CATECHOLAMINERGIC COMPONENT IN THE ACTION OF A HEPTAPEPTIDE ACTH$_{4-10}$ ANALOG ON OPEN FIELD BEHAVIOR OF RATS

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Fragments of adrenocorticotrophic hormones of the ACTH$_{4-7}$ and ACTH$_{4-10}$ types are known to stimulate the formation [1] and to delay extinction [6] of conditioned reflexes. A new synthetic heptapeptide, the ACTH$_{4-10}$ analog Met-Glu-His-Phe-Pro-Gly-Pro which, by modification of the C-terminal structure, evidently becomes more resistant to enzymic degradation than ACTH$_{4-10}$ itself, and has a prolonged action [2], possesses similar activity. It is suggested that a definite role in the realization of the behavioral effects of this group of ACTH fragments is played by their influence on the biogenic amine system of the brain [10].

Accordingly, in the investigation described below the action of this heptapeptide was studied on the effects produced by pharmacologic analyzers of catecholaminergic processes on concentrations of biogenic amines in the forebrain, and also on the rate of hydroxylation tyrosine — the key stage of dopamine (DA) biosynthesis in brain synaptosomes.

EXPERIMENTAL METHOD

Behavioral effects of the substances were studied in experiments on non-inbred male albino rats weighing 200 g. The substances were injected intraperitoneally in aqueous solution. To modify the functional state of the brain catecholaminergic systems we used haloperidol (0.05 mg/kg) 15 min before, apomorphine (1 mg/kg) 3 min before, and amphetamine (1 mg/kg) 30 min before the test. The heptapeptide was given in a dose of 0.015 mg/kg 5 min before the test. Control animals were given an injection of distilled water (1 ml/kg). The level of the animals' orienting-investigative activity (OIA) was assessed in the open field (OF) test [3]; the total distance covered, the number of standings on the hind limbs (standing posture), and the number of groomings were taken into consideration. After 7 days extinction of the behavioral responses was tested when the animals were placed again in the same experimental situation. Monoamine concentrations in the rats' forebrain were examined spectrofluorometrically, using column chromatography in Amberlite G-50 [5]. To determine the rate of hydroxylation of tritium-labeled tyrosine (2.8 Ci/mmole, from IZINTA, Hungary) in synaptosomes (the P_2 fraction), of the corpus striatum and hypothalamus of the rats, the method [8] was used in the modification [4]. The control for nonenzymic formation of labeled water consisted of tests with the corresponding amount of labeled tyrosine which, like the experimental samples, was incubated for 15 min at 37°C. Under these circumstances the synaptosomes were added after incubation, immediately before 400 µl of 3.5% TCA. The results were subjected to statistical analysis by Student's test or the nonparametric Wilcoxon–Mann–Whitney test.

EXPERIMENTAL RESULTS

Haloperidol, in the dose tested on rats placed for the first time in OF, caused a significant decrease in the values of parameters reflecting the level of the animals' OIA (distance covered, standings). Apomorphine, administered in this way, also inhibited this response (Table 1). The heptapeptide had no significant action on the effects of apomorphine or haloperidol. For control animals placed for the first time in OF, the average distance

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TABLE 1. Changes in Parameters of Behavioral Activity (in %) of Rate in Open Field Test after Administration of Substances

Substance	Number of rats	First placing in OF			Second placing in OF		
		total distance	standings	groomings	total distance	standings	groomings
Haloperidol	16	81[a]	79[a]	83	101	156[a]	136
Haloperidol + heptapeptide	16	70[a]	85	100	77[b,r]	96	136
Apomorphine	16	67[a]	31[b]	12	143[b]	140	143
Apomorphine + heptapeptide	14	74	31	35	133[B]	93	105
Amphetamine	16	132[b]	150[a]	225[a]	123	72	128
Amphetamine + heptapeptide	15	123	110[B]	60[B]	103	96	54
Heptapeptide	26	94	125	92	85	67	62

Note. Differences from control: a) $P < 0.05$; b) $P < 0.01$; differences between experimental groups: c) $P < 0.05$; d) $P < 0.01$.

covered was 1100 cm, which decreased on retesting to 640 cm, i.e., extinction amounted to about 42%. Extinction based on the number of standings was about 50%. The behavioral parameters of the control animals during first testing and retesting, given in Table 1, are taken as 100%.

It will be clear from Table 1 that on replacement of the animals in OF haloperidol and apomorphine disturbed the normal extinction of the orienting reaction and increased the value of its parameters. On combined administration of the peptide with haloperidol or apomorphine, the total distance covered was significantly less than after administration of only one of these substances. A tendency was observed in this case for the number of standings to decrease. This means that, according to the results of retesting, the heptapeptide improved extinction of the response, disturbed by haloperidol or apomorphine, and this effect was probably linked with memorizing of the situation.

On the first day of the experiments amphetamine intensified the OIA of the rats and increased the values of all parameters recorded. The increase in the number of acts of grooming can evidently be taken to indicate the development of stereotypy [9]. The heptapeptide definitely counteracted the effect of amphetamine, reducing the number of standings and acts of grooming. When animals which received amphetamine before the first test were placed again in OF, no significant changes of their behavior were observed, just as in the case of rats receiving the heptapeptide + amphetamine. The heptapeptide itself did not change any of the above parameters and did not affect extinction of OIA on retesting.

The results of experiments to determine concentrations of biogenic amines in structures of the forebrain showed that only apomorphine caused a significant rise of the DA level in the forebrain, whereas the peptide caused no significant changes in biogenic amine concentrations. The effect of apomorphine may evidently be due to inhibition of DA release [7]. In a concentration of 10^{-4} M the heptapeptide caused a moderate decrease in the rate of this process, and the effect decreased with an increase in concentration of tyrosine, the reaction substrate. In a lower concentration (10^{-6} M) the peptide had no appreciable effect.

Data in the literature on the effect of ACTH fragments, administered systematically, on tyrosine hydroxylase activity in the brain indicate that they may have both an activating and an inhibiting action, and they indicate that the effect of the peptide may depend on the original state of the animals [10]. In the present experiments no significant changes could be found in concentrations of DA, noradrenalin, and serotonin in the rats' forebrain. Previous investigations also showed that in many cases no changes in biogenic amine concentrations under the influence of ACTH or its fragments could be found in brain structures, although in some studies elevation of the DA level was observed in the corpus striatum and midbrain [4]. According to our own data, the heptapeptide abolished disturbances of extinction of OIA caused by apomorphine or haloperidol, and also weakened the effect of amphetamine on behavior of the rats in OF. These results, in our opinion, are definite evidence of the existence of a catecholaminergic component in the action of the heptapeptide. Meanwhile the neurochemical mechanisms of these effects require detailed study.

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RED BLOOD CELL TARGETING TO HUMAN AORTIC SMOOTH MUSCLE CELLS

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Targeted transport of drugs and other biologically active substances provides a new approch to the treatment of several diseases [3, 4, 11-13]. Liposomes and red blood cells, filled with the corresponding agent and injected into the blood stream, can be used as containers for transporting drugs to injured or pathologically changed parts of organs and tissues [1, 5]. Targeting is effected by means of vector molecules. Antibodies make good vectors [4, 11], because the antigen–antibody reaction is highly specific.

Many cardiovascular diseases are based on a disturbance of integrity of the endothelial lining of the vessel wall [9, 10]. The damaged endothelium provides access to underlying layers of the wall for substances circulating in the blood which affect metabolism, proliferation, and secretory activity of the cells of the subendothelial layer. Monoclonal antibodies, recognizing surface antigens of subendothelial cells, in conjunction with liposomes or red blood cells, could effect targeted transport and selective action on foci of atherosclerosis. For instance, it was recently reported that monoclonal antibodies, interacting specifically with the surface of rat smooth muscle cells (SMC) can be used to transport immonotoxins to target cells [6]. The writers have obtained monoclonal antibodies interacting with an antigen with mol. wt. of 330,000 daltons, located on the surface of human aortic SMC [2]. Endothelial cells of the aorta and umbilical vein do not contain this antigen.

The aim of this investigation was to study the possibility of using IIG10 monoclonal antibodies as vector for targeted transport of drugs to the subendothelial layer of a damaged region of vessel wall. Experiments were carried out *in vitro* in a model system: SMC, growing on a plastic support, were treated with IIG10 monoclonal antibodies, after which red blood cells, previously conjugated with antibodies to mouse immunoglobulins, were added to them. The results of these tests were analyzed spectrophotometrically and with the scanning electron microscope.

EXPERIMENTAL METHOD

A culture of human aortic SMC was obtained by the method in [8]. Preparation and the properties of IIG10 monoclonal antibodies, and culture of the SMC were described previously [2]. To obtain a mixed culture, endothelial cells and SMC were cocultivated for 3 days. The cells were incubated with monoclonal antibodies (0.002-30 μg/ml) for 30 min at 37°C in an atmosphere of 94% air and 6% CO_2, after which the free antibodies were removed by a triple change of medium.

Rabbit antibodies to mouse immunoglobulin light chains were conjugated with washed human red blood cells with the aid of $CrCl_3$ [7]. About $2 \cdot 10^5$ IgG molecules were attached

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