Chemistry of Natural Compounds, Vol. 37, No. 1, 2001

THE EFFECT OF TRITERPENE GLYCOSIDES ON ELECTRICAL ACTIVITY CHANGES OF IDENTIFIED MOLLUSK NEURONS

O. V. Kostyuchenko, V. I. Grishkovets, E. A. Sobolev, and I. I. Korenyuk

UDC 547.918:612.822

The effect of mono- and bisdesmoside triterpene glycosides on the background activity of the grape snail Helix pomatia identified neurons was studied. Monodesmoside glycosides at concentrations of 10^{-3}-10^{-4} M elicit stable hyperpolarization of neurons by increasing the membrane permeability for K^+ ions. At concentrations of 10^{-5}-10^{-7} M, they briefly change the neuronal impulse activity. The type of monodesmoside triterpene glycoside activity on neurons depends on the structure of the hydrocarbon. The glycoside effect on neuron membranes is related to the hemolytic activity.

Key words: triterpene glycosides, neuron electrical activity, glycoside hyperpolarizing effect.

The functional role and mechanism of action of exogenic glycosides are studied because of their wide spectrum of biological activity [1], which includes involvement in the nervous system [2, 3]. The effect of glycosides [4-6], in particular, holothurin, on neurons of the CNS consists of an increase of the permeability of the cell membrane for Na^+ ions, leading to its stable depolarization. It is also known that triterpene glycosides (TTG) can form complexes with membrane cholesterol in the lipid environment of ATP-ase. This alters the conformation of the membrane proteins [7, 8] and, consequently, inhibits the permeability of specialized channels for various ions (Na^+, K^+, Ca^{2+}, Cl^-). We suggest that this effect depends on the structure and concentration of the substances [8]. Therefore, changes in the structural features of the aglycone and the nature of the TTG hydrocarbon chains can increase or decrease the cellular permeability for certain ions. This leads to depolarization or hyperpolarization of neurons [8] or the lack of an effect on membrane processes [9].

Therefore, we investigated the effect of plant mono- and bisdesmoside TTGs (**1**-**22**) of oleanolic acid (R_2 = H) and hederagenin (R_2 = OH) on the background activity of known neurons PPa1, PPa2, and PPa7 [10] and several unidentified neurons of the grape snail.

The study of the biological activity of glycosides on neurons found that all bisdesmoside glycosides **12**-**22** at concentrations of 10^{-3}-10^{-2} M have no effect on the background activity of neurons regardless of the number of sugars in the hydrocarbon chain bound via an acylglycoside bond to the aglycone (from 1 to 3 in the studied series).

On the other hand, application of monodesmoside glycosides **1**-**11** at a concentration of 10^{-3} M had a definite effect on the electrical activity of both identified and unidentified neurons. The effect of these substances developed over 1-2 min from the time of application and consisted of stable hyperpolarization of the neuron membrane from -60 to -65 mV [the initial membrane potential (MP) was -50 ± 2 mV]. After rinsing the preparation with standard Ringer solution, the pulse activity of the neurons either was not restored or irreversible changes in the nature of the background activity were noted. These were manifested as the appearance of akson-dendridic potential actions (AP) for neurons PPa2 and PPa7, which exhibit single rhythmic activity and as a two-fold decrease in the AP amplitude in the bundled PPa1 neurons. However, AP generation gradually decreased up to complete inactivation of neurons and development of stable hyperpolarization despite the apparent restoration. The MP increased to (-68)-(-70) mV. Induced depolarization of the neuron did not generate AP.

Obviously, the presence of a free carboxyl on C-17 in the aglycones of the studied glycosides is responsible for the effect of the monodesmoside glycosides on the neuronal electrical activity. This correlates with the fact that bisdesmoside glycosides have no hemolytic activity whereas glycosides with a free carboxyl on C-17 are active [1].

V. I. Vernadskii Tavricheskii National University, 195007, Simferopol′, ul. Yaltinskaya, 4. Translated from Khimiya Prirodnykh Soedinenii, No. 1, pp. 39-42, January-February, 2001. Original article submitted June 6, 2000.

0009-3130/01/3701-0043$25.00

TABLE 1. Hyperpolarizing and Hemolytic Activity of Triterpene Glycosides

Compound	Threshold concentration for hyperpolarizing effect, M	Hemolytic activity HC_{50}*, M
1	1×10^{-4}	1.6×10^{-5}
2	1×10^{-3}	1.5×10^{-5}
3	2×10^{-3}	5.6×10^{-5}
4	5×10^{-5}	2.0×10^{-5}
5	1×10^{-4}	1.2×10^{-5}
6	1×10^{-5}	6.0×10^{-6}
7	1×10^{-4}	1.0×10^{-5}
8	1×10^{-4}	2.0×10^{-5}
9	2×10^{-6}	2.0×10^{-6}
10	2×10^{-6}	4.0×10^{-6}
11	1×10^{-5}	6.0×10^{-6}
12-22	Inactive	Inactive

*HC_{50} is the concentration causing 50% hemolysis under conditions described in Experimental.

Table 1 gives the threshold concentrations of the substances that cause brief hyperpolarization on the studied neurons.

The results indicate that monodesmoside glycosides **1**-**11** have a definite effect on the functional condition of the described neurons.

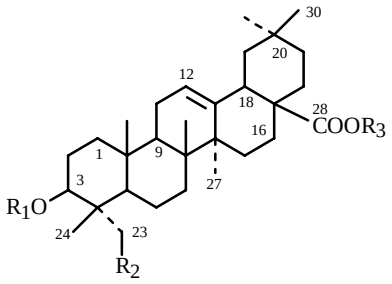
Clearly, the most active glycosides were those with three carbohydrates that have a branched chain on C-3 of the aglycone (glycosides **9** and **10**). Glycosides **2** and **3** exhibited the least activity for nerve cells.

Glycoside **11** has a nonbranched chain of three carbohydrates and the same activity as the bioside glycoside **6**, which differs from **11** by the lack of a terminal xylose. Biosides **7** and **8** are an order of magnitude less active than **6** and **11** and comparable with monoside **5** (hederagenin arabinoside). Obviously, adding glucose to arabinose on C-2 does not increase the activity. On the other hand, placing a deoxysugar, rhamnose (in glycoside **6**), in the same position markedly increases the activity. Furthermore, bonding glucose instead of arabinose to the aglycone in glycoside **2** significantly decreases the activity. Bioside glycoside **3**, which contains 1→2 bound glucoses, is even less active. Glycoside **1** with the glucuronic acid has the same activity as arabinoside **5**. Forming the sulfate ester of the aglycone on C-3 hydroxyl (glycoside **4**) give quite high activity that approaches that of bioside **6**.

A comparison of the activities of oleanolic and hederagenin glycosides shows that the nature of the aglycone plays a secondary role although the nature and number of monosaccharide residues in the chain on C-3 have a definite effect on the TTG activity.

A correlation between the magnitude of the effect on neuronal electrical activity and the hemolytic activity of the glycosides was observed (Table 1). Furthermore, it is interesting that a 1:4 combination of glycosides **6** and **17** (concentrations 10^{-3} and 4×10^{-3} M) and even 1:1 (both concentrations 10^{-4} M) completely relieved the hyperpolarization caused by separate perfusion of glycoside **6** at concentrations of 10^{-3} or 10^{-4} M. Such an effect can be explained by the protective action of bisdesmoside glycosides, which was noted previously for several similar glycosides in protecting liver cells against damage by CCl_4 [11].

We hypothesized that TTG are relatively strongly bound by their carbohydrate residues to carbohydrates on the neural membrane surface, which increases the K^+ permeability and leads to stable hyperpolarization. For this, we determined the effect of high TTG concentrations on the total K^+ current, which approximately doubled in several minutes after applying the glycoside solution but disappeared in 5-7 min when the neurons died. Addition in Ringer solution of tetraethylammonium chloride (TEA-Cl), a known nonselective K-channel blocker, decreased the average amplitude of the K^+ current by 1.4 nA compared with a control. However, application of a glycoside in the experimental cell suppressed the blocking action of TEA-Cl on the K^+ current. The current amplitude increased by 1.2 nA relative to the starting value. Threshold concentrations of glycosides had no effect on the total K^+ current.



	R_1	R_2	R_3
1:	GlcUA→	H	H
2:	Glc→	OH	H
3:	Glc-(1→ 2)-Glc→	OH	H
4:	^-O_3S→	H	H
5:	Ara→	OH	H
6:	Rha-(1→ 2)-Ara→	OH	H
7:	Glc-(1→ 2)-Ara→	H	H
8:	Glc-(1→ 2)-Ara→	OH	H
9:	[Rha-(1→ 2)]-[Glc-(1→4)]-Ara→	H	H
10:	[Rha-(1→ 2)]-[Glc-(1→4)]-Ara→	OH	H
11:	Xyl-(1→3)Rha-(1→2)-Ara→	OH	H
12:	GlcUA→	H	←Glc
13:	Glc→	OH	←Glc-(6←1)-Glc
14:	Glc-(1→ 2)-Glc→	OH	←Glc-(6←1)-Glc
15:	^-O_3S→	H	←Glc-(6←1)-Glc-(4←1)-Rha
16:	Ara→	OH	←Glc-(6←1)-Glc-(4←1)-Rha
17:	Rha-(1→ 2)-Ara→	OH	←Glc-(6←1)-Glc-(4←1)-Rha
18:	Glc-(1→ 2)-Ara→	H	←Glc-(6←1)-Glc-(4←1)-Rha
19:	Glc-(1→ 2)-Ara→	OH	←Glc-(6←1)-Glc-(4←1)-Rha
20:	[Rha-(1→ 2)]-[Glc-(1→4)]-Ara→	H	←Glc-(6←1)-Glc-(4←1)-Rha
21:	[Rha-(1→ 2)]-[Glc-(1→4)]-Ara→	OH	←Glc-(6←1)-Glc-(4←1)-Rha
22:	Xyl-(1→3)Rha-(1→2)-Ara→	OH	←Glc-(6←1)-Glc-(4←1)-Rha

Fig. 1. Structures of TTGs: GlcUA, β-D-glucuronopyranosyl-; Glc, β-D-glucopyranosyl-; Ara, α-L-arabinopyranosyl-; Xyl, β-D-xylopyranosyl-; Rha, α-L-rhamnopyranosyl-.

Thus, our experimental results suggest that monodesmoside triterpene glycosides exhibit hyperpolarizing action on neuron membranes that depends on the structure of the hydrocarbon chain and the nature of the monosaccharides. Obviously, this is due to the degree of binding with components in the lipid layer of the cell membrane, which releases K^+ and causes cell hyperpolarization.

EXPERIMENTAL

We used the following TTGs, which we isolated from plants of the Araliaceae family: **1**-**6**, **13**, **17**, from *Hedera taurica* [12]; **9**, **10**, **20**, **21**, from *Hedera colchica* [13]; **11**, **22**, from leaves of *Acanthopanax sieboldianus* [14]; **12**, from *Brassaia actinophilla* [15]; and **7**, **8**, **18**, **19**, from *Fatsia japonica* [16].

Experiments were performed on neurons of the grape snail *Helix pomatia* PPa1, PPa2, and PPa7 and unidentified neurons of the subglottic ganglia of the grape snail.

An isolated glottic ring was bonded to nerves by tungsten needles on the bottom of the experimental flow cell (0.5 mL). After mechanical removal of the external membrane, the subglottic ganglia were treated with Pronasa E enzyme (Sigma) for 45 min with subsequent washing with Ringer solution (pH 7.5) of ionic composition (mM): NaCl, 100; KCl, 4; $CaCl_2$, 10; $MgCl_2$, 4; tris-HCl, 10. Microelectrodes were filled with KCl solution (2.5 M). The resistance was 10-30 MΩ. The

experiments were performed at 18-21°C.

Currents were recorded by monitoring potential in a "whole cell" configuration [17]. Neurons were isolated using multiple passage of the right ganglion through glass pipettes (0.5-1 mm diameter). Microelectrodes were filled with (mM): KCl, 120; $MgCl_2$, 5, HEPES, 10; Na_2EDTA, 5 (pH 7.4). The resistance was 2-4 MΩ. TEA-Cl (15 mM) was added to the external solution in some experiments.

Bisdesmoside glycosides were dissolved in Ringer solution and diluted to the required concentrations. Monodesmoside glycosides were first converted with heating to the sodium salts by ~100% excess of 2% Na_2CO_3 solution and subsequent dilution by Ringer solution to the required concentration.

The hemolytic activity was determined by the literature method [18] using a 2% suspension of swine erythrocytes washed with physiological solution in isotonic phosphate buffer at pH 7.6 (300 mL 0.1 M NaH_2PO_4 and 200 mL 0.1 M Na_2HPO_4) and an equal volume of glycoside solution in the same buffer. The mixture was stored for 30 min. Unhemolyzed erythrocytes were removed by centrifugation. The hemoglobin concentration in the supernatant was determined photometrically at 577 nm. Complete hemolysis was attained by a significant excess of glycosides until the maximum optical absorbance was reached. The concentrations producing half the optical absorbance cause 50% hemolysis and are numerically equal to HC_{50}, which are listed in Table 1.

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