EFFECT OF YAKUT HORSE BRAIN FRACTION (1-10 kD) ON KINETIC PARAMETERS OF Ca^{2+} TRANSPORT SYSTEMS IN CARDIOMYOCYTE SARCOLEMMAL VESICLES

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In a previous study [7] of the mechanism of the antiarrhythmic and antifibrillary action of a (1-10 kD) fraction from the brain and intestine of the Siberian souslik *Citellus undulatus,* in a state of hibernation, showed that addition of this fraction to the incubation medium modifies membrane permeability of heart cells or K^+, Na^+, and Ca^{2+} ions. The action of substances contained in the (1-10 kD) fraction on cells of frog atrial fibers leads to reduction of K^+ and Na^+ ion transport and to an increase in the K^+-current at high membrane voltages [7].

It is evident that biologically active substances similar to those contained in the (1-10 kD) fraction of the brain and intestine of *C. undulatus* also accumulate in the corresponding organs of other animals adapted by evolution to cold and, in particular, of the Yakut horse, which falls into a state of torpidity when the ambient temperature falls to between −40 and −50°C. This is shown by the ability of a test fraction of the Yakut horse brain, like that of similar fractions of the brain and intestine of the souslik *C. undulatus,* to induce significant hypometabolic and hypothermic effects when injected into warm-blooded animals [1-3, 6, 11].

In connection with the facts described above, it was decided to study the effect of the (1-10 kD) fraction from the Yakut horse brain on membrane permeability of the cardiomyocytes of warm-blooded animals and, in particular, for Ca^{2+} ions, which are regulators of the excitability and contractility of heart muscle [8].

EXPERIMENTAL METHOD

Vesiculated preparations of the sarcolemma were isolated by the method in [9]. The protein concentration was determined by Lowry's method [10]. Passive Ca^{2+} transport in cardiomyocyte sarcolemmal vesicles (inflow of $^{45}Ca^{2+}$ into the vesicles) was carried out in incubation medium containing: 10 mM Tris-HCl, pH 7.4, 100 mM KCl, 100 μg protein, and an aliquot of the (1-10 kD) fraction of Yakut horse brain, dissolved in bidistilled water. The reaction was triggered by the addition of an aliquot of a solution containing different assigned amounts of $CaCl_2$, but in order to study the kinetics of active transport, besides different concentrations of $CaCl_2$ the aliquot also contained EGTA and ATP in final concentrations of 0.1, 2, and 1.5 mM respectively. The incubation time corresponded to the time when the graph of the rate of Ca^{2+} accumulation flattened out on a plateau, which in our experiments was 7 min. The reaction was stopped by filtration of the incubation medium through "Millipore" filters (USA) followed by washing with 5 mM Na^+-phosphate buffer, pH 8.7, containing 1 mM $CaCl_2$. The radioactivity of the filters was determined by means of a "Beckman LS-7000" liquid scintillation spectrometer (USA). The experimental results were subjected to statistical analysis by Student's test [4].

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TABLE 1. Effect of Different Concentrations of (1-10 kD) Fraction of Yakut Horse Brain on Rate of Passive Ca^{2+} Transport into Cardiomyocyte Sarcolemmal Vesicles (M ± m)

Concentration of (1-10 kD) fraction, M	Rate of passive Ca^{2+} transport, nmoles/mg protein			
	I	II	III	IV
$1 \cdot 10^{-9}$	2,26±0,25	3,29±0,47	10,50±1,33*	31,79±4,48
$1 \cdot 10^{-8}$	2,37±0,42	3,92±0,52	8,84±1,03	29,58±0,45*
$1 \cdot 10^{-7}$	2,48±0,56	3,40±0,26	10,90±0,14*	30,44±0,19*
$1 \cdot 10^{-6}$	2,63±0,56	3,48±0,49	10,03±1,24*	23,00±3,31*
$3 \cdot 10^{-6}$	2,68±0,38	4,11±0,25*	11,39±1,85*	26,02±1,71*
$1 \cdot 10^{-5}$	2,60±0,38	4,33±0,41*	11,03±0,84*	25,54±1,27*
$3 \cdot 10^{-5}$	2,61±0,34	4,53±0,61*	11,89±1,07*	24,89±0,31*
Control (H_2O)	2,27±0,18	3,19±0,38	8,20±0,49	32,00±1,78

Legend. Initial Ca^{2+} concentration in incubation medium was (in mM): I) 0.2, II) 0.5, III) 1.0, IV) 3.0. $p \leq 0.05$ compared with control.

TABLE 2. Effect of Different Concentrations of (1-10 kD) Fraction of Yakut Horse Brain on Rate of Active Ca^{2+} Transport into Cardiomyocyte Sarcolemmal Vesicles (M ± m)

Concentration of (1-10 kD) fraction, M	Rate of passive Ca^{2+} transport, nmoles/mg protein			
	I	II	III	IV
$1 \cdot 10^{-9}$	2,78±0,82	6,70±0,71	6,83±0,47	59,12±7,78
$1 \cdot 10^{-8}$	3,29±0,35	6,84±1,02	7,50±1,38	63,47±1,57*
$1 \cdot 10^{-7}$	3,00±0,02*	6,67±0,43	6,78±2,79	63,02±8,24*
$1 \cdot 10^{-6}$	3,33±0,24*	6,80±1,43	8,30±0,33*	58,76±5,03*
$3 \cdot 10^{-6}$	3,40±0,19*	7,46±0,21*	8,46±0,51*	71,99±12,03*
$1 \cdot 10^{-5}$	2,81±0,55	7,86±0,27	11,00±2,14*	67,83±9,94*
$3 \cdot 10^{-5}$	3,55±0,08*	8,52±0,50*	11,72±3,07*	68,59±6,55*
Control (H_2O)	2,72±0,22	6,57±0,36	6,70±0,03	49,61±2,12

Legend. Initial Ca^{2+} concentration in incubation medium was (in M): I) $1 \cdot 10^{-7}$, II) $3 \cdot 10^{-7}$, III) $1 \cdot 10^{-6}$, IV) $3 \cdot 10^{-6}$ Remainder of legend as to Table 1).

EXPERIMENTAL RESULTS

Investigation of the effect of different concentrations of the Yakut horse brain (1-10 kD) fraction on the velocity of passive Ca^{2+} transport (nmoles/mg protein) into cardiomyocyte sarcolemmal vesicles yielded the results shown in Table 1.

The following general conclusions were established: for all concentrations of the test (1-10 kD) fraction, the substrate concentration for Ca^{2+} transport being 1 mM, the velocity of passive Ca^{2+} inflow into the vesicles increased significantly, but with a substrate concentration for Ca^{2+} transport of 3 mM, which is the physiological level, a significant decrease in the velocity of transport was recorded for all concentrations of the (1-10 kD) fraction of Yakut horse brain.

In the next experiments the effect of the (1-10 kD) fraction of Yakut horse brain on the kinetics of active Ca^{2+} transport in the presence of ATP was studied (Table 2).

It can be concluded from analysis of these results that for all concentrations of the test (1-10 kD) Yakut horse brain fraction (from 10^{-9} to $3 \cdot 10^{-5}$ M). if the Ca^{2+} concentration in the medium was between 10^{-7} and $3 \cdot 10^{-6}$ M, values of the rate of Ca^{2+} accumulation in the reaction of energy-dependent transport exceeded the control values. Significant changes were observed on a Ca^{2+} concentration of $1 \cdot 10^{-6}$ M in the incubation medium, within the range of concentrations of the (1-10 kD) fraction of Yakut horse brain from 10 to $3 \cdot 10^{-5}$ M, but at a physiological Ca^{2+} concentration in the incubation medium of $3 \cdot 10^{-6}$ M, significant changes were observed over a wider range of concentrations of the (1-10 kD) fraction, namely from $1 \cdot 10^{-8}$ to $3 \cdot 10^{-5}$ M.

Since the method used in this investigation can be used to obtain inverted vesicles [9]. The increased inflow of Ca^{2+} into them under the influence of the (1-10 kD) fraction recorded for intact cardiomyocytes assumes a decrease in the intracellular concentration of the cation.

If the results are compared with data in the literature two observations may be made: first, the effect of the (1-10 kD) fraction from Yakut horse brain on Ca^{2+} transport in bovine cardiomyocyte membranes is the same as that of the corresponding fraction from the brain of hibernating sousliks (*C. undulatus*) on Ca^{2+} transport in trabeculae of the frog (*Rana ridibunda*) atrium and the renal tubule of the smooth newt (*Triturus vulgaris*) [5, 7], indicating absence of any species-specific differences in the physiological activity of the fraction under investigation; second, the physiological effects of the (1-10 kD) fraction from Yakut horse brain and its effect which we recorded on the Ca^{2+}-transport system of the cardiomyocyte sarcolemma are observed within about equal limits of concentration of the fraction, namely from 10^{-7} to $3 \cdot 10^{-5}$ M, possible evidence that these phenomena are interconnected.

It can thus be concluded from the results of these experiments that the (1-10 kD) fraction obtained from Yakut horse brain during natural adaptation to cold (in winter) induces a decrease in passive Ca^{2+} transport and an increase in the rate of active Ca^{2+} transport in inverted cardiomyocyte sarcolemmal vesicles in the presence of a physiological Ca^{2+} concentration in the incubation medium, and this may lead to a decrease in the intracellular concentration in the cardiomyocytes of this cation, which is a universal regulator of the excitability, contractility, and metabolism of muscle cells [8].

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