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Relationship between cardiotonic effects and inhibition on cardiac sarcolemmal Na^+, K^+-ATPase of strophanthidin at low concentrations[1]

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KEY WORDS strophanthidin; myocardial contraction; Na^+-K^+-exchanging ATPase; guinea pigs

ABSTRACT

AIM: To evaluate the effects of strophanthidin (Str) on cardiac contractile function and sarcolemmal Na^+, K^+-ATPase activities in isolated guinea-pig hearts. METHODS: Isolated guinea-pig hearts were perfused through aorta in a Langendorff mode. Heart rate (HR), left ventricular pressure (LVP), and first derivatives ($\pm dp/dt_{max}$) of LVP were recorded by eight-channel physiological instrument. Cardiac sarcolemmal Na^+, K^+-ATPase activities were determined with colorimetry. **RESULTS:** Str 0.1 nmol/L stimulated the Na^+, K^+-ATPase activities ($P<0.05$), but had no effect on HR, LVP, and $\pm dp/dt_{max}$. Str 1 nmol/L increased $+dp/dt_{max}$ ($P<0.05$) and Na^+, K^+-ATPase activities ($P<0.01$). Str 10 and 100 nmol/L significantly increased both LVP ($P<0.05$) and $+dp/dt_{max}$ ($P<0.05$ or $P<0.01$), and had no significant effects on Na^+, K^+-ATPase activities. However, Str 1-100 μmol/L at first enhanced the LVP and $\pm dp/dt_{max}$ ($P<0.01$), then reduced them resulting from irregular contraction, and effects of Str on Na^+, K^+-ATPase activities revealed a concentration-dependent inhibition ($P<0.01$). **CONCLUSION:** The positive inotropic effects and irregular contraction produced by Str at higher concentrations result from the inhibition of Na^+, K^+-ATPase activities, and the positive inotropic effects of Str at lower concentrations are not related to the inhibition of the Na^+, K^+-ATPase activities.

INTRODUCTION

Cardiac glycosides have been thought to produce positive inotropic actions in failure heart via the inhibition of Na^+, K^+-ATPase activities[1]. However, the therapeutic concentrations (1-10 nmol/L) of cardiac glycosides in patients with the heart failure are much lower than those shown to inhibit the Na^+, K^+-ATPase *in vitro*[2,3]. Hougen *et al*[4] and Gao *et al*[5] have also demonstrated that cardiac glycosides 1 to 10 nmol/L stimulated Na^+, K^+-ATPase activities. Thus, the inhibition theory of Na^+, K^+-ATPase seems to be inadequate in explaining the inotropic action of glycosides at therapeutic levels. The reports about the effects of glycosides at low concentrations on cardiac performance were contradictory, which showed a positive inotropic effect in some cases[6], a negative inotropic, or no effect in other experiments[7,8]. So the relationship between the inotropic actions and effects on Na^+, K^+-ATPase activities of Str needs further to be confirmed. To assess the relationship, we measured the positive inotropic actions and the changes

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of Na^+, K^+-ATPase activities produced by strophanthidin (Str, a kind of cardiac glycosides) at the concentration range from 0.1 nmol/L-100 μmol/L in Langendorff guinea-pig hearts.

MATERIALS AND METHODS

Drugs and chemicals Str (Sigma) was dissolved in dimethyl sulfoxide (Me_2SO) as stock solution. LiBr was from Beijing Chemical Co. Na^+, K^+-ATPase kit was purchased from Nanjing Jiancheng Study Co. All reagents were of AR grade.

Preparation of the isolated hearts Guinea pigs of either sex, weighing 250±20 g, were provided by Experimental Animal Center of Hebei Medical University (Grade II, Certificate No 04064). The hearts were rapidly excised, mounted through aorta, and perfused on a modified Langendorff apparatus at a constant perfusion pressure (10 kPa). A polyethylene cannula containing saline was introduced into the left ventricle cavity and was connected with a pressure transducer, by which heart rate (HR), left ventricular pressure (LVP), and its first derivatives ($\pm dp/dt_{max}$) were recorded on a polygraph system (RM 6000, Nihon kohden). The hearts were perfused with K-H buffer solution (37 ºC, pH 7.4, saturated with 95 % O_2 and 5 % CO_2). The K-H buffer solution contained the following (in mmol/L): NaCl 118, $NaHCO_3$ 25.0, KCl 4.7, KH_2PO_4 1.2, $MgSO_4$ 1.2, $CaCl_2$ 2.5, and glucose 11.0.

All the hearts were first perfused with K-H solution for 20-30 min for stabilization in a Langendorff apparatus and then were randomly divided into 8 groups (6-8 hearts each group). The hearts were perfused with K-H buffer solution in control group, and in seven Str groups with K-H solution containing different concentrations of Str (0.1, 1, 10, 100 nmol/L or 1, 10, 100 μmol/L). HR, LVP and $\pm dp/dt_{max}$ were continuously recorded for 20 min.

Preparation of cardiac sarcolemma Guinea-pig hearts were taken out and placed in ice-cold 10 mmol/L Tris-HCl buffer solution (pH 7.4) containing 1 mmol/L ethylene-diaminetetracetate. The left ventricles were minced with scissors, and homogenized in a homogenizer. The resultant suspension was filtered through 4 layers of gauze and centrifuged for 10 min at 1000×*g*. After the supernatant was discarded, the pellet was resuspended in 10 mmol/L Tris-HCl buffer solution and subjected to a sequence of Tris-HCl washing buffers and a further KCl extraction step[9,10]. A part of the final pellets were used to observe the purity of sarcolemma with electron micrograph, the others were resuspended in 1 mmol/L Tris-HCl (pH 7.0) to determine the activities of Na^+, K^+-ATPase. All procedures were performed at 0-4 ºC. The protein content was measured by Coomassie brilliant blue method.

Observation of sarcolemmal purity A part of the pellets were fixed overnight in 4 % glutaraldehyde-phosphate buffer (pH 7.4), diced into pieces of about 0.5 mm×0.5 mm×0.5 mm, post fixed for 1 h in 1 % osmium tetraoxide. Then the pellet was dehydrated in a graded acetone series (50 %-100 %) and embedded in 812-epoxy resin. Thin sections were made with LKB-V# ultramicrotome (Sweden) and double-stained with uranium acetate and lead citrate. The sections were examined and photographed using H-7500 electron microscope (Nihon kohden).

Determination of the cardiac sarcolemmal Na^+, K^+-ATPase activities The cardiac sarcolemmal Na^+, K^+-ATPase activities were determined by colorimetry. The suspension of cardiac sarcolemma (50-70 μg proteins per mL) was divided into 8 groups (8 samples each group) and incubated with saline or different concentrations (0.1, 1, 10, 100 nmol/L and 1, 10, 100 μmol/L) of Str at 37 ºC for 10 min according to the method provided by manufacturer. Enzyme active unit was expressed as mmol inorganic phosphate per gram sarcolemmal protein per hour ($mmol \cdot h^{-1} \cdot g^{-1}$ protein).

Statistics Data were expressed as mean±SD. Statistical analysis was performed using paired *t*-test.

RESULTS

Effects of Str on cardiac contractility In the control group, perfused guinea-pig hearts were continuously observed for 50 min and showed no changes in HR, LVP, and $\pm dp/dt_{max}$. Str 0.1 nmol/L did not affect the cardiac contractile function; Str 1 nmol/L increased $+dp/dt_{max}$ ($P<0.05$), but had no effects on other parameters; Str 10 and 100 nmol/L increased both LVP and $+dp/dt_{max}$ ($P<0.05$ or $P<0.01$). The peak time of LVP and $+dp/dt_{max}$ was about 10-15 min, and after that the plateau of increase was sustained for whole observation. There was no obviously change in HR and $-dp/dt_{max}$; Str 1, 10, and 100 μmol/L caused the increase ($P<0.01$) and subsequent decrease in LVP and $\pm dp/dt_{max}$ resulting from irregular contraction (the incident rate of irregular contraction were 57 %, 75 %, and 100 %, respectively). A peak effect was reached in 10 min. A concentration-dependent decrease in HR was observed ($P<0.05$ or $P<0.01$) (Fig 1).

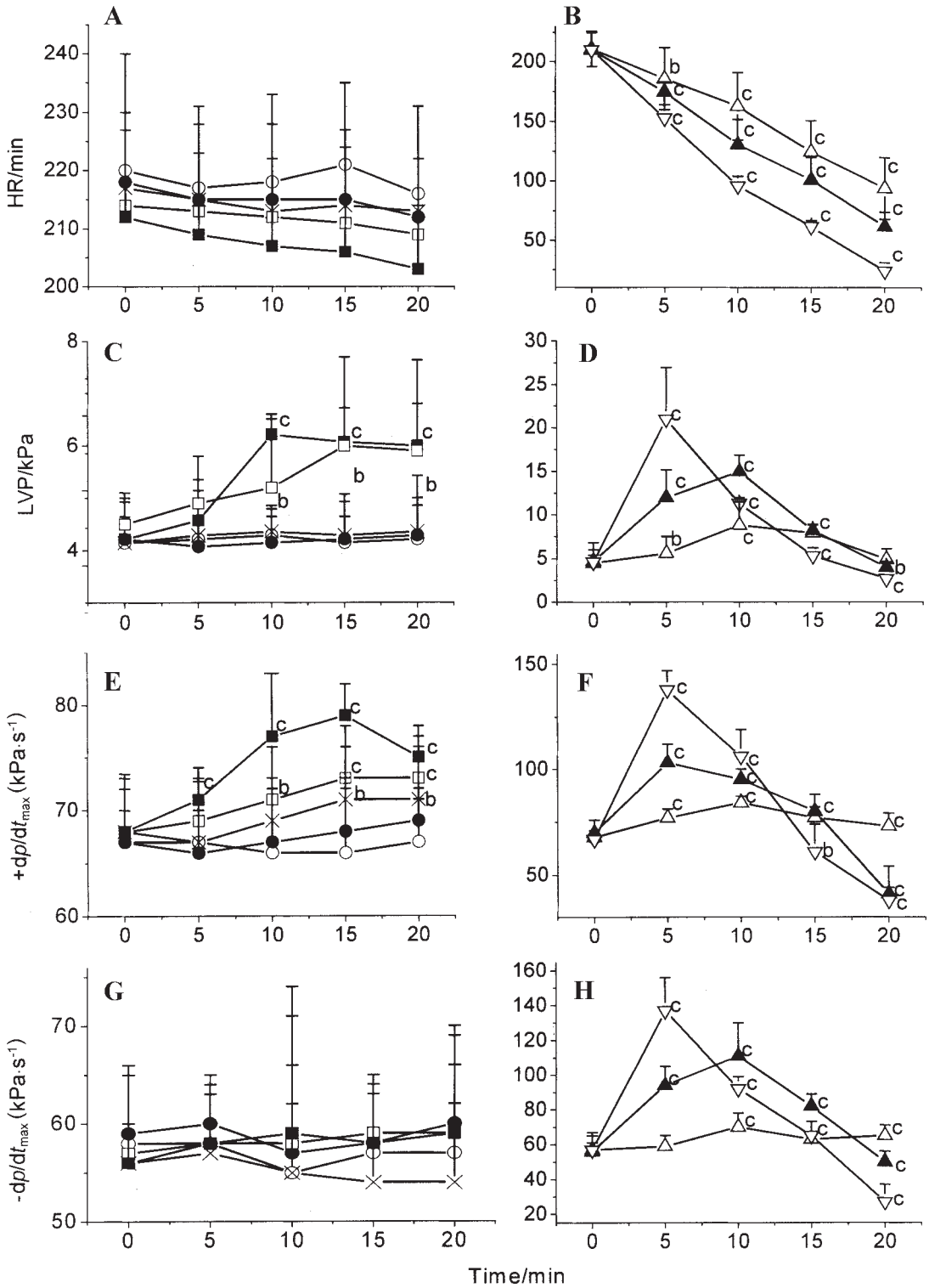

Fig 1. Time course of HR (A, B), LVP (C, D), +dp/dt_{max} (E, F), and -dp/dt_{max} (G, H) in the absence and presence of various concentrations of Str in isolated guinea-pig hearts. n=6-8 hearts. ^bP<0.05, ^cP<0.01 *vs* control.

Effects of Str on cardiac sarcolemmal Na^+,K^+-ATPase activities The electron microscopic examination showed that the membrane fraction was consisted of membranous sacs of varying shapes and sizes. No intact mitochondria, nuclei or myofibrils were found (Fig 2). Str 0.1 and 1 nmol/L increased (P<0.05) and 1-100 μmol/L inhibited the Na^+, K^+-ATPase activities (P<0.01). But Str 10 and 100 nmol/L did not affect

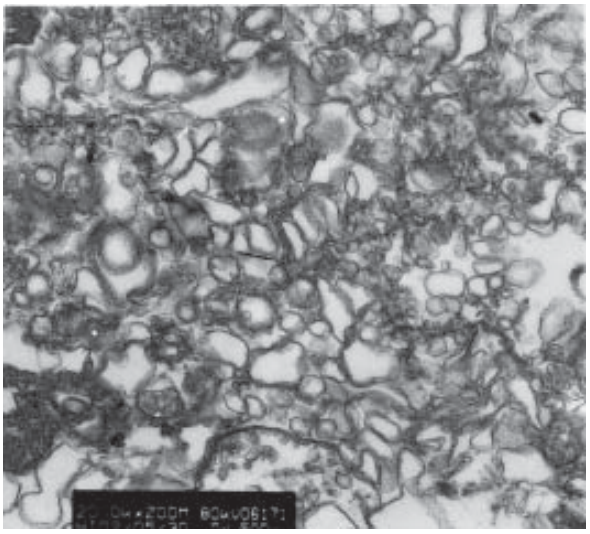

Fig 2. Electron micrograph of the guinea-pig heart sarcolemmal pellet (×10 000).

Na^+, K^+-ATPase activities (Tab 1).

Tab 1. Effects of Str on cardiac sarcolemmal Na^+, K^+-ATPase activity in guinea-pig hearts. *n*=8. Mean±SD. $^bP<0.05$, $^cP<0.01$ *vs* control.

Doses	Na^+,K^+-ATPase activity /mmol·h^{-1}·g^{-1} protein	Change rate/%
0	23.2±2.8	0
0.1 nmol/L	25.9±1.6^b	13
1 nmol/L	27.4±2.0^c	20
10 nmol/L	22.9±2.4	2
100 nmol/L	22.6±2.5	-6
1 mmol/L	19.1±2.7^c	-18
10 mmol/L	15.5±1.6^c	-25
100 mmol/L	6.3±1.4^c	-72

The relationship between the cardiac function parameters and the inhibition of Na^+, K^+-ATPase

The correlation analysis between the maximum of cardiac contractile parameters or incident rate of irregular contraction and the percent inhibition of Na^+, K^+-ATPase of Str at higher concentrations (1-100 μmol/L) were summarized in Tab 2. The results showed that there were a close correlation between LVP, $+dp/dt_{max}$ or $-dp/dt_{max}$, percentage of irregular contraction and the percent inhibition of Na^+, K^+-ATPase activities.

Tab 2. Correlation analysis between the cardiac function parameters and the inhibition of Na^+, K^+-ATPase.

	Slope(b)	Intercept(a)	*r*	*P*
LVP (*Y*) *vs* I-Na (*X*)	0.19	7.64	0.92	<0.01
$+dp/dt_{max}$ (*Y*) *vs* I-Na (*X*)	0.9	73.6	0.97	<0.01
$-dp/dt_{max}$ (*Y*) *vs* I-Nz (*X*)	1.0	68.0	0.86	<0.01
Percentage of irregular contraction (*Y*) *vs* I-NA (*X*)	0.7	50.5	0.95	<0.01

Y=a+b*X*, *r*: correlation coefficent.

DISCUSSION

Our results showed that Str 1-100 nmol/L increased the cardiac contractile function. Str 0.1, 1 nmol/L stimulated and 10,100 nmol/L did not affect the Na^+, K^+-ATPase activities. These results suggest that the positive inotropic actions of Str at lower concentrations are not related to the changes of Na^+, K^+-ATPase activities, either inhibition or stimulation, for Str 0.1 nmol/L increased the Na^+, K^+-ATPase activities without any effects on the cardiac contractile function. There was a close correlation between the increase of cardiac contractile function and the inhibition of Na^+, K^+-ATPase at higher concentrations of Str (1-100 μmol/L), suggesting that the inotropic actions result from inhibition of Na^+, K^+-ATPase activities. So there are at least two coexisted inotropic mechanisms of cardiac glycosides: one related to Na^+, K^+-ATPase inhibition, and the other, occurring at lower concentrations, involved a mechanism other than Na^+,K^+-ATPase inhibition. Our results is agreement with other researcher's work[6,11,12]. The present experiment further confirmed the inotropic effects of Str at lower concentrations are not the result of Na^+, K^+-ATPase inhibition.

Since intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) plays a crucial role in the excitation-contraction coupling, and Na^+, K^+-ATPase is the receptor of cardiac glycosides, it is very interesting to know how cardiac glycosides at lower concentrations induced the $[Ca^{2+}]_i$ elevation. In general, inhibition of the Na^+, K^+-ATPase activities caused the increase of intracellular Na^+ concentration ($[Na^+]_i$) and subsequent increase of $[Ca^{2+}]_i$ through Na^+-Ca^{2+} exchange. Obviously, the positive inotropic effects of Str at lower concentrations is not related to increased $[Na^+]_i$ secondary to the inhibition

of Na^+, K^+-ATPase. In fact, cardiac glycosides at lower concentrations did increase $[Ca^{2+}]_i$ without any change in $[Na^+]_i$[13-15]. What is the mechanism of positive inotropic effects of Str at lower concentrations? Santana *et al*[16] reported that nanomolar concentrations of cardiac glycosides activated the slip-mode conductance of the TTX-sensitive Na^+-channels by which Ca^{2+} enters the cell, leading to an increase in $[Ca^{2+}]_i$. Sagawa *et al*[17] also reported cardiac glycosides at lower concentrations activated the cardiac ryanodine receptors and resulted in Ca^{2+} release from sarcoplasmic reticulum. Mounting evidence suggests that there exists an endogenous ouabain-like factor which has hormone-like properties, and can relay message through various signal transducion pathways[18-20]. Recently, Tian *et al*[21] demonstrated that ouabain's effect on $[Ca^{2+}]_i$ in rat cardiac myocytes is throuth the signal transducing function of Na^+, K^+-ATPase activities. Yin *et al*[22] proved that low concentration of dihydroouabain increased $[Ca^{2+}]_i$ by Ca^{2+} influx via L-type Ca^{2+} channels, TTX-sensitive Na^+ channels or/and by directly triggering intracellular calcium release. Thus, the effects of glycosides might be involved in more complex mechanisms. More studies are necessary to further understand the molecular basis of the action.

In conclusion, Our results suggest that Str at lower concentrations still has the positive inotropic effects, which involve a mechanism other than Na^+, K^+-ATPase inhibition.

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