Stabilizing Effect of *Silene* Pectin Polysaccharide on Electrical Activity of the Sinoatrial Area in Frog Heart

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Silenan, a pectin polysaccharide from common catchfly (*Silene vulgaris*), corrects disorders in the conduction of action potentials between cells of the sinoatrial area of frog heart forming a functional syncytium. Recovery of action potential conduction in the sinoatrial cells was recorded in long-term experiments (>8 h). The effect of silenan manifested mainly against the background of arrhythmic generation and impaired propagation of action potentials.

Key Words: *pectin; polysaccharide; silenan; myocardium; action potential*

Ion currents across the sarcolemmal membrane play an important role in the generation of electrical and contractile activity of the myocardium. Long-lasting changes in the transport of even one potential-forming ion lead to disorders in the homeostasis of other ions, which can cause arrhythmia and ischemia. The search for nontoxic long-acting drugs of plant origin correcting the feedback mechanisms of ion transport regulation via the cell sarcolemma in heterogeneous tissue, for example, in the myocardium, is now in progress.

Silene vulgaris is a perennial medicinal plant belonging to the family of pinks, growing in many regions of the European Russia and widely used in medicine as a sedative, antiinflammatory, and antitoxic agent [6]. Silenan, a *Silene* pectin, is a branched pectin polysaccharide [3]. The pectin has virtually no toxic effect on mouse macrophages upon intraperitoneal injection. *Tanacetum* pectin structurally similar to silenan slightly and reversibly increases slow outward K^+ current in pond snail neurons [3]. However, the mechanisms of pectin effects on stimulated cells remain unclear.

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We studied the effect of silenan during arrhythmia development and in disorders of cell-cell interactions in the zone of contact between the venous sinus and atrial cells.

MATERIALS AND METHODS

Silenan was isolated from the catchfly above-ground part by extraction with ammonium oxalate water solution [2]. The structure of silenan was evaluated using methods of structural carbohydrate chemistry, including NMR spectroscopy and methylation with subsequent chromatography mass-spectrometry of methylated sugars [2].

Electrical activity of myocardial cells was studied on spontaneously contracting strips from the sinoatrial area of *Rana temporaria* heart. The animals were anesthetized in ether vapor and decapitated, the hearts were carefully removed and placed into normal solution (mmol/liter): 112 NaCl, 1.9 KCl, 0.9 $CaCl_2$, 2.4 $NaHCO_3$, 1.0 $MgSO_4$, 5 Tris-maleate-NaOH, pH 7.4±0.1 with aeration.

Strips from the sinoatrial area (n=17) were fixed on a frame with capron threads and placed into a perfusion box. Silenan was dissolved in distilled water, small aliquots from the stock solution were added into standard solution to the needed concentration (1-10 μg/ml). Silenan concentration was

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by two orders of magnitude lower than LD_{50} for mouse macrophages. Control strips were perfused without polysaccharide.

Action potentials (AP) were intracellularly recorded using glass microelectrodes. The signal was presented simultaneously to the oscilloscope and autorecorder (type H-3030) input. The signals were then scanned and digitized on a computer. Transmembrane AP were processed if the electrode was present in the cell for at least 15 min. The appearence of a notch wave in the rapid polarization phase of AP served as the marker of disordered diffusion of electrical current among the cells.

The results were statistically processed using Student's *t* test.

RESULTS

The main components of the Silene pectin carbohydrate chain ($[\alpha]_D^{25}$+167.3°(*c* 0.1; water), $[\eta]^{25}$=2.3 dl/liter, M_{SD}=23 kDa) includes the residues of galacturonic acid (63%, 2.3% methoxyl groups), arabinose (4.2%), galactose (3.2%), and rhamnose (2.2%) [2]. Like many pectins [7], silenan carbohydrate chain has a block structure (Fig. 1), belongs to the rhamnogalacturonane-I (RG-I) type, and consists mainly from sites of linear area, presented by α-1,4-D-galactopyranosyluronane (1) and α-1.2-rhamno-α-1,4-D-galacturonane (2), which is also the main carbohydrate chain of branched area of the pectin [2]:

$$\cdots\rightarrow 4)\text{-}\alpha\text{-D-Gal}p\text{A-}(1\rightarrow 4)\text{-}\alpha\text{-D-Gal}p\text{A-}(1\rightarrow_n 4)\text{-}\alpha\text{-D-Gal}p\text{A-}(1\rightarrow\cdots \quad (1)$$

$$\cdots\rightarrow 2)\text{-}\alpha\text{-Rha}p\text{-}(1\rightarrow 4)\text{-}\alpha\text{-Gal}p\text{A-}(1\rightarrow_n 2)\text{-}\alpha\text{-Rha}p\text{-}(1\rightarrow 4)\text{-}\alpha\text{-D-Gal}p\text{A}\rightarrow\cdots \quad (2)$$

The lateral carbohydrate chains of branched silenan area are attached to the main carbohydrate chain of rhamnogalacturonane via 1,4-bond to rhamnopyranose residues and are constructed from residues of α-1,5-bound arabinofuranose and β-1,3-, β-1,4-, and β-1,6-bound galactopyranose. The sites of branching of the lateral chain are residues of 3,5-di-O-substituted arabinofuranose and 2,3-, 3,6-, 4,6-di-O-substituted galactopyranose.

Addition of silenan for 3 h into bathing solution in the presence of spontaneous rhythmic activity did not change the main electrophysiological parameters.

Study of silenan (10 μg/ml) effect on cell-cell interactions in the cardiac sinoatrial area showed clear-cut changes in AP generation 30-40 min after its addition (Fig. 2). Notch disappeared in 60% cases (*n*=9) and did not appeared again after prolongation of the experiment with these strips. Spontaneous recovery of cell-cell interactions was described, but the probability of this outcome is significantly lower (25%; *n*=8). The observed phenomenon of normal current passage between sinoatrial cells can be attributed to the presence of negatively charged carboxyl groups of galacturonic acid residues in silenan, which facilitates absorption the pectin (at least partial) on the endothelium or basal membrane and increases electrical resistance of tight junctions. This stabilizes the polarized lipid backbone of the sarcolemma containing dynamic protein molecules of ionic channels and ionic pumps and reduces leakage currents [3]. Presumably, due to the long molecule the pectin acts extracellularly.

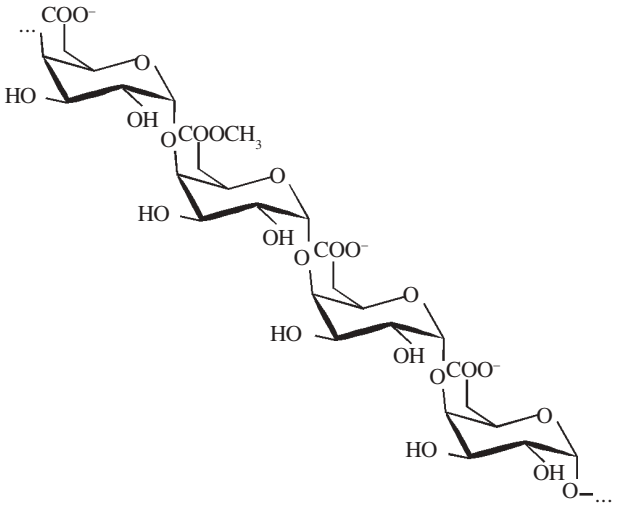

Fig. 1. Scheme of α-1,4-D-galactopyranosyluronane fragment, the main silenan chain.

In experiments on isolated spontaneously contracting strips from the sinoatrial area, the contractions were asynchronous in 35% (*n*=19). After addition of silenan (1-10 μg/ml) the process synchroni-

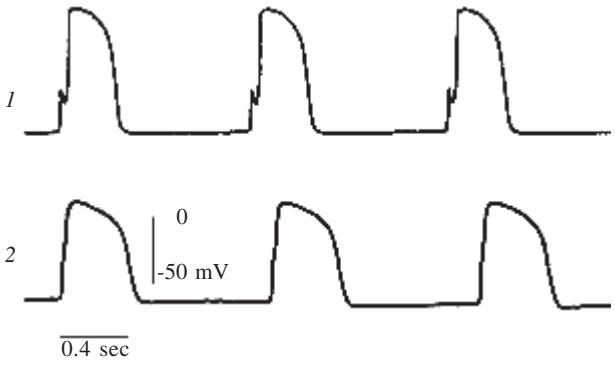

Fig. 2. Effect of silenan in disordered cell-cell interactions in the borderline area at the base of the frog heart sinoatrial valve. *1*) preparation in standard solution; *2*) AP of the same cell 60 min after exposure in solution with silenan (10 μg/ml).

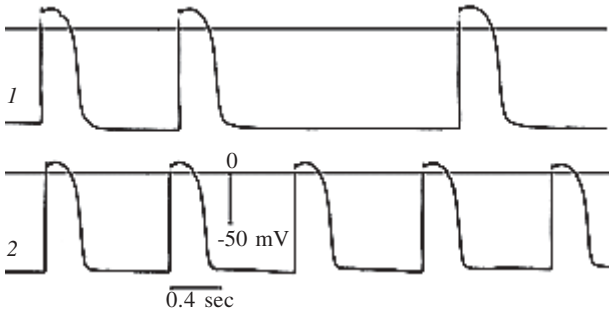

Fig. 3. Silenan effect in arrhythmic generation of AP in the frog heart sinoatrial cells. *1*) preparation in standard solution, long perfusion; *2*) recovery of rhythmic generation of AP after 60-min perfusion with silenan (10 μg/ml).

zed almost 2-fold more rapidly than in the standard solution.

"Perforation" of AP was sometimes observed in some of myocardial strips in normal solution (Fig. 3), and therefore the effect of silenan on the strips (n=13) was then studied in long-term experiments. Silenan solution (10 μg/ml) was added into perfusate after registration of irregular generation of AP, and rhythmic activity was restored in 50% strips (n=7) after 30-40 min (Fig. 3).

In control series (n=10), electrical activity was restored in 10% cases.

The results indicate that galacturonane, the main component of the silenan carbohydrate chain, provides the work of pectin as the natural ion-exchange material. Presumably, silenan increases the viscosity of saline and forms hydrophilic complexes in amounts proportional to the number of free carboxyl groups. It seems that the stabilizing effect on electrical activity of the cardiac sinoatrial area during disordered cell-cell interactions and arrhythmic generation of AP in long-term (more than 8 h) experiments is due to this property of the pectin. Previous studies showed that carnosine modified physiological processes in muscle contraction disorders [1].

Hence, pectin macromolecule modulates the entire surface of the tissue with which it comes in contact and therefore, it can be referred to extracellular additional "volume regulators" [5] participating in directed signal transfer. Free electrons can flow along the molecular pectin threads thus creating charge flow in the direction of intermolecular bonds. The effect of pectin manifests mainly in the presence of disordered electrical activity of myocardial cells.

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