Activating Effect of *Tanacetum Vulgare L.* Pectin Polysaccharide on Ionic Channels of Neuronal Membrane

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The membranotropic effects of TVF tanacetan pectin polysaccharide derived from *Tanacetum vulgare L.* was studied by the voltage-clamp method on isolated neurons of *Lymnaea stagnalis* mollusk. TVF in concentrations of 0.1-10.0 μg/ml nonselectively activated the outward potassium and total inward (sodium and calcium) ionic currents (slightly dose-dependently and reversibly increased their amplitude by 5-10%) and decreased nonspecific leakage current.

Key Words: *Tanacetum vulgare L.; pectin; isolated neurons; ionic currents; membrane leakage currents*

Pectins are heteropolysaccharides, building blocks of any plant cell wall and important components of animal and human nutrition. Recent studies demonstrated that many pectins are physiologically active compounds producing immunomodulating, antitumor, gastroprotective, antihypoxic, and antitoxic effects [4,6,8].

Tanacetum vulgare L. is actively used in popular medicine [3]. Pectin polysaccharide, which received the name of TVF tanacetan, was isolated from *Tanacetum* flowers [5]. The main components of its carbohydrate chain are D-galacturonic acid, L-arabinose, D-galactose, and L-rhamnose residues. The structure of the main carbohydrate chain of tanacetan was deciphered: it is a linear α-1,2-L-rhamno-α-1,4-D-galacturonane [7].

Voltage-operated ionic channels and chemoreceptors integrated in surface cell membranes provide generation of bioelectrical pulses in the nervous system and serve as targets for many drugs. However, the direct effects of pectin polysaccharides on the membrane of excitable cells were never described.

Here we studied the effects of TVF tanacetan on ionic channels of isolated neuronal membranes of *Lymnaea stagnalis* mollusk.

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MATERIALS AND METHODS

Tanacetum vulgare L. was collected during flowering in the Syktyvkar region, Republic of Komi. TVF was isolated from *Tanacetum vulgare L.* flowers by extraction with ammonium oxalate aqueous solution [5].

Experiments were carried out on isolated unidentified neurons of *Lymnaea stagnalis* mollusk at 20-22°C. For isolation of neurons the peripharyngeal ganglia were removed and treated with 0.25% trypsin in pond snail normal saline (Table 1) for 40-60 min.

We used method of intracellular dialysis and whole-cell voltage clamping technique [2]. Perfusion solution was delivered into the cell with the neuron on a polyethylene micropipette and the dialysing solution was let inside this pipette. Summary inward (sodium and calcium) and outward (fast and slow potassium) transmembrane ionic currents were recorded. Nonspecific (leakage) currents were automatically subtracted from recorded ionic currents (were compensated). Ionic currents were separated using perfusion (extracellular) and dialysis (intracellular) solutions with different ionic composition (Table 1) and by maintaining appropriate membrane potentials [1].

Tanacetan was dissolved in distilled water (1 mg per 10 ml) and successively diluted 10-fold in external (perfusing) salines to concentrations of 0.1, 1.0, and 10.0 μg/ml. The effect of TVF developed rapidly and stabilized after 2-3 min; the preparations were then

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washed for 7-10 min. The initial values of ionic currents were taken for 100%, and the currents developing under the effect of pectin were expressed in percent of the initial value.

Histograms of the dose-effect relationships were plotted. Changes in the amplitude and kinetics of ionic currents under the effect of TVF were evaluated visually on an oscillograph monitor and on a PC monitor using special software and Excel graphic editor.

The data were processed using Statistica 5.0 software. Differences between the mean values (n=5-6) were evaluated using Student's t test.

RESULTS

In series I the mean initial amplitude of potassium slow ionic currents in the control was 35 nA. Addition of TVF in concentrations of 0.1 to 10 μg/ml to extracellular solution lead to a concentration-dependent increase in the amplitude of slow outward potassium current (Fig. 1). The effects were reversible. After 7-10-min washout in control saline K^+ current returned to the initial level. The kinetics of current development under the effect of TVF virtually did not change (Fig. 2, *2-4*).

Changes in nonspecific currents of membrane leakage under the effect of TVF were biphasic, slightly increased during the first 1-2 min and then decreased. This confirmed improved stability of the neuronal membrane.

Other series of experiments showed that TVF activation of the summary sodium and calcium and fast potassium currents was similar to the effect on slow potassium currents, that is, the amplitude of ionic currents slightly and reversibly increased without changes in their kinetics.

A specific feature of our study of membranotropic activity of pectin polysaccharide tanacetan is a wide range of its concentrations (0.1-10.0 μg/ml), registration of a series of transmembrane ionic currents and nonspecific membrane leakage currents, study of the concentration-effect relationship, and evaluation of changes in the ionic currents kinetics.

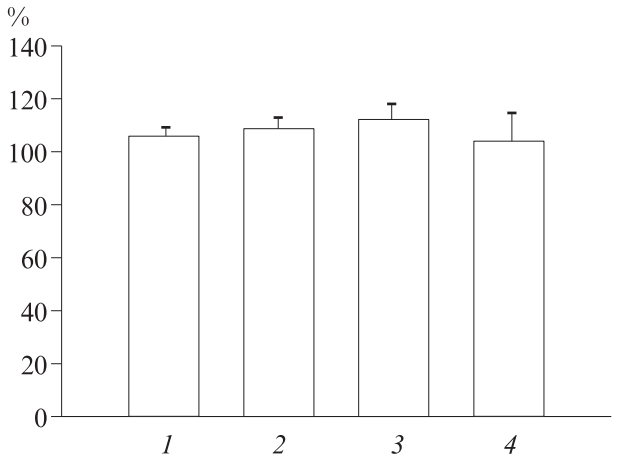

Fig. 1. Concentration-effect relationship under the effect of tanacetan in different concentrations on outward potassium slow currents of snail neurons. Ordinate: ratio (in percent) of tanacetan-modified potassium current amplitude to its initial value (control). Tanacetan doses: *1*) 0.1 μg/ml; *2*) 1.0 μg/ml; *3*) 10.0 μg/ml; *4*) washout.

The activating effect of TVF on ionic currents of the neuronal electrostimulated membrane was demonstrated for the first time. TVF rapidly, reversibly, and in a dose-dependent manner stimulated the outward potassium and summary inward ionic currents through potential-regulated ionic channels of snail neurons. Such an increase of all ionic currents and a decrease of the neuronal membrane leakage currents should be regarded as electrophysiological correlation between the activating and membrane-stabilizing effects of the plant polysaccharide. We found that the kinetics of ionic currents development remained virtually stable under the effect of TVF. This indicates that this pectin does not react with the portal mechanisms of ionic channels. Reversible effects of TVF indicate its poor fixation to structural elements of the membrane. The observed approximately equal increase of all ionic currents resultant from exposure to membranotropic TVF indicates its nonspecific (nonselective) effect on ionic channels. This can be due to common membrane mechanism of action, *e.g.* intensive phosphorylation of cell proteins regulating membrane permeability, and

TABLE 1. Ionic Composition (in mM) of Solutions for Snail Neurons

Solutions, currents	NaCl	CsCl	$CaCl_2$	$MgCl_2$	KCl	Tris-OH	pH
Extracellular normal (perfusion)							
total inward	100	—	2	1.5	5	2	7.5
potassium outward	100	—	2	1.5	5	2	7.5
Intracellular (dialysis)							
inward	—	120	—	—	—	2	7.4
potassium outward	—	—	—	—	120	2	7.4

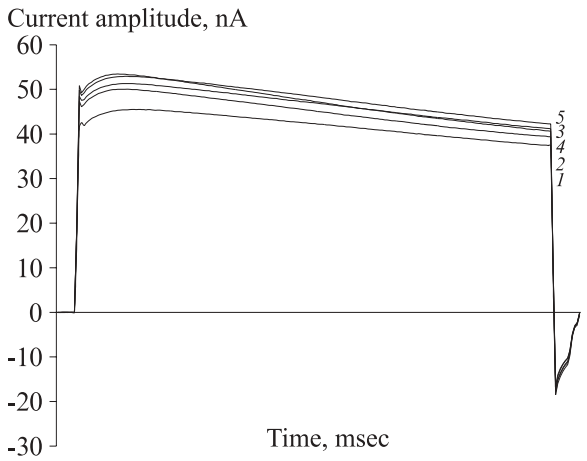

Fig. 2. Amplitude and kinetics of slow potassium current in a snail neuron in control (*1*), under the effect of tanacetan in concentrations of 0.1 μg/ml (*2*), 1.0 μg/ml (*3*), 10.0 μg/ml (*4*), and after washout (*5*). Abscissa: *t*=1000 msec.

universal changes in the functioning of all ionic channels.

Ionic mechanisms of electrogenesis, regularities of the mollusk neuron functioning, and their pharmacological characteristics are in principle similar to those of mammalian neurons [2], and hence, our findings can be partially extrapolated to warm-blooded animals and man.

The results persuasively attest to a membranotropic effect of TVF on transmembrane ionic currents of pond snail neurons. The physiological effect of the substance on cell membrane is determined by the specific structure of its molecule and physicochemical characteristics, as well as by specific features of its interaction with the membrane structures (the structure-activity relationship). Chemical modification of tanacetan and studies of physiological activity of its fragments seem to be desirable. We hope that our methods and findings will be useful in this future study.

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