Effect of Nitroglycerin on Pharmacological Properties of Strophantin *In Vitro* and *In Vivo*

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Tests of nitroglycerin for its impact on pharmacological properties of strophanthin *in vivo* using two rat models of heart failure and *in vitro* on isolated frog atria showed that nitroglycerin does not alter the toxicity or the negative chronotropic action of strophanthin while somewhat weakening its cardiotonic effect.

Key Words: *strophanthin; pharmacological properties; rats; nitroglycerin*

The pharmacotherapy of cardiac decompensation based on cardiac glycosides is not infrequently complicated by glycoside intoxication, which not only makes the prognosis of the underlying heart disease markedly worse, but also poses a real threat to the patient's life [3,8]. In recent years, peripheral vasodilators have been finding increasing application in clinical cardiologic practice along with glycoside cardiotonics in the treatment of the failing heart [7]. There remains, however, the question of how these vasodilators influence the pharmacological properties of cardiac glycosides.

The purpose of the present study was to see how the toxicity and chronoinotropic properties of strophanthin might be affected by the vasodilator nitroglycerin.

MATERIALS AND METHODS

The study was conducted on 162 Wistar rats (body weight 160-260 g) anesthetized with thiopental sodium (40 mg/kg intraperitoneally) and 60 freshly prepared specimens of isolated frog (*Rana ridibunda*) atria. Tolerance for the arrhythmogenic and systemic toxic actions of strophanthin was evaluated *in vivo* on rat models of acute and subacute heart failure (HF) using biological titration of minimal arrhythmogenic and lethal doses (MAD and LD) of this glycoside cardiotonic [4].

Acute HF was produced by forcing rats to swim to the point of complete exhaustion with a weight attached to the body. Subacute HF was induced by histotoxic activity of the β-adrenomimetic isadrine administered in large doses [4]. The criteria used to confirm that cardiac decompensation had set in included hemodynamic disturbances, anatomicohistological changes in viscera, and shifts in the myocardial electrolyte balance typical of this condition, as well as the development of hypersensitivity to the arrhythmogenic and systemic toxic actions of strophanthin. For assessment of the effect of nitroglycerin on the tolerance of rats for strophanthin toxicity, the vasodilator was injected intravenously (0.5 mg/kg) 5 min before the biological titration of strophanthin doses (MAD and LD) was started. In addition, the impact of nitroglycerin on chronoinotropic properties of strophanthin was evaluated in *in vitro* tests with isolated frog atria.

The force and frequency of isometric contractions by strips of myocardial tissue were recorded with a mechanotron [4]. As a "therapeutic" strophanthin concentration, we chose 10^{-3} g/liter because in our previous tests it had exerted the greatest inotropic effect during a 10-min exposure to this glycoside cardiotonic. For nitroglycerin, the "therapeutic" concentration was considered to be 5×10^{-4} g/liter, i.e., the ratio of "therapeutic" car-

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TABLE 1. Effect of Nitroglycerin on Tolerance for Arrhythmogenic and Systemic Toxic Actions of Strophanthin in Intact (Healthy) Rats and Rats with Simulated Cardiac Decompensation

Experimental conditions	№ of tests	Strophanthin dose, mg/kg	
		MDA	LD
Intact rats			
Control	16	7.6±0.11	14.6±0.20
After premedication with nitroglycerin	6	7.2±0.26*	14.2±0.35*
Rats with acute heart failure			
Control	25	5.0±0.10	11.4±0.14
After premedication with nitroglycerin	13	5.3±0.34*	11.5±0.40*
Rats with subacute heart failure			
Control	10	5.0±0.22	11.7±0.58
After premedication with nitroglycerin	8	5.1±0.11*	10.8±0.38*

Note. MAD = minimal arrhythmogenic dose; LD = lethal dose. *$p>0.05$ in comparison with the respective controls.

diac glycoside to vasodilator concentrations in the *in vitro* tests with frog atria was the same as in the *in vivo* experiment on rats.

The data obtained were subjected to statistical analysis using Student's t test.

RESULTS

Swimming to complete exhaustion and the β-adrenomimetic isadrine in large doses led to acute HF and subacute HF, respectively, with characteristic changes in hemodynamics, myocardial electrolyte balance, and tolerance for strophanthin toxicity. Thus, both methods of HF production caused significant (nearly twofold) decreases in stroke and minute volumes, appreciable reductions in the cardiac and stroke volume indexes, a rise in total peripheral vascular resistance, and a more than fivefold drop in the K^+/Na^+ ratio (which is an indicator of ionic balance in cardiomyocytes).

Rats with HF were markedly less tolerant of strophanthin toxicity than intact rats (Table 1), as evidenced by 34.3% and 19.9% decreases in the MAD and LD, respectively ($p<0.001$), in the group with subacute HF and 34.3% and 22% decreases in these doses in the group with acute HF.

Nitroglycerin premedication had little or no effect on strophanthin tolerance in the intact controls and in the rats with HF: these still showed hypersensitivity to the arrhythmogenic and systemic toxic actions of strophanthin, which is a characteristic feature of HF.

As follows from the data in Table 2, strophanthin exhibited a well-defined negative chronotropic effect and a biphasic effect on the amplitude of isometric contractions by frog atria during their 10-min exposure to this drug. Thus, after an initial rise, the cardiotonic effect of strophanthin progressively declined as the exposure was continued, so that after the 7th minute the contractile activity of the myocardial strips virtually did not differ from its initial value. When added alone (without strophanthin) to the cuvette containing a frog atrium, nitroglycerin had no effect on its contraction amplitude throughout the 10-min exposure period and caused a moderate and significant inhibition of contraction frequency only in the last 4 min of exposure (Table 2). When added together with strophanthin, nitroglycerin showed only a tendency to lower the positive inotropic effect of the cardiotonic initially (in the first 3 min) and reduced this effect significantly at minutes 4 and 5; in contrast, starting with minute 6 of exposure, nitroglycerin tended to delay the rate at which the cardiotonic effect of strophanthin was weakened during the second phase of its action. It should be noted that nitroglycerin did not significantly change the negative chronotropic effect of this cardiac glycoside.

The experimental findings presented above agree well with the well-known clinical observation that patients with HF have appreciably reduced tolerance for cardiac glycoside toxicity. In our two different animal models of cardiac decompensation closely mimicking the corresponding clinical situations, the venous vasodilator nitroglycerin, which is particularly indicated in cases of coronary insufficiency combined with HF [3,7], virtually did not affect the tolerance for the arrhythmogenic or systemic toxic actions of strophanthin: the animals still showed heightened sensitivity to the latter. This should be borne in mind when prescribing drugs from these two groups in combination.

Interestingly, whereas intact (healthy) rats displayed somewhat reduced strophanthin tolerance following premedication with nitroglycerin (which can probably be attributed to the peripheral sympathomimetic action of this drug and the inhibition of phosphodiesterase by it [7]), nitroglycerin-premedicated rats with HF tended, on the contrary, to exhibit increased resistance to strophanthin cardiotoxicity, apparently because of the nitroglyc-

TABLE 2. Effects of Strophanthin, Nitroglycerin, and Their Combination in "Therapeutic" Concentrations on Chronoinotropic Parameters of an Isolated Myocardial Strip

Exposure time, min	% Change in parameter after dosing relative to predosing level		
	strophanthin	nitroglycerin	strophanthin+ nitroglycerin
Amplitude of isometric myocardial contractions			
1	106.7±0.9*	100.4±1.7	104.9±2.9
2	118.3±1.8*	100.1±0.6	114.4±4.0*
3	129.6±2.6*	99.2±1.4	121.1±4.3*
4	138.5±2.3*	100.3±1.9	125.5±3.4*+
5	146.7±2.3*	101.5±1.8	132.6±4.0*+
6	128.7±6.5*	100.6±2.2	134.3±4.1*
7	119.1±6.0*	98.9±2.2	120.3±2.6*
8	109.3±5.5	99.4±2.1	114.4±2.0*
9	104.8±6.3	101.4±2.4	106.8±2.1*
10	98.6±6.0	102.6±2.0	103.2±3.7
Contraction frequency of myocardial strip			
1	99.0±0.6	100.0±0.0	100.0±0.0
2	97.0±1.5*	100.0±0.0	96.2±2.9
3	94.8±2.1*	97.2±2.6	92.8±4.0
4	92.0±2.3*	95.2±3.6	91.3±4.9
5	82.6±3.0*	93.0±5.2	86.8±5.1*
6	74.6±4.2*	91.0±5.0	82.7±4.2*
7	71.7±4.7*	89.0±4.1*	81.0±5.0*
8	69.3±4.6*	86.3±5.7*	78.2±3.5*
9	69.3±4.6*	84.2±6.6*	76.7±3.3*
10	67.7±5.2*	82.2±5.5*	74.5±1.8*

Note. The asterisk denotes a significant difference from the predosing level taken as 100%, while the plus sign denotes a significant difference from the effect of strophanthin.

erin-induced diminution of the preload and afterload on the myocardium with a resultant lessening of the acidosis and hypoxia which have been reported to potentiate the toxicity of cardiac glycosides [1].

Our *in vitro* findings concerning the impact of strophanthin on the force of isometric myocardial contractions lend strong support to the hypothesis that the response to cardiac glycosides is biphasic [2,9]. This response appears to result from the superposition of two distinct effects produced by these cardiotonics in the myocardium, namely, a positive inotropic effect and a toxic effect which have unequal time constants. Indeed, when the cardiac glycoside strophanthin was added to the cuvette containing a myocardial strip, a gradient of its concentrations in the direction from the periphery of the strip to its center was formed as a result of strophanthin diffusion in the intercellular space. It has been stated [2] that as a glycoside cardiotonic spreads over the cross section of a myocardial strip, its positive inotropic effect will give way to toxic action. The negative chronotropic effect of strophanthin observed in our tests with isolated frog atria provides further evidence for the ability of glycoside cardiotonics to exert a direct inhibitory effect on the sinus node [1]. It should be noted that in these tests nitroglycerin significantly reduced the cardiotonic effect of strophantin when this effect was at its height (minutes 4-5 of exposure). An important role in the mechanism by which cardiac glycosides exert their inotropic action is ascribed to increases in the intracellular concentration of Ca^{2+} ions as a critical factor in the coupling of electrical and mechanical processes occurring in the cardiac muscle [6,10,11]. It has also been shown that nitroglycerin, in contrast, possesses calcium-blocking properties [5], and these apparently account for the diminished positive inotropic effect of strophanthin observed under the experimental conditions used.

It is to be hoped that the findings from this study regarding the effects exerted by nitroglycerin on the toxicity and chronoinotropic parameters of strophanthin will prove useful in predicting the pharmacological effects of combination therapies using glycosides in cases of cardiac decompensation.

To summarize, we found that:

– premedication with the venous vasodilator nitroglycerin did not alter the tolerance of rats with acute or subacute heart failure for the arrhythmogenic activity or systemic toxicity of strophanthin.

– exposure of isometrically contracting isolated frog atria to nitroglycerin, while not affecting the negative chronotropic effect of strophanthin, did weaken its cardiotonic activity during the period when the latter was expected to be at its peak.

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