Effects of Bioglycans Isolated from Birch Fungi *Inonotus obliquus* on Electric Activity of Venous Sinus Cells in Frog Heart

V. A. Golovko

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Bioglycans isolated from chaga in a concentration of 0.0001% reduced frequency of action potential in venous sinus cells of frog heart during the first 15-30 min of exposure, then this parameter increased by 10% per hour over 3.5 h, and was 41±3 min^{-1} from the 4th to the 20th hour of incubation. The frequency of action potentials in heart strips in the chaga extract was 40% higher than in Ringer's solution. The effect of chaga bioglycan is probably associated with adsorption on myocyte membranes. Binding of Ca^{2+} to bioglycans observed during the first 30 min inhibited efflux of intracellular Ca^{2+}.

Key Words: *chaga; bioglycans; action potential; pacemaker*

Chaga (birch fungi *Inonotus obliquus*) are used in Russian medicine for centuries. The preparation Befungin produced from these fungi since 1955 normalized blood content of cholesterol and improved functions of the cardiovascular system [2,4] [4]. However, the mechanisms of this effects remain unclear.

Here we studied the effects of chaga bioglycans on spontaneous rhythmic activity and membrane potentials of venous sinus cells of frog heart.

MATERIALS AND METHODS

Experiments were performed on sinoatrial strips isolated from grass frogs *Rana temporaria* (n=27) in February-March. After destruction of the spinal cord, the animals were decapitated, and the heart was immediately removed. Two preparations from the right and left parts of the sinoatrial ring were produced. The effects of 24-h incubation with bioglycan were studied on the right part. The left part was incubated in control Ringer's solution containing (in mM) 112 NaCl, 1.9 KCl, 0.9 $CaCl_2$, and 2.4 $NaHCO_3$ (pH 7.5-7.7 was maintained by aeration with air and Tris-maleate-NaOH buffer). The preparations were perfused at 21±2°C.

Chaga fungi were gathered in Syktyvkar environs in August. Air-dried raw materials were preliminary treated with methanol and chloroform to remove low-molecular-weight admixtures. Bioglycan precipitate was obtained after 5-h extraction with hot distilled water (70°C) followed by ethanol precipitation [3]. Bioglycans were isolated by centrifugation, dissolved in water, dialyzed, and lyophilized (total yield 1.6%). Glucose was assayed after hydrolysis with 2 M trifluoroacetic acid at 100°C for 3 h [3]. Bioglycan was added to standard Ringer's solution to a concentration of 0.0001%.

Action potentials (AP) were recorded intracellularly with glass microelectrodes (initial resistance no less than 40 MΩ) and imputed into a C1-114*1 oscilloscope coupled with an H-3030 automatic recorder. The results were statistically analyzed and presented as mean±standard error. The differences were significant at $p<0.05$ [1].

RESULTS

Bioglycans of 0.0001% chaga extract induced various effects on spontaneous activity of the right sinoatrial

Laboratory of Physiological Researches, Department of Biotechnology and Molecular Immunology, Institute of Physiology, Komi Research Center, Ural Division of the Russian Academy of Sciences, Syktyvkar

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strips (Fig. 1): the contraction frequency decreased during the first 15-30 min of exposure, then increased by 10% per hour over 3.5 h, and was 41±3 min^{-1} (n=14) from the 4th to the 20th hour of incubation.

Strips from the left sinoatrial ring were incubated in standard Ringer's solution. Initially, the frequency of their spontaneous contractions did not differ from that of right part strips (30±4 *vs.* 31±3 min^{-1}). The frequency of AP slightly increased during the first 30 min of perfusion, decreased to 32±3 min^{-1} over the next 60 min, and remained at this level for 6 h, then monotonously decreased to 25±5 min^{-1} by the 20th hour (n=16). The frequency of AP in strips incubated in control Ringer's solution was 40% lower than in the presence of chaga polysaccharide extract (p<0.01).

Chaga bioglycans are colored light brown. Venous sinus preparation changed to the same color a few minutes after perfusion with bioglycan extract, which implies adsorption of bioglycans on the sarcolemmal membrane. The intensity of staining was most pronounced in the region of atrial ring scarification. After a 3060-min incubation, the adsorption became irreversible.

Bioglycans added in a concentration of 0.0001% induced negative (initial) and positive (late) chronotropic effects on venous sinus strips. Overshoot and spontaneous depolarization decreased after 5-h incubation, while the duration of AP (APD) corresponding to 100% repolarization decreased. In 4 of 10 experiments, APD_{100} increased due to delayed final repolarization, while APD_{50} decreased in comparison with the control. At the same time, the preparations kept in Ringer's solution (without chaga bioglycans) demonstrated a 20-30% increase in APD_{100} due to a noticeable plateau phase. The rate of diastolic depolarization deceased by 30% compared with that registered in the bioglycan-containing solution (Fig. 2).

During the first 30-60 min, the effects of bioglycan on AP generation in venous sinus cells of frog heart were comparable with those of hypocalcium solutions [1]. However, the positive chronotropic effects of hypocalcium solutions usually persist for no more than 10-15 min and are generally followed by negative chronotropic effects [1,5]. By contrast, the positive chronotropic effects of chaga extract persisted 50-100 times longer.

These data and results of other investigators [6] suggest the following mechanism of bioglycan effects: Ca^{2+} ions partly bind to bioglycans adsorbed on myocyte membranes, thus inhibiting efflux of intracellular Ca^{2+}. These properties of bioglycans determine their ability to improve electrical characteristics of membranes and their positive chronotropic effects.

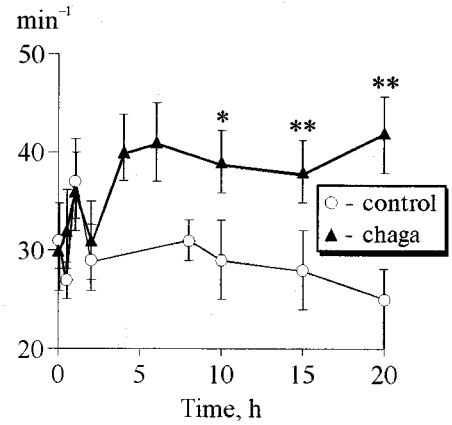

Fig. 1. Effects of bioglycans isolated from birch fungi (chaga) on the frequency of spontaneous contractions of venous sinus strips from frog heart. *p<0.05 and **p<0.01 compared with the control.

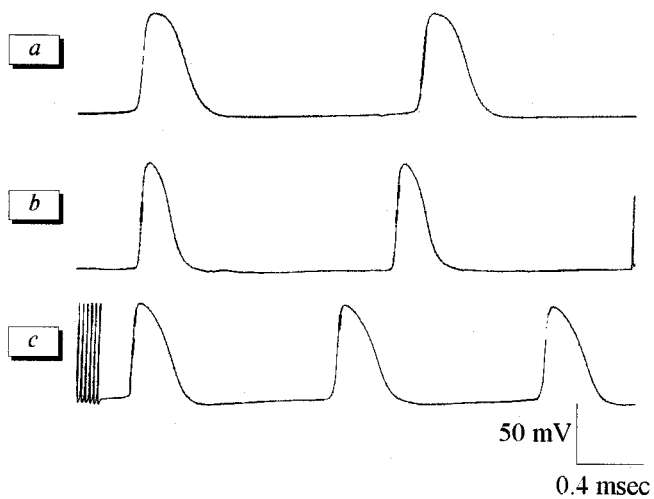

Fig. 2. Effects of bioglycans isolated from birch fungi on action potentials of venous sinus cells in frog heart. Action potential by the 6th hour of incubation in standard Ringer's solution (*a*), 10 min after addition of 0.0001% chaga bioglycans (*b*), and after 15-h perfusion with chaga extract (*c*).

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