CHANGES IN PROTEIN METABOLISM IN BRAIN NEURONS DURING THE WITHDRAWAL SYNDROME IN CHRONIC ALCOHOLIC RATS (HISTOAUTORADIOGRAPHIC AND INTERFEROMETRIC STUDY)

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The writers showed previously [4] that administration of alcohol to rats for 8 months with periodic withdrawal of ethanol induces chronic toxic encephalopathy in brain structures, associated with an overlay of acute circulatory disturbances and dystrophic-destructive changes in the animals during the period of development of features of a "withdrawal syndrome." During chronic experimental administration of alcohol, especially in the period of ethanol withdrawal, significant disturbances of protein metabolism take place in the brain tissue, as revealed by biochemical investigations [1, 7, 12]; however, their results are somewhat contradictory.

The aim of this investigation was to study changes in metabolism of neuronal proteins with the aid of cytochemical investigations conducted in different periods of development of the "withdrawal syndrome" after 8 months of intermittent alcohol administration to rats.

EXPERIMENTAL METHOD

Experiments were carried out on noninbred male rats. The technique of chronic alcohol administration and the model of abstinence were described previously [4]. Altogether 16 rats were used, of which four were intact, of the corresponding age. The rats were decapitated immediately after withdrawal of the last dose of ethanol, and 12 and 24 h later. Each group of animals, including the control, consisted of four rats. The experimental and control rats received an intraperitoneal injection of ^{3}H-leucine in a dose of 1 μCi/g body weight 2 h before sacrifice. Pieces of brain were fixed in Carnoy's fluid and embedded in pairs (experiment and control) in the same paraffin wax block. Frontal sections 7-10 μ thick, glued to slides, were coated with type M photographic emulsion and exposed for 3 weeks at 4°C. After development of the autoradiographs the sections were stained with thionine and mounted in balsam. Grains of reduced silver were counted under the microscope (×640) above neurons in layers III and V of the sensomotor cortex and above hippocampal pyramidal cells in areas CA1-CA2. The intensity of incorporation of the label into the neurons was estimated from the number of grains per 100 μ^2 cross section of the neuron, determined by the usual method. The content of solid matter (proteins) in the nucleus and cytoplasm of the neurons was investigated in unstained dewaxed sections in a BINAM-211 interference microscope. The phase shift in the nucleus and cytoplasm relative to the medium was determined in monochromatic light at a wavelength of 535 nm. The results, with the thickness of the section and the value of the area of cross section, were substituted in the appropriate formulas [2]. The results were subjected to statistical analysis by Student's test.

EXPERIMENTAL RESULTS

It will be clear from Fig. 1 that incorporation of labeled leucine conformed to a phasic pattern at different stages of development of the withdrawal syndrome. Whereas immediately after withdrawal of ethanol synthetic activity was considerably

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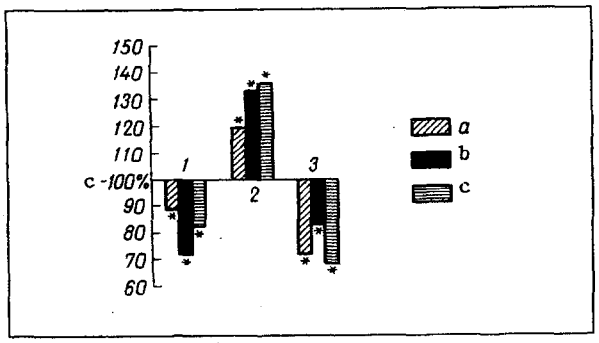

Fig. 1. Dynamics of changes in rate of incorporation of ^{3}H-leucine into brain neuronal proteins of chronic alcoholic rats during development of withdrawal syndrome. Here and in Fig. 2: C) control, 1) immediately after withdrawal of ethanol, 2) 12 h, 3) 24 h later; a) neurons of layer III, b) large neurons of layer of V of sensomotor cortex, c) hippocampal pyramidal neurons. Asterisks indicate significant changes.

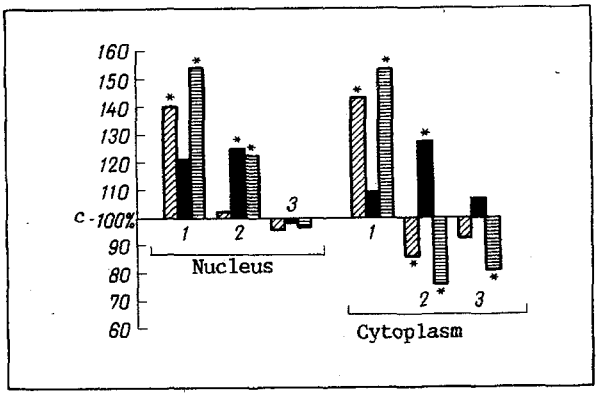

Fig. 2. Dynamics of changes in content of solids in nucleus and cytoplasm of brain neurons of chronic alcoholic rats during development of withdrawal syndrome.

depressed compared with the control, 12 h later it showed a significant rise, amounting to 20% or more above the control values, but by 24 h it again fell sharply, below the control level. The direction of these changes was clearly the same in all structures studied.

A different picture was found in the dynamics of the protein concentration. In this case (Fig. 2), immediately after withdrawal the values recorded exceeded the control level in both nucleus and cytoplasm, but not to an equal degree in different regions. However, during development of a state of abstinence, the dry weight of the neurons decreased, especially that of the cytoplasm of neurons in layer III of the sensomotor cortex and the hippocampal pyramidal cells, which fell below the control level. Only in large neurons of layer V did the protein content remain almost at its previous level. By 24 h, a further tendency for the neuronal protein content to fall could be seen in all the regions tested.

The high content of solids in the neurons and low synthetic activity in the period preceding development of the "withdrawal syndrome" reflect definite disturbances of neuronal protein metabolism. Chronic alcoholic poisoning depressed the animals' functional activity; they became apathetic, disinclined to move, and drowsy. Against the background of depressed general activity of the animals with chronic alcoholic poisoning, metabolic disturbances developed, including the metabolic activity of the brain neuronal proteins, utilization and synthesis of which were slowed, as our observations indicate. Meanwhile in chronic experimental alcohol poisoning a considerable number of hyperchromic neurons, with depressed metabolic activity, could be seen in the cortex of the rats [4, 8]. This was revealed by electrocytochemical investigations [5, 6]: disturbances in the RNA-protein-synthesizing system were discovered in the hyperchromic neurons, with depression of synthesis and transport of nucleopro-

teins. During development of the "withdrawal syndrome," the maximum of which occurs between 12 and 24 h after withdrawal of ethanol [4, 9, 11, 12], the protein content in the neurons fell appreciably until 12 h, probably due to intensification of their breakdown in this period. The increasing deficiency of neuronal proteins caused a sharp increase in the rate of incorporation of labeled leucine (Fig. 1), evidence of activation of protein metabolism at the height of the "withdrawal syndrome." In our view, an important role in the genesis of development of abstinence is played not only by exposure to toxic products of ethanol metabolism, especially acetaldehyde, but also the marked stressor reaction, for considerable activation of the pituitary–adrenal system is found in this period [11]. The changes in protein metabolism which we found must be regarded as mobilization of compensatory mechanisms in the nervous system in response to stress at the time of maximal intensity of the "withdrawal syndrome."

The absence of any strict parallel, and sometimes even an opposite direction of the changes in synthetic activity and in the content of solids in the neurons can be explained by differences in the functional lability of these parameters, the high plasticity of nerve tissue structures, and also the definite phasic pattern of metabolic processes [2]. These properties of nerve tissue are manifested in both normal and pathological states. The protein content in the structures is a more stable parameter in these cases, whereas synthetic activity is more dynamic, more prone to fluctuations even in the course of the 24-h period [3]. This distinguishing feature of metabolic processes in the CNS during the development of abstinence after withdrawal of ethanol was confirmed by our own data.

Thus during development of a "withdrawal syndrome" in chronic alcoholic rats significant disturbances of protein metabolism take place in brain structures and are characterized by a phasic pattern of changes in different periods of abstinence, reaching maximal intensity in the period from 12 to 24 h after withdrawal of ethanol.

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