EFFECT OF COLCHICINE-INDUCED DISTURBANCE OF AXOPLASMIC TRANSPORT ON MEDIATOR LIBERATION IN MYONEURAL SYNAPSES OF THE FROG SARTORIUS MUSCLE

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Application of colchicine to the nerve innervating the sartorius muscle in frogs reduces differences in the functional state of the myoneural synapses of the muscle as reflected in the quantum composition of the end-plate potential (EPP) and the dynamics of EPP amplitudes during high-frequency stimulation. Meanwhile the frequency of the miniature EPP and its amplitude are reduced, the latter evidently as a result of a decrease in the effective resistance of the postsynaptic membrane. Changes in evoked and spontaneous secretion of mediator are connected with a disturbance of axoplasmic transport in somatic nerve fibers.

KEY WORDS: axoplasmic transport; colchicine; synaptic transmission.

Recently in connection with the study of axoplasmic transport (AT) it has been shown that substances synthesized in the perikaryon of the motoneuron and carried by rapid AT into presynaptic structures [7, 16], and also their transport across the neuromuscular junction into skeletal muscle can be activated [6]. The alkaloid colchicine has the specific property of binding tubulin, a component of the cytoplasmic microtubules of the axon [8], of destroying the microtubules, and thereby stopping fast AT [17]. Chronic colchicine poisoning of nerve fibers has been shown to modify the structural organization and function of the synaptic apparatus of the rat optic nerve [10], and in the motor nerve—skeletal muscle system of warm- and cold-blood animals it leads to the development of denervation-like changes in muscle fibers although the transmission of excitation in the neuromuscular synapse remains intact [3, 5]. However, the functional state of synapses of the peripheral nervous system and, in particular, of the neuromuscular apparatus of the frog under conditions of a deficiency of the substances carried by fast AT has not been finally settled.

Accordingly the object of the present investigation was to study spontaneous and evoked mediator secretion in synapses of the frog sartorius muscle at various times after treatment of its motor nerve with colchicine.

EXPERIMENTAL METHOD

Experiments were carried out on frogs (Rana ridibunda) in the fall and winter. A 30 mM solution of colchicine (from Merck) was applied on a cotton swab for 30 min to the isolated motor nerve to the sartorius muscle [3]. In some experiments desympathization of the sartorius muscle was carried out by unilateral extirpation of the paravertebral abdominal sympathetic chain. All the frogs were kept at 20°C. A nerve-muscle preparation of the sartorius muscle and sciatic nerve was used in the experiments. The preparation was kept in a bath with a continuous flow of Ringer's solution of the following composition (in mmoles/liter): NaCl 115, KCl 2.5, $CaCl_2$ 1.8, $NaHCO_3$ 3. Action potentials in the muscle fiber in response to repetitive stimulation were blocked by the addition of D-tubocurarine chloride (1×10^{-6} g/ml) to the Ringer's solution. End-plate potentials (EPPs) and miniature end-plate potentials (MEPPs) were recorded by a standard microelectrode technique. EPPs in five to seven muscle fibers were recorded in each nerve-muscle preparation by moving the microelectrode successively from one border of the muscle to the other. The quantum composition of EPP (m) and the quantum value were calculated by an indirect method [9, 13, 19]. To calculate the value of m the motor nerve was stimulated by 100 pulses with a frequency of 0.5 Hz. If the amplitude of the EPP exceeded 5 mV,

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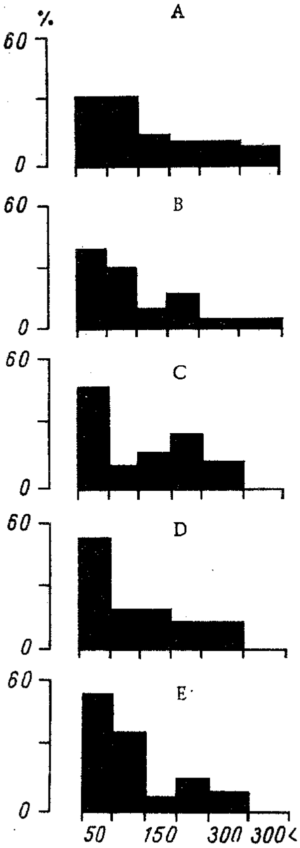

Fig. 1. Histogram of sartorius muscle synapses according to quantum composition of EPP in control and at various times after application of colchicine to motor nerve. Abscissa, quantum composition of EPP; ordinate, number of synapses (in %). A) Control; B) 1 day, C) 3 days, D) 6-8 days, and E) 13-15 days after application of colchicine. Data shown are based on 37-49 experiments.

the amplitude of each EPP was corrected for nonlinearity of MEPP summation [19]. The magnitude of frequency potentiation and depression of EPP during repetitive nerve stimulation with a frequency of 100 Hz was determined as the ratio of the amplitude of the second and subsequent EPPs to the amplitude of the first, taken as 100%. To determine the mean amplitude of MEPP, 100 consecutive MEPPs were recorded. Nerve-muscle preparations from intact frogs served as the control. The experimental results were subjected to statistical analysis. All calculations were carried out on the Odra-1204 computer.

EXPERIMENTAL RESULTS

Judging from the level of the quantum composition of the EPPs, three types of synapses can be conventionally distinguished in the frog sartorius muscle [2]. The first type includes synapses whose quantum composition of EPP exceeds 200 quanta. The rhythmic activity of these synapses is distinguished by short-term potentiation, followed by progressive depression. Synapses of the second type have a quantum composition of EPP of between 100 and 200 quanta. These synapses respond to stimulation at 100 Hz by potentiation, which does not change to depression until 80 EPPs. The third type consists of synapses with a quantum composition of EPP of under 100 quanta. The rhythmic activity of these synapses is characterized by the prolonged development of EPP potentiation.

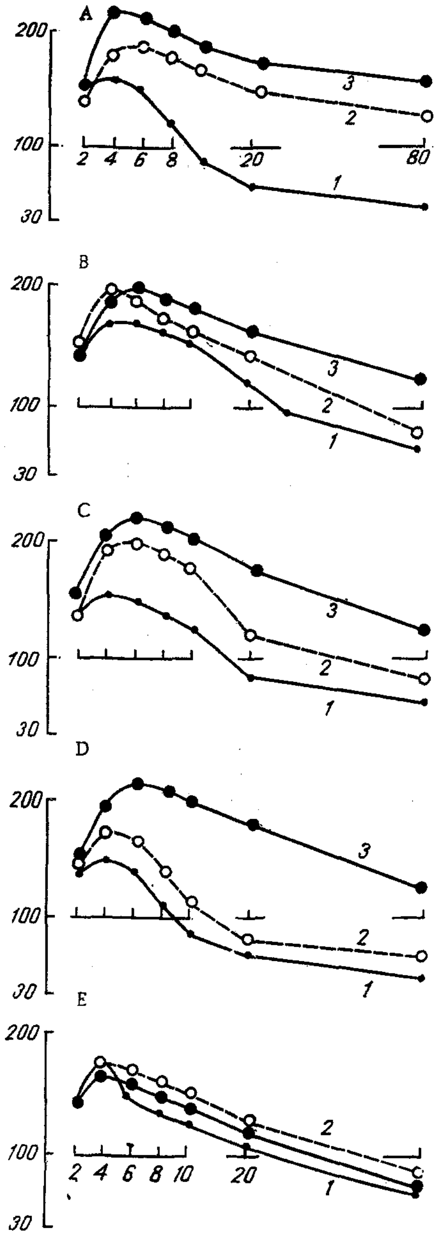

Fig. 2. Dynamics of EPP amplitudes during stimulation of three types of synapses at a frequency of 100 Hz in control and at various times after application of colchicine to motor nerve. Abscissa, stimulus No.; ordinate, amplitude of EPP (in %). 1) 1st, 2) 2nd, 3) 3rd type of synapse. Remainder of legend as in Fig. 1.

The experiments showed that during the first day after application of colchicine to the motor nerve the number of synapses with the lowest quantum composition of EPP (under 100 quanta) in the sartorius muscle increased from 58 to 69% (Fig. 1A, B). On the 3rd and 7th days after application of colchicine these changes were more marked still (Fig. 1C, D). On the 14th-15th day the proportion of neuromuscular synapses with the lowest quantum composition of EPP was 84% (Fig. 1E).

Perfusion of a nerve-muscle preparation with colchicine solution is known to reduce the quantum composition of EPP and, it is important to note, this effect is reversible [4]. Presumably when colchicine is applied to the nerve it may be carried either along the perineurium or with the flow of lymph to nerve endings.

TABLE 1. Amplitude (in μV) and Frequency (in Hz) of MEPPs in Frog Sartorius Muscle under Normal Conditions and at Various Times after Application of Colchicine to Motor Nerve (M ± m)

Statistical index	Control		After application					
			1st day		7th day		14th day	
	amplitude	frequency	amplitude	frequency	amplitude	frequency	amplitude	frequency
M	199,59	3,20	176,66	0,54	158,14	1,21	131,72	0,81
±m	6,04	1,13	7,73	0,12	8,37	0,23	4,66	0,14
P	—	—	<0,05	<0,02	<0,01	<0,05	<0,001	<0,05

It may rightly be asked, therefore, whether the changes observed in the value of m are connected with the direct action of the alkaloid on the nerve ending. If this is so, the greatest changes ought to be found at the earliest times (the colchicine concentration in the tissue fluid must then be highest, and the effect is reversible), but this in fact was not observed.

As regards the subepineural diffusion of the alkaloid, this must be extremely small [14]. The changes observed are thus evidently the result of a disturbance of fast AT.

Analysis of the dynamics of EPP amplitudes during high-frequency stimulation showed that, starting from the 1st day after colchicine application, synapses which by their m value belonged to the second and third types, but with the characteristic dynamics of EPP amplitudes of synapses of the first type, appeared. With an increase in the time after application of colchicine these changes became more marked (Fig. 2A-D). On the 14th-15th day after treatment of the nerve trunk with colchicine the dynamics of the EPP amplitudes became virtually identical regardless of the group to which the synapses belonged on the basis of their m value (Fig. 2E). Depression is a feature of synapses in which the rate of mobilization of mediator is less than the rate of its liberation [18]. Hence, on the basis of a qualitative assessment of the dynamics of EPP amplitudes during high-frequency stimulation it can be postulated that, because of a disturbance of fast AT, both the rate of mobilization of the mediator and the reserves of available mediator are reduced. However, this does not rule out a simultaneous change in the probability of liberation of each quantum of acetylcholine.

Evidence has been obtained that desympathization of the frog sartorius muscle leads to a reduction in the quantum composition of its EPP [1]. With the method used in this case to disturb AT (application of colchicine to a branch of the sciatic nerve) the proximal-distal AT of noradrenalin-containing granules and enzymes participating in noradrenalin synthesis is disturbed in sympathetic nerve fibers also [11]. This may have a definite effect on the quantum composition of the EPP. It was therefore necessary to compare the character of changes arising in the synapses after blocking of the sympathetic innervation (desympathization) and after blocking AT.

On the 7th-9th day no redistribution of neuromuscular synapses in accordance with the value of m or with the dynamics of EPP amplitudes could be observed in the desympathized muscle. After application of colchicine to the nerve, however, distinct changes were found in the above-mentioned parameters at these same times.

Consequently, the changes observed in the functional state of the myoneural apparatus of the frog sartorius muscle were due primarily to a disturbance of AT in somatic nerve fibers. As early as on the 1st day after application of colchicine to the motor nerve a decrease in amplitude of the MEPP and a simultaneous increase in frequency compared with the control were observed. Later in the course of the experiments the amplitude of MEPP continued to fall progressively while its frequency remained low (Table 1). These results suggest that the neuron soma, with the aid of AT, constantly controls the character of the spontaneous acetylcholine secretion.

The changes detected in the amplitude of MEPP are most likely to be connected with changes in the properties of the postsynaptic membrane [15], although a change in the value of the quantum of mediator cannot be ruled out [12]. The experiments showed that the maximal sensitivity of the postsynaptic membrane to acetylcholine remained virtually constant at all times, but the effective resistance of the membrane was significantly smaller 1 and 2 weeks after application of colchicine. Consequently, the decrease in the amplitude of MEPP was evidently due to a decrease in the input resistance of the postsynaptic membrane.

Treatment of the motor nerve with colchicine, disturbing the fast phase of AT, thus reduces differences in the functional state of the myoneural synapses of the frog sartorius muscle as reflected in the quantum composition of the EPP and the dynamics of the EPP amplitude during repetitive stimulation, and this effect coincides with a reduction in the heterogeneity of the muscle fibers under these conditions as revealed histochemically [3]. The decrease in the frequency of MEPP is also evidence of a disturbance of spontaneous acetylcholine secretion by the nerve endings.

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STRUCTURAL CHANGES IN ENTEROCYTE MEMBRANES IN ACHOLIA WITH SPECIAL REFERENCE TO MICROTOPOGRAPHICALLY DIFFERENT CARBOHYDRASES

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Activity of surface and intracellular carbohydrases was compared in rats with chronic total loss of bile. The function of adsorbed amylase, a glycocalyx marker, is disturbed by a much greater degree than the function of invertase, a marker of the plasma membrane proper.

KEY WORDS: small intestine; hydrolysis of carbohydrates; bile.

The physiological role of bile is not yet fully understood. The results of membrane studies point to a possible role of the components of bile in the regulation of the metabolic activity of the enterocyte and, in particular, in the metabolism of its membrane structures.

The properties of the plasma membrane proper and of the glycocalyx were assessed in rats with acholia with special reference to the corresponding marker enzymes: invertase [2] and adsorbed amylase.

EXPERIMENTAL METHOD

Albino rats (37 animals) were fed in the ordinary way on a mixed diet. A fistula of the bile duct was formed as described by Shlygin and Vasilevskaya [3]. Fasting rats were decapitated on the 4th and 7th days after the operation.

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