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CYTOCHEMICAL MANIFESTATIONS OF SHORT- AND LONG-TERM ACTIVATION OF THE RAT BRAIN DOPAMINERGIC SYSTEM

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Changes in the state of the catecholaminergic system and, in particular, in the dopamine (DA) concentration in the brain, are among the causes of many pathological states of the CNS [8, 9]. The creation of an experimental model using dihydroxyphenylalanine (L-dopa) as the precursor of DNA synthesis has made possible the study of CNS function in the presence of hyperfunction of the dopamine system.

The aim of this investigation was to discover correlation between changes in morphochemical parameters of neurons belonging to individual brain formations and changes in the functional state of animals during activation of the dopaminergic system by short- or long-term L-dopa administration.

EXPERIMENTAL METHOD

Experiments were carried out on mature male Wistar rats weighing 240-270 g, selected beforehand by the open field (OF) test, and characterized by high horizontal motor activity (140-200 squares crossed or more). The rats were given an intraperitoneal injection of 25.5 mg/kg body weight of the preparation madopar-125, containing L-dopa, together with benserazide, an inhibitor of its peripheral decarboxylation. This dose of the preparation corresponds to the action of 50 mg/kg of pure L-dopa. Control animals received the same volume (0.3 ml) of physiological saline. Throughout the experiment the rats were tested in OF 30 min after each injection. The sensomotor cortex (SMC) (layers III and V), caudate nucleus (CN), and nucleus accumbens (NA) were taken for investigation. The experimental and control animals were decapitated after 3 and 14 days and the left cerebral hemisphere fixed in Carnoy's solution followed by embedding in paraffin wax, and interferometry [4], whereas the right hemisphere was frozen, and sections cut in a cryostat to a thickness of 20 μ, which were used to determine aminopeptidase (APase) activity. Protein concentrations in the test structures were determined by interferometry in monochromatic light, at a wavelength of 535 nm, on a BINAM-L-212 microscope, in the cytoplasm and nuclei of the neurons, The largest and smallest axes of ellipses inscribed inside the outlines of the cells and nuclei were measured simultaneously with the MOV-1-15 screw-acting ocular micrometer, and the areas of their cross sections were calculated. To calculate the dry mass of solid substances, values of the shifts along the axis and the area of cross section of the cell structures were substituted in the corresponding formulas [2]. APase activity was demonstrated in cryostat sections, using D,L-alanine-2-naphthylami [3] as the substrate, and was measured in optical density units on the LYUMAM-IZ microscope at a wavelength of 550 nm. The experimental data were subjected to statistical analysis by Student's test.

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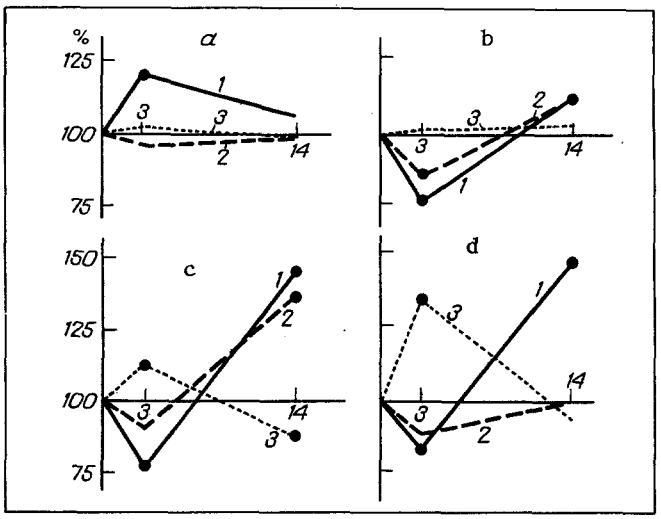

Fig. 1. Changes in morphochemical parameters of neurons of layers III (a) and V (b) of sensomotor cortex, caudate nucleus (c) and n. accumbens (d) in brain of rats with disturbed motor activity after administration of L-dopa for 3 and 14 days. Abscissa, days of experiment; ordinate, changes compared with control, in % 1) Protein concentration in cytoplasm, 2) area of cross section of cytoplasm, 3) aminopeptidase activity.

EXPERIMENTAL RESULTS

Under the influence of the doses of L-dopa mentioned above motor activity of the rats in OF was restricted. This decrease continued 3 days later and until the end of the experiment. Besides behavioral changes in OF, most of the rats after the 1st week of the experiment, on being held in the hand exhibited great unease, but without any sign of aggressiveness. These behavioral changes are evidently the result of strengthening of the feeling of anxiety associated with hyperactivation of the brain dopamine system, as is confirmed by data in the literature [1].

The results of the cytochemical investigation showed that behavioral changes are connected with changes in structural metabolism in neurons both of SMC and of deep brain structures (CN and NA) However, despite similar changes in the animals' behavior, the character of the morphochemical changes differed significantly after short- and long-term administration of the drug.

A decrease in protein concentration in the cytoplasm of the neurons by 23 and 15 respectively was found in neurons of CN and NA after administration of L-dopa for 3 days (Fig. 1). The changes discovered can be regarded as an increase in the intensity of protein breakdown compared with normal, as shown by the raised level of APase activity in these cells. After administration of L-dopa for 14 days, normalization of APase activity was observed in the same brain formations, but the protein concentration now exceeded the control level, suggesting that protein accumulation was taking place in the absence of utilization, matching the increased synthesis.

In SMC, after administration of L-dopa for 3 days, neurons of layer III were characterized by a 20% higher protein concentration in the cytoplasm of the neurons, accompanied by a normal level of APase activity. In these same neurons, with administration of L-dopa to the animals for 14 days, the parameters studied were virtually restored to normal. This time course of the morphochemical changes in response to injection of L-dopa suggests that neurons of associative type respond actively to hyperactivity of the dopaminergic system in the initial stages of the experiment.

Neurons in layer V of SMC, of the efferent-projection type, were characterized in the early stages (3 days) by a reduction of 23% in protein concentration in the cytoplasm compared with the control. APase activity under these circumstances corresponded to the control level, but the area of cross-section of the cytoplasm showed a tendency to decrease. Administration of L-dopa for 14 days caused an increase in protein concentration with a simultaneous increase in size of the neurons. Under these circumstances APase activity corresponded to the control level. It can be tentatively suggested that the rate of protein synthesis in neurons of layer V was increased during this period, as was confirmed by autoradiogra-

phic data showing increased incorporation of [^{3}H]-leucine into the proteins of these neurons during long-term L-dopa administration [7].

The results are thus evidence that administration of L-dopa, reducing motor activity, was accompanied in the early stages by opposite changes in structural metabolism, which were most marked in neurons of deep brain formations (CN and NA): in the early stage (3 days) this was manifested as a decrease in protein concentration and activation of APase, whereas long-term administration of the compound led to protein accumulation in the cytoplasm as a result of a decrease in its realization, possibly accompanied by activation of synthesis. Opposite changes during short- and long-term exposure to L-dopa also have been found in mediator systems [5, 6]; specifically, during activation of the DA-system, inhibition of serotoninergic and acetylcholinergic mediation was observed. We also know from the published data that long-term administration of L-dopa in clinical practice leads to the development of late dyskinesia, which can be abolished only by substances blocking the synthesis of DA exhausting its reserves [10].

It can be postulated on the basis of these experimental results that a definite connection exists between the mechanism of the change in horizontal motor activity of animals and the state of their structural metabolism in brain neurons. The particular features of these changes depend on the duration of L-dopa administration.

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