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EFFECT OF D,L-DOPA ON PROTEIN ANTIGEN CONTENT IN SOME RAT BRAIN STRUCTURES

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KEY WORDS: brain, protein antigens, neurospecific proteins, dopa.

The problem of the role of neurotransmitter systems in trans-synaptic regulation of protein synthesis in postsynaptic structures is a key problem in neurobiology. A decisive role has been ascribed to the possible selective effect of neurotransmitters on post-translation modification of proteins in the conversion of short-term changes in unit activity into stable, long-term changes. Induction of modifications of individual brain-specific proteins (antigens) by mediators undoubtedly has a direct bearing on the mechanisms of plasticity [9].

Data on the mechanisms of concrete interaction between neurotransmitters and protein-synthesizing brain systems are very scanty and are concerned chiefly with synthesis. Changes in RNA and protein synthesis in the brain have been demonstrated under the influence of noradrenalin (NA) and its analogs [2, 3, 14]. For example, stimulation of adrenoreceptors by amphetamine inhibited incorporation of radioactive label in RNA and proteins, but with an increase in the dose of the drug their synthesis was activated [2, 3]; D-amphetamine inhibited protein synthesis in the rat brain [14]; a decrease in the NA content in the brain by diethylthiocarbonate and reserpine reduced, whereas elevation of the NA level increased, the intensity of RNA synthesis [4].

Changes in nucleic acid and protein metabolism under the influence of NA may also be an important stage in the mechanism of regulation of synaptic efficiency [7]. Evidence of this is given by reorganization of the chemoreactive properties of cerebral neuron membranes by microiontophoretic application of NA and by stimulation of the locus coeruleus, the principal site of concentration of noradrenergic neurons [5, 13]. Clearly the role of individual proteins in this process may be very considerable. An electrophoretic study of water-soluble proteins of the cerebral and cerebellar cortex revealed divergent changes in individual protein fractions in response to electrical stimulation of the locus coeruleus [8]. However, the question of the functional role of different individual, including tissue-specific, proteins in the mechanisms of action of NA remains unsolved.

The aim of the present investigation was to study (by crossed immunoelectrophoresis) the character of the effect of the NA precursor D,L-dopa, on the content of protein antigens in the hypothalamus, cerebellum, and frontal cortex of the rat brain.

EXPERIMENTAL METHOD

An antiserum obtained by immunizing rabbits with water-soluble extract of rat brain by the scheme described previously [11] was used for the immunochemical investigations. To remove antibodies against serum protein the immune serum was exhausted with rat blood serum [6].

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TABLE 1. Content of Antigens in Rat Brain Structures after Injection of D,L-Dopa (in % of control)

Antigen	Brain structure		
	cortex	cerebellum	hypothalamus
1	98	106	100
2	—	—	113*
3	94	105	107
4	105	95	105
5	111	110	126
6	100	95	96
7	102	104	102
8	—	121*	141**
9	109	107	111
10	105	110*	125*

Legend. *P < 0.05; **P < 0.01. Twelve experiments each in control and experiment.

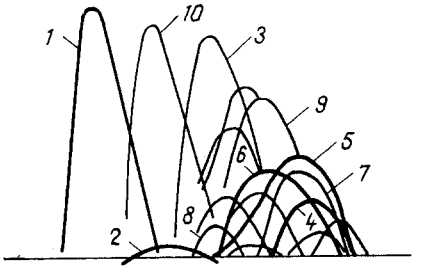

Fig. 1. Crossed immunoelectrophoresis of rat cerebellum antigens (shown diagramatically on right). Explanation in text.

Experiments were carried out on male Wistar rats weighing 200-300 g. There were two series of experiments, and 12 animals were used in each series. The rats were given an intraperitoneal injection of D,L-dopa (from Reanal, Hungary) in a dose of 40 mg/kg. The solvent was injected into control animals. The rats were decapitated 1.5 h later and the frontal cortex, cerebellum, and hypothalamus were removed in the cold. The brain structures were homogenized in barbital buffer, pH 8.6, with ionic strength 0.035, in the ratio of 3 ml buffer to 1 g tissue. The homogenate was centrifuged at 8000 rpm for 50 min and the supernatant was investigated by crossed immunoelectrophoresis [12].

Samples 5 μl in volume were fractionated electrophoretically in 1% agarose gel in the same buffer with a field intensity of 12 V/cm for 20 min. Electrophoresis was then continued in gel containing 120 μl/cm^2 of antiserum, with a potential gradient of 2 V/cm for 18 h at 14°C. After immunoelectrophoresis the plates with gel were washed in 0.15 M NaCl, dried at 60°C, and stained for proteins with Coomassie bright blue R-250 (from Farak, East Germany).

The content of protein antigens in the brain structures was estimated from the area of the precipitation peaks, which is directly proportional to the concentration of the corresponding antigen in the test mixture [12]. The results were subjected to statistical analysis by Student's t test and dispersion analysis.

EXPERIMENTAL RESULTS

The antiserum used, which reacts in crossed immunoelectrophoresis with extracts of rat brain structures, revealed about 20 precipitation peaks differing in position, shape, and intensity of staining (Fig. 1). The ten

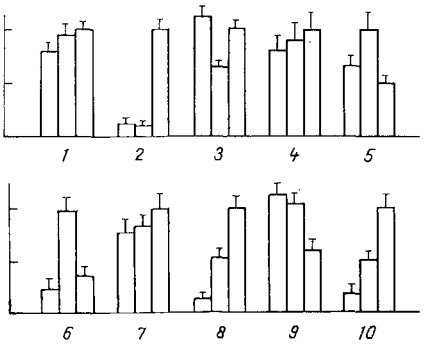

Fig. 2. Content of antigen 1-10 in rat brain structures (in conventional units). First column in each group represents frontal cortex, second – cerebellum, third – hypothalamus (n = 6).

antigens which could be identified in all test structures, and with which the later work was done, were numbered from 1 to 10. Antigens 1-10 were absent in rat blood serum, but antigens 4-10 were found in liver extracts, i.e., they are not specific for brain.

The investigation showed that these brain structures differed quantitatively in their content of antigens revealed. The content of antigens 1, 4, and 7 were only equal in the cortex, cerebellum, and hypothalamus; the remaining antigens were unevenly distributed among the structures.

The results in Table 1 indicate that injection of D,L-dopa into the animals affected metabolism of individual proteins (antigens) of the brain structures to a different degree. In most cases the changes were in the direction of an increase in their content, and the changes were greatest in the hypothalamus. A significant increase in the content of antigens 8 and 10 was observed in the cerebellum and hypothalamus, and of antigen 2 in the hypothalamus; the latter was present in much smaller quantities in the cerebellum and frontal cortex.

Attention is drawn to the fact that locations of antigens 8 and 10 correlate with the distribution of NA in the cortex, cerebellum, and hypothalamus [1, 15]; the content of antigen 2 in the hypothalamus, like that of NA, was several times greater than in the other two structures (Fig. 2).

It can be concluded from the results that NA participates in the regulation of metabolism of water-soluble antigens 2, 8, and 10 in the brain, and one of its mechanisms may be selective stimulation of their synthesis or post-translation modification by the neurotransmitter.

This hypothesis gives added conviction to our previous data [10] indicating that injection of disulfiram, an inhibitor of NA synthesis, into rats selectively affects the content of brain-specific (P_1) and general-tissue, crossed (P_2) antigens in the central structures. Changes in their content were linked with stages of learning: P_1 with the recording of information, P_2 with its fixation in the brain. The results of the present investigation, obtained by quantitative immunoelectrophoresis, allow these hypotheses to be developed further and suggest the possibility of "translation" of the new biochemical protein phenotype induced by monoamines (NA) into an elementary behavioral act through a system of NA – secondary messenger – individual protein (antigen). Post-translation modification of this antigen (these antigens) may also lie at the basis of fixation of an acquired skill and its subsequent utilization and improvement.

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CIRCADIAN RHYTHM OF ACTIVITY OF THE CAUDATE NUCLEUS IN CATS AND ITS SENSITIVITY TO PSYCHOTROPIC DRUGS

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Circadian changes in brain function may be reflected significantly in the action of psychotropic drugs. The specific properties of individual psychostimulants and neuroleptics largely depend on changes in activity of the basal ganglia and, in particular, of the caudate nucleus [2, 3].

It was accordingly decided to assess the character of caudate nucleus activity at different times of the 24-h period and to assess the role of this factor in the effect of the psychostimulant amphetamine and of the neuroleptic haloperidol.

EXPERIMENTAL METHOD

Altogether 96 experiments were carried out on seven cats of both sexes weighing 2-3.5 kg. Under pentobarbital anesthesia bipolar nichrome electrodes (diameter 0.2 mm) were first inserted into different parts of the caudate nucleus. One week after the operation the experiments were repeated, with the conditions of keeping and feeding standardized as much as possible. Natural illumination was used during the summer months (June to August). The level of general activity of the cats was assessed by their response to adequate test stimuli (calling by name, petting, playing) by means of a point scale [8]. To characterize stereotyped behavior the number of head movements was counted and abnormal activity of the animals was recorded cyclographically [5]. The caudate nucleus was stimulated by square pulses (frequency 2 pulses/sec, strength of current 2-20 μA, duration of stimulus 0.5 msec, of stimulation 10-15 sec). Restraint of movements arising as a result was analyzed by the method described previously [4].

In three cats (Nos. 6, 7, and 8; eight experiments) general activity and thresholds of the restraining response were determined during the 24-h period every 3 h starting at 9 a.m. In the other cases the caudate response and effect of the drugs were assessed at midday (11 a.m.-1 p.m.) and midnight (11 p.m.-1 a.m.) at intervals of 2-3 days. The drugs were injected intraperitoneally at the specified times 25-30 min before determination of the effect. The numerical results were subjected to statistical analysis by Student's t test ($P < 0.05$). After the end of the experiments and fixation of the brain, the position of the stimulating electrodes was determined in frontal sections and compared with data in the atlas [13].

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