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SOME BIOCHEMICAL CHARACTERISTICS OF MUSCLE PERFUSATE AND ITS EFFECT ON TRANSMITTER RELEASE IN THE NEUROMUSCULAR SYNPASE

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It was suggested previously that the muscle metabolite histidine may participate directly or indirectly in antidromic regulation of motor nerve ending function [8, 10]. Exogeneous histidine significantly increases the quantum composition of end-plate potentials (EPP) through an increase in the reserves of release-ready quanta and it reduces the frequency of miniature EPP (MEPP) [12]. Meanwhile it has been shown that resting and, in particular, synaptically activated skeletal muscles secrete into the incubation medium a substance giving a positive diazo reaction, and presumed to contain in its composition a histidine residue [10, 13], but not identical with free histidine or the histidine-containing muscle dipeptide carnosine [4]. Data have been obtained which suggest that carnosine may be the source of this substance [10].

As long ago as in 1935, Kibyakov [6, 7] found that muscle perfusate abolishes fatigue in a nerve–muscle preparation, and, as is now known, this fatigue is presynaptic in nature [11]. The active principle of the perfusate was found to have motor nerve endings as its target, and it is evidently neither a protein nor a catecholamine [6, 7]. Incidentally, fatigue in the nerve–muscle preparation is also abolished by exogenous histidine and carnosine [1, 4].

The aim of this investigation was to attempt to solve two problems: 1) are histidine-containing substances released into the medium during perfusion of the vascular bed of skeletal muscles; 2) does the perfusate have a presynaptic action similar to the action of histidine?

EXPERIMENTAL METHODS

The hind limbs of a donor frog were perfused in situ through the dorsal aorta and perfusate was collected from the abdominal vein. Ringer's solution, with the addition of 12 mM $MgCl_2$ (normal concentrations of Na^+, K^+, and Ca^{++}) was used as the perfusion medium. This solution was identical with that in which the control parameters of synaptic transmission of the recipient preparation (the dermo-pectoralis muscle of another frog) were recorded; Mg^{++} was used to reduce the quantum composition of EPP. A working portion of perfusate (about 50 ml without blood) was collected over 30-40 min. Before and after perfusion the pH of the solution was determined and its K^+ ion concentration measured with the aid of a K^+-selective valinomycin electrode, with a sensitivity of 28-30 mV to a tenfold change in K^+

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Table 1. Concentrations of Substances in Perfusate and Its Fractions as Shown by Diazo Reaction and Reaction with DEPC ($\times 10^{-6}$ mg/ml of initial perfusate)

Test	Initial perfusate	Perfusate after gel-filtration		
		first peak	second peak	total quantity
Diazo react.	2,45±0,04	0,46±0,10	1,71±0,16	2,17±0,21
React. with DEPC	—	0,45±0,11	1,55±0,24	2,00±0,31

Legend. Initial perfusate — six experiments; after gel filtration — three experiments. From each experiment mean value for two to four tests is shown.

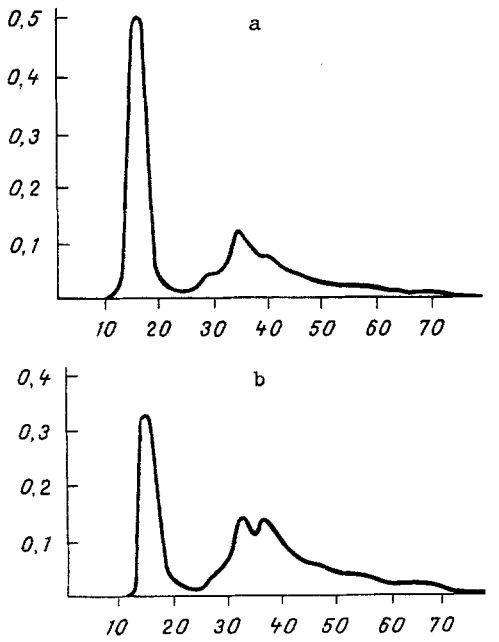

Fig. 1. Elution profiles during gel filtration of perfusate on Sephadex G-25 in two experiments (a, b). Abscissa, volume of eluate (in ml); ordinate, absorbance at wavelength of 280 nm (relative units). Elution in continuous-flow cuvette. Eluent — trifluoroacetic acid. Rate of elution 60 ml/h.

concentration [5]. For the biochemical test, Pauli's diazo reaction was carried out (detection of the imidazole ring) in the original perfusate [3, 13]. The perfusate was then fractionated by gel-filtration on Sephadex G-25 Fine (column from LKB, Sweden). The diazo reaction and the reaction with specific reagent for histidine (diethylpyrocarbonate — DEPC, from Baycovin, West Germany) were carried out in the fractions thus obtained [15]. The yield of the substances revealed by both tests was calculated as histidine. L-histidine (Reanal, Hungary) was used as the standard.

MEPP and EPP evoked by nerve stimulation (0.5 sec^{-1}) were recorded intracellularly in fibers of the recipient muscle in the control solution and every 10 min after its replacement by the perfusate. The mean quantum composition of EPP was determined from the ratio between the mean amplitudes of EPP and MEPP, and the binomial parameters p (probability of quantum release) and n (number of quantum release sites) also were determined by the known formulas [9]. Altogether there were 11 experiments.

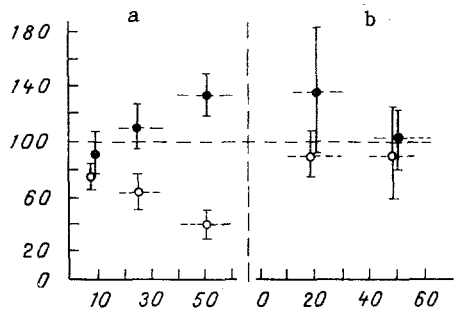

Fig. 2. Effect of perfusate on quantum composition of EPP (closed circles) and on frequency of MEPP (open circles). Abscissa, time after beginning of change of solutions (in min); ordinate, value of parameter (in % of control). a) Under the influence of perfusate, b) after rinsing. Vertical lines denote 95% confidence intervals of means. Values of parameters in each experiment obtained by averaging for two or three consecutive 10-min intervals (for smoothing out differences in the times of development of maximal effects), limits of averaging are shown by horizontal broken lines.

EXPERIMENTAL RESULTS

The pH of the perfusate was very slightly reduced (by 0.17-0.37) compared with initially (7.4-7.7). The K^+ concentration showed a very small increases (on average by 1.14 ± 0.54 mm).

Diazo-reacting compounds were found in the perfusates (Table 1). After gel filtration of the perfusates the elution profiles contained two main peaks (Fig. 1). Substances reacting with the diazo reagent and with DEPC were represented mainly in the fraction forming the second peak, where their content was about 80%. The molecular weight of the substances forming this peak was under 5 kilodaltons.

The quantity of diazo-reacting compounds in each fraction agreed closely with the quantity of compounds reacting with DEPC. This suggests that the two reactions detect the same compounds, with histidine in their composition. The concentration of histidine residues in the perfusate, calculated from data in Table 1, has an order of magnitude of 10^{-8} M.

The frequency of MEPP and quantum composition of EPP in the control to the different experiments varied only a little (3.9 ± 0.9 sec^{-1} and 4.8 ± 0.8 respectively). During the action of the perfusate the quantum composition of EPP in 85% of experiments increased by 30-100%, whereas in the remaining experiments it was unchanged or showed a small decrease (by 15-35%). On average the quantum composition was increased by about 40% (Fig. 2). The greatest change in quantum composition of EPP was observed with different experiments at different times of action of EPP of the perfusate (30-60 min).

Values of the parameters p and, in particular, n in different experiments varied considerably both in the control (0.3 ± 0.1 and 25.7 ± 10.4) and at different times of action of the perfusate. After averaging of the parameters in each experiment for the whole period of action (1 h) the value of n was found to increase to 176 ± 30% ($p < 0.05$), whereas there was no significant change in p. In a sample including all times of action of the perfusate in all experiments, positive correlation was found between relative changes in quantum composition of EPP and the parameter n ($r = 0.53$; $p < 0.01$). There was no correlation with changes in p. These data indicate that the increase in the quantum composition of EPP under the influence of the perfusate took place on account of an increase in n.

In all experiments the frequency of MEPP fell by 40-60% under the influence of the perfusate (Fig. 2), whereas the amplitude of MEPP was unchanged. After rinsing of the preparation with the control solution, the initial values of frequency of MEPP and quantum composition of EPP, as well as of the parameter n were virtually completely restored.

The investigation thus showed that, first, muscle perfusate contains substances which possess histidine in their composition (these substances, moreover, are mainly low-molecular-weight proteins), and second, their perfusate can increase the efficiency of transmission in neuromuscular synapses with a lowered level of transmitter secretion. This mechanism

may perhaps lie at the basis of the stimulating effect of perfusate on the fatigued nerve–muscle preparation [6, 7].

Under the influence of perfusate, presynaptic function was found to change qualitatively, just as during the action of exogenous histidine [12].

It can accordingly be postulated that K^+ ions (in the case of damage to cells during perfusion) and catecholamines may be secreted into the perfusion medium. These factors may stimulate presynaptic function and abolish fatigue [2, 8, 14]. However, the increase in the K^+ concentration in the perfusate observed in our own experiments was clearly insufficient to evoke these effects [2, 8]. So far as catecholamines are concerned, according to the literature [6] they are not the active principle of the perfusate which abolishes fatigue. Moreover, both these factors, unlike the perfusate, increase the frequency of MEPP [8, 14].

One possibility is that the active factor of the perfusate, stimulating evoked transmitter secretion and thereby abolishing fatigue, is a histidine-containing compound. If this is true, it is much more effective than free histidine, for the concentration of this compound in the perfusate is almost four orders of magnitude lower than the threshold concentration of exogenous histidine (about 10^{-4} M), at which the latter exhibits its presynaptic action [12].

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