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INFLUENCE OF A NUMBER OF DITERPENE ALKALOIDS ON THE CALCIUM HOMEOSTASIS OF RAT CARDIOMYOCYTES. STRUCTURE–ACTIVITY RELATIONSHIP

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It has been established that, under the conditions of calcium overload induced by ouabain, the spontaneous liberation of Ca^{2+} from the sarcoplasmic reticulum (SR) is suppressed by songorine, 1-benzoylnapelline, dihydroatisine, hetisine, and benzoylheteratisine. The relationship between the structures of the alkaloids and their activities is discussed.

At the present time, throughout the world more than 600 diterpene alkaloids (DAs) have been isolated and their chemical structures have been determined. The sources of these DAs are plants of the genera *Aconitum, Delphinium, Garrya, Spiriae, Inulae, Thalictrum*, and others, numerous species of which grow in almost every continent [1].

Investigations of the pharmacological properties and the elucidation of structural–functional features of DAs have revealed a broad spectrum of their biological action. Substances have been found that possess pronounced spasmolytic and anti-inflammatory [2], local anesthetic [3], and neurocardiotoxic [4] properties. Their antiarrhythmic activity has been studied the most widely [5]. On various models of arrhythmia leading to the calcium overloading of the myocardium, representatives of the class of DAs have proved to be effective antiarrhythmics [6], which presupposes their direct action on the liberating functions of the SR.

We have investigated the action of a number of DAs with various types of structure on the liberation of Ca^{2+} from the SR under the conditions of induced calcium overload and have elucidated the dependence of the activity of an alkaloid on its structure.

The recordings of one of three similar experiments are given. The arrows show the moments of adding ouabain (A), 1-benzoylnapelline (B), and ryanodine (C).

Figure 1 (curve 1) shows the increase in $[Ca^{2+}]_i$ on the addition of ryanodine. Preliminary treatment of a suspension of rat cardiomyocytes with ouabain led to an increase in $[Ca^{2+}]_i$ in response to the addition of ryanodine, which shows a ouabain-induced increase in the reserves of Ca^{2+} in the SR (Fig. 1, curve 2). At effective concentrations of ryanodine, calcium overload may set in and a trigger activity leading to arrhythmia may develop. On this is based the arrhythmogenic action of ouabain and digitalis preparations, which permits their wide use for modeling a calcium overload [8].

In the model under consideration, DAs were active agents preventing the liberation of Ca^{2+} from the SR under the experimental conditions. It can be seen in Fig. 1 (curve 3) that in the presence of benzoylnapelline the response to the addition of ryanodine was considerably lowered in comparison with a control. Table 1 gives results on the change in $[Ca^{2+}]_i$ when a suspension of cardiomyocytes was treated with the corresponding DA. Such an action of DAs on the myocardium, together with their reported capacity for modifying the functioning of the Na^+ channels [9], may serve as an explanation of their high antiarrhythmic activity both under the conditions of a disturbance of the conductivity and excitability of the myocardium, leading to arrhythmia of the reentry type, and also in calcium overloading, which is the main step in the generation of arrhythmias of various geneses, especially the trigger types [10].

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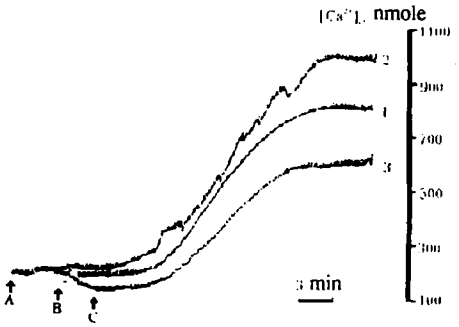
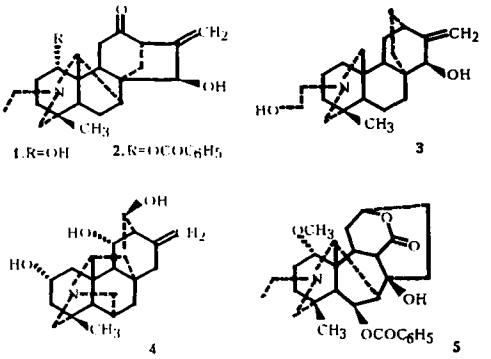


Fig. 1. Ryanodine-induced increase in the intracellular concentration of Ca^{2+} in isolated rat cardiomyocytes: 1) action of ryanodine (10 nM) on the liberation of Ca^{2+} from the SR; 2) under conditions of calcium overload (ouabain, 10 μM + ryanodine, 10 nM); 3) influence of 1-benzoylnapelline (10 μM) on the ryanodine-induced increase in the intracellular concentration of Ca^{2+} under the conditions of calcium overloading of the cardiomyocytes.

The results that we have obtained agree well with those of a study of the antiarrhythmic properties of DAs of various types and of the elucidation of the structure-activity relationship. Thus, the compounds investigated are effective antiarrhythmic agents, although fairly considerable variations in activity determined by a series of structural differences are also observed.

One of the main approaches to the elucidation of a structure-activity relationship is the analysis of the efficacies of a series of closely related chemical compounds, and also a comparison of such series.



The alkaloids considered belong to several types of DAs: the napelline type — songorine (**1**) and benzoylnapelline (**2**); the dihydroatisine type — dihydroatisine (**3**); the hetisine type — hetisine (**4**): and the heteratisine type — benzoylheteratisine (**5**). In spite of certain differences in the structure of the nitrogen-carbon skeleton, almost all the representatives of the types of DAs studied possess pronounced antiarrhythmic properties. Starting from the analysis given previously [6] of conformational calculations by the MMX86 program using the results of an x-ray structural analysis [7], in the series of DAs and their analogs, on the whole, the carbon skeletons with fixed oxygen atoms retain their geometry and are similar in structure in various classes of DAs. The analysis shows that the qualitative direction of the cardiorhythmic and cardioprotective action of alkaloids is ensured by the system of substituents. In spite of differences in the number and locations of the substituents among the five alkaloids considered, common to all of them is the same high basicity of the nitrogen atom, the presence of free hydroxy groups capable of forming hydrogen bonds, and the presence of a hydrophobic grouping at C4 forming intermolecular bonds.

With respect to their capacity for blocking the spontaneous liberation of Ca^{2+} from the SR under the conditions of a calcium overload, the compounds considered form the following sequence: songorine < hetisine < dihydroatisine < 1-benzoylnapelline < benzoylheteratisine, which corresponds to the sequence of their antiarrhythmic actions (see Table 1) [11]. The key factor is the presence of an aromatic substituent in the DA molecule (compare the values for songorine and 1-benzoyl-

TABLE 1. Action of a Series of Diterpene Alkaloids on Ryanodine-induced $\Delta[Ca^{2+}]_i$ under the Conditions of Calcium Overloading in Comparison with Their Antiarrhythmic Activities

Compound	$\Delta[Ca^{2+}]_i$, nM	LD_{50}/ED_{50}
1	607 ±103	19.4
2	423 ± 62	133.4
3	564 ± 72	38.0
4	586 ± 79	26.0
5	404 ± 59	142.9
Control	850 ± 115	

In the control, the fluorescence on the addition of ryanodine in the presence of ouabain was measured.

napelline). The determining role of an aromatic group may be connected with the following factors. Investigations of local anesthetics have shown that in a series of compounds with closely related structures the relative activity indices correlate with the values of the distribution coefficients between organic solvents. For compounds with greatly differing chemical structures, activity correlates with the effectiveness of their sorption on lipid membranes or proteins [12]. The presence of the aromatic groups in 1-benzoylnapelline and benzoylheteratisine raises the total hydrophobicity of the alkaloid molecule and facilitates diffusion through the plasmatic membrane and adsorption on the surface of the intracellular targets [13]. This probably explains their increased cardiorhythmic activity and their capacity for blocking the liberation of Ca^{2+} from the SR.

The slight differences in the activities of dihydroatisine, songorine, and hetisine may be connected with the presence of additional hydroxy groupings ensuring the formation of a larger number of hydrogen bonds. This may have definite functional significance in the interaction of the alkaloid with the corresponding cell structures. The rise in activity from 1-benzoylnapelline to benzoylheteratisine is due to the presence of a mobile oxygen substituent, as has been confirmed by the results of pharmacological analysis [14]. Thus, the results of the investigations witness the presence of general laws of the structure-activity relationship for DAs, which is of great theoretical and practical importance. On the basis of the results obtained it may be stated with high probability that it is most desirable to pursue the search for new antiarrhythmic drugs possessing high activity and great breadth of therapeutic action among monoaromatic derivatives of DAs. By varying the nature of the aromatic substituent in these derivatives and also the positions in the molecule of hydroxy groups and free oxygen atoms it will be possible to obtain a series of highly effective new antiarrhythmic drugs differing in the spectrum and nature of their cardiorhythmic action.

EXPERIMENTAL

The diterpene alkaloids were obtained in the alkaloid chemistry laboratory of the Institute of the Chemistry of Plant Substances, Academy of Sciences of the Republic of Uzbekistan, from *Aconitum sp.* [1] and were kindly supplied by B. T. Salimov and M. N. Sultankhodzhaev.

Cardiomyocytes from Wistar rats weighing 150-180 g were isolated with collagenase in a solution containing (in mM): NaCl — 120; KCl — 5.8; $MgSO_4$ — 1.5; KH_2PO_4 — 1.4; glucose — 14.1; $NaHCO_3$ — 4.3; HEPES — 10; pH 7.0; at 37°C by the method described in [15]. As a result of isolation we obtained 70-75% of viable calcium-tolerant cells of rectangular shape. The measurement of $[Ca^{2+}]_i$ was made with the use of the fluorescent probe Quin-2 as described in [16]. The fluorescence parameters were measured in a medium containing the DA in a concentration of 10 μM, ryanodine 10 nM, and ouabain 10 μM.

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