INCORPORATION OF ^{3}H-LEUCINE INTO ACID BRAIN PROTEIN OF HIBERNATING ANIMALS

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UDC 612.822.1:547.96]-06:612.592]-087.45

KEY WORDS: hibernating animals; synthesis of acid brain proteins.

Many investigations have recently been undertaken to study metabolism of nerve tissue proteins in animals in different functional states and during excitation and inhibition. One of the most adequate models of a state of inhibition in the CNS in the intact animal is hibernation in mammals, which is characterized by a sharp decrease in the intensity of respiration, of the circulation, excretion, and metabolism. Palladin et al. [4] have shown that the turnover rate of brain proteins is sharply reduced in hibernating susliks. The present writer [2] studied the dynamics of incorporation of radioactive label in total protein in certain parts of the brain at different stages of awakening, when electrogenesis is observed to recover in the brain of the awakening animals.

However, to study more precisely the mechanisms of activity of brain structures information is necessary on the role of individual protein fractions, whose intensity of renewal is determined by the function they perform, in these processes. Since an important role in brain function is ascribed to acid proteins, the aim of the present investigation was to study the intensity of synthesis of the most acid protein fraction with mobility corresponding, under similar conditions of fractionation, to nerve-specific protein S-100 [6].

EXPERIMENTAL METHOD

Experiments were carried out on red-cheeked susliks into which 4,5-^{3}H-leucine (from the Radiochemical Centre, Amersham, England) was injected intraperitoneally in a dose of 3 μCi/g body weight 3 h before decapitation. Extracts of water-soluble proteins of the cerebral cortex, reticular formation, and hippocampus were prepared by the method described in [2], and then subjected to electrophoretic fractionation in plates of 7.5% polyacrylamide gel in the system described by Davis [3]. The duration of electrophoresis was about 3 h, with a current density of 18.2 mA/cm^2 in the concentration stage and 13.7 mA/cm^2 in the separation stage. Bromphenol blue was used as marker. After fractionation the gel plate was taken from the chamber, stained with Amido black 10B (from Reanal, Hungary), washed in acetic acid, and examined densitometrically, after which, to determine the intensity of incorporation of label into each fraction, the plate was cut into strips 2 mm wide parallel to the lie of the protein fraction. Pieces of gel were then placed in flasks for counting radioactivity, dried in a thermostat at 50°C, and treated with 30% H_2O_2 at 65°C overnight to destroy the gel structure, after which scintillation fluid based on dioxan was added and radioactivity was counted in an SL-30 liquid scintillation counter (from Intertechnique, France). The results were expressed in units of relative radioactivity, a unit being the ratio between the count in a separate fraction and the total radioactivity of the gel, obtained by adding the counts in all the strips. Since counting was done in equal volumes of gel each time, quenching was disregarded. The results were subjected to statistical analysis by Student's t-test ($P \leq 0.05$).

EXPERIMENTAL RESULTS

The percentage of incorporation of label into the acid fraction of the susliks' reticular formation was less than in all other structures investigated (Table 1). On the first day of awakening it increased sharply. On the 7th day maximal incorporation was observed, amounting to 4.68% of all other proteins represented on the gel. After 2 weeks of awakening the intensity of synthesis decreased, although it still remained higher than its level in sleeping animals.

Institute of Clinical and Experimental Medicine, Siberian Branch, Academy of Medical Sciences of the USSR, Novosibirsk. (Presented by Academician of the Academy of Medical Sciences of the USSR V. P. Kaznacheev.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 91, No. 5, pp. 546-548, May, 1981. Original article submitted October 15, 1980.

 0007-4888/81/9105-0600$07.50

TABLE 1. Incorporation of Labeled ^{3}H-Leucine (in units of relative radioactivity) into Most Acid Protein Fractions of Different Brain Structures of Hibernating Animals (M ± m)

Structure	Hibernation	Awakening	Wakefulness	
			1 week	2 weeks
Cortex	1,86±0,24 $n=11$	3,50±0,22 $n=11$	4,16±0,46 $n=11$	2,62±0,23 $n=10$
Reticular formation	1,42±0,20 $n=10$	3,18±0,37 $n=7$	4,68±0,43 $n=7$	2,23±0,27 $n=6$
Hippocampus	3,44±0,27 $n=9$	3,71±0,35 $n=6$	4,29±0,22 $n=9$	3,83±0,26 $n=10$

The character of changes in incorporation of ^{3}H-leucine into protein of S-100 type in the cerebral cortex was similar to that described above, although the changes were less marked. The intensity of synthesis of this fraction was significantly lower in the hibernating animals than in susliks which had just awakened. Incorporation also reached a maximum on the 7th day.

The acid water-soluble protein of the hippocampus is characterized by a high level of synthesis in the sleeping state, close to the level observed in the cortex and reticular formation of awakened animals. Later, on the 7th day of wakefulness there was a small increase in the level of its synthesis. A tendency was then observed for the intensity to fall, and in the second week the intensity of synthesis of this protein fraction was close to that in the sleeping animals.

The hippocampus is known to perform a "guarding" function in hibernating animals. Electrical activity can be recorded in it even when the brain temperature of the animals reaches 8-9°C and electrogenesis is totally suppressed in all other brain structures. This attaches particular importance to the high intensity of incorporation of label into acid hippocampal protein in sleeping animals and it suggests that this protein plays a direct role in maintenance of the functional activity of this structure during hibernation.

The sharp increase in the intensity of incorporation of label into protein of the reticular formation and cortex was undoubtedly linked with awakening of the animals and activation of all their vital processes, although the physiological significance of this intensification may differ. Previous observations [1] show that, despite intensive synthesis of this protein, there is little change in its content in the cortex, and not until the 7th day does it differ from its value during hibernation. However, if a protein is intensively synthesized and equally intensively utilized, its content in a structure may not change, reflecting dynamic equilibrium between processes of synthesis and breakdown. It can thus be concluded that acid protein of the S-100 type synthesized in the cortex is utilized more rapidly on the first day of awakening. In the reticular formation immediately after awakening this protein evidently accumulates, for intensification of its synthesis is accompanied by an increase in its relative content. A different picture is observed 1 week after emergence from hibernation: incorporation of the label continues to increase rapidly, but its content does not change significantly, indicating increased utilization of the protein. After 2 weeks protein utilization evidently decreases; despite a reduction of more than half in incorporation, its content remains at a high level and may even show a tendency to rise. When the term utilization of protein is used, this is taken to mean not only its breakdown, but also its possible conversion from the water-soluble into the insoluble form, for example, in the case of imbedding into the membrane, from which it could not be removed by the method of extraction used.

Even a brief period of hypothermia is known to cause serious disturbances of memory in homoiothermic animals. Hibernating animals, on the other hand, have a remarkable ability to store and recall information acquired before falling asleep. A study of the unique features of metabolism of acid brain proteins in hibernating animals, a group which includes most of the nerve-specific proteins, could shed light on the mechanisms of this phenomenon.

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