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Time-dependent Striatal Dopamine Depletion after Injection of 6-Hydroxydopamine in the Rat. Comparison of Single Bilateral and Double Bilateral Lesions

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Abstract

For future investigation of possible perturbation of circadian rhythm in animal models of Parkinson's disease we needed an animal model providing lasting 80–100% striatal dopaminergic depletion in rats, but without induced mortality. We have thus compared the effects of a single hydroxydopamine bilateral striatal lesion (SB-hydroxydopamine) with those of a double hydroxydopamine bilateral lesion (DB-hydroxydopamine) at the same dose (16 μg/striatum) by HPLC determination of dopamine and 3,4-dihydrophenylacetic acid (dopac) levels in the striatum.

Two weeks after neurosurgery, SB-hydroxydopamine and DB-hydroxydopamine induced dopaminergic depletion of at least 81% compared with control groups. After eight weeks striatal dopaminergic depletion was only 60·97% in SB-hydroxydopamine rats, suggesting a compensatory phenomenon, whereas in DB-hydroxydopamine rats dopaminergic loss was stable at 88%. For the DB-hydroxydopamine group the dopac/dopamine ratio was significantly increased at week 2 only, whereas no significant change was observed for other groups. This increase might be explained by increased dopamine turnover.

We have demonstrated that striatal DB-hydroxydopamine injection induces lasting 80–100% neuronal loss, close to that observed in the disease in man, without induced mortality, and provides a tool which meets our experimental requirements.

As demonstrated for conditions such as cancer and asthma (Bruguerolle 1998), the time of administration of drugs can affect circadian rhythm. As far as we are aware, progressive modification of circadian rhythm in Parkinson's disease has never been investigated, even though motor fluctuations and daily variations (on–off phenomenon, fluctuation of end dose, circadian akinaesia, etc) are often evoked in daily clinical practice (Hoehn & Yahr 1968; Hornykiewicz 1972; Hoehn 1983)– indeed, a chronobiological and a chronopharmacological approach to investigation of Parkinson's disease might be of interest. However, an animal model of Parkinson's disease is needed, and might be a basis for the future study of the chronopharmacology of antiparkinsonian drugs.

For investigation of this hypothesis in future experiments with rats, the aims of this study were to characterize an animal model with the characteristics 80–100% striatal dopaminergic depletion in both hemispheres, lasting for at least eight weeks, and a lesion without induced mortality.

Future telemetric study of circadian rhythms requires stable dopaminergic depletion lasting for several weeks–80–100% dopaminergic degeneration is needed, to reproduce the neuronal loss observed in the pathology in man (Bernheimer et al 1973; Agid et al 1987), but without excessive mortality. Other animal models of Parkinson's disease, for example 6-hydroxydopamine bilateral lesions in the substantia nigra and methylphenyltetrahydropyridine lesion, induce quasi-total loss of dopaminergic neurons, but lead to 50% mortality (Sakai & Gash 1994; Gerlach & Riederer 1996; Salin et al 1996). A 6-hydroxydopamine bilateral lesion in the striatum is followed by 60% dopaminergic depletion only of the nigro-striatal bundle (Amalric et al 1995). Thus, for these reasons, it was supposed that injection of 6-hydroxydopamine at

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two different sites of the striatum, e.g. anterior and posterior, would provoke greater dopaminergic depletion of the whole structure. Nevertheless, such an animal model, if validated, must be a compromise between lack of induced mortality, sufficient dopaminergic depletion and lack of compensation, i.e. with lasting neuronal loss. The aim of this work was to compare the effects of single bilateral (SB) and double bilateral (DB) injection of the neurotoxin into the striatum by determination (by HPLC) of dopamine and 3,4-dihydrophenylacetic acid (dopac) levels in this tissue.

Material and Methods

Animals

For a minimum of 3 weeks before use, 24 Wistar AF IOPS adult male rats (Iffa–Creddo, St Germain-sur-l'Arbresle, France), 220–240 g at the beginning of the experiment, were housed in individual transparent polypropylene cages under controlled environmental conditions (relative humidity 50–55%; temperature $24 \pm 1^{\circ}$C) and synchronization of light–dark cycle (12 h–12 h; light from 0600 h to 1800 h) with free access to food and water. Experiments were performed in accordance with European Communities Council directives (86/609/EEC).

Hydroxydopamine lesions

At least three weeks after arrival, rats were anaesthetized by intraperitoneal (i.p.) administration of chloral hydrate ($400\ mg\ kg^{-1}$) and placed in a Kopf stereotaxic instrument with the tooth bar set $-3{\cdot}3$ mm above the interneural line. Lesions were made by means of a single bilateral injection, at $1\ \mu L\ min^{-1}$, into each striatum of hydroxydopamine ($16\ \mu g$) diluted with saline ($4\ \mu L$) containing ascorbic acid (1%). These animals (SB-hydroxydopamine; $n = 8$) received the neurotoxin at the coordinates (from the atlas of Paxinos and Watson) AP $+0{\cdot}20$ mm, L ± 3, DV $-5{\cdot}4$ mm from the bregma. The sham group receiving a single bilateral injection (SB-Sham) received injections of normal saline ($4\ \mu L$) containing ascorbic acid (1%). A second group of animals (DB-hydroxydopamine; $n = 8$) received a double bilateral injection, into the anterior and posterior striata, of hydroxydopamine ($8\ \mu g$), as the free base, in saline ($2\ \mu L$) containing ascorbic acid (1%). The coordinates used (Paxinos and Watson's atlas) were AP $+1{\cdot}70$ from the bregma, L $\pm 2{\cdot}8$, DV $-5{\cdot}6$ for anterior injection; AP $-0{\cdot}92$ from Bregma, L ± 4, DV $-5{\cdot}5$ for posterior lesion. Four rats (DB-Sham) received two injections of saline ($2\ \mu L$) containing ascorbic acid (1%) in the striatum, at the same coordinates. The direct injection into the striatum, inducing secondary dopaminergic degeneration of striatal neurones, used in this model is well established in other animal models of Parkinson's disease (Amalric et al 1995; Baunez et al 1995; Saade et al 1997). To determine the time-dependency of dopamine depletion rats were killed by decapitation two, four, six and eight weeks after creation of the lesions. Brains were quickly removed, and the striata were removed by dissection, weighed, frozen on dry ice and stored at -80°C.

Analysis of dopamine and dopac

Striatal determination of dopamine and dopac, enabling evaluation of the rate of dopamine turnover as expressed by the ratio dopac/dopamine, was used to provide an index of dopamine release and turnover to compare the efficiency of DB-hydroxydopamine and SB-hydroxydopamine lesions, taking into account the possible time-dependency of the depletion.

Two, four, six and eight weeks after production of the 6-hydroxydopamine lesions dopamine and dopac levels in the striata of each hemisphere were determined by reversed-phase high-performance liquid chromatography (RPHPLC). The chromatograph was equipped with a Precision Instruments (France) model 201 amperometric detector (electrochemical detection) and PCIP2 computer software (Kontron Instruments, France) for calculation and integration. Compounds were separated on a Spherisorb (Interchim, France) ODS2 C_{18} column; the mobile phase (pH 4·5) was sodium acetate (13·6 g), EDTA (212 mg), octyl sulphate (30 mg) and 7% methanol in 1 L water.

Tissues were homogenized with perchloric acid (0·5 M; $100\ \mu L$ (mg tissue)$^{-1}$) and 3-methoxy-4-hydroxybenzylamine (MHBA) was added as internal standard. After sonication the homogenates were centrifuged at 10 000 *g* and the supernatant was diluted with 20 vols mobile phase and injected ($20\ \mu L$) directly into the chromatograph. Detection limits were 50 pg for both dopamine and dopac (Dusticier & Nieoullon 1987).

Data analysis

Striatal levels of dopamine and dopac ($ng\ mg^{-1}$), expressed as means ± s.e.m. for each group, were compared by three-way analysis of variance. The three factors monitored were location (side of the lesion, i.e. right or left), time (experimental period,

i.e. 2 to 8 weeks after lesions) and treatment (hydroxydopamine or NaCl). If no interactions were detected between location and period, between location and treatment and among location and time and treatment, comparisons, by one-way analysis of variance, were made between week 2 and week 4, between week 4 and week 6 and between week 6 and week 8 for SB-sham, DB-sham, SB-hydroxydopamine and DB-hydroxydopamine rats, and also between the sham groups (SB- and DB-sham), between SB-sham and SB-hydroxydopamine and between DB-sham and DB-hydroxydopamine for weeks 2 to 8. If any difference was significant, analysis of variance was followed by Fisher's protected least significant difference test for multiple comparisons.

Results

As expected, animals showed evident motor disturbances after hydroxydopamine lesion for the first two weeks after surgery. Mean ± s.e.m. concentrations of dopamine and dopac, after stereotaxic surgery, are shown in Table 1. Concentrations of dopamine and dopac in the striata of SB-sham and DB-sham groups did not change significantly during the duration of the experiment and dopamine and dopac levels were no different for the respective control groups. Two weeks after surgery dopaminergic depletion for the SB-hydroxydopamine and DB-hydroxydopamine groups was 81·37% and 86·02%, respectively, compared with controls. Whereas from two to eight weeks after surgery striatal dopaminergic depletion in the DB-hydroxydopamine group was maintained at ca 85-91%, dopaminergic depletion in the SB-hydroxydopamine rats was 86·00% and 60·97% at weeks 2 and 8, respectively. Table 1 also shows that the dopac/dopamine ratio was significantly highest at week 2 for the DB-hydroxydopamine group (compared with both DB-sham and SB-hydroxydopamine groups).

Discussion

As expected, single bilateral and double bilateral intrastriatal administration of hydroxydopamine induced degeneration of the dopaminergic terminals of the nigrostriatal bundle. This study reports for the first time that injection of hydroxydopamine into the striatum at two different sites, i.e. the anterior and posterior, induced more dopaminergic depletion in the whole structure than did a single injection of the neurotoxin at the same dose (16 μg (striatum)$^{-1}$). Thus, eight weeks after surgery we observed 60% loss of striatal dopaminergic neurons in the SB-hydroxydopamine model whereas depletion in the DB-hydroxydopamine rats was ca 88% compared with controls. The time-dependency of striatal dopamine depletion in SB-hydroxydopamine rats was such that the loss at week 2 (81%) was more pronounced than that at week 8 (61%), which might be indicative of a compensatory phenomenon. Such an effect was not observed in the DB-hydroxydopamine group, striatal dopaminergic depletion being ca 85–91%, similar to the

Table 1. Levels of dopamine and dopac and the dopac/dopamine ratio for sham-operated and hydroxydopamine lesion rats for each period of the protocol.

Experiment		Single injection		Double injection	
		Hydroxydopamine	Sham	Hydroxydopamine	Sham
Week 2	Dopamine	2·37 ± 0·48*	12·75 ± 0·14	1·82 ± 0·43*	13·00 ± 0·87
	Dopac	0·40 ± 0·77*	1·54 ± 0·54	2·61 ± 0·53*†	1·15 ± 0·87
	Ratio	0·169 ± 0·02	0·120 ± 0·04	2·170 ± 1·030*†	0·088 ± 0·0001
Week 4	Dopamine	6·68 ± 1·07*‡	13·25 ± 1·76	1·92 ± 0·22*†	13·00 ± 0·40
	Dopac	1·13 ± 0·34*‡	2·12 ± 0·39	1·77 ± 0·32‡	1·39 ± 0·05
	Ratio	0·166 ± 0·05	0·174 ± 0·05	0·935 ± 0·120†	0·107 ± 0·0001
Week 6	Dopamine	3·15 ± 0·73*§	13·43 ± 0·46	1·17 ± 0·05*†	13·20 ± 0·26
	Dopac	0·60 ± 0·11	1·267 ± 0·003	0·94 ± 0·12§	1·267 ± 0·003
	Ratio	0·211 ± 0·02	0·095 ± 0·003	0·806 ± 0·09	0·096 ± 0·002
Week 8	Dopamine	4·63 ± 0·66*	11·85 ± 0·03	1·44 ± 0·10*†	12·11 ± 0·05
	Dopac	0·66 ± 0·02*	1·75 ± 0·03	0·99 ± 0·09*¶	1·84 ± 0·14
	Ratio	0·159 ± 0·02	0·148 ± 0·003	0·728 ± 0·100	0·152 ± 0·120

Results (μg (g tissue)$^{-1}$; means ± s.e.m.) were compared by one-way analysis of variance. If a statistically significant difference was detected, this was followed by Fisher's protected least significant difference test. *$P < 0·05$ hydroxydopamine compared with NaCl for each period. †$P < 0·05$ SB-hydroxydopamine compared with DB-hydroxydopamine for the same period. ‡$P < 0·05$ 2 weeks compared with 4 weeks for the same group. §$P < 0·05$ 4 weeks compared with 6 weeks for the same group. ¶$P < 0·05$ 6 weeks compared with 8 weeks for the same group.

neuronal loss observed in the pathology of man (Bernheimer et al 1973; Agid et al 1987).

The compensatory phenomenon observed in the SB-hydroxydopamine group might be explained by a neuroplasticity phenomenon such as ''sprouting'', i.e. emission of collateral terminals from the remaining dopaminergic neurons as evoked in several studies. Other mechanisms, such as an increase in the number of postsynaptic receptors might also be responsible for these phenomena (Zigmond et al 1989). Measurement of dopac, the main metabolite of dopamine, expressed as the widely used ratio dopac/dopamine, might provide a good index of dopamine release and turnover. Our data revealed an increase of this ratio in the two groups, compared with controls, and significantly higher values in the DB-hydroxydopamine rats. This finding is also in good agreement with the greater efficiency of DB-hydroxydopamine lesion compared to SB-hydroxydopamine lesion. These data are in good agreement with results from the literature. Thus, for instance, Onn et al (1986) observed a dramatic increase in the dopac/dopamine ratio one week after lesion, and lower values four months after surgery. An increase in this ratio was also described by Zigmond et al (1998) and explained in terms of an increase in dopamine turnover. These authors reported that passive loss of high-affinity dopamine-uptake sites might be observed with the degeneration of dopamine terminals. As reported by Rausch et al (1988), increased tyrosine hydroxylase activity was also reported in remaining dopamine neurons after hydroxydopamine lesions, in accord with the observation that tyrosine hydroxylase is the rate-limiting enzyme in the biosynthesis of catecholamines.

We have demonstrated in this study that striatal injection of DB-hydroxydopamine induces lasting 80–100% neuronal loss, comparable with that observed in Parkinson's disease in man, without inducing mortality. This model, a combination of lack of induced mortality, sufficient dopaminergic depletion, and lack of compensation with lasting neuronal loss, provides a good tool which meets the experimental need outlined above.

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