# EXPERIMENTAL BIOLOGY

# Catecholamines and Their Metabolites in the Brain and Urine of Rats with Experimental Parkinson's Disease

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Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny,* Vol. 130, No. 8, pp. 223-227, August, 2000
Original article submitted January 24, 2000

The content of catecholamines and their metabolites in the brain and the relationship between cerebral catecholamine levels and their urinary excretion were studied in rats with 6-OHDA-induced hemiparkinsonism. 6-OHDA reduced brain concentrations of dopamine, DOPAC, and homovanilic acid and urinary excretion of dopamine, dioxyphenilalanine, and DOPAC by more than 90%. A positive correlation was found between the concentrations of these metabolites in the urine and striatum. Measurement of urinary catecholamines and their metabolites is a perspective test for evaluating the status of the dopaminergic nigrosostriate system of the brain in experimental parkinsonism.

**Key Words:** *experimental parkinsonism; catecholamines*

Investigation of the pathogenesis of Parkinson's disease determines new trends in the development of perspective approaches to its diagnosis and therapy [8]. The priority of this problem is dictated by high incidence of this disabling disease essentially decreasing patients' quality of life [1]. Inefficiency of traditional therapy with L-DOPA necessitates the search for new treatment of this disease [7].

Identification of the main biochemical substrate of Parkinson's disease (decreased dopamine concentration in the striatum due to disturbances in the dopaminergic nigrostriate system — DNS) and pathogenetic mechanisms of dopaminergic neuron death (oxidative stress, apoptosis) created prerequisites for possible therapeutic interventions in the pathogenesis of neurodegeneration [1,5,8]. Such pathogenetic approaches to the treatment of Parkinson's disease, *e. g.* transplantation of embryonal nervous tissue and therapy with neurotrophic factors along with antioxidants are now actively used in clinical and experimental studies [7].

In experimental studies of Parkinson's disease the concentration of the striatal dopamine during treatment is an important criterion of treatment efficiency [3,6-8]. However *in vitro* catecholamine assays in the brain tissue are very difficult. We tried to develop a dynamic test for *in vivo* evaluation of the brain catecholamines in rats with 6-OHDA-induced hemiparkinsonism by measuring daily catecholamine excretion with the urine. Decreased urinary concentrations of dopamine, norepinephrine, and their metabolites in patients with Parkinson's disease were reported previously [4, 5], but the relationship between central and peripheral catecholamines has not been investigated.

Here we studied cerebral concentrations of catecholamines in rats with hemiparkinsonism and the relationship between cerebral content of catecholamines and their urinary excretion in rats with DNS pathology caused by injection of 6-OHDA.

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0007-4888/00/0008-805$25.00 

## MATERIALS AND METHODS

Experiments were carried out on adult male Wistar rats (250-300 g) kept under standard conditions with the natural day/night cycle and free access to food and water.

DNS was damaged by stereotaxic administration of 6-OHDA into the right ascending mesostriatal dopaminergic tract by means of Narishige stereotaxic device under ketamine narcosis (80 mg/kg). The first dose of 6-OHDA (2.5 μl, 4 μg/μl, Sigma) with 0.25% ascorbic acid was injected into a point with the following coordinates: Ap=4.4, L=1.2, V=7.5, the upper canine plane was positioned 2.3 mm below the interaural line. The second dose of neurotoxin (1.5 μl) in the same concentration was injected according to coordinates Ap 4.0, L 1.0, and V 7.6, with the upper canine plane being 3.4 mm above the interaural line. 6-OHDA was injected into the brain with a microsyringe connected to a Sage-instruments pump at a rate of 1 μl/min.

On day 8 after denervation before sacrifice the animals were injected with a nonselective D-receptor agonist apomorphine. Rotation was tested in an automated rotometer for 40 min. Experimental group consisted of 10 animals rotating at a rate of no less than 7.5 rpm, which indicated a high degree of DNS damage [7,9]. Control group consisted of 10 animals administered similar stereotaxic injections of 0.25% ascorbic acid.

Fourteen days after injection of 6-OHDA daily portion of the urine was collected in metabolic cages (Techiplast). On days 14-20 after injury, 6 and 5 rats from the experimental and control groups, respectively, were decapitated, the brain was rapidly removed, and the area containing the striatum was isolated separately for each hemisphere. All samples for biochemical analysis were rapidly frozen at -80°C. In other animals the brain was perfused with 4% neutral paraformaldehyde routinely on day 30 of the experiment and serial sections of *substantia nigra* were prepared and stained with cresyl violet.

The concentration of 3,4-DOPA, dopamine, 3,4-DOPAC, homovanilic acid (HVA), norepinephrine and its metabolite 3,4-dioxyphenylethylene glycol (DOPEG) in the brain and urine were measured by high-performance liquid chromatography [2].

The data were analyzed using Student's paired and two-sample *t* test and Pearson's correlation analysis.

## RESULTS

Injection of 6-OHDA into the mesostriatal dopaminergic tract led to the development of motor asymmetry characterized by postural disorders (inclination of the head and tail to the injured side at rest) and spontaneous rotation around the damaged side. These disorders were compensated within 7-10 days. Unlike spontaneous rotation, apomorphine-induced rotation caused by hypersensitivity of D receptors due to essential decrease of dopamine concentration in the damaged striatum persisted for the entire animal's life. Mean values of apomorphine-induced rotation in experimental group were 9.6±1.5 rpm. No rotations were observed in the control group.

Histological analysis of the midbrain showed gliosis at the site of 6-OHDA injection and neuronal death in *substantia nigra* pars compacta in all animals with high degree of rotation (Fig. 1). A more than 90% decrease in dopamine, DOPAC, and HVA concentrations and a negligible decrease of norepinephrine level were observed in the striatum of such animals, which indicated predominant suppression of DNS function in the presence of relatively intact noradrenergic structures on the damaged side (Table 1). Examination of the brain from controls showed a decrease in dopamine and norepinephrine concentrations (by 24 and 35%, respectively). The concentrations of HVA in the brain hemispheres were virtually the same, while DOPAC level was higher in the right hemisphere (Table 1).

**TABLE 1.** Changes in Catecholamine Concentrations (μg/g Wet Tissue) in the Brain of Rats with 6-OHDA Denervation (*M*±*m*)

| Catecholamines | Control group | | 6-OHDA-denervated rats | |
|---|---|---|---|---|
| | S | D | S | D |
| Dopamine | 738.83±139.54 | 564.61±167.93 | 447.62±64.77* | 28.47±8.79* |
| DOPAA | 93.67±28.94 | 107.22±36.35 | 221.57±81.82* | 15.84±5.25* |
| Norepinephrine | 619.58±54.41 | 408.70±87.05 | 383.40±31.05 | 193.98±60.19 |
| Epinephrine | 4.77±1.61 | 3.54±1.96 | 2.95±1.78 | 1.83±0.29 |
| DOPEG | 36.02±10.42 | 35.49±11.79 | 19.14±1.63 | 15.56±4.77 |
| HVA | 62.1±16.8 | 45.10±19.26 | 53.20±9.07* | 5.53±2.30* |

**Note.** *S*: left (intact hemisphere); *D*: right (denervated) hemisphere. *$p<0.01$ *vs.* intact hemisphere.

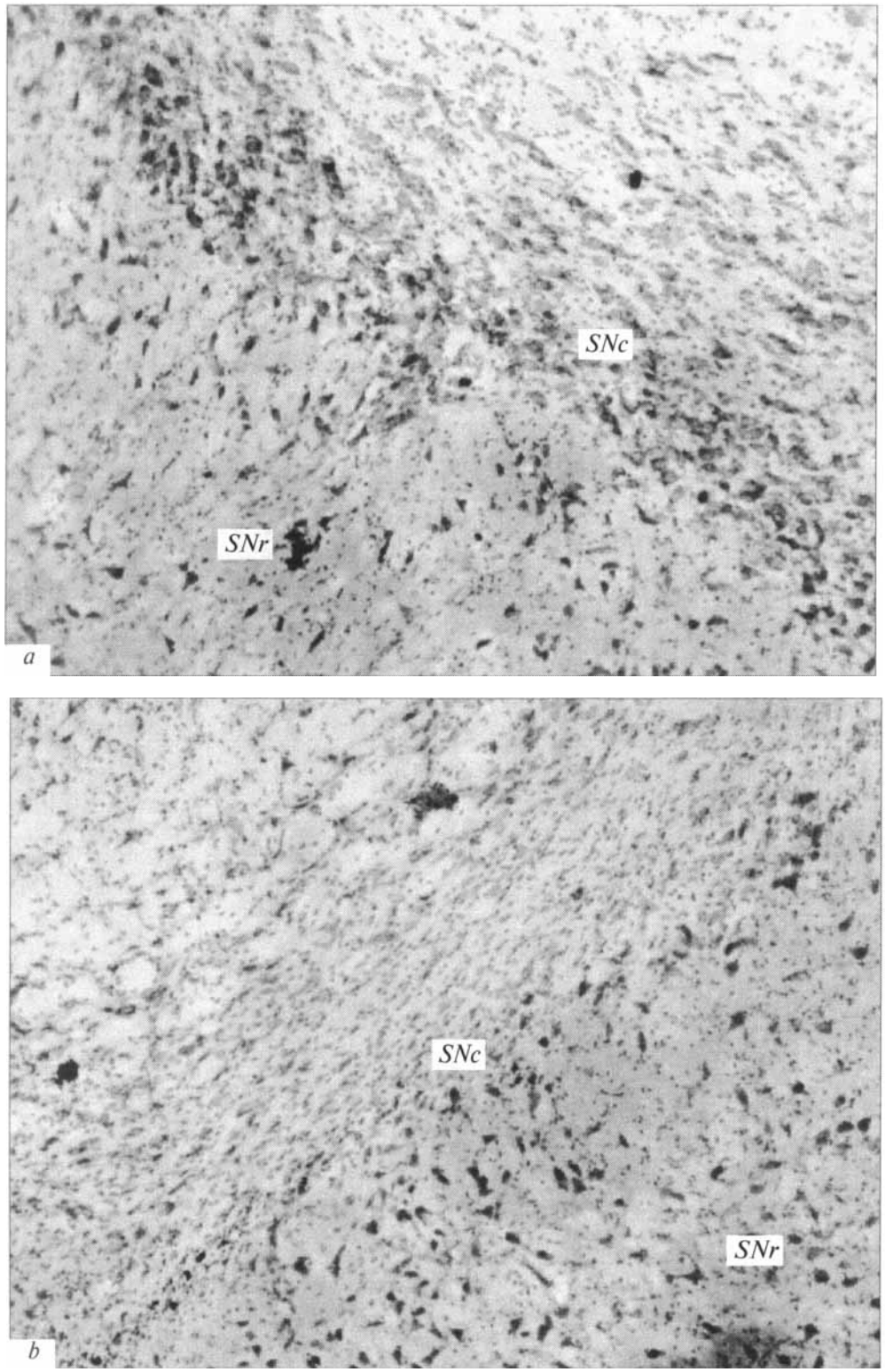


**Fig. 1.** Pathomorphological changes in *substantia nigra pars compacta* (*SNc*) 30 days after injection of 6-OHDA in intact hemisphere (*a*) and on the damaged side (*b*), ×100. Cresyl violet staining. Death of dopaminergic neurons, infiltration of the compact zone with microglial cells. *S. nigra pars reticulata* (*SNr*) is unchanged.

These changes in catecholamine spectra in the control can be regarded as a reaction to intervention. It is noteworthy that dopamine and norepinephrine concentrations in the intact left hemisphere were essentially lower in experimental rats, which indicates a possible involvement of the contralate-

**TABLE 2.** Coefficients of Correlation between Catecholamine Concentrations in the Brain and Urine in Rats with Hemiparkinsonism

| Urine | Brain | | | | | |
|---|---|---|---|---|---|---|
| | DOPEG | DOPAA | norepinephrine | epinephrine | dopamine | HVA |
| DOPA | 0.16 | 0.67* | 0.14 | 0.16 | 0.37 | 0.35 |
| DOPEG | -0.06 | -0.25 | -0.27 | -0.34 | -0.34 | -0.32 |
| DOPAA | 0.26 | 0.48* | 0.16 | -0.07 | 0.11 | 0.09 |
| Norepinephrine | 0.39 | 0.34 | 0.22 | 0.00 | 0.03 | 0.02 |
| Epinephrine | -0.17 | -0.25 | -0.39 | -0.25 | -0.13 | -0.12 |
| Dopamine | 0.43 | 0.45 | 0.60* | 0.34 | 0.14 | 0.16 |
| HVA | 0.08 | 0.25 | -0.04 | -0.31 | -0.23 | -0.33 |

**Note.** *$p<0.05$.

ral hemisphere in the pathological process after injection of 6-OHDA.

Urinary excretion of DOPA, DOPAA, dopamine, and norepinephrine in denervated rats decreased significantly. The most pronounced decrease in the excretion after 6-OHDA injection was observed for DOPA and DOPAA, whose concentrations in daily urine of animals with hemiparkinsonism decreased by 80 and 56%, respectively ($p<0.01$ *vs.* before 6-OHDA and control). None the less, daily DOPA concentration normally varied within a wide range and tended to decrease in the control group (Fig. 2), while changes

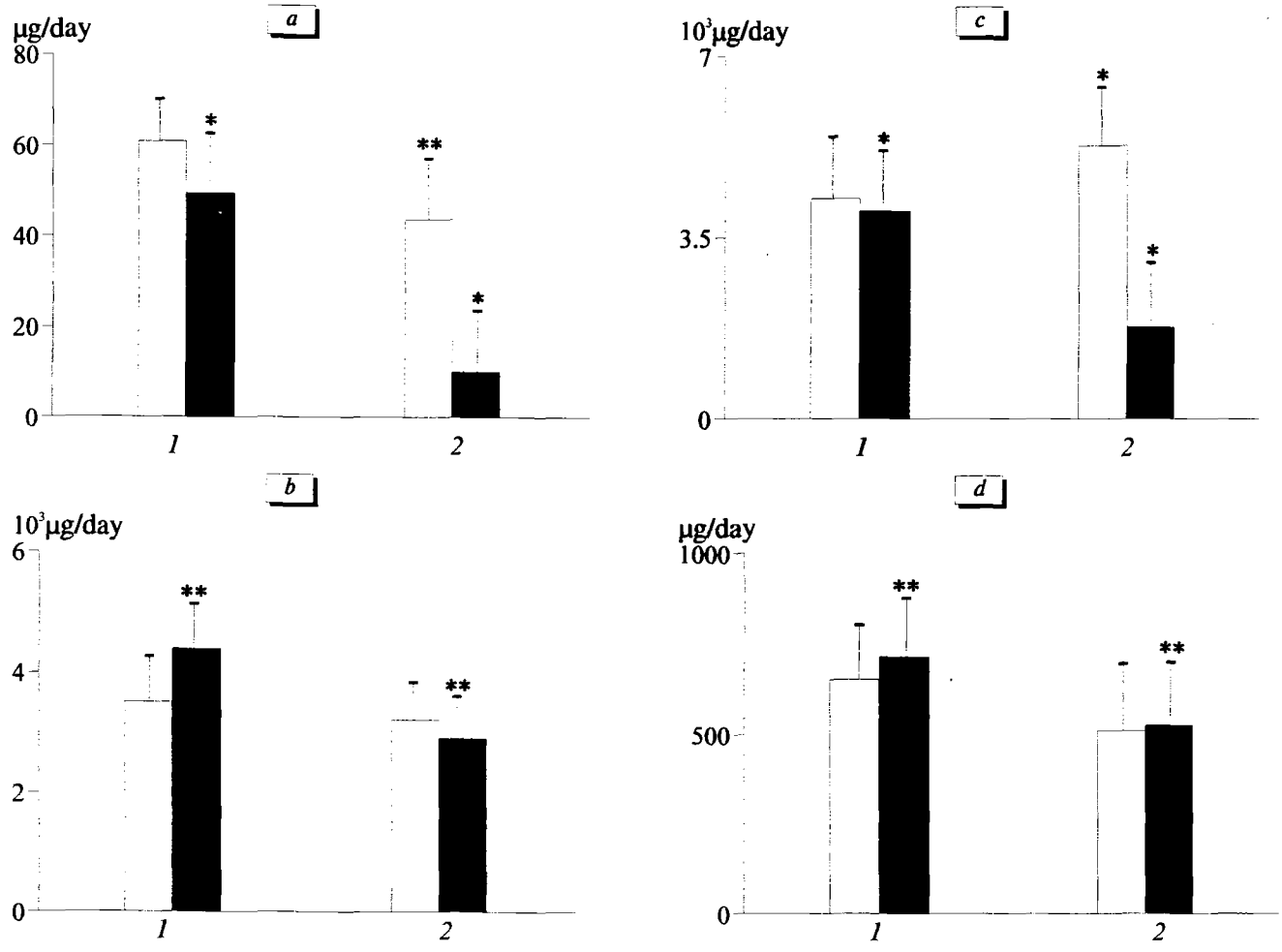


**Fig. 2.** Effect of DNS denervation on the content of DOPA (*a*), dopamine (*b*), DOPAC (*c*), and norepinephrine (*d*) in daily urine of rats before (*1*) and after the injury (*2*). Light bars: control, dark bars: experiment. *$p<0.01$, **$p0.05$ *vs.* the control.

in DOPAC excretion in experimental and control rats were opposite. Dopamine and norepinephrine concentrations in the experimental group before and after denervation differed significantly, but after denervation the differences between the control and experimental groups were insignificant. Differences in urinary DOPEG and HVA concentrations were insignificant both in the experimental and control groups.

Pronounced changes in DOPA and DOPAA concentrations in the urine cannot be explained solely by local effect of 6-ODHA in one hemisphere. Probably we observed a result of systemic effect of DNS impairment on the production and excretion of peripheral catecholamines. This hypothesis is confirmed by well-known alteration of catecholamine production in the adrenals of patients with Parkinson's disease [1,8].

Analysis of correlations demonstrated a relationship between the decrease in catecholamine concentrations in the urine of rats with hemiparkinsonism (Table 2). The highest positive correlation was revealed for DOPA and DOPAC. On the other hand, dopamine concentration in the urine significantly ($p<0.05$) correlated with norepinephrine level in the brain. Other parameters did not correlate, which seems to be due to the effect of catecholamines excreted by the adrenal medulla.

Hence, unilateral injury to the mesostriatal dopaminergic tract by 6-OHDA produces complex behavioral and biochemical effects in rats, characterized by stable contralateral apomorphine-induced rotation and significant decreases in the concentrations of dopamine, DOPAC, and HVA in the homolateral hemisphere and by decreased excretion of catecholamines with urine, the decreases in DOPA and DOPAC concentrations being significant. The concentrations of DOPA, DOPAC, and dopamine in the urine positively correlate with striatal concentrations of DOPAA and norepinephrine. Measurement of these catecholamine metabolites in the urine holds good promise for evaluation of the cerebral DNS status in experimental parkinsonism.

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