EFFECT OF HYPO- AND HYPERTHYROIDISM ON CORTICAL NEURONS IN RATS INVESTIGATED BY INTERFEROMETRY

G. N. Moskovkin and A. M. Aref'eva

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A marked decrease in the dimensions and dry weight of the cytoplasm and nucleus of neurons in the ganglionic layer of cortical area FP^m was found in rats developing under the conditions of hypo- and hyperthyroidism.

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A deficiency of thyroid hormone in the early stages of mammalian ontogenesis causes marked delay in development of the central nervous system [10, 11, 15]. It has been shown that the rate of DNA-dependent RNA-polymerase reactions in the liver cells of young animals with hypothyroidism is much slower than normal. One result of this is a lower rate of protein synthesis [17]. It has also been shown that protein and lipid synthesis play an important role in growth and differentiation of the developing brain.

The object of this investigation was to determine, by means of interferometry, changes in the dry weight of cortical neurons in the early postnatal development of rats with hypo- and hyperthyroidism and to compare them with normal. Since the dry weight of the cell consists principally of protein material, changes in the dry weight of the neurons in animals with hypo- and hyperthyroidism could indicate the presence of disturbances of protein synthesis.

EXPERIMENTAL METHOD

Experiments were carried out on albino rats in a normal state aged 1, 7, and 24 days, on rats with hypo- and hyperthyroidism aged 24 days. Hypothyroidism was produced in newborn rats by intraperitoneal injection of methylthiouracil into lactating females as an aqueous suspension in a daily dose of 100 mg for 7 days after parturition and then in a daily dose of 70 mg until the end of the experiment. Hypothyroidism in the young rats was judged from the presence of a characteristic goiter response and the histological picture of the thyroid gland. Hyperthyroidism was produced by daily subcutaneous injection of 2 μg L-thyroxine into the young rats from the first day after birth until the end of the experiment.

Pieces of brain were fixed for 2.5 h in a mixture of basic formalin, 96° alcohol, and glacial acetic acid in the ratio 3:1:0.3 [2], which preserves the structure of tissues well and causes little change in the dry weight of cells. With comparatively rapid processing before embedding in paraffin wax (4 h) little deformation was caused of the cell structures, which were well preserved. Serial sections were cut to a thickness of 5 μ.

Neurons in the ganglionic layer of cortical area FP^m, using Svetukhina's classification [7], were studied. For each stage of the experiment, three animals were sacrificed and 100-120 cells were measured. The phase shift was determined in an interference microscope [2]. The area of the cells was measured by a planimeter from drawn projections of cells stained by Nissl's method with cresyl violet. The dry weight (m) was determined in picograms (10^{-12} g) by the formula [2]:

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$$m = \frac{\delta \cdot S}{100\rho},$$

where δ is the phase shift of the light ray; S the area of the cell or nucleus; ρ the specific increase in refractive index, amounting to 0.0018 for protein.

EXPERIMENTAL RESULTS

The results given in Table 1 show that the dimensions of the cells and also their dry weight under normal conditions increase progressively with the animal's age. These results, indicating an increase in the protein content of the neurons, agree well with relative quantitative histochemical data [1]. Under normal conditions the dimensions and mass of the nucleus in neurons of animals aged 1 and 7 days are greater than the dimensions and mass of the cytoplasm. At the age of 24 days these proportions are equal, and in fact, so far as mass is concerned, they may be reversed. This is evidently a characteristic feature of many developing cells and of the neuron in particular [5].

In animals aged 24 days and with hypothyroidism, a sharp decrease was observed both in the size of the cells, in agreement with the results of earlier investigations [11], and in their dry weight. These values were also lower in animals with hyperthyroidism than in the control, indicating either that high doses of thyroxine were given or that the velocity of synthetic processes taking place under normal conditions in neurons is close to the optimum.

Similar results were obtained in a study of the dimensions of cortical cells in the brain of rabbits with hypothyroidism [4]. However, when guinea pigs were investigated under the same conditions [3], no significant differences were observed between the control and experimental animals in the structure and size of their cortical cells, presumably due to species-specific properties of these animals.

Metabolic effects discovered in the brain tissue of rats with hyperthyroidism are so varied that it is difficult at present to suggest any single mechanism to account for them. Many changes in the developing nervous system in hypothyroidism are explained, at least in part, by disturbance of the ability of nerve tissue to synthesize protein [14]. In more recently published papers this view has been confirmed; it has been shown that in hypothyroidism the incorporation of labeled amino acids into nerve tissue proteins is considerably reduced [13, 17].

The present investigation also showed that the protein content in neurons was considerably reduced. Demonstration of a decrease in the RNA content under these same conditions [12] gives a more definite idea of the mechanism of disturbance of protein synthesis in hypothyroidism.

It is interesting to compare the results described above with other effects of hypothyroidism. For instance, several workers have shown that in hypothyroidism the content of amino acids of the glutamine group [17] and of N-acetyl-L-aspartic acid [16] in the brain is reduced, and activity of certain enzymes is depressed: succinate dehydrogenase, NADP-H_2-cytochrome c-oxidoreductase, acetylcholinesterase and nonspecific cholinesterase [6, 12, 15], γ-aminobutyric acid transaminase and glutamate decarboxylase [9], and aspartate aminotransferase [17]. The view that in hypothyroidism many changes in the developing nerve tissue are based on a disturbance of protein synthesis does not explain the fact that not all the enzymes studied react to this factor in hypothyroidism [9, 17]. There are no grounds at the present time for suggesting any specific relationships between the level of thyroid hormone in the body and synthesis of particular enzymes in the neuron.

TABLE 1. Effect of Experimental Hypo- and Hyperthyroidism on the Dry Weight and Dimensions of Cells in the Ganglionic Layer of the Rat Cerebral Cortex

Group	Age of rats in days	Phase shift of light ray (in deg)			Area (in conventional units)			Dry weight (in pg)	
		nucleus	cytoplasm	fiber	cell	nucleus	cytoplasm	nucleus	cytoplasm
Control	1	31,49± 0,97	39,15± 0,77	17,44± 1,0	79,5± 1,51	51,5± 1,53	28,0± 0,985	17,4± 0,75	11,7± 0,47
Control	7	29,10± 0,72	43,74± 0,72	22,06± 0,86	147,6± 2,33	103,75± 4,01	43,85± 0,85	32,23± 1,3	22,5± 0,62
Control	24	41,66± 1,85	64,38± 1,07	40,70± 1,38	369,5± 9,18	189,5± 4,37	180,0± 6,125	84,24± 1,32	124,20± 5,3
Hypothyroidism	24	69,85± 1,81	80,70± 1,10	40,45± 2,4	173,6± 4,7	87,6± 2,58	96,0± 3,84	65,37± 2,54	82,77± 3,48
Hyperthyroidism	24	55,85± 1,45	67,52± 1,0	40,28± 0,96	278,0± 5,23	128,6± 4,16	149,4± 5,52	76,73± 3,01	108,20± 3,9

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