# Effects of L-DOPA and 6-Hydroxydopamine on the Intracellular cAMP Content in Rat Kidney with Neurotrophic Dysfunction of the Organ

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It has been established that chronic stimulation of the sciatic nerve in rats, accompanied by a disturbance in the neurotrophic supply of the kidneys, results in damage to the mineralocorticoid receptors (MCR) of the tubule cells due to a pathological effect of the nerve stimuli arriving from the focus of nerve injury in the sympathetic nerves and via the brain adrenergic system and hypothalamus-endocrine glands system. Pharmacological stimulation of the sympathetic nervous system aggravates the functional disturbances in the kidney MCR in reflex dystrophy (RD), whereas pharmacological desympathization normalizes the receptor function [1,2,4]. In this connection the role of different levels of adrenergic activity in the regulatory mechanisms of aldosterone reception is a matter of interest. It is well known, that the sympathetic nerves, their neurotransmitters, and neuropeptides act upon the target cells via cAMP, which initiates the protein metabolic processes in the cytoplasm, nuclei, chromatin, and, possibly, among them, the metabolism of MCR protein-subunits (9,11,13,14). Thus, it seemed expedient to measure the concentration of cAMP in the kidneys under RD conditions against the background of different levels of adrenergic activity. The aim of this study was to examine the cAMP content in normal and in RD kidneys treated with L-DOPA and 6-hydroxydopamine (6-OHDA).

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## MATERIALS AND METHODS

Experiments were carried out on male albino rats weighing 180-200 g. Kidney RD was achieved by transecting the left sciatic nerve in the upper third of the femur followed by the administration of 0.2 ml 2% formalin in its central portion. To reinforce adrenergic activity L-DOPA (1-3,4-dioxyphenylalanine) was used as a precursor of catecholamine synthesis. L-DOPA (Serva, Germany) injected daily in a dose of 1 mg/100 g i.p. from the day of the nerve injury resulted in a stable elevation of the norepinephrine concentration in the urine [7]. 6-OHDA was used as a sympathetic inhibitory agent, because it destroys the adrenergic nerve endings and the neurons themselves, leading to a marked and prolonged decrease of the content of catecholamines in the peripheral organs and tissues [10,12,15]. 6-OHDA (Serva, Germany) was also injected i.p. on the day of nerve injury and 7 days after the operation in a dose of 10 mg/100 g. The influence of RD, 6-OHDA, and L-DOPA treatment on the kidney cAMP concentration was assessed 14 days after the onset of their

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TABLE 1. Effect of L-DOPA and 6-OHDA on cAMP Content (pmol/g) in Rat Kidney in the Control and on the 14th Day of Reflex Dystrophy.

| Experimental conditions | Control | 14th day of RD |
|---|---|---|
| Background | 1126±43 ($n$=20) | 1325±58 ($n$=20) $p$<0.05* |
| Adrenalectomy | 991±62 ($n$=18) $p$>0.05* | 1167±69 ($n$=17) $p$>0.05** |
| Adrenalectomy + L-DOPA | 1307±56 ($n$=20) $p$<0.05* | 1561±67 ($n$=20) p<0.02** |
| L-DOPA | 1405±80 ($n$=19) $p$<0.02* | 165375 ($n$=20) $p$<0.01** |
| Adrenalectomy+6-OHDA | 860±77 ($n$=18) $p$<0.01* | 1050±70 ($n$=18) $p$<0.01** |
| 6-OHDA | 912±70 ($n$=18) $p$<0.02* | 1103±61 ($n$=17) $p$<0.02** |

Note. One asterisk: significance of differences compared to control, two asterisks: significance of differences compared to RD.

effects. One half of the experimental animals (the control and treated rats surviving 14 days of RD) underwent a bilateral adrenalectomy 3 days prior to the experiment to prevent receptor binding with endogenous aldosterone. To assess the effect of adrenalectomy alone, again performed 3 days before the experiment, the kidney cAMP content was measured for control in only adrenalectomized animals and in rats with adrenalectomy on the 14th day of RD. Bilateral adrenalectomy was performed as a single-stage operation under ether anesthesia. All adrenalectomized animals received a 0.9% NaCl solution for drinking. Sham-operated animals were the controls. The kidney cAMP content of the sham-operated rats both in the control and on the 14th day of RD was the same as the cAMP level in intact animals and in rats with injured sciatic nerve. Intracellular cAMP was assayed by radioimmune kits (Amersham, England). The animals were decapitated. The kidneys were immediately frozen in liquid nitrogen. Extraction of tissue cAMP was performed with ethanol in a weight: volume ratio of 1:10. The radioactivity of the samples was counted in a ZhS-8 scintillator with an SL-4000 counter (Intertechnique, France). The concentration of cAMP was expressed in pmol/g wet tissue. A total of 230 rats were used in all the experiments. The results were processed by the method of variational statistics using the Fisher-Student test of the reliability of differences [8].

## RESULTS

The cAMP level in the rat kidney was elevated as compared to the control (the animals with intact sciatic nerve) on the 14th day of RD following sciatic nerve injury (Table 1). Daily injection of L-DOPA for 14 days in adrenalectomized control rats also produced a cAMP increase in the organ. Kidney RD together with L-DOPA administration in adrenalectomized animals resulted in a higher cAMP production. The rise of the cAMP level as an effect of L-DOPA was more expressed both in the control and under RD conditions in rats without adrenalectomy as opposed to operated animals, because the bilateral adrenalectomy itself either in the control or under RD produces an insignificant drop of the cAMP level in the kidneys according to our present data (Table 1) and a previous study [3].

6-OHDA administration both in the animals with intact sciatic nerve and in rats on the 14th day after denervation was accompanied by a decline of cAMP in the kidneys. Adrenalectomized rats, regardless of nerve injury, demonstrated a pronounced cAMP decrease after 6-OHDA injections in comparison with intact rats. This phenomenon stems from the summated depressive effect on the cAMP level in the kidneys of 6-OHDA-induced selective degradation of catecholaminergic structures and adrenalectomy.

Thus, the degree of cAMP increase was successively elevated in 3 experimental series, as follows: 1) on the 14th day of RD in comparison with the control; 2) in control animals with L-DOPA injections alone; 3) and, particularly, in RD rats with an L-DOPA-activated sympathetic system. The more pronounced elevation of kidney intracellular cAMP in rats with injured sciatic nerve in comparison with intact animals was due to an increase of the catecholamine level in the kidneys and some other tissues in animals with cut sciatic nerve, as demonstrated in previous investigations [5,6]. It is known that RD in the kidneys is accompanied by a decrease of the specific accumulation of aldosterone with MCR of tubular cells as a result of the pathological sympathetic impulses emanating from the injury focus [1,2,4]. Moreover, the reinforcement of the adrenergic activity by L-DOPA injections results in further damage to receptor function. It may be assumed that MCR dysfunction in kidney RD and, particularly, under RD against the background of L-DOPA is attended by an elevation of cAMP, which mediates the metabolic effects of catecholamines. Moreover, 6-OHDA injections in rats with RD prevented the development of trophic disturbances leading to impaired aldosterone receptor function in the kidneys [4]. In our experiments, however, pharmaco-

logical deprivation of the sympathetic function by 6-OHDA administration was accompanied by a decrease of cAMP in the kidneys with RD to the control level.

The data obtained lead to the conclusion that the cause of aldosterone reception impairment under RD (as result of sympathetic hyperfunction) and an improvement of receptor function under pharmacological desympathization may be catecholamine-induced variations of cAMP, which switches on a protein kinase-initiated system of intracellular metabolism, yielded the alterations in the protein spectra of cytoplasm, nuclei, and chromatin in the rat tubule cells, including the aldosterone receptor complex and its molecular environment.

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# Inotropic Responses of Human Myocardium in Ischemic Heart Disease

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Surgical management markedly improves the efficacy of treatment of ischemic heart disease (IHD), although it may be necessary to perform adjunct therapy by administering a fairly broad spectrum of pharmacological preparations [5]. This is all the more important, because surgical intervention may provoke heart dyskinesia and complicate the ischemic damage to the myocardium. At the same time, experimental results have been reported on a pronounced adaptogenic effect of brief repeated periods of ischemia or

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