ELECTROPHYSIOLOGICAL ANALYSIS OF THE EFFECT OF CORTISONE ON THE NEUROMUSCULAR APPARATUS IN RATS

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UDC 612.816.014.46:615.357.453

The action of cortisone on the neuromuscular apparatus was studied in situ in experiments on rats. Combined recordings were made of the action potentials of nerve (AP_n) and muscle (AP_m) and also of intracellular resting membrane potentials (MP) and miniature end-plate potentials (MEPP). Cortisone was injected in a dose of 1.5 mg/100 g body weight daily for 10 days. In the animals receiving the hormone, MP of the muscle fibers was reduced compared with the control, the amplitude of the MEPP was reduced and their frequency increased, the time of neuromuscular transmission was lengthened, and its reliability was reduced (the amplitude of AP_m fell faster in the course of tetanus).

KEY WORDS: neuromuscular transmission; cortisone.

Morphological, histochemical, and pharmacological data on the "dystrophic" and inhibitory effect of large doses of glucocorticoids on muscle tissue and on neuromuscular synapses have recently been published [10-13]. Electrophysiological data on the effect of glucocorticoid hormones on the neuromuscular apparatus are few in number and contradictory in nature [5, 6, 8, 10].

The object of this investigation was the electrophysiological assessment of the action of a large dose (1.5 mg/100 g body weight*) of the glucocorticoid cortisone on the neuromuscular apparatus of a warm-blooded animal (rat) in situ.

EXPERIMENTAL METHOD

Male Wistar rats weighing 95-115 g and aged about 2 months were used. The dose of cortisone (Adreson, N. V. Organon Oss, Holland) for daily intramuscular injection was calculated by the equation $X = 0.59 \cdot 4/n$, where n is the body weight (in kg) [3]. Physiological saline was injected into the control animals. The experimental and control animals were kept under identical conditions. Before the experiment the animals were anesthetized with urethane (0.8 ml of 15% solution/100 g body weight) and fixed to a dissecting table with the spine uppermost. The sciatic and peroneal nerves and the extensor digitorum longus muscle were dissected as the test object. The space formed as a result of the operation was filled with physiological saline (37°C) for warm-blooded animals, with the following composition (in g/liter): NaCl 9.00, $CaCl_2$ 0.24, KCl 0.42, $NaHCO_3$ 0.20, glucose 1.0. During the experiment for combined recording of the action potentials of the nerve (AP_n) and muscle (AP_m) this solution was replaced by mineral oil, and in the experiments with intracellular recording, the physiological saline was left in the space. Combined recording of

*This dose of cortisone, if injected daily for 10 days, can give rise to some reduction in liberation of the hormone from the adrenal cortex of the experimental animals; this probably reduces the actual rise in the cortisone concentration.

Laboratory of Neuromuscular Physiology, Physiological Institute, Leningrad University. Department of General Biology, First Leningrad Medical Institute. (Presented by Academician V. N. Chernigovskii.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 81, No. 4, pp. 387-390, April, 1976. Original article submitted November 28, 1974.

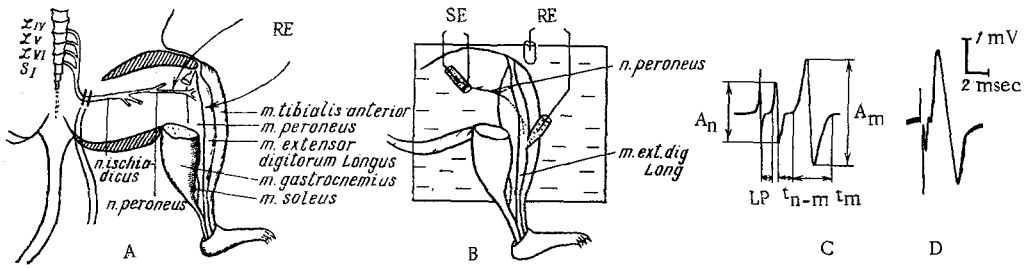

Fig. 1. Scheme showing methods of combined recording of action potentials of nerve (AP_n) and muscle (AP_m) and of intracellular recording of potentials of muscle fibers, and example of record of combined AP_n and AP_m. A and B) Scheme of stimulation and recording, respectively, for combined and intracellular methods: SE) stimulating electrodes; RE) recording electrodes. C) Scheme of measurement of parameters of AP_n and AP_m (stimulus artifact in front of AP_n). LP) Latent period of AP_n; t_{n-m}) time of neuromuscular transmission; t_m) duration of AP_m; A_n) amplitude of AP_n; A_m) amplitude of AP_m. D) Example of record of AP_n and AP_m.

TABLE 1. Changes in Parameters of AP_n and AP_m during Administration of Cortisone (20 experiments)[1]

Parameters	Experimental animals	Control animals	P
Threshold (in V) of AP_m	0,22±0,05	0,51±0,09	<0,05
Latent period of AP_n (in msec)	0,42±0,04	0,55±0,05	<0,01
Duration of AP_m (in msec)	6,15±0,38	6,38±0,35	>0,05
t_{n-m} (in msec)	0,95±0,13	0,63±0,19	<0,05
Time for amplitude of AP_m to fall by 50% in tetanus (in sec)	9	13	

[1]Mean values and 95% confidence limits shown in Tables 1 and 2.

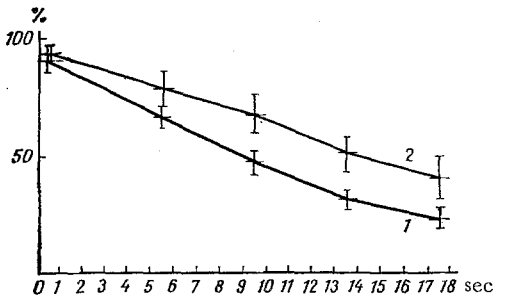

Fig. 2. Changes in electrical activity of nerve and muscle during tetanus with stimulation of sciatic nerve at a frequency of 100/sec. Changes in mean amplitude of AP_n during tetanus: 1) experimental animal; 2) control. Abscissa, time of stimulation (in sec); ordinate, amplitude of muscular response (in % of its maximal value at beginning of stimulation).

AP_n and AP_m was carried out by Samoilov's method [4, 7]. A scheme of the investigation and an example of a record are shown in Fig. 1. During tetanization the nerve was stimulated at a frequency of 100/sec by stimuli, each 0.02 msec in duration and with a strength of 1.5-4 thresholds. With stimuli of this duration the stimulation artifact was minimal and the pattern of AP_n was not distorted. The resting membrane potential of the muscle fibers (MP) and the miniature end-plate potentials (MEPP) were recorded intracellularly by the usual method, using microelectrodes filled with 3 M KCl.

EXPERIMENTAL RESULTS AND DISCUSSION

The character of the electrical response of the nerve and muscle was similar in the experimental and control animals. The results of the corresponding measurements, given in Table 1, show that the threshold of electrical stimulation was reduced by about half in the animals receiving the hormone. The latent period of AP_n of the experimental animals was one-third shorter than that of the controls. The duration of AP_m did not differ significantly in the experimental and

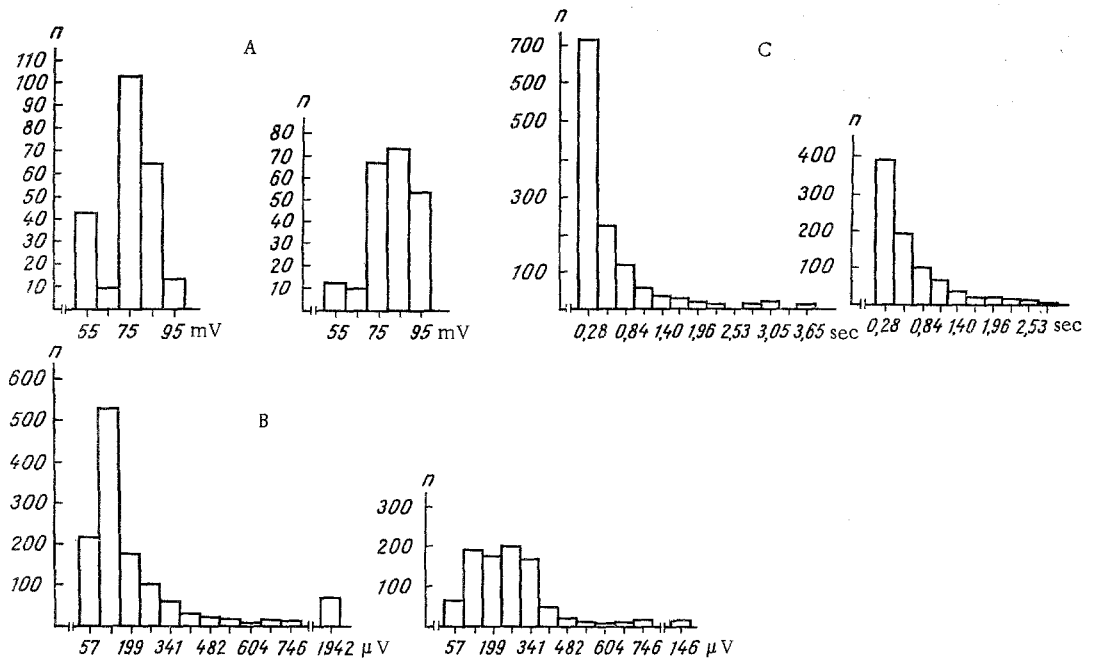

Fig. 3. Changes in values of MP and amplitude and intervals of MEPP following administration of cortisone: A) histograms of distribution of MP in experimental (left) and control (right) rats. Abscissa, amplitude of MP (in mV); ordinate, number of fibers tested (n). B) Distribution of amplitude of MEPP in experimental (left) and control (right) rats. Abscissa, amplitude of MEPP (in μV); ordinate, number of MEPP (n). C) Histograms of distribution of MEPP intervals in experimental (left) and control (right) rats. Abscissa, interval between MEPP (in sec); ordinate, number of MEPP (n).

TABLE 2. Changes in Parameters of MP and MEPP during Administration of Cortisone (5 experiments)

Parameters	Experimental animals	Control animals	Experiment/control (%)	P
MP (in mV)	69,9±4,3	79,6±4,3	88	<0,001
Amplitude of MEPP (in μV)	161,1±9,5	245,8±12,2	67	<0,001
Frequency of MEPP (in spikes/sec)	1,95±0,13	1,63±0,15	116	<0,001

control animals. The time of neuromuscular transmission (t_{n-m}) in the experimental animals was almost half as long again as in the controls. The amplitude of AP_n and AP_m in the experimental animals was about two to three times greater than in the controls. During prolonged stimulation of the sciatic nerve at a frequency of 100/sec, a 50% decrease in the amplitude of AP_m took place faster in the experimental animals than in the controls (Fig. 2), i.e., the reliability of the neuromuscular system in animals receiving the hormones was reduced.

The results of the experiments with intercellular recording are given in Table 2. The decrease in the mean value of MP was connected with the shift of the main peak of the histogram of MP distributions (Fig. 3A) of the experimental animals toward lower values. The mean amplitude of MEPP in animals receiving the hormone was 67% of their amplitude in the control animals, and the main peak of distribution of amplitudes for the experimental animals (Fig. 3B) was shifted correspondingly into the region of lower values. In both groups of animals an additional smaller peak was observed in the region of high amplitudes of

MEPP, reflecting the contribution of "giant" miniature potentials. When the mean amplitude of MEPP was calculated, the amplitude of these potentials was disregarded. The middle part of MEPP in the experimental animals was 16% greater than in the controls. The peak corresponding to minimal intervals was more marked in the distribution of intervals between MEPP in the experimental animals (Fig. 3C).

The results of this investigation are evidence of the varied action of cortisone on the neuromuscular apparatus of the rat.

The increase in excitability of the nerve to electrical stimuli in animals receiving glucocorticoids agrees with the observation [11] that the excitability of nerve is reduced in adrenalectomized animals. In the present case there was a true increase in electrical excitability, evidently in connection with depolarization of the fibers, as shown by a decrease in the latent period of the nerve response, i.e., an increase in the conduction velocity. However, the effect of an increase in excitability of the nerve was evidently partly due also to reduction of the connective-tissue membranes. The reducing effect of glucocorticoid hormones on connective tissue is well known [2]. The increase in amplitude of AP_m against the background of depolarization can evidently be explained only by reduction of the connective-tissue membranes, i.e., by reduction of the connective-tissue shunts, for according to existing data [9], large doses of glucocorticoids reduce the amplitudes of intracellular APs of muscle fibers against the background of a fall of their MP. The same explanation most probably holds good also for the increase in amplitude of AP_n. The lowering of MP of the muscle fiber could be due to a reduction in the concentration of K^+ ions in the cells, as has been shown under the influence of glucocorticoids [9]. Cortisone evidently has a depolarizing action on nerve endings also, and it thereby increases the frequency of MEPP. The decrease in amplitude of MEPP through the action of cortisone is explained by the fall in MP and also, probably, by the decrease in sensitivity of the cholinoceptors. This last effect of cortisone is described in [5]. The increase in the time of neuromuscular transmission and the decrease in its reliability in the animals receiving the hormone may be due to the decrease in the response of the postsynaptic membrane to a quantum of mediator and, correspondingly, to a reduction in the EPP. Elevation of the critical level of depolarization of the muscle fibers on account of the prolonged fall of MP may also play a significant role.

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