STATE OF NEUROMUSCULAR TRANSMISSION IN EXPERIMENTAL THYROTOXICOSIS

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The functional state of the neuromuscular synapse was investigated in anesthetized rats after administration of thyroid extract by mouth for 3 weeks. In rats with thyrotoxicosis the amplitude of the action potential of the muscle was reduced, the threshold strength of stimulation was lowered to an indirect stimulus, and the response latency and the absolute and relative refractory phases were shortened. The appearance of a depressed response in rats with thyrotoxicosis occurred at lower frequencies and the appearance of a transmission block at higher frequencies than in the control animals. After administration of neostigmine to rats with thyrotoxicosis, the indices of development of depression approximated to those recorded in the control group. The results of this investigation indicate a disturbance of neuromuscular transmission in thyrotoxicosis.

The study of changes in the functional state of the neuromuscular synapse during an increase in the content of thyroid hormones in the body is not only of special importance to the understanding of the pathogenesis of motor disorders in patients with thyrotoxicosis, but is also of great interest to neuropathophysiology in general.

In an earlier paper results were given showing changes in synaptic conduction at the spinal level in animals with experimental thyrotoxicosis [2]. The results suggested that in hyperthyroid states, besides the disturbances of metabolism in muscle tissue demonstrated by many investigators, changes could also take place in neuromuscular transmission. The investigation described below was carried out to study this problem.

EXPERIMENTAL METHOD

Rats were anesthetized with pentobarbital (40 mg/kg). Electrical responses of the gastrocnemius muscle to single and repetitive stimulation of the peripheral end of the divided tibial nerve by square pulses of supramaximal strength and 0.1 msec in duration were investigated in the animals. To record the muscle potentials the recording electrode was placed at the point of entry of the nerve into the muscle, while the second electrode was inserted into the tendon. The muscle was flooded with mineral oil warmed to 37° C.

The time of neuromuscular transmission (latent period of the response), the duration of the absolute and relative refractory phases, and the development of fatigue (from the moment of onset of fatigue to the complete blocking of conduction) were determined. To obtain fatigue, frequencies of between 10 and 500 Hz were used. In a special series of experiments the action of neostigmine (0.25 mg/kg, intravenously) on neuromuscular transmission was studied in animals with thyrotoxicosis. Thyrotoxicosis was induced by feeding the animals

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TABLE 1. State of Neuromuscular Transmission in Experimental Thyrotoxicosis

Group of animals	Latent period (in msec)			Amplitude of action potentials (in mV)			Refractory period (in msec)					
							absolute			relative		
	n	M ± m	P	n	M ± m	P	n	M ± m	P	n	M ± m	P
Control	15	1,3±0,03	<0,01	15	10±1,16	<0,01	16	2,34±0,15	<0,02	16	9,7±0,45	<0,01
With thyrotoxicosis	15	1,1±0,02		15	7,5±0,56		13	1,70±0,08		13	8,0±0,43	
With thyrotoxicosis + neostigmine (0.25 mg/kg)	12	1,25±0,14		12	7,9±0,11		14	4,36±0,56		14	13,9±0,9	

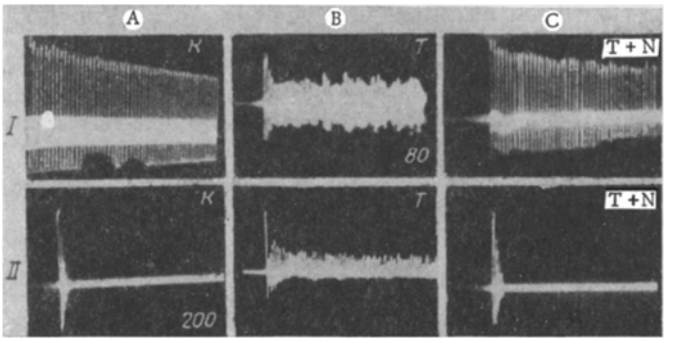

Fig. 1. Responses of muscle to indirect stimulation of nerve in rats of control group (A), rats with thyrotoxicosis (B), and rats with thyrotoxicosis after administration of neostigmine (C). Stimulation of peripheral end of divided nerve at frequencies of 80 (I) and 200 (II) pulses/sec.

with thyroid extract in a daily dose of 0.2-0.4 g for 3 weeks. The rats lost up to 40% of their initial body weight while the concentration of iodine bound with the plasma proteins increased from 3.5 ± 1.2 to 10 ± 1.7 μg%.

EXPERIMENTAL RESULTS

A decrease in the threshold strength of stimulation to 0.3 ± 0.03 V ($P < 0.01$) was found in the rats with thyrotoxicosis and all the parameters tested were reduced (Table 1): the amplitude of the action potential and its latent period ($P < 0.01$), and also the absolute and relative refractory phases ($P < 0.02$)

Investigation of the fatigue effect by stimulating the nerve at frequencies of between 10 and 500/sec also showed substantial differences between the responses of the control rats and of the rats with thyrotoxicosis. The beginning of fatigue in the control group of animals was recorded when the frequency of stimulation was between 50 and 80/sec, and a complete block to the transmission of excitation occurred within frequencies of 100 and 300/sec (Fig. 1). In response to stimulation at a high frequency, the block occurred after the first stimuli. In animals with thyrotoxicosis the onset of fatigue was shifted toward lower frequencies (from 30 to 50/sec), but a complete block of transmission occurred during stimulation at frequencies of 400/sec or higher. In most cases the application of stimuli at a high frequency evoked a series of low-amplitude potentials, varying in magnitude, after a relatively high response, although smaller than normal to the first stimulus.

It was concluded from these results that thyrotoxicosis leads to a disturbance of neuromuscular transmission. It could be postulated that the changes which arose were due to a deficiency of mediator in the neuromuscular synapse. The decrease in amplitude of the combined action potential of the muscle during its indirect stimulation and the shift of the fatigue effect toward lower frequencies demonstrate that some fibers do not respond to a single stimulus or to repetitive stimulation at low frequency. Meanwhile, preservation of the responses of the muscle to high-frequency stimulation of the nerve, together with the variability and general decrease in amplitude of the muscle responses, suggests that new muscle fibers participate in the response as a result of potentiation of the presynaptic apparatus and the liberation of mediator in synapses which become activated during repetitive stimulation of the nerve. An effect of intermittent participation in the response of different muscle fibers with different degree of distur-

bance of their neuromuscular transmission arose [4, 6]. An effect of this type has also been observed in other forms of reversible disturbance of neuromuscular transmission connected with a deficiency in the liberation of mediator (poisoning with tetanus toxin) [4, 6].

To test these hypotheses experiments were carried out using the cholinesterase inhibitor neostigmine. The grounds for this procedure were as follows: if the observed changes in neuromuscular transmission were due to a relative deficiency of acetylcholine, the use of neostigmine must to some extent improve neuromuscular transmission and bring its state nearer to what is observed normally. Conversely, if the disturbance of transmission was the result of an excess of mediator, as some workers consider [9], the use of neostigmine should aggravate the disturbances of synaptic conduction described above.

The results of an investigation of the parameters of neuromuscular transmission after administration of neostigmine showed (Table 1) that under these conditions the latent period of the action potential was prolonged in the animals with thyrotoxicosis, the duration of the absolute and relative refactory phases was increased, and the times of onset and development of fatigue and the appearance of a transmission block were changed (Fig. 1). These indices more closely resemble those recorded in the control animals. Neostigmine thus normalized, in a certain sense of the term, neuromuscular transmission in the animals with thyrotoxicosis. The increase in the acetylcholine concentration in the region of the end-plates evidently compensated for its relative deficiency during indirect stimulation of the muscle in the animals with thyrotoxicosis and facilitated the recruiting of more motor units of the muscle into the response, thus leading to relative normalization of the response. Consequently, the results are evidence of a deficiency of mediator in the synapse of the skeletal muscle in thyrotoxicosis. Similar results with the use of neostigmine have been obtained in the analysis of vagal effects on the heart in rabbits after prolonged administration of thyroid extract [8] and also in the analysis of disturbances of neuromuscular transmission in poisoning with tetanus toxin [5, 6], when the liberation of mediator is disturbed [4, 7].

These results do not accord with the view that "hypersynapsia" arises [1] in hyperthyroid states, nor do they confirm the suggestions of some investigators that acetylcholine synthesis is increased in neuromuscular synapses of the skeletal muscle of rats receiving L-thyroxine [9].

The results described above raise once again the question of assessment of the parameters of lability [3]. Some time ago, in an investigation using tetanus toxin, one of us [3-6] showed that shortening of the refractory period and the ability of a muscle to reproduce responses to a high frequency of indirect stimulation are not always indices of high lability. Conversely, under certain conditions they must be interpreted as indices of latent pathology inducing functional dispersion of the neuromuscular units. The results of the present investigation confirm this conclusion.

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