CHANGES IN CHOLINESTERASE ACTIVITY IN SPINAL MOTONEURONS OF RATS IN EXPERIMENTAL THYROTOXICOSIS

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Thyrotoxicosis in rats causes a decrease in the number of cells with high cholinesterase activity and a corresponding increase in the number of cells with low cholinesterase activity in the spinal motoneurons.

Electrophysiological investigations in the author's laboratory have demonstrated changes in the conduction of nervous impulses in spinal neuronal structures in experimental thyrotoxicosis [3]. The principal mediator of transmission of the nervous impulse is acetylcholine. Together with other factors, its concentration determines the level of nervous conduction. Existing information regarding the state of the acetylcholine–cholinesterase system in thyrotoxicosis is concerned mainly with the blood concentrations of these substances [1, 2, 5, 6].

It was decided to use histochemical methods to determine changes in the cholinesterase activity in experimental thyrotoxicosis directly in the spinal motoneurons.

EXPERIMENTAL METHOD

Sexually mature male rats weighing 150-180 g were studied. Eight rats received a single injection of L-thyroxine in a dose of 40 μg/100 g body weight. Thyrotoxicosis was induced in the animals of another group by oral administration of dried thyroid by the method usually adopted in the writer's laboratory [4]. The animals were decapitated 2 h after a single injection of L-thyroxine, on the 15th day (10 rats) and 30th day (7 rats) of thyroid administration, and also one month after the end of administration of thyroid by mouth for 30 days (10 rats). Pieces of spinal cord from the experimental animals were mounted in a single block with the spinal cord of the control animals.

Cholinesterase activity was studied in the spinal motoneurons at the level C_5-C_6 by Gomori's modification of Kelly's method. In this method, a dark brown precipitate of copper thiocholate is deposited wherever cholinesterase is located. Proof that this method in fact detects cholinesterase is given by the total decolorization of sections first treated with eserine in a concentration of 10^{-6} M. The reaction was carried out with preliminary fixation of the tissue in formalin, and sections were cut to a thickness of 25 μ in the cryostat. At least 50 motoneurons were studied in each experimental group.

Cholinesterase activity was estimated by indirect cytophotometry on the MF-4 microphotometer. No other reference could be found in the literature to the possibility of quantitative estimation of cholinesterase by the method now used. It was therefore considered that only the intensity of staining and, consequently, the relative cholinesterase activity could be estimated from the cytophotometric results, and this activity was accordingly classified as follows: high, medium, and low.

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TABLE 1. Changes in Cholinesterase Activity in Spinal Motoneurons of Rats after Administration of L-Thyroxine, on 15th and 30th Days of Thyroid Feeding, and 1 Month after End of Thyroid Feeding for 30 Days

Group of animals	Cholinesterase activity		
	high	medium	low
	% of cells		
Control	56	39	5
Single administration of L-thyroxine	47	38	15
Thyroid feeding for 15 days	7	62	31
" " " 30 days	12	36	52
One month after end of thyroid feeding for 30 days	35	50	15

EXPERIMENTAL RESULTS

The results are given in Table 1.

They show that a single injection of L-thyroxine caused no significant changes in cholinesterase activity in the motoneurons. In the early stages of toxicosis produced by oral administration of thyroid for 15 days, changes took place in the relative proportions of cells in the spinal cord with high, medium, and low cholinesterase activity: the number of cells with high activity was sharply reduced and there was a corresponding increase in a number of cells with medium and low activity. The further development of thyrotoxicosis (administration of thyroid for 30 days) led to an even greater decrease in cholinesterase activity in the motoneurons: a decrease in the percentage of cells with high cholinesterase activity by comparison with the control resulted from a marked increase in the number of cells with low activity (5% in the control, 52% in the experimental series); there was no change in the number of cells with medium activity. A period of recovery for 1 month after oral administration of thyroid for 30 days led to restoration of the normal relative proportion of cells with the different levels of activity.

The change in conduction of nervous impulses at the spinal level observed in thyrotoxicosis can thus be attributed, besides other factors, to changes in the acetylcholinesterase system. This is shown by the reduced cholinesterase activity detected in the present investigation in experimental thyrotoxicosis.

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