergic terminals, and γ-aminobutyric acid (GABA) was measured in the globus pallidus, to determine putative L-DOPA effects on D2 receptors in striato-pallidal projection neurones.

Materials and methods

Animals and treatment

Male Sprague-Dawley rats (Harlan Olac Ltd., Bicester, UK) of 270–320 g body weight at the time of stereotaxic procedures were used in all experiments. The studies were carried out by licensed investigators in accordance with the Home Office's "Guidance on the Operation of the Animals (Scientific Procedures) Act 1986".

In the acute L-DOPA treatment, L-DOPA methyl ester was given i.p. at a dose of either 20 or 100 mg/kg, 60 min after carbidopa (25 mg/kg, i.p.), an inhibitor of peripheral AADC. In some studies, m-hydroxybenzylhydrazine (NSD-1015), an inhibitor of central AADC, was given at a dose of 100 mg/kg i.p. immediately before L-DOPA bolus.

In the long-term experiments, two groups of rats were considered: carbidopa alone i.e. vehicle control (n = 6), and L-DOPA + carbidopa (chronic L-DOPA group, n = 6). In these experiments the starting body weight was 210–230 g. Carbidopa solution or L-DOPA/carbidopa mixture was given in drinking water according to Blunt et al. (1992). Fluid intake and body weight were measured twice a week and drug concentrations were adjusted accordingly to achieve target doses of 200 mg/kg/day and 25 mg/kg/day for L-DOPA and carbidopa, respectively. The actual amount of L-DOPA received by rats was

170 ± 18 mg/kg/day and that of carbidopa was 21 ± 2 and 24 ± 3 mg/kg/day in the L-DOPA/carbidopa and carbidopa alone groups, respectively. At 4 and 5 weeks of the long-term treatment, two consecutive PET scans were performed on each animal, the first scan without and the second scan with an acute L-DOPA treatment (20 mg/kg, as described above). Two days before each of the scans, the long-term L-DOPA/carbidopa or carbidopa administration was discontinued, to assure a drug-free interval.

In microdialysis experiments, three groups of rats were considered: control, acute L-DOPA 20 mg/kg and acute L-DOPA 100 mg/kg (4–6 animals per group).

In PET studies, the following groups of rats were included: intact control, acute L-DOPA 20 mg/kg, acute L-DOPA 20 mg/kg + NSD, acute L-DOPA 100 mg/kg, acute L-DOPA 100 mg/kg + NSD, chronic carbidopa, chronic carbidopa + acute L-DOPA 20 mg/kg, chronic L-DOPA, chronic L-DOPA + acute L-DOPA 20 mg/kg (5–10 animals per treatment group).

Carbidopa was provided by Merck, Sharp & Dohme (Hoddesdon, UK). All other chemicals were purchased from Sigma Chemical Co. Ltd (Poole, UK). All chemicals used for chromatography analysis were of analytical or HPLC grade.

PET

Rats were anaesthetised with isoflurane in a mixture of N_2O and O_2, and, using a heated pad, body (rectal) temperature was maintained at approximately 37°C. In experiments with acute pre-treatment, L-DOPA was given at ~90 minutes before [^{11}C]raclopride. Prior to scanning, rats were stereotaxically positioned within a small-animal scanner (Bloomfield et al., 1995) such that the striata were at the centre of the field of view (FOV). [^{11}C]Raclopride, prepared routinely (Farde, 1988), was injected i.v. at a dose of ~10 MBq per rat. The range of the concentrations of co-injected stable raclopride varied from 0.35 to 5.7 nmol/kg in the intact rats and from 0.13 to 5.53 nmol/kg in the various treatment groups. These variations reflected the specific activities of [^{11}C]raclopride at the time of injection which ranged from 5 to 223 GBq/μmol. Dynamic scans were performed over 60 minutes with 21 frames (3 × 5s, 3 × 15s, 4 × 60s, 11 × 300s). Scans were reconstructed using a ramp filter with Nyquist cut-off, resulting in a spatial resolution of 2.3 × 2.3 × 4.3 mm full width at half maximum at the centre of the FOV.

Using Analyze software (Robb and Hanson, 1991), the image volumes were interpolated to give cubic voxels of 0.47 mm dimension. Three regions of interest (ROI) which corresponded to left and right striata and cerebellum were sampled as described previously (Hume et al., 1996). Assuming cerebellum to be a region devoid of D2 receptors, the individual cerebellum ROI time-radioactivity curves were used as an indirect input function in a reference-tissue compartmental model (Hume et al., 1992). The measure of the specific binding of [^{11}C]raclopride is the striatal ROI binding potential (BP), defined as the ratio of k3 : k4, where k3 and k4 are, respectively, the rate constants for transfer to and from the specifically bound striatal compartment. For an infinitely low concentration of stable ligand, BP is a measure of B_{max}/K_d, where B_{max} represents the total number of binding sites and K_d is the apparent dissociation constant.

Microdialysis

Dialysis probes of a vertical concentric design were constructed (Hutson et al., 1986) using a tubular dialysis probe (200 μm diameter, GFE-9 Gambro) of either 2 or 3 mm length, for globus pallidus and striatum, respectively. The probes were implanted under isoflurane anaesthesia at the following coordinates relative to bregma and dura: AP +0.7 mm, ML −3.0 mm, DV −6.0 mm (striatum) and AP −1.3 mm, ML −3.0 mm, DV −7.0 mm (globus pallidus) (Paxinos and Watson, 1986). With the probes fixed to the skull, the animals were allowed to recover for approximately 18 h. All experiments were conducted on the day after probe implantation. Microdialysis was performed under isoflurane/N_2O with O_2 anaesthesia for direct comparison with the PET studies. Body

(rectal) temperature was maintained at 37°C. Microdialysis probes were perfused with artificial CSF (composition in mmol/L: NaCl 125; KCl 2.5; $MgCl_2$ 1.18; $CaCl_2$ 1.26; pH 6.5) at a flow rate of 2.0 μL/minute using a Carnegie Medicine microdialysis pump, model CMA/140 (Biotech Instruments Ltd, Kimpton, Herts, UK). Baseline was monitored for up to 2 h before pharmacological manipulations. Samples were collected every 20 min into vials containing 10 μl 0.1 M H_3PO_4, then frozen on dry ice and stored at −70°C until analysed. The position of the probe was verified post mortem.

HPLC

Amino acid analysis

The dialysates were analysed for amino acids using HPLC with fluorescence detection after pre-column derivatisation with ortho-phtaldialdehyde (OPA). A Gilson HPLC gradient system equipped with a 231–401 auto-sampling injector was used. The derivatisation procedure was controlled by means of Gilson ASTED software (Anachem, Luton, Beds, UK). The (OPA) reagent for derivatisation was prepared as follows: 50 mg ortho-phtaldialdehyde were dissolved in 1 mL of methanol, then 19 mL of 100 mM borate buffer pH 9.5 and 100 μL of mercaptoethanol were added. This solution was stable for up to 2 weeks when kept in the dark. 30 μL dialysate was mixed with 30 μL of 20 nM norleucine as an internal standard. 50 μL of this mixture was incubated with 70 μl OPA reagent and 100 μL of this incubate was injected onto the HPLC column.

A reverse phase 150 mm × 4.6 mm C18 ODS5 analytical column was used at room temperature with a flow rate of 1 mL/min. The mobile phase consisted of two solvents: 10% methanol and 0.05 M NaH_2PO_4 adjusted to pH 5.6 with 4 M NaOH (solvent A) and a mixture of 28% acetonitrile, 24% methanol and 48% water (solvent B). A continuous gradient starting at 10% B and 90% A and reaching 95% B at 25 minutes was applied.

The fluorescent detector (LS 30, Perkin Elmer, Beaconsfield, UK) was set at 340 nm excitation and 440 nm emission wave lengths, respectively, and amounts of amino acids were estimated using a PE Nelson integrator software (Perkin Elmer, Beaconsfield, UK). The retention times for glutamate and GABA were 6.5 and 16.3 min, respectively, and the detection limits were approximately 20 fmol.

Dopamine analysis

Dopamine (DA) content in striatal dialysates was measured by means of HPLC with high sensitivity electrochemical detection (ESA Coulochem 5100A, Severn Analytical, Macclesfield, UK) essentially as described previously (Opacka-Juffry et al., 1991).

Statistical analysis

Changes in amino acid levels over time as a function of treatment conditions were analysed using a two-way analysis of variance (ANOVA) for repeated measures, followed by Dunnett's post hoc t-test. The level of significance was set at $P < 0.05$.

The PET data could not be compared by ANOVA due to a variable specific activity of the radiotracer. For this reason a non-parametric analysis (Mann-Whitney U – Wilcoxon rank sum W test) was applied to the residuals of the experimental values relative to the fitted normal binding potential curve.

Results

PET

As reported previously (Hume et al., 1995), [^{11}C]raclopride BP values for rat striatum were influenced by the range of doses of stable raclopride associated

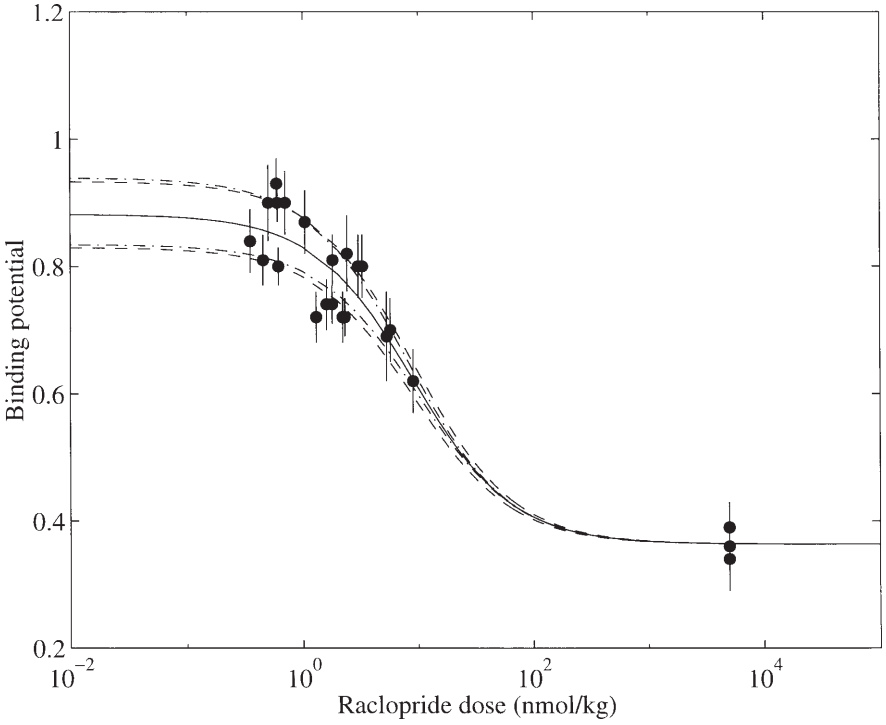
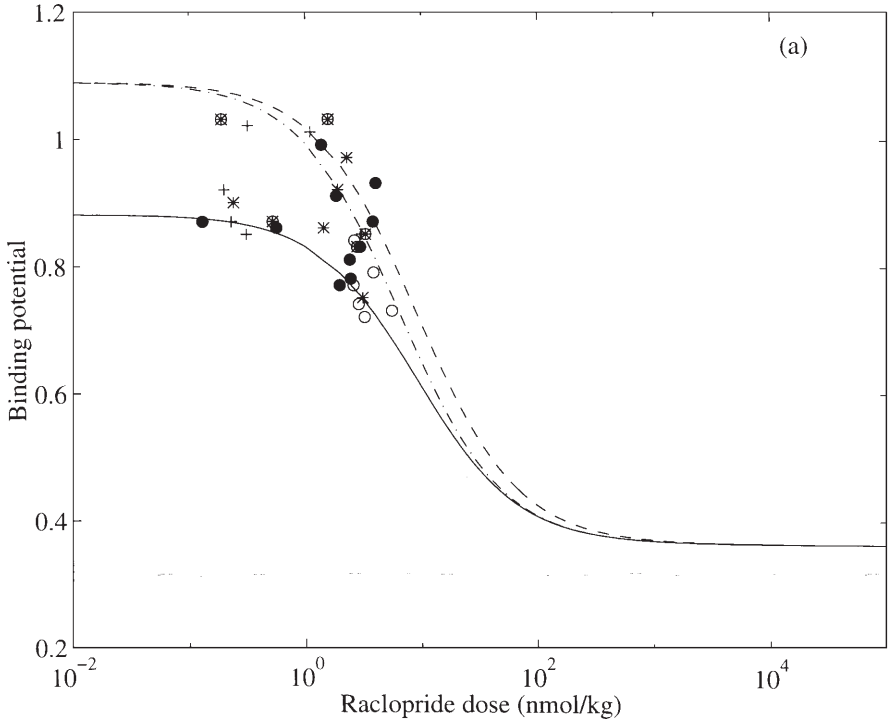


Fig. 1. Striatal BP values for [^{11}C]raclopride expressed as a function of stable raclopride dose injected to intact control rats. Each datum point is an average of BP calculated for left and right striatal ROI from a single rat. The error bar is the average standard error on the 2 fits. The solid line is the best fit to a single-site binding model, as described in the text. The dashed and dashed-dot lines represent a 10% simulated change in B_{max} or K_d, respectively

with the radiochemical synthesis and typically co-injected during a PET scan. To account for this in treatment group comparisons, all the BP data obtained are presented as a function of the dose of nonradioactive raclopride. Figure 1 shows control BP data for the striatum of intact, anaesthetised rats, obtained in the present study, together with BP values from further studies (unpublished) where additional doses of stable raclopride have been added to the injectate, in order to obtain sufficient data to adequately describe a saturation curve. The solid line is the best fit to the data, using iterative nonlinear regression to a single-site binding model for ligand-receptor interactions, as described by Hume et al. (1995). The curve fits the relationship

$$BP = [appB_{max}/(C + appK_d)] + NS$$

where $appB_{max}$ is proportional to the number of binding sites, C is the concentration of injected raclopride, $appK_d$ is the concentration of injected ligand required to occupy 50% these sites, and NS is a measure of the nonspecific binding estimated in vivo when D2 receptors are occupied by the saturating dose of raclopride used. Fitted parameters were as follows: $appB_{max} = 4.66$, $appK_d = 8.99$ and $NS = 0.36$.


(a)
Binding potential
Raclopride dose (nmol/kg)
1.2
1
0.8
0.6
0.4
0.2
10^{-2}
10^{0}
10^{2}
10^{4}

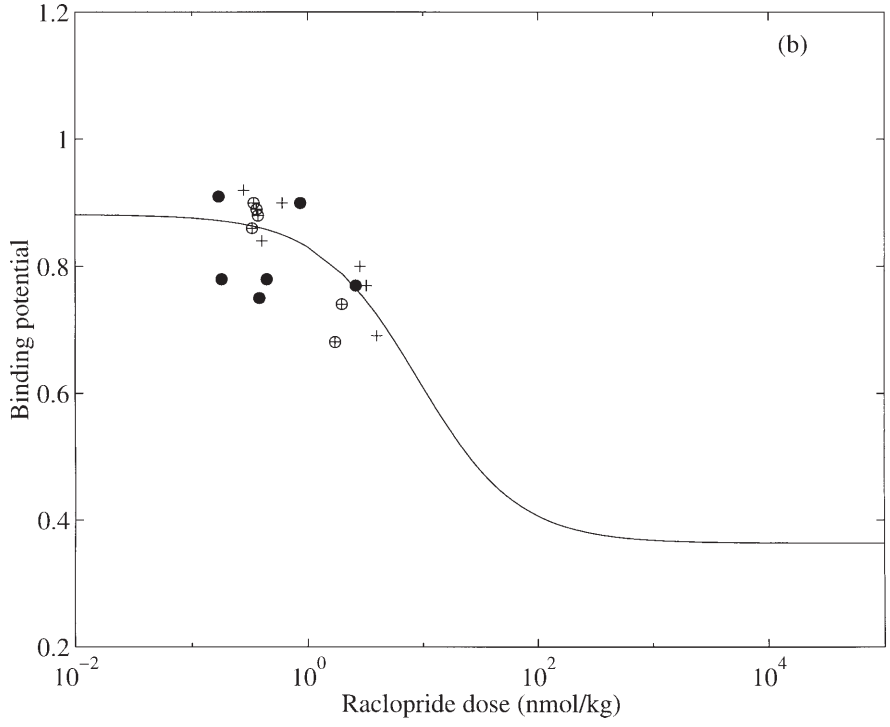

(b)
Binding potential
Raclopride dose (nmol/kg)
1.2
1
0.8
0.6
0.4
0.2
10^{-2}
10^{0}
10^{2}
10^{4}

Figure 2(a) shows individual data from those L-DOPA treatment groups which had striatal BP values on or above the control saturation curve. Results were variable but the maximal BP values attained were consistent with a 40% increase in B_{max} or K_d compared with controls. The treatment groups were all four groups of acute carbidopa/L-DOPA (both doses of L-DOPA, 20 or 100 mg/kg, with or without NSD-1015) and the chronic carbidopa plus acute carbidopa/L-DOPA group. Statistical analysis (Mann-Whitney U – Wilcoxon rank sum W test) showed a significant difference between the residuals of the pooled experimental data and the control values (corrected for ties, 2-tailed $P < 0.001$).

Figure 2(b) shows data from those groups whose BP values fell within or below the control range. They included the long-term carbidopa treatment, the chronic L-DOPA group and the chronic carbidopa/L-DOPA plus acute L-DOPA group, indicating that the long-term L-DOPA treatment abolished the sensitising effect of L-DOPA on D2 receptors. Statistical analysis (Mann-Whitney U – Wilcoxon rank sum W test) showed no significant difference between the residuals of the pooled experimental data and the control values (corrected for ties, 2-tailed $P = 0.76$).

Microdialysis

For ease of comparison, all microdialysis results are expressed as relative values i.e. percentage of baseline collected for 2 hours prior to the L-DOPA bolus. Since carbidopa given at a dose of 25 mg/kg i.p. had no observable effect on either DA or amino acids measured, the values of a stable intact baseline measured for one hour prior to carbidopa pre-treatment and those obtained during one hour between carbidopa and L-DOPA injections were averaged.

Acute L-DOPA treatment at the doses chosen resulted in a dose dependent increase in striatal extracellular DA levels, as shown in Fig. 3. The lower dose of L-DOPA (20 mg/kg) caused a maximal 60% increase in extracellular DA levels, while the higher dose (100 mg/kg) resulted in a nearly eight-fold increase, with the highest values seen between 100 and 140 min after i.p. injection of L-DOPA. The time course of these extracellular events was the criterion which determined the interval between L-DOPA administration and

Fig. 2. Striatal BP values for [^{11}C]raclopride expressed as a function of stable co-injected raclopride, (**a**) data from those L-DOPA treatment groups which showed BP values on or above the control saturation curve (solid line from Fig. 1). The dashed and dashed-dot lines represent a 40% simulated change in B_{max} or K_d, respectively. Groups are: (•) acute DOPA 20 mg/kg; (✳) acute DOPA 20 mg/kg + NSD; (o) acute DOPA 100 mg/kg; (⊗) acute DOPA 100 mg/kg + NSD; (+) chronic carbidopa + acute DOPA 20 mg/kg; (**b**) data from those treatment groups which showed BP values within or below the control range. Groups are: (+) chronic carbidopa; (⊕) chronic DOPA; (•) chronic DOPA + acute DOPA 20 mg/kg. Each datum point is an average of BP calculated separately for left and right striatal ROI from a single rat. The errors in the estimations were similar to those shown in Fig. 1. The solid line represents the control saturation curve

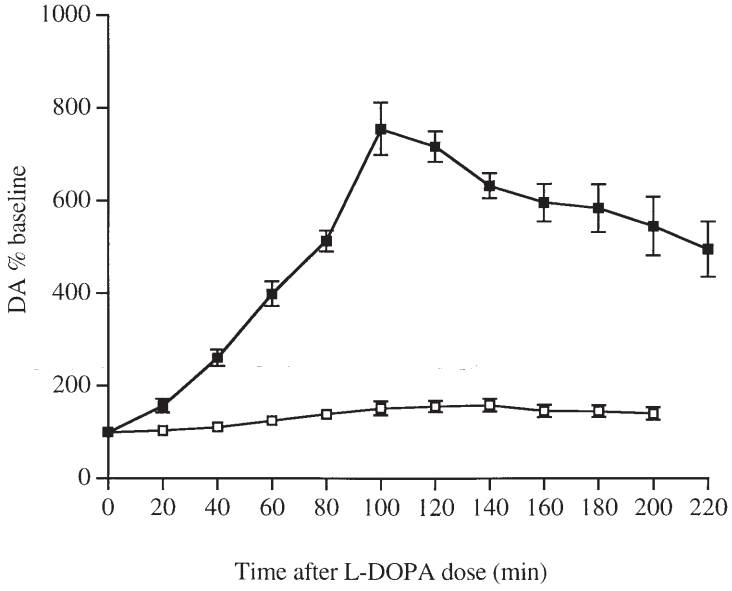


Fig. 3. Effects of different L-DOPA doses on extracellular levels of striatal dopamine, as measured by microdialysis in isoflurane anaesthetised rats. The data (mean ± SE) are expressed as a percentage of the baseline, collected for 2 h prior to the L-DOPA injection. L-DOPA was given at time zero, at a dose of 20 mg/kg (open squares, n = 6) or 100 mg/kg (closed squares, n = 4), i.p., 1 h after carbidopa (25 mg/kg, i.p.). The basal levels were 0.12 ± 0.02 pmol/20 min sample (mean ± SD; n = 10) and were not affected by the carbidopa pretreatment

[^{11}C]raclopride injection in the PET studies (~90 min), so that the 60 minute scanning period would coincide with the greatest dopaminergic response. Effects of L-DOPA acute treatment on amino acid neurotransmitters were assessed following the lower L-DOPA dose of 20 mg/kg i.p., which was chosen for its small effect on endogenous striatal DA (Fig. 3). NSD-1015 was not included in this treatment as it caused a decrease in extracellular DA (Hume et al., 1995).

Acute L-DOPA treatment did not significantly affect striatal extracellular glutamate when compared with rats predosed with carbidopa alone (Fig. 4a). An essentially similar finding applied to extracellular GABA monitored in the striatum (Fig. 4b).

On the other hand, in the globus pallidus, extracellular GABA levels were significantly reduced in the course of L-DOPA acute treatment ($P < 0.01$) whilst carbidopa only pretreatment had no significant effect during the time tested (Fig. 5). The maximal reduction in pallidal GABA was within the range of 20% baseline.

Discussion

It has been previously shown in vivo, with the use of a clinical PET scanner that acute administration of L-DOPA causes an increase in [^{11}C]raclopride

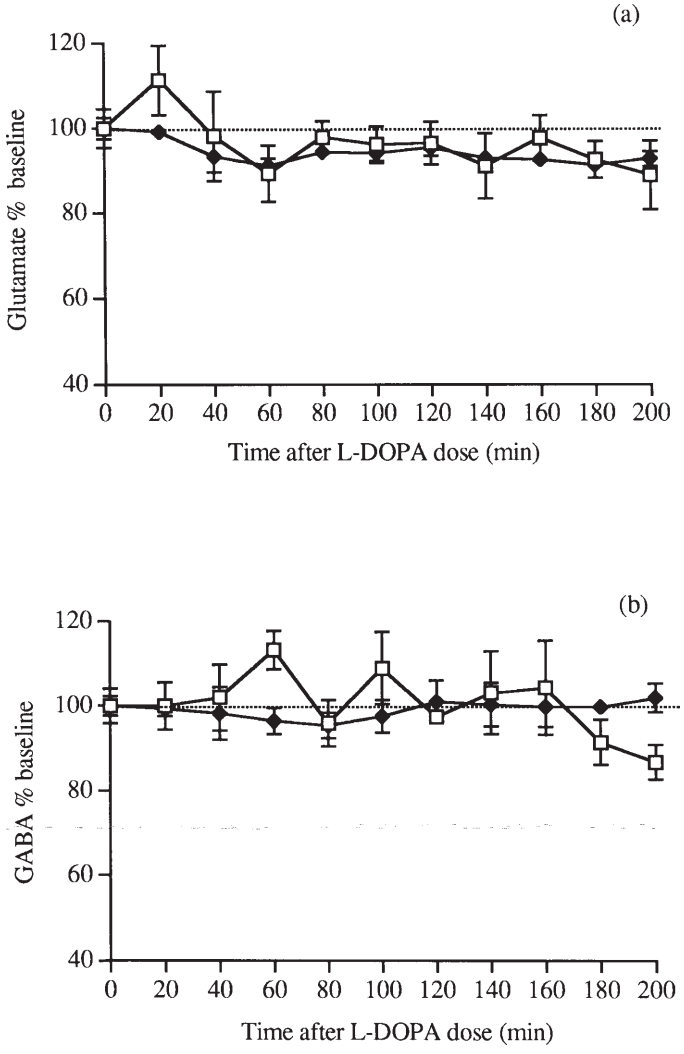


Fig. 4. Extracellular levels of glutamate (**a**) and GABA (**b**) as measured in isoflurane anaesthetised rat striatum after injections of L-DOPA, 20 mg/kg (open squares) i.p., given 1 h after carbidopa (25 mg/kg i.p.; closed diamonds represent carbidopa only control). The data (mean ± SE) are expressed as a percentage of the baseline, collected for 2 h prior to the L-DOPA injection. The basal levels of glutamate and GABA were 5.15 ± 1.66 pmol/20 min sample and 0.452 ± 0.099 pmol/20 min sample (mean ± SD; n = 12), respectively

binding in normal rat striatum, independent of DA (Hume et al., 1995). That finding suggested a rapid modulation of D2 receptors by L-DOPA and was generally compatible with the hypothesis that L-DOPA can play a neuromodulatory role in the central nervous system (Misu and Goshima, 1993). It was also consistent with the report of Murata and Kanazawa (1993) on direct acute effects of L-DOPA on D2 receptors in rats as measured ex vivo. Murata and Kanazawa (1993) additionally observed a pronounced increase in striatal D1 receptor binding, which correlated with a significant elevation in D1 receptor mRNA levels after a single L-DOPA dose. This

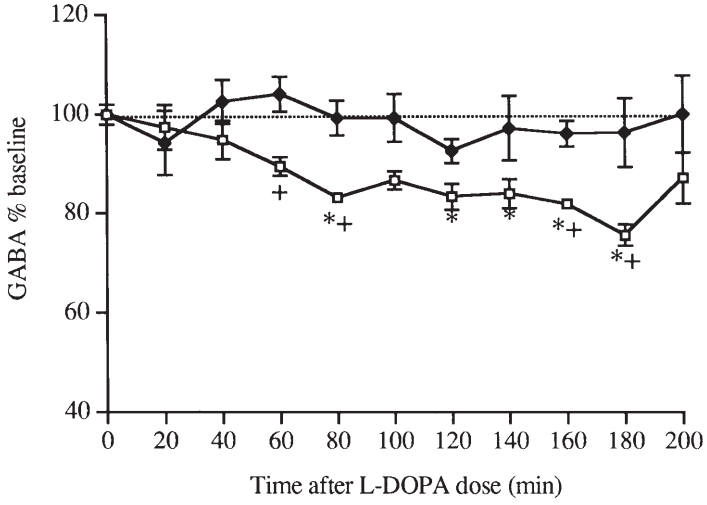


Fig. 5. Extracellular levels of GABA measured in globus pallidus of isoflurane anaesthetised rat after injections of L-DOPA 20 mg/kg i.p., (open squares), given 1 h after carbidopa (25 mg/kg i.p.). Closed diamonds represent carbidopa only control. The data (mean ± SE; n = 4 per group) are expressed as a percentage of the baseline, collected for 2 h prior to the L-DOPA injection. The basal levels of GABA in the globus pallidus were 0.623 ± 0.120 pmol/20 min sample (mean ± SD; n = 12). A two-way ANOVA with repeated measures indicated significant effects; for treatment differences, $F = 35.459$, $p = 0.001$; for time, $F = 3.133$, $p = 0.003$. *significantly different from the last baseline sample ($p < 0.01$; post-hoc t-test); +significantly different from carbidopa only control at the time point shown ($p < 0.05$; post-hoc t-test)

"supersensitive response" of both D1 and D2 dopamine receptors was abolished in the course of long-term (28 days) L-DOPA administration. The fluctuating response of rat striatal dopamine receptors to L-DOPA which depends on L-DOPA regimen has obvious connotations with PD clinical treatment.

In the present study, we have addressed this issue, extending our earlier observations by assessing long-term effects of L-DOPA treatment on striatal D2 receptor binding in vivo and D2 receptor supersensitivity which follows acute L-DOPA treatment in rats. We applied a recently developed PET technology more adequate to experimental applications, namely a dedicated small animal scanner with an improved spatial resolution (Bloomfield et al., 1995; Hume et al., 1996).

With this methodology, we have highlighted the observation that striatal BP for [^{11}C]raclopride depends upon the dose of co-injected nonradioactive raclopride. Thus, a reduction in BP was observed when the raclopride dose was higher than ~1 nmol/kg i.v. Recently, Morris and London (1997), in simulation studies of the saturation of [^{11}C]raclopride binding in vivo based on human and non-human primate PET data, have calculated a "greatest allowable dose" of stable raclopride of ~0.5 nmol/kg before the BP estimate is compromised. Because of this phenomenon, the present PET data have been

expressed as a function of the dose of stable raclopride, and have not been tested using conventional statistical approaches using experimental groups.

The data demonstrate that acute L-DOPA leads to an increase in striatal BP for [^{11}C]raclopride in normal rats. This was detected not only at the lower dose of L-DOPA (20 mg/kg i.p.) used previously (Hume et al., 1995) but also at the higher dose of 100 mg/kg i.p. The acute L-DOPA doses differ in the levels of striatal extracellular dopamine produced. Under conditions comparable with those used in the PET studies, the higher dose of 100 mg/kg i.p. led to an approximately 8-fold increase in extracellular striatal DA, whereas the lower dose of L-DOPA resulted in only 60% increase in striatal DA. It has been known for several years that there is a competition between raclopride and endogenous dopamine and that radiolabelled raclopride can be displaced by elevated endogenous DA (Hume et al., 1992; Dewey et al., 1993; Volkow et al., 1994; Seeman et al., 1996). In order to eliminate the possible influence of endogenous dopamine on [^{11}C]raclopride binding to D2 receptors, we assessed acute L-DOPA effects in animals with or without pretreatment with the central AADC inhibitor NSD-1015. The increase in striatal BP observed at either dose of L-DOPA was not affected by the inhibition of central AADC. This suggests that the increase in [^{11}C]raclopride binding to striatal D2 receptors following acute L-DOPA treatment is independent of endogenous DA and reflects direct modulatory effects of L-DOPA on these receptors.

The observation that L-DOPA induced D2 receptor supersensitivity was not affected by NSD-1015, as confirmed at this stage of PET studies, helped to decide on the L-DOPA acute treatment further applied in both long-term PET investigations and microdialysis studies on potential functional implications of D2 receptor sensitisation. Thus, the lower L-DOPA dose of 20 mg/kg i.p. was chosen because of its only mild effect on endogenous striatal DA. It was combined with carbidopa and no NSD-1015 to limit the severity of pharmacological manipulations in rats maintained under anaesthesia for up to ten hours, and to avoid causing a reduction in extracellular striatal DA which we observed after NSD-1015 administration (Hume et al., 1995).

In order to investigate whether D2 receptor response to an acute dose of L-DOPA was altered by long-term L-DOPA treatment, rats were given L-DOPA plus carbidopa for 5 weeks, at an average dose of L-DOPA of 170 mg/kg/day, and then were challenged with a L-DOPA bolus of 20 mg/kg i.p. The duration of repeated L-DOPA administration used in numerous experimental studies on rats ranges from 14 days to 12 months (Jenner and Marsden, 1987). The previously reported in vitro results on long-term effects of L-DOPA on rat striatal D2 receptors also vary. It has been shown, for example, that a prolonged L-DOPA exposure causes either no change, a slight increase or a decrease in D2 receptor binding density in intact rat striatum (Jackson et al., 1983; Jenner and Marsden, 1987; Reches et al., 1982; Rouillard et al., 1987; Wilner et al., 1980). In contrast, most papers on long-term L-DOPA treatment of rats with a unilateral nigrostriatal lesion have suggested that the treatment tends to reverse the lesion-induced D2 receptor supersensitivity (Parenti et al., 1986; Reches et al., 1984; Schneider et al., 1984). In our case,

the present long-term treatment either with carbidopa only or L-DOPA plus carbidopa did not affect striatal BP for [^{11}C]raclopride measured in vivo.

Interestingly, in those rats which underwent a long-term exposure to L-DOPA, the subsequent acute L-DOPA challenge did not cause any obvious elevation in striatal BP for [^{11}C]raclopride. In contrast, chronic treatment with carbidopa alone did not attenuate the L-DOPA acute effect. This finding suggests that the L-DOPA-evoked in vivo striatal D2 receptor sensitisation can be abolished by a long-term L-DOPA treatment and is consistent with the ex vivo results obtained by Murata and Kanazawa (1993). At this stage, we do not attempt to speculate as to the mechanism of the L-DOPA direct effects on D2 striatal receptors.

Irrespective of the cause of the increased [^{11}C]raclopride binding to striatal D2 receptors after acute L-DOPA, functional implications of this phenomenon are of interest. Assuming that the increased ligand binding indicates supersensitivity of D2 receptors coupled with the second messenger system, the L-DOPA sensitising action should translate into functional effects within the neuronal pathways concerned, i.e. should affect neuronal activity of those basal ganglia neurones which are regulated by D2 receptors.

D2 receptor-like protein localisation in rat neostriatum has been well documented (Levey et al., 1993; Sesack et al., 1994; Fisher et al., 1994). Its presence was detected in three populations of neostriatal neurones: the medium spiny projection neurones, and the medium- and large-sized aspiny interneurones. Immunoreactivity for the receptor protein was also detected in presynaptic terminal boutons and in small thinly myelinated axons (Fisher et al., 1994). Thus, D2 receptor-like protein localization is consistent with postsynaptic actions on GABA-ergic projection neurones (striatopallidal and striatonigral) and cholinergic interneurones, and with autoreceptor functions at the level of presynaptic nigrostriatal axon terminals in the striatum (Sesack et al., 1994).

There is also some evidence of D2-like immunoreactivity representing heteroreceptors on the class of corticostriatal and thalamostriatal asymmetric axospinous synapses (Levey et al., 1993; Sesack et al., 1994), and there is an abundant physiological and pharmacological evidence of D2 receptor-mediated inhibition of glutamate release from corticostriatal terminals (Mitchell and Doggett, 1980; Calabresi et al., 1992; Yamamoto and Davy, 1992).

In the the present studies, we focused on D2-dependent presynaptic control of cortico-striatal glutamatergic terminals and on postsynaptic control of GABA-ergic striatopallidal projection neurones. It has been well documented that activity of the latter group of neurones remains under an inhibitory control of D2 receptors (Gerfen, 1992).

In the globus pallidus (the projection area of striatopallidal neurones), extracellular GABA levels were significantly reduced in the course of L-DOPA acute treatment. This effect is consistent with an activation of D2 receptors on striatopallidal neurones and corresponds to the previously published observations on the increase in GABA-ergic activity of striatopallidal neurones after destruction of the nigrostriatal pathway when striatal dopam-

ine inhibition is removed (Jian et al., 1993; Linderfors, 1993; Scheel-Krüger, 1986). The degree of reduction in pallidal GABA levels reported here (approximately 20% baseline) is modest but it is probable that only a portion of extracellular pallidal GABA measured under the present conditions results from neurotransmission, as deduced from microdialysis studies on ventral pallidum of awake rats (Bourdelais and Kalivas, 1992) and caudate-putamen of anaesthetised rats (Campbell et al., 1993). An indirect evidence that L-DOPA itself and not striatal DA was responsible for the changes observed in GABA levels in the pallidum following L-DOPA dose of 20 mg/kg, comes from a pilot microdialysis study performed in awake rats (unpublished data) where this L-DOPA dose (plus carbidopa, no NSD 1015) caused no effect on striatal DA efflux. In those rats, L-DOPA administration was followed by similar changes in GABA levels in the globus pallidus as those discussed in the present paper.

In contrast, extracellular GABA monitored in the striatum did not undergo any significant changes following L-DOPA acute treatment. It is known that striatal GABA originates predominantly from axonal collaterals of both striatopallidal and striatonigral projection neurones (Kita and Kitai, 1988). The striatonigral pathway, contrary to the striatopallidal one, remains under stimulatory control of D1 dopamine receptors (Gerfen, 1992). Assuming that Murata and Kanazawa's (1993) in vitro findings concerning D1 receptor supersensitivity following acute L-DOPA treatment can be extended into an in vivo situation, it is conceivable that L-DOPA effects on striatal GABA release from both D1 and D2 controlled collaterals of GABA-ergic projection neurones mutually counteract and therefore remain undetectable in our present experimental paradigm.

The fact that the acute L-DOPA treatment did not significantly affect striatal extracellular glutamate while it decreased GABA release in the globus pallidus might imply some kind of selectivity of L-DOPA acute action towards D2 receptors of striatopallidal projection neurones. The lack of corticostriatal response to L-DOPA may, however, have different reasons. Firstly, a large proportion of extracellular glutamate originates from metabolic pools (Daly and Moghaddam, 1993), and this could mask small fluxes related to the neuronal activity. Secondly, it is possible that corticostriatal glutamate release responds to D2 receptors only under conditions of stimulated and not basal release (Yamamoto and Davy, 1992).

With the present suggestion that L-DOPA may exert a sensitising effect on D2 receptors localised on GABA neurones projecting to the globus pallidus, it is appropriate to emphasise that GABA-ergic projection neurones are essential for the expression of striatum-related behaviour and some forms of dyskinesia may be related to a deficit of GABA in the striatopallidal system (Scheel-Krüger, 1986). The fact that long-term L-DOPA treatment reversed the striatal D2 receptor supersensitivity evoked by a single L-DOPA dose is likely to further disturb the dopaminergic-GABAergic interactions within the basal ganglia.

In conclusion, the present data confirm that acute L-DOPA treatment leads to increased in vivo binding of [^{11}C]raclopride, a selective D2 receptor ligand, in rat striatum. Additionally, they demonstrate that this in vivo D2

receptor sensitisation can be abolished by a long-term L-DOPA treatment. Considering such fluctuating L-DOPA effects on striatal D2 receptors, it seems interesting that the L-DOPA-induced increase in striatal D2 receptor binding can be associated with functional consequences such as reduced GABA-ergic output in the globus pallidus. It is likely that the effects reported here contribute to the phenomenon of fluctuating responses to L-DOPA therapy observed in end-stage Parkinson's disease.

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Authors' address: Dr. J. Opacka-Juffry, MRC Cyclotron Unit, Hammersmith Hospital, DuCane Road, London W12 OHS, United Kingdom.