PARKINSON'S SYNDROME INDUCED IN CATS BY THE USE OF 6-HYDROXYDOPAMINE. OBSERVATIONS OF BEHAVIOR AND MOTOR DISORDERS

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Key words: parkinsonism, 6-OHDA microinjections, substantia nigra, corpus striatum, EMG

Abstract. The possibility of inducing Parkinson's syndrome in cats was investigated in three kinds of lesions: by microinjection of 6-hydroxydopamine (6-OHDA) into the pars compacta of substantia nigra (SNC), bilateral injection into the SNC and globus pallidus (GP) and into the SNC and caput nuclei caudati (NC). In all three kinds of lesions of the dopaminergic system disturbances of behavior involving specially the motor system were obtained, corresponding to the parkinsonism syndrome — in the form of bradykinesia-akinesia, increased muscle tonus of plastic type, vegetative disorders (sialorrhea, pupils) and psychic disorders such as lack of interest in the surroundings and food. The character of the enhanced muscle tonus typical for extrapyramidal disturbances was confirmed by EMG examination. The parkinsonism-like syndrome induced in the cats was transient and receded after several weeks.

INTRODUCTION

The aim of the study was to evoke pathological symptoms imitating Parkinson's disease in cats by means of neurotoxin 6-hydroxydopamine, in order to obtain a model for future experimental electrophysiological studies. An incentive to those investigations were the reports that 6-OHDA
has neurotoxic properties towards the neurons of the dopaminergic system.

It follows from the data in the world literature that a pathological syndrome similar to Parkinson's disease in humans and monkeys, but less pronounced and without tremor, could be evoked experimentally in mice and rats (1, 3-5). There were, however, only a few reports on the induction of such a pathological syndrome in cats.

Since in our conditions cats are the best object for electrophysiological investigations, experiments were undertaken in order to obtain a model similar to the parkinsonism syndrome in cats.

METHODS

Six cats weighing on the average 3 kg each were used after a six-week quarantine. Three kinds of stereotaxic lesions were performed under general hexobarbital anesthesia in a 80 mg/kg i.p. dose. A microinjection of 6-hydroxydopamine was given with a Hamilton syringe. Two cats were used in each group. The following lesions were produced:

1. Bilateral lesion of the pars compacta of the substantia nigra of the brain (SNC) (two microinjections on each side).
2. Bilateral lesion of the substantia nigra — SNC and globus pallidus (GP).
3. Bilateral lesion of the SNC and head of the nucleus caudatus (NC).

The target points for the stereotaxic microinjection were found according to the Atlas of Snider and Niemer (9).

The microinjection of 6-OHDA at each target point consisted of 0.2 μl of the toxin dissolved in 0.2% vitamin C, introduced at a rate of 0.5 μl/min in a dose of 10 μg/2 μl.

The two microinjections of 6-OHDA for the caudate nucleus head were larger, amounting to 10 μ/10 μl each, so that the lesions merged into one of about 3 mm in diameter just before the anterior edge of the commissura anterior. Thirty minutes prior to the microinjection of 6-OHDA, the cats received disipramine hydrochloride (DMI) i.p. in a dose of 25 mg/kg body weight. In the postoperation period the animals received, by way of protection, 600,000 U/24 h of penicillin and an infusion solution for the first 2 or 4 days. For a histological verification of the lesions the cats were perfused intracardially with an 0.9% physiological saline solution until the blood was completely washed out of the vascular system. Then they were perfused with 10% formalin, the skull was opened and the extracted brain was placed in 10% formalin for two weeks. The fixed brains were embedded in paraffin and cut into 20 μm sections, which were further stained by Niessl’s method.
Results and postoperative observations

Observation of the cats in the postoperative period disclosed certain differences in the general and motor behavior depending on the kind of anatomical lesion in the given experiment. Therefore the observations are described successively for each of the three kinds of experiments.

Postoperative observations of the cats after a bilateral SNC lesion

The cats remained in the lethargic sleep for 48 h. They lay with half-closed eyelids and widely distended pupils. They received an infusion solution. Within the 3rd day they began to move their paws and heads but did not stand up, the pupils were still widened. They began to swallow fluid introduced into the mouth. On the fourth day the animals attempted to raise their heads by distinctly saccadic movements; the movements of the extremities were sluggish and uncoordinated. The cats swallowed fluids introduced into the mouth but did not swallow solid food and salivated profusely. On the fifth day the cats exhibited uncoordinated movements in attempts to stand up. These movements were distinctly slowed down, and the muscles weakened. The muscle tonus in the extremities was enhanced in the stretch reflex on the side of the flexors and extensors. Wagging of the head to the sides was noted. On the sixth day the cats began to walk. The movements were slow and there was a tendency to reel to the sides. They set the posterior paws wide apart. The muscle tonus in the extremities was enhanced, more in the posterior than in the anterior extremities. The pupils were narrowed. They drank fluids and swallowed mashed food introduced into the mouth. Between the seventh and the tenth day the legs were put less wide apart during walking, but the movements continued to be slow. The head was held erect, the tendency to stagger to the sides gradually disappeared. The cats did not clean themselves. There was profuse sialorrhea. Between the tenth and the fourteenth day the cats took interest in the environment, approached the food presented to them and began to take it by themselves. The slow bradykinesia continued, and the muscle tonus was still markedly increased. The animals began to make sounds. After three weeks a normalization of movements was manifested: there was no tendency to stagger to the sides in walking and attempts were made to run. The animals had a fairly good appetite, and began to clean themselves. The increase of the muscle tonus receded. About two months after the operation the behavior of the animals was identical with that of intact cats, the movements of the extremities were quite efficient and rapid, the cats took a lively interest in their surroundings and cleaned themselves. The sialorrhea ceased.
Postoperative observations of cats after lesions of substantia nigra (SNC) and globus pallidus (GP)

The animals remained in the lethargic sleep for full three days (72 h) with semiclosed eyes. The pupils were dilated with weak reaction to light. The animals made no movements and were artificially hydrated. On the 4th day the pupils began to narrow, the animals made sluggish movements with a single extremity. They did not sit up or try to stand. They started to swallow fluids introduced into the mouth. The sialorrhea continued. On the fifth day the pupils were still distended, the eyes open, the animals looked around. They started making saccadic movements, inclining at the same time their heads to the sides. They did not stand up or eat and were not interested in food. When fed with a spoon they swallowed the food. The muscle tonus was distinctly enhanced in the extremities, both on the side of the flexors and on the side of the extensors. Between the sixth and the tenth day the cats were still fed with a spoon and showed no interest in food. When attempting to stand up and walk they reeled to the sides and frequently fell over. At the same time they inclined their heads to the sides. The movements of the extremities were distinctly slowed down and uncoordinated. They set their feet wide apart, both the anterior and the posterior ones, and dragged the latter. The muscle tonus of the extremities was enhanced and of plastic type. The pupils were narrowed. Between the eleventh and the fourteenth day the cats began to eat food by themselves but in small portions and they preferred drinking. The movements of the extremities continued to be slowed down, insecure and with marked ataxia, with inclination of the head. An enhanced muscle tonus of a plastic type continued in the extremities. There was a tendency to rotary movements about the body axis and a periodic tension of the posterior extremities. After three weeks the animals of this group behaved similarly to those in experimental group I.

Postoperative observations of cats after lesions of substantia nigra (SNC) and nucleus caudatus (NC)

For two and a half days the cats remained in lethargic sleep with half-closed eyes, the pupils dilated and a weak reaction to light. No spontaneous movements of the extremities were noted. During that time the animals were artificially hydrated. On the third and the fourth day the animals sporadically moved their extremities, then tried to sit up clumsily and still remained lying. The movements of the extremities were slowed down and uncoordinated. Tremor of the head and inclination to the sides were noted. The cats began to swallow fluids introduced into the
mouth through a tube. In the stretching reflex of the extremities an enhanced tension of the muscles was found, both on the side of the flexors and on the side of the extensors. On the fifth and the sixth day the animals began to take interest in the surroundings. Their pupils were still slightly distended (anisocoria). They tried to stand up and walk, but their movements were uncoordinated, with feet wide apart. In attempts to walk they reeled and staggered to the sides, frequently falling. The movements of the extremities were distinctly sluggish and the muscle tonus enhanced in both groups of muscles, both on the side of the extensors and on the side of the flexors. During the attempts to move there appeared wagging movements of the head to the sides of saccadic character. The animals made no sounds. They did not ingest food unaided, but swallowed liquids or semiliquid food. The pupils narrowed gradually but anisocoria was noticeable with a weak reaction to light. Between the seventh and the fourteenth day the animals continued to show no interest in drink or food which they swallowed automatically only when introduced into the mouth. The pupils became narrow. The cats continued to walk with feet wide apart with a tendency to lean to the sides. The movements of the extremities were slowed down, and the hind extremities had a tendency to drag. In the standing position stretching of the posterior extremities was observed rather frequently with a stiff erection of the tail (Fig. 1) lasting several seconds. The animals showed no interest in games (artificial mouse). In the third week the animals took more interest in the surrounding and food.

Fig. 1. Stretching the hind extremities with erection of the tail, after 6-OHDA microinjection into SNC.
They did not emit any sounds. The movements continued to be slow and there was a tendency to rotary movement; sometimes there was reeling to the sides with sporadic falls. Stretching of the posterior extremities and tails was also observed. The muscle tonus of plastic type continued to be increased in the extremities, particularly in the posterior limbs. Wagging of the head was still noted. In the fourth and the fifth week the behavior of the animals gradually improved, they became more lively and more interested in the surrounding, started to make sounds asking for drink, and ate the food set before them more readily by themselves. Their walk was more and more steady, but the movements continued to be slow. Sporadically there occurred retraction of the hind limbs with straining but lasted for a shorter time. The muscle tonus seemed to be still slightly increased. The cats began to clean themselves. After 6 weeks their behavior and motor efficiency became normal, resembling that of intact cats.

Electromyographic examination

In the polygraphic records of cats in the experiments with the neurotoxin 6-OHDA we can regard, as exponents the disturbances in the coordination of movements and the enhanced muscle tonus expressed by the changes presented in Figs. 2 and 3.

![Electromyogram](image)

Fig. 2. Persisting tonic activity both in the flexors and in the extensors (standing position of the cat). SR, m. soleus right; TR, m. tibialis right; SL, m. soleus left; TL, m. tibialis left.

Figure 2 presents the electromyographic (EMG) record of a cat with lesion II in a standing position with needle electrodes in the antagonistic muscles of both posterior extremities (m. soleus and m. tibialis). It was
noteworthy that the tonic activity persists both in the flexors and in the extensors of the extremities. When the tonic activity disappears in the extensors (the cat sits down), the activity continues on the part of the flexors, although it should also disappear (state of rest).

Figure 3 concerning lesion III presents the EMG record of a cat at rest (sitting posture). Instead of being extinguished the activity continues in the flexors. This means that the regulation of the muscle tonus in the extremities between flexors and extensors is disturbed.

Fig. 3. In the resting position of the animal tonic activity continues in the flexors (TR and TL).

**Histological examination**

An example of the destructive action of neurotoxin 6-OHDA on cells of the dopaminergic system is given in Fig. 4. The microinjection of 6-OHDA (trace of micropipette — E) caused the loss of the cells of pars compacta substantiae nigrae (SNC) visible in the microphotograph between markings A and B. At marking D a group of cells containing melanin survived at the boundary of pars reticularis substantiae nigrae (SNR). Below SNC gliosis is visible (C).

In histological preparations, after a 6-OHDA injection into the globus pallidus and caput nuclei caudati such pronounced structural lesions as in the SN were not found. Loss of cells with patches of gliosis was visible. Microinjections into the globus pallidus were asymmetric, this being due to the deviation of the micropipette endings in passing through the compact capsula interna fibres to the globus pallidus.
Fig. 4. Loss of the melanine cells in SNC between A and B. Trace of micro-pipette — E; group of surviving melanine cells — D; gliosis — C.
DISCUSSION

An analysis of the character of the observed disturbances in all the three groups indicates that they were similar to the symptoms characteristic for Parkinson's syndrome in humans. This syndrome exhibits akinesia-bradykinesia, an increased muscle tonus of plastic type, tremor of the extremities and psychomotor bradykinesia. These features, except tremor, could be noted in the cats in all the three variations of experiments with lesions of the dopaminergic system. The period immediately following the operation was characterized by several days (3-5) of akinesia which then passed to bradykinesia, a great slowing down of movements, and an undoubted behavioral component consisting in lack of interest in the surrounding and food. The next period of bradykinesia was associated with disturbances in motor coordination and an increased muscle tonus of plastic type, involving the groups of flexors and extensors in the extremities. This imitated the disorders appearing in Parkinson's syndrome.

Iversen (5) demonstrated that 6-OHDA is a suitable drug for developing an animal model of parkinsonism. There appeared further communications that the lesion of the substantia nigra due to 6-OHDA causes a fall in the dopamine level (striatal DA). The fall on the side of the lesion is, ten days after the microinjection, proportional to the degeneration of the dopaminergic neurons. This has been demonstrated in electronmicroscopic studies (10) after an electrolytic damage to the SN (3, 6, 7), and histochemical observations after local 6-OHDA injections into the SN of mice and rats (11). In investigations on monkeys (8) it appeared that a single injection of 6-OHDA at the level of the posterior thalamus through which passed a bundle of nigrostriatal axons produces a severe loss of neurons in the pars compacta substantiae nigrae and a considerable fall of the dopamine level reaching 80% (2) on the side of the lesion. These disturbances were accompanied by motor disorders in the form of bradykinesia with an enhanced muscle tonus, torticollis, circling around the body axis and tremor. After the administration of L-dopa doses (1.0-2.5 mg/kg) the disturbances receded. This observation confirms the conjecture that the symptoms observed represented Parkinson's syndrome in monkeys (induced by the destruction of the nigrostriatal pathway with loss of neurons in the SN due to the neurotoxin 6-OHDA).

In the light of data from the literature and the authors' own observations it may be supposed that the severe pathological symptoms observed in the experimental cats, occurring immediately after surgical treatment, were connected with an acute phase of the neurotoxin action
on the dopaminergic neurons. The extrapyramidal symptoms doubtlessly belonged to the parkinsonism syndrome. They are a transient phenomenon, and, according to the results reported in the literature, may be related to the drastic fall of the dopamine level under the influence of 6-OHDA. The gradual recession of those symptoms and the return of the animals to a normal state may be explained by the survival of part of the cells of the dopaminergic system, which continued dopamine production, gradually compensating for the loss up to the level indispensable for the normal functioning of the various parts of the nervous system.

The final conclusion resulting from the investigations described is that it is possible to evoke the parkinsonism syndrome in cats by means of 6-OHDA neurotoxin. The resulting model of this disease is, according to most reports not durable. To obtain in cats a more stable model, which could serve for electrophysiological studies further experiments are indispensable, requiring a suitable choice of the neurotoxin dosage and of the extent of the selective lesions of the dopaminergic system together with checking simultaneously the level of the catecholamines and enzymatic investigations.

We wish to thank Professor A. Romaniuk and Mrs. M. Strzelczuk for their help and critical assessment of the manuscript. The investigation was supported by Project 06-02.IV.2. of the Polish Academy of Sciences.

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