EXPERIMENTAL RESULTS

On the basis of data showing that the large ducts are located mainly in the center of the pancreas and the small ducts closer to its surface [1], values of MP of the cells were studied at various depths: 0-150 μ, 150-500 μ, and 500-900 μ (the total thickness of the guinea pig pancreas is 1-1.2 mm). It will be clear from Fig. 1 that the largest number of cells with a polarization level not exceeding 30 mV was recorded in the surface layers of the dorsal regions of the pancreas. Cells with MP of up to 50 mV constituted a smaller group, and cells with MP up to 70 mV formed the smallest group. The character of the histogram changed at mid-depth. There, the number of cells with MP up to 30 mV decreased appreciably, the number of cells with MP up to 50 mV was virtually unchanged, but the number of cells with MP of over 50 mV increased. In the deep layers of the pancreas, lying next to the serous membrane, the number of cells differed appreciably from that in the middle and dorsal regions. In the deep layers the smallest number of cells with MP of up to 30 mV was recorded, and the number of cells with MP of up to 50 mV was virtually the same as in the other layers. Meanwhile, in the deep layers, the number of cells with high MP was increased. As regards cells of the islets of Langerhans, they have a low MP [3], and when the track of Me passed outside the zone of these islets on the surface of the pancreas, only a few of them were recorded in the deeper zones.

Thus MP of different types of cells can easily be recorded in the pancreas in vivo and a laminar analysis of their number undertaken. In this respect the pancreas resembles the salivary glands. On the other hand, the distribution of cells with a high MP level predominantly on the ventral surface shows that ducts are evidently most numerous in this region.

LITERATURE CITED

1. D. E. Brockman, Cell. Tissue Res., 205, 445 (1980).
2. J. S. Davison and G. T. Petersen, J. Physiol. (London), 310, 44 (1981).
3. P. M. Dean and E. K. Matthews, J. Physiol. (London), 210, 255 (1970).
4. A. Nishiyama and O. H. Petersen, J. Physiol. (London), 238, 145 (1974).
5. L. H. Scheyer and C. A. Scheyer, Am. J. Physiol., 209, 1304 (1965)
6. J. Singh, J. Physiol. (London), 358, 469 (1985).

CHANGES IN WATER-SOLUBLE ANTIGEN LEVELS IN THE RAT BRAIN DURING BILATERAL AVOIDANCE TRAINING

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Despite many publications on the role of protein metabolism in learning and memory processes [1] the role of individual proteins in the mechanisms of conditioned reflex formation and consolidation still remains a comparatively unresearched problem.

The aim of this investigation was to study metabolism of individual water-soluble antigen proteins using methods of rocket and crossed immunoelectrophoresis during active avoidance training in animals. Attention was concentrated on analysis of the content of a water-soluble brain antigen which we designated antigen 2, which has the electrophoretic mobility of albumins and is not found in the liver and in various other organs [2]. The content of this antigen is several times higher in the hypothalamus, mesencephalon, and medulla (i.e., in re-

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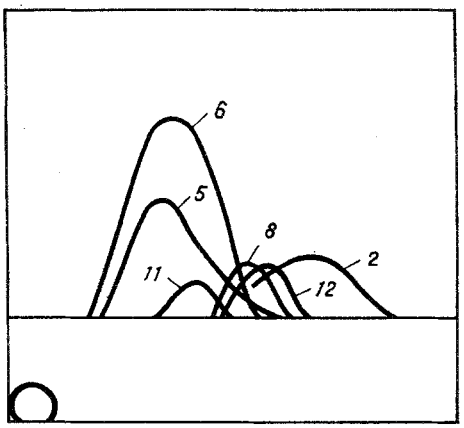

Fig. 1. Scheme showing arrangement of test antigens (numbers) in crossed immunoelectrophoresis.

TABLE 1. Content of Antigens in Rat Brain Structures 1 h after Training (M ± m)

Antigens	Group of animals	Frontal cortex	Temporal cortex	Hippocampus	Mesencephalon
2	Control	100±3	100±7	100±5	100±5
	1	100±5	122±8*	106±11	93±6
	2	106±3	97±6	98±6	95±4
5	Control	100±5	100±7	100±12	100±7
	1	100±11	112±7	108±16	96±12
	2	95±5	90±7	92±10	91±13
6	Control	100±3	100±6*	100±7	100±11
	1	105±3	96±5	100±8	103±8
	2	102±5	86±2	91±4	95±8
8	Control	100±8	100±9	100±14	100±6
	1	98±3	108±3*	100±9	98±11
	2	91±5	94±4	88±9	81±13
11	Control	100±6	100±7	100±11	100±5
	1	135±19	141±22	115±11	101±9
	2	124±8	102±9	97±9	91±9
12	Control	100±8	100±9	100±9	100±9
	1	129±11	118±7*	109±8	100±7
	2	112±7	99±6	97±6	90±10

Legend. *p < 0.05 compared with group 2. Control group and group 1 contained six animals, group 2 contained eight animals.

gions rich in monoamines) than in other brain structures such as, for example, the neocortex, cerebellum, hippocampus, and caudate nucleus.

It is very probable, due to these last two circumstances, that antigen 2 participates in learning processes for, first, it possesses definite tissue specificity and, second, it is evidently controlled by the catecholaminergic systems of the brain, the role and place of which in the mechanisms of memory is being intensively studied [1].

EXPERIMENTAL METHOD

Antiserum for the immunochemical investigation was obtained by intradermal immunization of rabbits with a water-soluble rat brain extract, by the scheme in [5]. The immune serum was exhausted with rat blood serum, and for rocket immunoelectrophoresis, with liver extract.

Experiments were carried out on 42 male Wistar rats weighing 130-180 g. Defensive bilateral avoidance reflexes formed in a shuttle box served as the learning model. The conditioned stimulus was an acoustic signal with a frequency of 625 Hz, the unconditioned stimulus an electric shock with a voltage of 25-45 V applied to the floor of the shuttle box 5 sec after the beginning of acoustic stimulation. Intervals between combinations were 30-90 sec.

TABLE 2. Content of Antigen 2 in Rat Brain Structures 1 Week after Training (M ± m)

Animals	Frontal cortex	Temporal cortex	Hippocampus	Caudate nucleus
Control (10)	100±6	100±4	100±3	100±4
With low score of avoidances (5)	105±5	122±6*	102±6	93±7
With high score of avoidances (7)	105±9	95±4	99±5	87±7

Legend. *p < 0.01 compared with other two groups. Number of animals given in parentheses.

In the experiments of series I, 1 week before training the animals' swimming activity was tested, by placing them for 12 min in a cylinder, 35 cm in diameter, filled with water. In the center of the cylinder, at a height of 1 mm from the water surface, electrical contacts connected to an electronic counter were provided, and were periodically closed by waves created by the animals during swimming. The animal's motor activity in water was assessed by the reading of the counter. The animals were trained in bilateral avoidance by presentation of 50 acoustic stimuli, they were decapitated 1 h later, and the content of water-soluble antigens studied by crossed immunoelectrophoresis in the frontal cortex, hippocampus, and mesencephalon.

In the experiments of series II the rats were trained in avoidance by the use of 60 acoustic stimuli, they were decapitated 1 week later and the content of antigen 2 determined by rocket immunoelectrophoresis in the temporal and frontal cortex, hippocampus, and caudate nucleus.

Specimens for immunochemical analysis were prepared, crossed immunoelectrophoresis carried out, and the antigen content estimated in the brain structures as described previously [2]. In the rocket version of immunoelectrophoresis [4] the antigens were separated in 1% agarose gel containing 40 μl/cm^2 of antiserum, in a voltage gradient of 2 V/cm for 18 h. The content of antigen 2 was estimated by measuring the area of the rocket-shaped precipitation peaks.

The results were subjected to statistical analysis by Student's t-test and by correlation analysis.

EXPERIMENTAL RESULTS

A scheme of crossed immunoelectrophoresis of the brain antigens tested is given in Fig. 1; antigens 2, 5 6, and 8 were described by the present writers [2] and, with the exception of antigen 2, are also found in rat liver.

In the experiments of series I analysis of the antigen content in the brain structures revealed no difference between the experimental animals and the passive control. In the learning experiments considerable variation was observed in the degree of formation of the avoidance skill in individual animals, and accordingly, for further analysis two groups of animals were compared: group 1 included badly trained animals with a low score of avoidance responses (7 ± 2, n = 6), and group 2 included well trained animals, with a high score of avoidance (23 ± 2, n = 8). Animals of group 1 were found to have a higher content of antigens 2, 8, and 12 in the temporal cortex than those of group 2 (Table 1). The content of antigens 6 in animals of group 2 was lower in this brain structure than in the control. A tendency was found toward correlation between the number of combinations of nociceptive and acoustic stimuli and the antigen content in the various structures, and it reached significance for antigen 2 in the temporal cortex ($\tau = 0.54$, $p < 0.05$) and for antigen 8 in the mesencephalon ($\tau = 0.55$, $p < 0.05$).

The results are in agreement with the view that intensification of functional activity of the CNS during reflex activity is accompanied by intensification of protein breakdown [3], whereas reinforcement, connected with cessation of the action of a nociceptive stimulus in that particular case, leads to accumulation of proteins by inhibition of their breakdown or intensification of their synthesis [6].

The content of antigen 2 in the temporal cortex 1 week after training remained higher in animals with a low score of avoidance (6 ± 3, n = 5) than in animals with a high score of avoidance responses (28 ± 4, n = 7), and also than in the control (Table 2); correlation was preserved between the combined application of conditioned and unconditioned stimuli and the antigen content in that structure ($\tau = 0.69$, $p < 0.05$).

These results indicate that antigen 2 is associated with long-term memory processes. Animals with a low score of avoidance responses can be regarded as being in the initial stages of learning. Consequently, the increase in their protein content preceded consolidation of the conditioned reflex and could reflect the formation of an association between the acoustic and nociceptive stimuli, whereas the preservation of these changes is evidence of stable changes in protein metabolism, which evidently lies at the basis of preservation of conditioned-reflex connections.

Evidence in support of the view that antigen 2 is involved in the mechanisms of long-term memory is given by the correlation, discovered in this investigation, between the animals' motor activity in water and the antigen content in the frontal cortex 1 week later ($\tau = 0.55$, $n = 20$, $p < 0.05$), which could reflect structural changes in the motor analyzer.

LITERATURE CITED

1. R. I. Kruglikov, Neurochemical Mechanisms of Learning and Memory [in Russian], Moscow (1981).
2. I. F. Pavlov and M. B. Shtark, Byull. Éksp. Biol. Med., No. 7, 67 (1983).
3. A. V. Palladin, Selected Works [in Russian], Kiev (1975).
4. Textbook of Quantitative Immunoelectrophoresis [in Russian], Moscow (1977), p. 43.
5. E. Bock et al., J. Neurochem., 18, 2435 (1971).
6. H. Hyden, Biosci. Commun., 4, 189 (1978).