EFFECT OF GANGLIOSIDES ON Na,K-ATPase ACTIVITY AND CONFORMATION OF MICROSOMAL MEMBRANES

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The effect of gangliosides on activity of Na,K-ATPase and K-dependent nitrophenyl-phosphatase was studied. Gangliosides in low concentrations were shown to activate Na,K-ATPase, but to inhibit it in high concentrations. In all concentrations used the gangliosides had only an inhibitory action on K-dependent nitrophenyl-phosphatase. Inhibition of the enzyme by gangliosides was reversible and competitive relative to K^+. Calculation of Hill's coefficient showed that gangliosides, whether their action was activating or inhibitory, behaved as allosteric effectors. To elucidate the mechanism of action of gangliosides on the enzyme their effect on microsomal membranes was studied by the fluorescent probe method. Gangliosides were found to produce marked conformational changes in microsomal membranes of the brain.

KEY WORDS: gangliosides; Na,K-ATPase; microsomal membranes; fluorescent probes.

Gangliosides, specific components of neuronal membranes, have a marked cerebral vasoconstrictor action of myotropic nature [7], and this served as the basis for the study of their inhibitory effect, in model experiments, on active transport Na,K-ATPase, the inhibition of which leads to depolarization of membranes with the development of muscular contraction [14].

EXPERIMENTAL METHOD

The preparation of Na,K-ATPase [10] and intact microsomes [1] were isolated from albino rat brain by differential centrifugation. Na,K-ATPase activity was determined from the increase in inorganic phosphate or potentiometrically; activity of K-dependent nitrophenyl-phosphatase was determined from the increase in p-nitrophenol, and protein by the method of Lowry et al. [13]. To study interaction between gangliosides and microsomal membranes, the anionic probe 1-anilinonaphthalene-3-sulfonate (ANS^-) and the cationic probe acridine orange (AO^+) were used. The parameters of fluorescence were recorded on the MPF-2A (Hitachi) spectrofluorometer with automatic correction for sensitivity. Effects of dilution and the internal filter [9] were taken into account. Absorption spectra were photographed on the USP-2 spectrophotometer. The quantum yield of fluorescence was measured by a relative method, using tryptophan as the standard [15]. Gangliosides were isolated from the gray matter of the human brain by Bogoch's method with certain modifications [8].

EXPERIMENTAL RESULTS

As Table 1 shows, gangliosides in a concentration of the order of $4 \cdot 10^{-4}$ mM or lower had a marked activating action on Na,K-ATPase. In higher concentrations they inhibited activity of the enzyme; the inhibitory effect increased during preincubation of the gangliosides with the membrane preparation (Fig. 1A). The study of the effect of gangliosides on activity of K-dependent nitrophenyl-phosphatase showed that, in both low and high concentrations, they had a similar action, namely inhibition, and this was seen particularly clearly when preincubation was used (Fig. 1B). These results suggest that the inhibitory action of gangliosides on activity of the enzyme was evidently connected with their effect on the phosphatase reaction, which is the third stage

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TABLE 1. Effect of Different Concentrations of Gangliosides on Na,K-ATPase Activity (in μmoles Pi/mg protein/min; M ± m)

Concentration of gangliosides, mM	Number of experiments	Control	Experiment	P
$2 \cdot 10^{-5}$	10	1,40±0,09	2,10±0,12	<0,05
$4 \cdot 10^{-5}$	10	0,51±0,06	0,94±0,01	<0,001
$1 \cdot 10^{-4}$	10	0,79±0,03	1,59±0,01	<0,001
$2 \cdot 10^{-4}$	10	2,10±0,02	4,80±0,004	<0,01

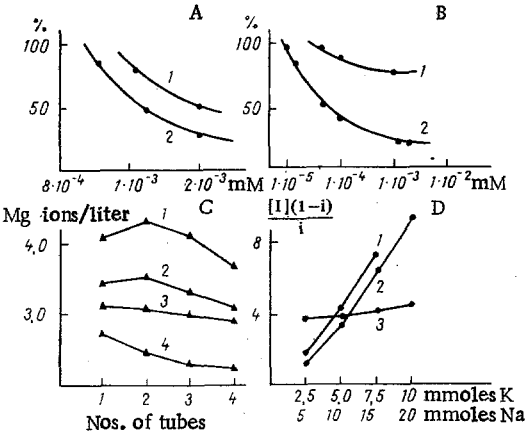

Fig. 1. Effect of gangliosides on Na,K-ATPase (A), K-dependent nitrophenyl-phosphatase of brain (B), and active outflow of Na^+ from sartorius muscle (C). A and B: 1) Without preincubation, 2) with preincubation of gangliosides. C: 1) Incubation in medium containing 10 mM KCl (without gangliosides); 2, 3) with ganglioside concentration of $2 \cdot 10^{-4}$ and $2 \cdot 10^{-3}$ mM, respectively; 4) in absence of KCl. D) Competition between gangliosides and K^+ for Na,K-ATPase (1) and K-dependent nitrophenyl-phosphatase (2); absence of competition with Na^+ (3).

of the Na,K-ATPase transport cycle [5]. Consequently, the inhibitory effect of the gangliosides was achieved through their action on the outer side of the membrane, where the potassium combining site of the enzyme is located; the activating effect, on the other hand, could be ascribed to their action on the first stages, namely the action on the Na^+ and ATP combining sites, which are concentrated on the inner side of the membrane [4].

The ability of gangliosides to effect their inhibitory action on Na,K-ATPase through the outer side of the membrane also was demonstrated by experiments to investigate active Na^+ transport in the sartorius muscle of the frog Rana camerani. After saturation of the muscle with Na^+ at 2-4°C, when virtually no Na,K-ATPase activity was present, subsequent preincubation of the muscle at 24°C was accompanied by active outflow of Na^+ into the medium. As will be clear from Fig. 1C, which demonstrates the results of determination of the Na^+ concentration in the incubation medium by flame photometry, gangliosides in all concentrations used inhibited the working of the sodium pump.

In subsequent investigations some aspects of the inhibitory action of gangliosides on Na,K-ATPase were studied. As the graphs of Hunter and Downs [12] show, gangliosides compete with K^+ for the potassium combining site of Na,K-ATPase and K-dependent nitrophenyl-phosphatase molecules: The value of the expression $[I](1 - i)/i$ (where I is the concentration of inhibitor, i that part of the activity of the enzyme which was inhibited by the ion in the given concentration) increased with an increase in the K^+ concentration in the medium. Com-

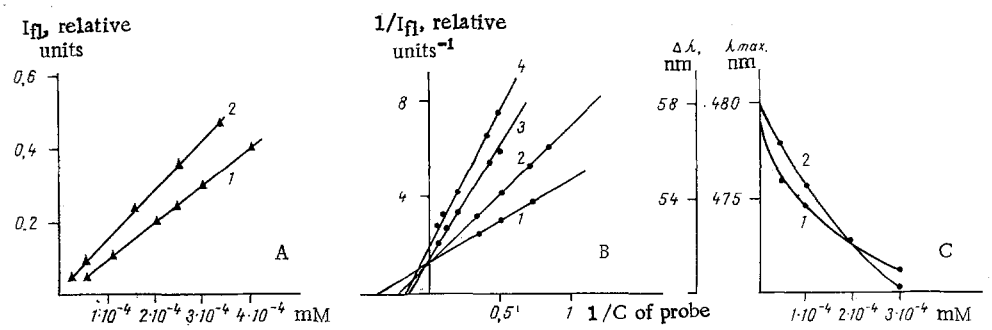

Fig. 2. Effect of gangliosides on parameters of fluorescence of probes bound with brain microsomal membranes. A) Increase in intensity of fluorescence of ANS^- (1) and AO^+ (2) in presence of different concentrations of gangliosides; B) fluorescence of ANS^- (1, 2), and AO^+ (3, 4) in presence of different concentrations of probe (reciprocal coordinates) and in absence (2, 4) and presence (1, 3) of gangliosides (10^{-4} mM); C) changes in maximum of fluorescence (1) and "half-width" of maximum (2) in presence of different concentrations of gangliosides.

petition of gangliosides with Na^+ was not found (Fig. 1D). The possibility thus cannot be ruled out that the mechanism of the inhibitory action of gangliosides on the enzyme under investigation is based on their ability to compete with K^+.

It was also discovered that the inhibitory effect of gangliosides is a reversible process, for with dilution of the incubation medium the degree of inhibition of Na,K-ATPase and K-dependent isophenyl-phosphatase activity decreased correspondingly. To study the cooperativeness of action of gangliosides on Na,K-ATPase by the difference method [6], Hill's coefficient was calculated; it was found to be 1.93 for activation and 1.51 for inhibition, evidence of the cooperative character of the action of gangliosides on the enzyme.

To elucidate the membrane component of the action of gangliosides on Na,K-ATPase by means of fluorescent probes the effect of gangliosides on the microsomal membranes was investigated. Gangliosides were shown to potentiate (Fig. 2A) fluorescence of ANS^- and AO^+ induced by brain microsomes; the intensity of fluorescence depended on the concentration of gangliosides. The results of measurement of fluorescence in the presence of changing concentrations of probes are plotted in Fig. 2B between reciprocal coordinates: $1/I_{fl} = f(1/C_{probe})$. This graph makes an intercept on the ordinate approximately equal (inversely proportional) to the number of combining sites, and on the continuation of the abscissa an intercept approximately equal to the combining constant [2]. As the graph shows, during the action of gangliosides the number of combining sites of ANS^- was almost unchanged whereas the number for AO^+ increased; meanwhile, the combining constant increased for ANS^- and was unchanged for AO^+. Consequently, the increase in fluorescence of ANS^- was evidently connected with an increase in the quantum yield, as a result of conformational changes induced by gangliosides in the membrane. In the case of AO^+ the potentiation of fluorescence may have arisen through an increase in the electronegativity of the membrane caused by gangliosides.

It will also be noted that the increase in fluorescence of ANS^- was accompanied by a short-wave shift of the maximum of wavelength (λ_{max}) and a decrease in $\Delta\lambda$ (Fig. 2C), which can be interpreted as the result of the ability of the gangliosides to reduce the polarity of the combining sites of the probe and the ability of molecules surrounding the probe to undergo reorientation during the lifetime of the excited state of ANS^- [3], and also to increase the rigidity of the microenvironment of the probe in the membrane [11].

The investigation thus showed that gangliosides, in comparatively high concentrations, are able to inhibit, Na,K-ATPase by producing conformational changes in the membrane. According to data in the literature, this enzyme leads to depolarization of membranes with activation of the contractile system and it may be one of the mechanisms of the vasoconstrictor effect possessed by gangliosides.

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CHANGES IN COMPOSITION OF HEART PROTEINS IN EARLY ONTOGENY UNDER NORMAL CONDITIONS AND WHEN THE UTERO-PLACENTAL BLOOD FLOW IS REDUCED

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The heart of rabbit embryos on the 11th, 12th, 14th, 16th, and 18th days of development was studied under normal conditions and when the utero-placental blood flow was reduced. Normally the ratio weight of heart–weight of embryo is particularly high in the period of rapid development of the hemodynamic functional system embryo–placenta–uterus (12th and 14th days), but later the ratio falls. The increase in total nitrogen in the heart and changes in the relative proportions of its fractions as a result of an increase in the content of contractile proteins and proteins of the stroma, was particularly great toward the 18th day. Under pathological conditions the weight of the embryo and the weight of the heart were reduced on all days of the experiments. The ratio weight of heart–weight of embryo toward the 18th day indicated the onset of spontaneous rehabilitation as a result of a gradual improvement in the utero-placental circulation. However, the total nitrogen content in the heart in all groups investigated remained the same as in the control or it increased, possible evidence of dehydration of the heart. Changes in the fractional composition of the heart proteins pointed to profound biochemical disturbances in the organ which could be one cause of the disturbance of its functional state.

KEY WORDS: embryogenesis; heart; contractile proteins; utero-placental circulation.

The development of the heart and, in particular, the formation of its contractile function have attracted and continue to attract the attention of biochemists and physiologists. In the accessible literature there is practically no information on the fractional composition of the heart proteins from the time when the organ begins to function in the embryos of placental animals under normal and pathological conditions.

The circulation in the utero-placental region is considered to be more than sufficient to supply the embryo with nutrients and oxygen [5]. However, as the writers' investigations have shown, there is a connection between the decrease in intensity of the utero-placental circulation and the development of the embryo, the mechanism of which is a disturbance of the coupled development of the hemodynamic functional system of embryo–placenta–uterus [1]. The data in this respect are of considerable interest not only for age and comparative biochemistry, but also for perinatal medicine, for an important problem at the present time is that of delayed development of the fetus [3, 6].

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