Effect of Panangin on Activity of Messengers Systems in Hypertrophied Rat Myocardium

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Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 134, No. 9, pp. 299-302, September 2002
Original article submitted February 4, 2002

The balance between the two major second messenger systems in hypertrophied myocardium was studied in rats receiving panangin for 16 days. Panangin producing stimulating and polarizing effects on cardiomyocyte membrane improved electrophysiological characteristics of hypertrophied myocardium (electrical stability, duration of supernormal excitability period, and action potential), activated the phosphoinositide exchange, and inhibited the adenylate cyclase system. The panangin-induced change in membrane potentials was accompanied by a pronounced inositol response, *i.e.* a decrease in the content of membrane polyphosphoinositides (phosphatidylinositol-4-phosphate and phosphatidylinositol-4,5-biphosphate) in the brain. It was concluded that function of ion channels depends on activity of phosphoinositide- and adenylate cyclase second messengers systems.

Key Words: *second messengers*; *polyphosphoinositides*; *cAMP*; *panangin; hypertrophied myocardium*

The adequacy of responses of various cell to external stimuli is regulated via complex interactions between the adenylate cyclase- and phosphoinositide second messenger systems. In addition, information can be transmitted into the cell by modulation of transmembrane ion currents. The regulatory pathway is associated with the so-called fast responses and usually discussed independently. However, the available data indicate that virtually all ion channels are in some or other way coupled with the phosphoinositide and adenylate cyclase systems. In particular, some receptors are coupled to ion channels via G-proteins [9], while functional activity of ion channels depends on phosphorylation of their proteins by protein kinase [5].

Hence, changes in ion channel conductivity induced by electrical pulse can activate or inhibit intracellular messenger pathways. To test this hypothesis, we studied the functional state of two intracellular messenger systems during *in vivo* panangin treatment. Panangin (Gedeon Richter) is a widely used drug containing potassium aspartate and magnesium aspartate. This preparation supplies ions to cardiomyocytes and aspartate serves as a ion transporter. Panangin enhances myocardial stability probably due to its stabilizing, polarizing, and antihypoxic effects on myofibril membranes [1]. Thus, it is important to elucidate the effects of panangin on membrane messenger functions under conditions of experimental left ventricular hypertrophy [7,10], which is a potent destabilizing factor.

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MATERIALS AND METHODS

Experiments were carried out on rats. Left ventricular hypertrophy was modeled by 2.5-2.7-fold controlled occlusion of the abdominal aorta. Nine months after aorta occlusion (at the stage of progressive cardiac sclerosis) panangin was administered intraperitoneally in a daily dose of 6 mg/kg for 16 days. Electrical stability of the myocardium was evaluated by the duration of supernormal excitability period and ventricular fibrillation threshold [10].

Endogenous phosphatidylinositol-4-phosphate (PIP) and phosphatidylinositol-4,5-bisphosphate (PIP_2) were

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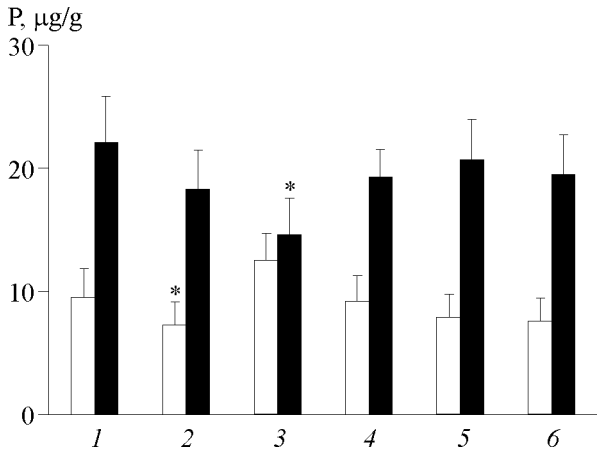

Fig. 1. Changes in the content of phosphatidylinositol-4-phosphate (light columns) and phosphatidylinositol-4,5-biphosphate (dark columns) in rat cerebral hemispheres during treatment with panangin. *1*) control; *2-6*) injection days 1, 4, 8, 12, and 16, respectively. *Significant differences from the control.

isolated from rat hemispheres using a modified method [13].

The concentration of cAMP in myocardial homogenate was measured after protein precipitation (6% trichloracetic acid) by thin-layer chromatography and radioimmunoassay using kits made at the Institute for Research, Production and Application (Czech Republic). Chromatography was performed on Silufol UV-254 plates in an isopropanol-water-ammonia system (7:2:2 v/v, R_f=0.61) [4].

Inositol-1,4,5-triphosphate (IP_3) and inositol-1,4-biphosphate (IP_2) were extracted from cardiomyocytes after protein precipitation (10% trichloracetic acid). Samples were neutralized with diluted NaOH, pH was adjusted to 8.0. The fractions were separated by ion-exchange chromatography, identified using standards (Sigma), and quantified by phosphate [11]. The control group consisted of animals with hypertrophied myocardium not treated with panangin.

Each experimental point is a mean of five replicates.

RESULTS

Hypertrophied myocardium is characterized by slower accumulation of potassium ions in the interstitial space compared to intact myocardium [10,12]. This results in prolongation of action potential and period of supernormal excitability of the myocardium [10]. In rats with hypertrophied myocardium, the duration of this period increased 3-fold: from 4 to 12.8 msec. In intact animals panangin had no effect on these parameters. In rats with hypertrophied myocardium receiving panangin for 16 days the fibrillation threshold remained unchanged, but the period of supernormal excitability decreased by 44% (to 7.5 msec, p<0.01).

This improvement in electrical stability of the myocardium can be achieved due to normalization of ionic transport after treatment with the membrane stabilizing drug panangin. Depolarization in the presence of K^+ increases Ca^{2+} entry into cells [2]. It should be kept in mind that calcium ions are a component of the phosphoinositide cascade and play an important role in coupling of the ionic channels with activation of the phosphoinositide system. It can be hypothesized that rapid K^+ entry into cells (through channels) determines the dynamic balance between activation of the phosphoinositide and adenylate cyclase systems. Panangin reduced the content of membrane-bound polyphosphoinositides PIP and PIP_2 in rat brain. The minimum concentration of PIP was found on day 1 postinjection, while the minimum concentration of PIP_2 was observed on day 4 of panangin treatment. A tendency to normalization of polyphosphoinositide content was observed at the end of treatment (Fig. 1). These changes resulted from enhanced degradation of polyphosphoinositides: inositol response.

The interactions between second messenger pathways can be subdivided into synergistic (potentiation, enhancement) and antagonistic (inhibition) [5]. Panangin-induced activation of polyphosphoinositide exchange accompanied by hydrolysis of PIP and PIP_2 (major precursors of the second messengers IP_2 and IP_3) led to a decrease in cAMP content. The content of IP_2 and IP_3 increased both in the myocardium and cerebral hemispheres (p<0.02). Thus, this was the case of antagonistic interaction between the two major second messenger systems. This effect can be mediated via activation of protein kinase C (the target of diacylglycerol, a product of polyphosphoinositide hydrolysis). Phosphorylation of regulatory GTP-binding proteins by activated protein kinase C can result in desensitization and uncoupling of receptor systems associated with adenylate cyclase [5].

However, all responses of inositol and cyclic nucleotide signaling systems are closely interconnected, and it is often difficult to determine whether the shifts in the levels of inositol phosphates were the first cause, which gives rise to changes in cAMP level, or, vice versa, cAMP level changed first, while shifts in IP_2 and IP_3 are a result of the subsequent protein phosphorylation [8].

The observed tendency to recovery of the levels of polyphosphoinositide and second messengers IP_2 and IP_3, and cAMP levels despite panangin treatment allowed us to distinguish two phases in these changes. The first phase is characterized by inositol response accompanied by polyphosphoinositide hydrolysis, accumulation of inositol phosphates, and a decrease in cAMP content. After adaptation to the drug (second phase) activity of the polyphosphoinositide second messenger system returned to the initial level (Fig. 2).

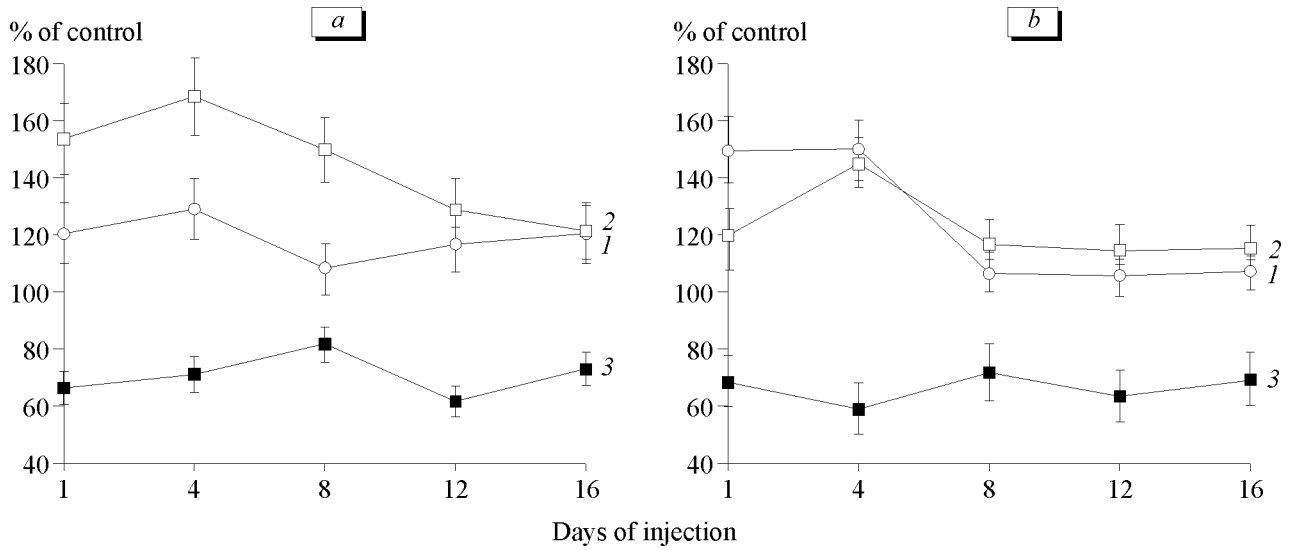

Fig. 2. Content of inositol bisphosphate (*1*), inositol trisphosphate (*2*), and cAMP (*3*) in hypertrophied myocardium (*a*) and cerebral hemispheres (*b*) during treatment with panangin.

This dynamics can be explained by the corresponding changes in target cell sensitivity. Desensitization of cells will necessarily result in recovery of the baseline activity of the second messenger system [6]. Other defense feedback mechanisms can also participate [3,6].

Both intracellular second messenger systems play the key role in biological effects of various hormones, neurotransmitters, bioactive substances, and electrical pulses. These systems are under incessant control of various regulators acting simultaneously in different directions. The rate of cell responses varies considerably. Opening of ion channels usually takes a few milliseconds, while the response mediated by polyphosphoinositide hydrolysis takes a few seconds [5]. Nevertheless, our findings suggest that biological processes involved in the regulation of the membrane potential should not be considered as only electrical events. Despite extremely high rate of ion channel mechanisms, their function is always intimately coupled with universal signaling second messenger systems. The dynamics of the concentration of second messengers implies the reciprocal character of the interplay between the adenylate cyclase and phosphoinositide second messenger systems.

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