BRAIN NUCLEIC ACID CONTENT OF INBRED RATS OF VARIOUS STRAINS

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Changes in the nucleic acid content were studied during defensive conditioning to electric shocks. In WAG rats a reflex was formed on average after 42.7 presentations of combined electrical and photic stimulation, compared with 14.09 combinations in August rats. Cytophotometric determination of the nucleic acid content in the neurons and perineuronal glia of the cortex revealed an increase in RNA only in August rats. It is suggested that the increased RNA synthesis in the animals of this strain may be the material basis of their greater ability to learn.

KEY WORDS: inbred animals; defensive conditioning; nucleic acids; neurons.

The RNA content of neurons has been shown to depend on their specific activity [1, 2]. This suggests that determination of the nucleic acid content in nerve tissue cells can be used to study the biochemical correlates of behavior. Inbred animals are convenient objects with which to investigate the biochemical basis of behavior. The work of Bovet et al. [5] and Cohen [7] has shown that behavioral characteristics such as spontaneous motor activity, sensitivity to acoustic stimulation, ability to learn, and so on, may be genetically determined. It has been shown, for example, that mice of strains CBA, C3H, and C57 have a much lower learning ability index than DBA and SEC mice, and these differences increase with an increase in the degree of inbreeding of the strain [6].

The object of this investigation was to study the DNA and RNA contents in the brain cells of inbred rats with genetically determined differences in behavior.

EXPERIMENTAL METHOD

Noninbred and inbred rats of strains WAG/GSto and August/LacSto weighing 140-160 g were used. Ten noninbred and 12 of each strain of inbred animals were used for the defensive conditioning experiments. The nucleic acid content was determined in six experimental rats and six control animals of each strain. Defensive conditioning to electric shock was produced in a T maze with an electrified floor. The animals were placed on the starting area of the main compartment of the maze and the right and left arms were simultaneously illuminated. After 5 sec a current of about 30 V was applied to the floor, which the animal could escape from only by running into the illuminated passage. The reflex was produced in 2 days. During the first day combinations of electrical and photic stimulation were presented 20 times, and during the second day they were repeated until the criterion of conditioned reflex formation was attained (five correct runs out of six). The control animals received the same number of electric shocks and flashes, but not in combination. The animals were decapitated 30 min after the end of the experiment.

The brain was removed and frozen with liquid nitrogen (the brains of the experimental and control animals were mounted in the same block). Frozen sections were cut to a thickness of 20 μ and stained with gallocyanin and chrome alum for the histochemical detection of total RNA and DNA and of DNA separately after preliminary treatment of the sections with ribonuclease. The RNA content was determined as the difference be-

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TABLE 1. Nucleic Acid Content in Cerebral Cortex of Control Inbred August and WAG Rats and of Same Rats Conditioned in a T Maze

Group of animals	August/LacSto				WAG/GSto			
	neurons		glia (nucleus)	neuropil	neurons		glia (nucleus)	neuropil
	nucleus	cytoplasm			nucleus	cytoplasm		
Control								
RNA+DNA	0,395±0,04	0,202±0,01	0,436±0,05	0,029±0,01	0,410±0,05	0,214±0,04	0,387±0,1	0,030±0,01
DNA	0,168	0	0.210	0	0,170	0	0,211	0
RNA	0,227	0,202	0,226	0,029	0,240	0,214	0,166	0,030
Experimental								
RNA+DNA	0,447±0,05	0,220±0,05	0,451±0,05	0,045±0,01	0,418±0,06	0,216±0,02	0,390±0,08	0,037±0,01
DNA	0,158	0	0,206	0	0,167	0	0,209	0
RNA	0,289	0,220	0,245	0,045	0,251	0,216	0,181	0,037
P (RNA+DNA)	<0,001	0,001	0,001	0,001	0,1	0,1	0,1	0,1

tween the total nucleic acids and DNA. Nucleic acids were determined quantitatively by direct cytophotometry on the MPM-0.5 cytospectrophotometer (Opton, West Germany), with a 40× ocular, a wavelength of 545 nm, and a probe diameter of 0.5 μ in the plane of the section. The content of RNA and DNA was expressed in conventional units. Their content was estimated in the nuclei and cytoplasm of the neurons, the nuclei of the perineuronal glia, and also in the neuropil of the frontal cortex.

EXPERIMENTAL RESULTS

Conditioning took place faster in August rats (on average after 14.09 combinations) than in WAG rats (42.7 combinations); noninbred rats occupied an intermediate position (24.2 combinations).

The results of determination of the nucleic acid content in the inbred animals are given in Table 1. They show that the control rats of the two strains were indistinguishable as regards the DNA content in the neurons of the glia of their brain. After conditioning the DNA content in these structures remained unchanged. Control August and WAG rats likewise were indistinguishable from one another in their RNA contents (the small differences for individual cortical structures were not statistically significant). However, after defensive conditioning these differences appeared relatively clearly because of a change in RNA content in all structures of the August rats studied.

Increased functional activity of the nervous system is accompanied by an increase in the RNA contents in the brain cells in the case of relatively weak and short stimulation [4, 8, 9]. After intensive function of the nerve cells their RNA content falls and this coincides with the development of fatigue and exhaustion of the nervous system [3, 4]. Experiments on spinal motoneurons of rats also have shown that the increase in RNA content is connected with excitatory synaptic activation of the neurons, whereas the decrease in the RNA content is connected with inhibitory synaptic influences on the neuron. Marx [10] and Cohen [7] postulated on the basis of McAfee's observations [11] that in this case the neuromediators act indirectly on the genetic apparatus of the cell and, consequently, on RNA and protein synthesis in the postsynaptic neuron. The stimulation used in the present investigation did not exceed normal physiological loads either in strength or in duration. It can therefore tentatively be suggested that the increase in the RNA content in functioning neurons takes place primarily through the activation of neuronal RNA synthesis itself. This hypothesis is confirmed by the greater increase in the RNA content in the nuclei of the neurons. Characteristically these changes were more marked in August rats, in which defensive conditioning took place much more rapidly. Probably neuron function in the animals of this strain takes place with a shift of excitatory-inhibitory equilibrium toward excitation, and the associated intensified RNA synthesis is the material basis for the better ability of the August rats to learn. In WAG rats, by contrast with August rats, no significant changes were observed in the RNA contents, probably because of the lower intensity of their excitatory postsynaptic processes.

The observed increase in the RNA content in the neuropil and nuclei of the glial cells of the experimental animals probably reflects the initial stage of neuroglial interrelations for subsequent compensation of the RNA content in the neuron.

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ELECTRON-MICROSCOPIC AUTORADIOGRAPHY OF RNA SYNTHESIS IN THE INJURED MYOCARDIUM

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A burn of the wall of the left ventricle was produced in newborn rats. Synthesis of RNA in the muscle cells of the heart at a distance from the site of the burn was investigated by electron-microscopic autoradiography 24 h after injury. The tissue was fixed 2 and 6 h after injection of uridine-^{3}H. The density of distribution of silver grains above the nucleus and cytoplasm of the cardiomyocytes was lower in the experimental animals than in the controls.

KEY WORDS: burn of myocardium; RNA synthesis; electron-microscopic autoradiography.

The ability of the heart to regenerate at the cellular level is very limited [2]. The main method of structural compensation of the myocardium after loss of part of it is by hyperplasia of the ultrastructures in the residual muscle cells [4]. This method of repair is known as intracellular regeneration. The view is held that in the early period of ontogeny, while natural mitotic division of the heart muscle cells is still taking place, the myocardium can replace a lost area by the intensification of this division [3, 7]. Experiments have shown [1] that an increase in mitotic activity of the muscle cells in newborn rats after injury does not continue throughout the period of healing of the heart. One year later elongated scars were found in all the rats at the site of injury. It must accordingly be concluded that in newborn animals in the period of mitotic division of the muscle cells, structural replacement takes place on a basis of intracellular regeneration. The experiments described below were carried out to study this mechanism.

EXPERIMENTAL METHOD

A measured burn of the heart was produced in newborn rats by applying the hot end of a broken needle for 1 sec to the wall of the left ventricle. The animals were given an injection of uridine-5-^{3}H in a dose of 2 mCi 24 h after injury and were killed 2 and 6 h after injection of the isotope. Newborn rats of the same litter, receiving the isotope alone and killed simultaneously with the experimental animals, served as the control.

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