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Pharmacological Properties of the Complexes of Plant Carbohydrate-Containing Metabolites with the Agents Affecting the Cardiovascular System

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Abstract

Antihypertensive and antiarrythmic properties of the complexes of glycyrrhizic acid (glycoside of licorice roots), glycosides of *Stevia rebaudiana* Bertoni (stevioside, enzymatically treated stevioside SWETA and rebaudioside), arabinogalactan (polysaccharide of Siberian Larch) with propranolol, amiodarone and nifedipine were investigated. It was established that all the studied complexes of nifedipine except the complex of nifedipine with rebaudioside at the molecular ratio of 1 : 4 are promising for further pharmacological examination. The complex of propranolol with glycyrrhizic acid at the molecular ratio of 1 : 4 in the dose of 0.0025 mg/kg is also distinguished; it demonstrated good antiarrythmic activity. In the case of complexes with amiodarone, it may be concluded that complexing of this pharmacon with carbohydrate-containing plant metabolites used in our experiments does not lead to the desirable decrease in efficient dose but, quite contrary, worsens its antiarrythmic effect.

Key words: complexes, plant metabolites, antihypertensive and antiarrythmic properties

INTRODUCTION

Arterial hypertension (AG) is a common problem for industrially developed countries and developing ones [1]. Five major classes antihypertensive preparations are recommended to treat it: inhibitors of angiotensin converting enzyme, blockers of receptors AT_1, calcium antagonists, β-adrenoceptor blockers, diuretics [2, 3]. All the presented classes of compounds possess definite undesirable by-effects [4–7]. In this connection, the problem of the development of efficient preparations to treat arterial hypertension possessing minimal by-effects remains urgent for pharmacologists and specialists in medical chemistry. One of the efficient approaches to solve this problem is to bind the active substance in a molecular complex with plant carbohydrate-containing metabolites, which provides the protection of the basic preparation from rapid metabolism in the organism and improves its transport through biological membranes [8].

In the present work we describe the results of screening of antihypertensive and antiarrythmic activity of the complexes of glycyrrhizic acid (GA) (glycoside of licorice roots), glycosides of *Stevia rebaudiana* Bertoni (stevioside (ST), enzyme treated stevioside SWETA (SW) and rebaudioside (RB), arabinogalactan (AG, polysaccharide of Siberian larch) with propranolol (PP), amiodarone (AMD) and nifedipine (NF).

EXPERIMENTAL

Laboratory animals

The study was carried out with the rats of the Wistar line with body mass 180–240 g. The animals were obtained from the Laboratory of Experimental Animals of the Institute of Cytology and Genetics, SB RAS (Novosibirsk) and were kept under standard conditions with free access to water and standard granulated food. Experimental groups were formed from 8–10

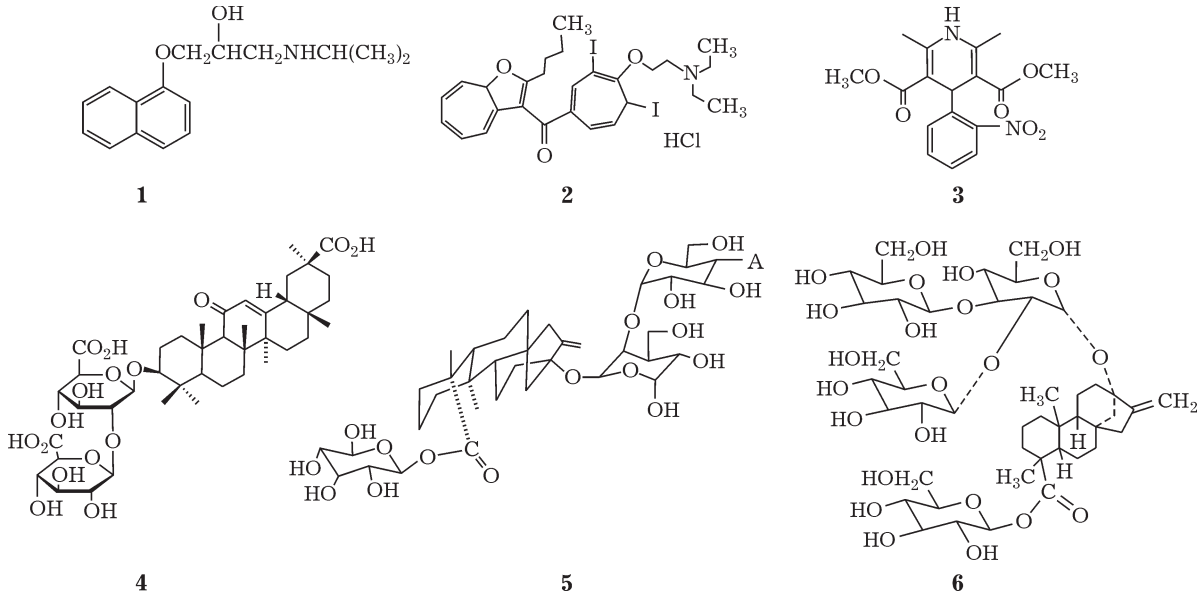

Fig. 1. Structural formulas of propranolol **1**, amiodarone **2**, nifedipine **3**, glycyrrhizic acid **4**, stevioside **5**, rebaudioside **6**.

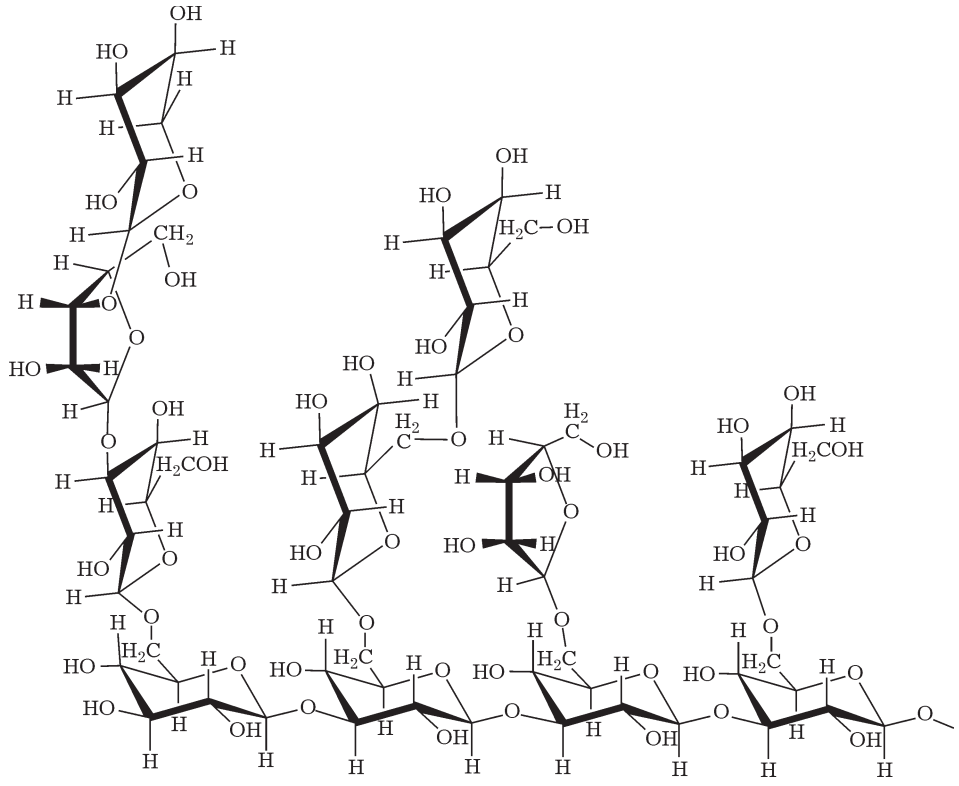

Fig. 2. Structural formula of arabinogalactan.

individuals of the same mass. Works with animals were carried out in accordance with the statements of the European Convention for the Protection of Vertebrate Animals used for experimental and other scientific purposes (Strasbourg, 1986).

Compounds under investigation

Complexes were synthesized using the mechanochemical method [9, 10] in the Group of Mechanochemistry of Organic Compounds of the Institute of Solid State Chemistry and Mechanochemistry, SB RAS (Novosibirsk).

Models and tests

Animals were narcotized with sodium thiopental (30 mg/kg, intraperitoneal introduction). Reference agents and compounds under investigation were introduced into the femoral vein in the form of aqueous solutions or a suspension with the addition of Tween 80. Arterial pressure was measured in the carotid artery through the cannula inserted preliminarily. To record ECG, hypodermic electrodes were placed at the second standard abduction. Registration of the parameters was carried out with the help of the electrophysiological complex Coulbourn LabLine (USA).

The model of calcium chloride arrhythmia was reproduced by a single introduction of the lethal dose of $CaCl_2$ (250 mg/kg) into the femoral vein of a rat before the introduction of the agent under investigation or within 1 min after its introduction. Adrenal arrhythmia was caused by a single introduction of the lethal dose of adrenaline (0.3 mg/kg) into the femoral vein of a rat before the introduction of the agent under investigation or within 1 min after its introduction.

Statistical treatment of the results

Statistical treatment of the data was carried out with the help of Statistics 7.0 software package. Results are presented as the average ± standard error. The reliability of the differences was assessed using Student's *t*-criterion.

RESULTS AND DISCUSSION

Complexes with glycyrrhizic acid

For the complex PP/GA in the molecular ratio of 1 : 4 and PP, the maximal dose was

TABLE 1

Hypotensive activity of the complexes of propranolol (PP) with GA, nifedipine (NF) with GA, NF with stevioside SWETA

Agents	Dose, mg/kg	Arterial pressure (AP)		Decrease in AP, %
		Initial	After the introduction of the agent	
PP/HA (1 : 4)	0.2	152.6±8.8	131.9±5.9*	14
	0.0025	139.2±4.9	123.6±8.1*	11
PP	0.2	133.9±5.7	119.0±3.9*	11
	0.0025	128.0±2.8	113.5±5.4*	11
NF/GA (1 : 4)	3.5	141.0±5.0	104.0±4.3*	26
NF/ST (1 : 4)	3.5	130.0±6.5	96.0±4.3*	26
NF/SW (1 : 4)	3.5	142.0±5.0	96.0±4.3*	32
NF	3.5	121.0±6.6	85.0±4.3*	30
	0.35	116.7±4.4	105.2±2.2*	9
ST	3.5	149.0±4.7	139.0±3.5*	7
SWETA	3.5	140.0±4.3	129.0±4.0*	8
GA	0.0025	135.6±2.8	136.4±3.5	0
	0.2	141.4±3.4	140.2±2.8	0
	3.2	126.5±1.3	129.6±2.0	0

*$p < 0.05$ with respect to initial AP.

established (0.2 mg/kg) for which the arterial pressure (AP) decreases by 27 mmHg, and the minimal dose (0.0025 mg/kg) for which the AP decreases by 15 mmHg. The same decrease in AP after the introduction of the complex and PP in the dose of 0.0025 mg/kg points to the stabilizing effect of complex formation. It is noteworthy that the PP content of the complex is 12 times lower. The GA itself has no effect on AP (Table 1).

The antiarrythmic activity of the complex PP/HA (1 : 4) was studied with the model of adrenal arrhythmia in the dose at which the maximal hypotensive effect is achieved (0.0025 mg/kg). One can see in the data presented in Table 2 that after the introduction of the complex in this dose at the background of arrhythmogen the survival of the animals was 100 %, while after the introduction of PP in the dose of 0.0002 mg/kg present in the complex, the survival decreased to 40 %. The GA itself does not possess antiarrythmic action in these doses.

For the complex of AMD with GA (1 : 4), we studied antiarrythmic activity with the calcium chloride model of arrhythmia. It was established that after the intravenous introduction of the complex in the dose of 0.005 mg/kg before arrhythmogen the survival of animals is 100 %, while after the introduction at the background of arrhythmia survival is 80 %. After the introduction of AMD in the same dose and in the dose contained in the complex (0.0008 mg/kg), 100 % antiarrythmic effect was observed after the similar schemes of introduction (Table 3). When smaller doses were introduced, no effect was observed, both for the introduction of the complex and for AMD alone.

TABLE 2

Antiarrythmic activity of the complexes of propranolol (PP) with glycyrrhizic acid (GA), nifedipine (NF) with rebaudioside (RB) (adrenal model of arrhythmia)

Agents	Dose, mg/kg	Percentage of survived animals, %
PP/HA (1 : 4)	0.0025	100
PP	0.0002	40
HA	0.0025	0
NF/RB (1 : 4)	0.0035	
	(NF 0.00035)	100
NF/RB (1 : 2)	0.0035	
	(NF 0.0007)	100
NF	0.00035	0
RB	0.12	50

TABLE 3

Antiarrythmic activity of the complex of amiodarone (AMD) with glycyrrhizic acid (GA) and arabinogalactan (AG), nifedipine (NF) with AG (calcium chloride model of arrhythmia)

Agents	Dose, mg/kg	Percentage of survived animals, %	
		Agent + $CaCl_2$	$CaCl_2$ + Agent
AMD/GA (1 : 4)	0.005	80	100
NF/GA (1 : 4)	0.12	80	90
NF	0.12	0	0
GA	0.005	0	0
NF/GA (1 : 4)	0.175	100	65
AMD/AG (1 : 4)	0.005	100	30
NF	0.017	0	0
AMD	0.005	100	100
	0.0008	100	100
AG	0.175	0	0

The obtained results provide evidence that in the case of AMD/GA complex (1 : 4) we failed to achieve the properties characteristic of glycoside complexing (a decrease in the dose with the conservation of the basic activity). In this situation, it should be stressed that after the introduction of the complex in the dose of 0.005 mg/kg the lethal termination is observed later by 1.5–2 min than that after AMD introduced in the same dose.

For the complex of NF with GA (1 : 4), we studied the hypotensive and pleiotropic antiarrythmic action. It was established that after the intravenous introduction of the complex and pure NF in equal doses (3.5 mg/kg) similar decrease of AP is observed: 26 % (37 mmHg) and 30 % (36 mmHg). It is noteworthy that the dose of NF in the complex is 10 times lower and equals 0.35 mg/kg. In the same dose, NF causes a decrease in AP by only 9 % (11.5 mmHg) (see Table 1). It is known that antiarrythmic action is not significant for NF [11]. In this connection, we studied the antiarrythmic effect of the complex in comparison with NF. With the model of calcium chloride arrhythmia, we established the dose of complex (0.12 mg/kg) which causes the maximal antiarrythmic effect for two schemes of the introduction of agents.

The survival of animals is 80 % in the case when the complex is introduced before the arrhythmogen and 90 % when the complex is introduced after the arrhythmogen. For NF in the dose of 0.12 mg/kg, antiarrythmic action was not detected (see Table 3). With the model of adrenal arrhythmia, no antiarrythmic action of NF was detected.

Thus, the complex NF/GA (1 : 4) is a new potential medicinal agent that has no analogs in the character of pharmacological action because it allows to pass from hypotensive action to antiarrythmic by changing the dose.

Complexes with the metabolites of Stevia rebaudiana Bertoni

In the studies of hypotensive action of the complexes of NF with ST and stevioside SWETA after intravenous introduction, their high basic activity was demonstrated for the same dose (3.5 mg/kg) as that of the complex NF/GA (1 : 4). Complexes NF/ST (1 : 4) and NF/SW (1 : 4) cause a decrease in AP by 26 (34 mmHg) and 32 % (46 mmHg), respectively. ST and SW alone in the doses of 3.5 mg/kg cause a decrease in AP only by 7 (10 mmHg) and 8 % (11 mmHg), respectively (see Table 1). Comparing the hypotensive action of two complexes, we should stress the difference in the decrease in AP (by 6 %) for the introduction of NF/SW (1 : 4) complex. Such a difference may be connected with an increase in the stability of the complex with stevioside SWETA.

The antiarrythmic action of the complex NF/ST (1 : 4) was studied with two models of arrhythmia: calcium chloride and adrenal ones. It was established that with the dose of 0.15 mg/kg the achieved survival is 100 and 40 %, after the introduction of the agent prior to arrhythmogen for calcium chloride arrhythmia and adrenal one, respectively. After the introduction of the agent in the same dose at the background of developed arrhythmia, the survival of animals was 20 % with calcium chloride arrhythmia; while no effect was observed with adrenal arrhythmia (Table 4).

The high antiarrythmic activity of stevioside itself should be stressed: it prevents the development of arrhythmia by 45 % as a mean for both models of arrhythmia. This property may be due to antagonism with calcium known for stevioside [12]. Relying on this, we may assume that complexation of stevioside with NF enhances the pleiotropic action of the latter.

Studying the hypotensive action of the complex NF/RB (1 : 4) in the dose of 3.5 mg/kg we established a decrease in AP by 9 %, while similar complexes of NF with GA, ST and SW cause a decrease in AP by 26 %. This difference in efficiency may be connected with the structural feature and stability of the complex. One of the ways to enhance the activity of this complex can be a decrease in the content of RB it its composition to the NF/RB ratio equal to 1 : 2. Investigation of the hypotensive activity of the complex NF/RB (1 : 2) showed a sharp (by a factor of 2.8) enhancement of the effect, which confirms the assumption about the dependence of the basic activity of complex on the number of RB molecules (Table 5).

In view of the low efficiency of complexes with NF for the adrenal model of arrhythmia, investigation of the antiarrythmic activity of NF/RB (1 : 4, 1 : 2) complex was performed only with the model of calcium chloride arrhythmia (Table 6).

It was established that in the dose of 0.0035 mg/kg both complexes introduced before arrhythmogen provide 100 % survival of animals.

TABLE 4

Antiarrythmic activity of the complex of nifedipine (NF) with stevioside (ST)

Agents	Dose, mg/kg	Percentage of survived animals, %			
		Complex + Arrhythmogen		Arrhythmogen + Complex	
		$CaCl_2$	Adrenalin	$CaCl_2$	Adrenalin
NF/ST (1 : 4)	0.15	100	40	20	0
NF	0.12	0	0	0	0
ST	0.12	40	50	0	0

TABLE 5

Hypotensive activity of the complex of nifedipine (NF) with rebaudioside (RB)

Agents	Dose, mg/kg	Hypotensive effect, %
NF/RB (1 : 4)	3.5 (NF 0.35)	9
NF/RB (1 : 2)	3.5 (NF 0.7)	26
NF	0.35	9
NF	3.5	27
RB	3.5	0

TABLE 6

Antiarrythmic activity of the complex of nifedipine (NF) with rebaudioside (RB) in a model of calcium chloride arrhythmia

Agents	Dose, mg/kg	Percentage of survived nimals, %
NF/RB (1 : 4)	0.0035 (NF 0.00035)	100
NF/RB (1 : 4)	0.0035 (NF 0.0007)	100
NF	0.00035	0
RB	0.12	50

When the complexes were introduced after arrhythmogen, we did not observe any effect. Nifedipine in the dose contained in the complex (0.00035 mg/kg) does not exhibit antiarrythmic activity.

Thus, complexes with rebaudioside differ from other studied complexes by the high basic activity of pharmacon at the ratio of 1 : 2 but not 1 : 4. An enhancement of the pleiotropic activity is conserved for both complexes (NF/RB = 1 : 2 and 1 : 4).

Complexes with arabinogalactan

To study the hypotensive and antiarrythmic activity, we obtained the complexes NF/AG with different AG content (1 : 4, 1 : 8, 1 : 16). As a result of screening, the dose-dependent effect of NF/AG complexes after intravenous introduction was established. It was demonstrated that after the introduction of complexes NF/AG (1 : 4, 1 : 8, 1 : 16) in the maximal doses 35, 70 and 140 mg/kg (which corresponds to the NF dose 3.5 mg/kg) the hypotensive activity increases from 41 to 54.6 %. After the introduction of complex NF/AG (1 : 4) in lower doses (14.7 and 35 mg/kg) AP decreases by 26 % as a mean. A similar decrease in AP was also observed after the introduction of complex NF/AG (1 : 8) in doses 14 and 7 mg/kg, and complex NF/AG (1 : 16) in doses 28 and 14 mg/kg. Nifedipine in the maximal dose (3.5 mg/kg) causes a decrease in AP by 31.4 % and in the minimal dose by 9 % (Fig. 3).

Thus, it was established that all the studied complexes NF/AG after intravenous introduction within the dose range 1.4 to 0.35 mg/kg (calculated for NF) exceed NF in the strength of action and in the stability of hypotensive action. AG itself does not cause statistically significant decrease in AP.

On the basis of the results obtained studying hypotensive activity, we chose the complex

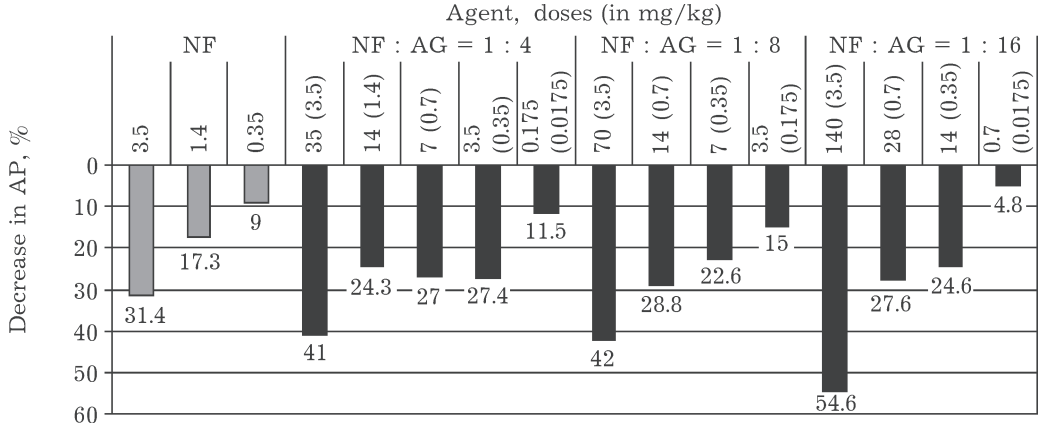

Fig. 3. Hypotensive activity of the complex of nifedipine (NF) with arabinogalactan (AG).

NF/AG (1 : 4) possessing stable hypotensive activity within a broad dose range for the investigation of antiarrythmic characteristics (see Table 3).

It was established that the complex at a dose of 0.175 mg/kg (NF dose 0.0175 mg/kg) introduced before arrhythmogen prevents the lethal heart rhythm disturbance in 100 % of cases, while its introduction after arrhythmogen prevents the lethal disturbance only in 65 % of cases. Nifedipine introduced at a dose of 0.017 mg/kg does not exhibit antiarrythmic action on the model of calcium chloride arrhythmia.

Thus, the results of NF complexation with polysaccharide AG are comparable with the results obtained for the complexes with plant glycosides characterized by high hypotensive and antiarrythmic activity with a 10-fold decrease in the dose of NF.

As stressed above, in the investigation of the pharmacological properties of complex AMD/GA (1 : 4) the effect typical for complexation, that is, a decrease in the dose of pharmacological agent with the conservation of high basic activity was not detected. In this connection, it was interesting to study the possibility of complexation AMD with AG. For this purpose, we used complex AMD/AG at the molecular ratio of 1 : 4 and the dose of 0.005 mg/kg, similarly to that used previously for complex AMD/GA (1 : 4).

It was demonstrated in the investigation of the activity of AMD/GA (1 : 4) complex that 100 % prevention of the development of arrhythmia occurs with the model of calcium chloride arrhythmia and its insignificant (30 %) blockage (see Table 3). It was established as a result of investigation that complexation of AMD with polysaccharide AG did not cause substantial changes of the pharmacological activity of the complex AMD/AG (1 : 4).

CONCLUSION

Generalizing the results obtained in this study, it should be stressed that the most promising substances for further pharmacological investigation are all the studied complexes of nifedipine except the complex NF/RB (1 : 4). Among other complexes, PP/GA (1 : 4) at the dose of 0.0025 mg/kg is distinguished. This complex exhibited good antiarrythmic activity. As far as the complexes with amiodarone are concerned, it may be concluded that complexation of this pharmacological agent with carbohydrate-containing plant metabolites used in our experiments does not lead to the desirable decrease of the effective dose but, quite contrary, worsens its antiarrythmic effect.

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