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RELATIONSHIP BETWEEN THE CHEMICAL STRUCTURE AND PHARMACOLOGICAL ACTION OF CARDIAC GLYCOSIDES OF THE STROPHANTHIDIN SERIES

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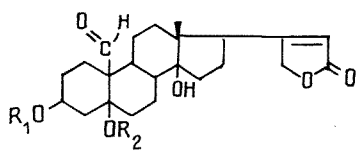
The possibility has been shown of finding and creating highly effective cardiotonic drugs for pediatric practice on the basis of cardenolides obtained by the chemical transformation of natural cardiac glycosides.

The problem of the interrelationship of the chemical structure of compounds and their action on the animal organism is one of the most interesting in modern science. The facts that have been gathered in its study are bringing us closer to an understanding of biochemical structures and processes forming the basis of physiological phenomena, and advances in this field are permitting the development of a theoretical basis for the purposeful synthesis of effective drugs.

Cardiac glycosides have already been used for more than 200 years to treat blood circulation insufficiency. It is known that the main carrier of their cardiotonic activity is the aglycon, while the sugar moiety attached to the aglycon exerts an influence on their activity, toxicity, and solubility and their fixation in the tissues. Thus, glycosides having a single sugar – monosides – are more active than diglycosides, while an aglycon having no sugar residue is less active than all the others [1, 2]. The aglycon of the strophanthin-like glycosides is strophanthidin. It is the bearer of the cardiotonic activity of strophanthin-K, and, although its biological activity is inferior to that of strophanthin-K, it is superior to the latter in its tranquilizing activity on the CNS. In view of this, the drugs acetylstrophanthidin, methylstrophanthidin, and others have been obtained from strophanthidin [2].



Our task was to study the products of the transformation of cardiac glycosides based on strophanthidin as aglycons that had been obtained in two directions: by the introduction of acyl groups into the aglycon and the sugar moiety and by the glycosylation of strophanthidin at C-3 and C-5 by various carbohydrate components. As early as the fifties, strophanthidin acetate, approved for clinical use, had been obtained by the acetylation of strophanthidin [3]. The possibility has been shown of the glycosylation of a tertiary hydroxy group of strophanthidin, and a number of new compounds have been obtained in this way [4, 5]. The condensation of strophanthidin and its acetate with acetobromorhamnose in the presence of silver carbonate has led to the synthesis of strophanthidin 3,5-bisrhamnoside hexaacetate and strophanthidin 3-acetate 5-rhamnoside triacetate, the subsequent saponification of which has yielded strophanthidin 3,5-bisrhamnoside and strophanthidin 5-rhamnoside with yields of 10-15% (Table 1). It has subsequently been found that the use of mercury cyanide in place of silver carbonate increases the yield of strophanthidin bisrhamnoside to 50%.

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TABLE 1. Comparative Biological Activities of the Cardenolides Studied and Their Effects on the Isometric Contraction of Papillary Muscles

Compound	Source	Number of FAU in 1 g of cryst. subs.	Number of CAU in 1 g of cryst. subs.	Inotropic effect in a conc. of $5 \cdot 10^{-7}$ g/ml
1. Strophanthidin $R_1=R_2=H$	Cotchorus olitorius [6]	40000	30.7	12,4±1,30
2. Strophanthidin 3-acetate (R_1=Ac, R_2=H)	Partial synthesis [3]	20000	5000	42,4±1,60
3. Strophanthidin 3-benzoate (R_1=Bz, R_2=H)	- [7]	—	—	—
4. Strophanthidin 3-β-*D*-riboside (R_1=β-*D*-Rib, R_2=H)	- [5]	68320	8400	36,5±3,18
5. Strophanthidin 5-α-*L*-rhamnoside (R_1=H, R_2=α-*L*-Rha)	Partial synthesis [4]	Inactive	1000	Inactive
6. Strophanthidin 3,5-bis-α-*L*-rhamnoside hexaacetate ($R_1=R_2$=α-*L*-Rha $(OAc)_3$)	" [4]	Inactive	Inactive	Inactive
7. Strophanthidin 3,5-bis-α-*L*-rhamnoside ($R_1=R_2$=α-*L*-Rha)	- [4]	14000	5400	66±1,57
8. Strophanthidin 5-α-*L*- rhamnoside 3-acetate (R_1=Ac, R_2=α-*L*-Rha)	- [4]	Inactive	2200	20±1,87
9. Strophanthidin 3-β-*D*-glucoside tetraacetate (R_1=β-*D*-Glc $(OAc)_4$, R_2=H)	- [8]	5230	1240	Inactive
10. Convallatoxin triacetate (R_1=α-*L*-Rha $(OAc)_3$, R_2=H)	- [9]	1350	1000	Inactive
11. K-strophanthin-β (R_1=β-*D*-Glc→β-*D*-Cim—; R_2=H)	Apocynum cannabinum L.	55000	8330	66±2,64

Pharmacological investigations have shown that cardenolides with acetylated sugar residues (**6**, **8**, **9**, **10**) exhibit either no or very weak activity in comparison with the initial glycosides. This is apparently explained by an increase in the hydrophobicity and total volume of the molecule. A more effective way of transforming natural cardiac glycosides proved to be their synthesis with different positions of the carbohydrate units in the aglycon. Cardenolides having the carbohydrate component in the third position exhibited the greatest biological activity (**4**, **11**), while compounds having sugar residues at C-3 and C-5 (**7**) had a lower activity, and substances with a carbohydrate component only at C-5 (**5**, **8**) exhibited practically no activity.

It has been established that together with strophanthidin acetate, among the newly obtained cardenolide derivatives strophanthidin *D*-riboside and strophanthidin bisrhamnoside possess pronounced biological activities and positive inotropic actions on the heart (see Table 1). In 1 g of crystalline strophanthidin bisrhamnoside there are 5400 CAUs and 14,000 FAUs while 1 g of K-strophanthidin-β contains 8330 CAUs and 55,000 FAUs. Consequently, strophanthidin bisrhamnoside, just like strophanthidin acetate [1] is inferior in biological activity to K-strophanthin-β. However, in its breadth of pharmacological action strophanthidin bisrhamnoside exceeds the new cardiac glycoside strophanthidin riboside [5] and strophanthin-K. Furthermore, after 72 h, the above-mentioned drugs have been eliminated from the organism completely. These strophanthidin derivatives possess a pronounced cardiotonic activity – they improve the contractile function of the myocardium and increase the stroke and minute volumes of the heart.

At the present time, a drug – rafantozid – has been developed from strophanthidin bisrhamnoside and has been approved for use in practical medicine as a cardiotonic agent. The change in chemical structure led to the situation that strophanthidin bisrhamnoside causes no stimulant action on prolonged intramuscular injection. This makes the more convenient intramuscular injection possible, which is an advantage for pediatric clinics, since the intravenous injection of glycosides, particularly to neonates and infants up to one year old, presents great difficulty.

The absence of cumulative properties and the instantaneous development of the cardiotonic action of strophanthidin acetate makes this drug promising for use in resuscitation divisions, particularly in pediatric resuscitations. In view of these features, we have studied the action of the drug on young animals. A comparative study has been made on the influence of strophanthidin acetate, strophanthidin bisrhamnoside, and strophanthin-K in the age aspect on experimental myocarditis, and investigations have been conducted of enzymatic activity and of some indices of carbohydrate-phosphorus metabolism, oxidative phosphorylation, and the phospholipid spectrum of the cardiac muscle.

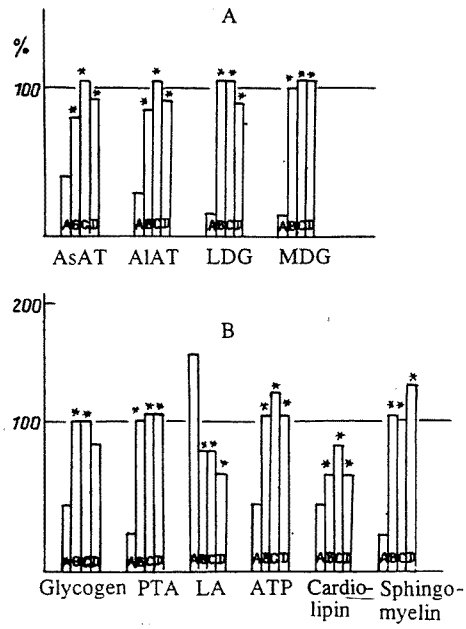

Fig. 1. A. Change in the levels of the enzymes AsAT, AlAT, LDG, and MDG (in relation to an intact animal taken as 100%) in the 7-day action of strophanthidin acetate (B), strophanthidin bisrhamnoside (C), and strophanthin-K (D), under the conditions of experimental myocarditis (controls – A), 30-day-old rats. B) Influence of the three-day treatment with strophanthidin acetate (B), strophanthidin bisrhamnoside (C), and strophanthin-K (D) of rats with experimental myocarditis (control – A) on the levels of glycogen, pyrotartaric acid (PTA), lactic acid (LA), ATP, the energy load, cardiolipin, and sphingomyelin in comparison with the same indices for intact animals (taken as 100%).

The results have shown that the drugs exert a unidirectional action – they normalize the enzymatic activities of AlAT, AsAT, LDG and MDG in 30-day-old rats (Fig. 1A). While in the hearts of control animals, the activities of these enzymes fell, the administration of strophanthin-K, strophanthidin acetate, and strophanthidin bisrhamnoside raised their activity, which indirectly showed the normalization of the plastic and energy metabolisms. Under the influence of the drugs, the level of glycogen and of ATP in the cardiac muscle increased and the energy potential of a pathologically altered myocardium rose (Fig. 1B).

In seven-day-old rats, the most pronounced effect was observed on the administration of strophanthin-K: in a dose of 2 mg/kg the drug raised the respiratory coefficient (RC) to 4.73 ± 0.17, while in the hearts of the control animals this index amounted to 3.05 ± 0.14. In seven-day-old rats with myocarditis the consumption of oxygen in the active state fell sharply. The administration of the drugs under investigation restored this to the initial level. Strophanthidin acetate and strophanthidin bisrhamnoside raised the degree of coupling of the processes of oxidative phosphorylation to a greater degree.

The reproduction of experimental myocarditis in 14-day-old rats was accompanied in the control experiments by a fall in the RC to 1.94 ± 0.11 (in the intact animals it amounted to 2.86 ± 0.13). Treatment with strophanthidin acetate and strophanthidin bisrhamnoside in doses of 1 mg/kg caused rises in the RC to 2.35 ± 0.16 (strophanthidin acetate) and 2.48 ± 0.13 (strophanthidin bisrhamnoside). Increasing the dose of the drug to 2 mg/kg enhanced the effect, the RC rising to 2.92 ± 0.12 and 3.01 ± 0.18, respectively.

Under the influence of the preparations concerned, the indices of the phospholipid spectrum also normalized and improved. The levels of cardiolipin and sphingomyelin rose, while the amount of lysophosphatidylcholine fell, which led to

the normalization of the ratio of the sum of neutral to the sum of the acid phospholipids. The most pronounced effect in 14-day-old animals was observed on treatment with strophanthidin bisrhamnoside and strophanthidin acetate.

The restoration of the qualitative and quantitative composition of the phospholipids under the influence of the drugs apparently led to a regularization of the structural organization of the membranes and the normalization of their structural function. Without denying the unidirectional nature of the action of the glycosides under investigation on metabolic processes in the myocardium during the experimental myocarditis of young animals, it must be mentioned that strophanthidin bisrhamnoside acetate caused a more pronounced normalization of the activity of the transamination enzymes in the myocardium and also of the indices of the phospholipid spectrum, while strophanthidin-K was more effective in relation to the enzymes of the carbohydrate and phosphorus metabolisms in the cardiac muscle.

Thus, the glycosides obtained by the introduction of acyl groups into the sugar moiety possess no pronounced biological activity. Glycosylation of the third hydroxy group of strophanthidin has given a number of new compounds of which the most interesting is strophanthidin bisrhamnoside. This transformation of the chemical structure of natural cardiac glycosides leads to a change in the pharmacological activity of the substances. Strophanthidin bisrhamnoside, like strophanthin-K, possesses a cardiotonic activity but is favorably distinguished from the latter by its lower toxicity and the absence of cumulative properties. Like strophanthin-K, strophanthidin bisrhamnoside and strophanthidin acetate normalize and improve metabolic processes in the myocardium in cardiac pathology. They exhibit a milder, gentler action on the metabolism of the myocardium of 7- to 14-day-old animals. Modification of the chemical structure has led to a cardiotonic drug for intramuscular injection which is of great practical value (rational and convenient use in practical medicine, particularly pediatric cardiology).

EXPERIMENTAL

Biological activity is expressed in CAUs (cat activity units) and FAUs (frog activity units) in accordance with the Tenth edition of the State Pharmacopeia. In *in vitro* experiments, inotropic activity was investigated on isolated guinea-pig papillary muscles as in [10].

Experimental myocarditis was reproduced in rats as described in [11, 12]; the drugs were administered intramuscularly in amounts of 3 CAU/kg. The animals were decapitated 2 h after the last injection and the activities of aspartate and alanine aminotransferases (AsAT and AlAT) and also of lactate and malate dehydrogenases (LDH and MDH) in the blood serum were determined with the use of a Chemapol set of reagents. The levels of glycogen [13], lactic acid [14], pyrotartaric acid [15], and adenyl nucleotides [16] were determined in heart homogenates. Phospholipid fractions were isolated by thin-layer chromatography on Chemapol LSB/40 silica gel, followed by quantitative determination [17]. The energy load of the system was calculated by Atkinson's method [18]. The ratio of the rate of respiration in the presence of a substrate to the rate of respiration after the use of ADP is expressed by the value of the respiratory coefficient and characterizes the degree of phosphorylation.

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