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Restraint stress changes heart sensitivity to arrhythmogenic drugs[1]

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AIM: To study the effects of acute restraint stress on ventricular electric stability (VES) and its mechanisms of action. **METHODS**: VES was evaluated both *in vivo* and *in vitro* by the changes of arrhythmogenic responses to icv or ip aconitine in rats and iv $BaCl_2$ or adrenaline in rabbits following restraint stress for different durations. Pretreatments and the assay of heart-specific enzymes were made. **RESULTS**: The heart sensitivity to these drugs was promoted after stress for 2 h, but obtunded after stress for 8 h (the latency of ventricular arrhythmia to icv aconitine was shortened from 4.1 ± 0.9 min in control rats to 2.9 ± 0.9 min after stress for 2 h, $P < 0.05$; but prolonged to 9.3 ± 3.8 min after stress for 8 h, $P < 0.05$). In Langendorff heart, the changes of VES induced by stress were similar to those *in vivo*, but to lesser degree. Pretreatment with adrenalectomy inhibited the descending phase of VES, while pretreatment with both aminophylline and vagotomy remarkably depressed the ascending phase at 8 h. In addition, the serum activities of lactate dehydrogenase (LDH), creatine kinase (CK), and aspartate aminotransferase and their isozymes, LDH_1 and CK-MB, were elevated at 2 h, and rose continuously at 8 h. **CONCLUSION**: Acute restraint stress causes biphasic changes of VES. The initial decrease of VES was related to adrenal catecholamine release, whereas the following increase of VES was ascribed to adaptive decrease of cAMP and vagal activation. The changes of VES did not always parallel the injury of heart.

KEY WORDS physical restraint; arrhythmia; aconitine; aminophylline; adrenalectomy; vagotomy; lactate dehydrogenase; creatine kinase

The relationship between mental stress and cardiovascular dysfunction has been studied[1,2]. Acute stress reduced ventricular electric stability (VES), and enhanced arrhythmia and sudden cardiac death[3,4]. However, the heart from animals subjected to repeated restraint stress developed an increased resistance to a broad spetrum of harmful factors[5], suggesting that cardioprotective mechanisms had been activated. In this study, we concentrate on two principal questions: (1) relationship between acute restraint stress and ventricular arrhythmia; (2) the possible mechanism responsible for stress-induced changes of VES.

MATERIALS AND METHODS

Sprague-Dawley ♂ rats ($n = 122$, $239 \pm s$ 25 g) and rabbits ♂ ($n = 36$, $2.4 \pm s$ 0.2 kg) were housed for >3 d, with free access to food and water.

Restraint stress The extremities and teeth of animal were fixed on a special frame with adhesive plasters, but not ligation. At the end of stress, none of animals had notable edematous extremities.

Drug-induced arrhythmia *in vivo* After stress treatment, the rat was immediately anesthetized with ip urethane 1.1 g kg^{-1}, then mounted on a stereotaxic apparatus, and injected icv aconitine 1.7 μg in 10 μL after 10 min stabilization. Aconitine (E Merck) was

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dissolved as described previously[4].

Conscious rats (aconitine, 200 μg kg^{-1}, ip) and rabbits ($BaCl_2$ 4 mg kg^{-1}, iv, or adrenaline 60 μg kg^{-1}, iv) were injected. The onset of ventricular arrhythmias were recorded.

Drug-induced arrhythmias *in vitro* After stress treatment, the rat was decapitated. Langendorff heart was perfused with Krebs-Henseleit solution. Electrocardiogram was recorded with 2 surface electrodes. After 5—10 min stabilization, chloroform solution of aconitine (5 μg in 2 μL) was injected into the anterior wall of left ventricle with a microsyringe.

Pretreatment (1) Adrenalectomy 1 wk before stress to abolish the facilitation of catecholamines at stress for 2 h; (2) vagotomy immediately after stress in combination with ip aminophylline (to inhibit the breakdown of cAMP) at the beginning of stress to diminish possiblely protective factors at stress for 8 h.

Serum enzyme assay The activity of lactate dehydrogenase (LDH), creatine kinase (CK), and aspartate aminotransferase (AST) in serum were determined with an automatic bioanalyzer (Monarch 1000, USA). LDH_1 was determined with plate electrophoresis, and CK-MB was determined after CK-MM was inhibited by its monoclonal antibody.

Statistical t test and exact probability were used.

RESULTS

Effects of stress on arrhythmogenesis to drugs *in vivo* In anesthetized rat, the arrhythmogenic response to icv aconitine was biphasically changed by acute stress. The occurrences of ventricular arrhythmias were promoted, and the transformation from ventricular premature to ventricular tachycardias was rapid after stress for 1 or 2 h, but postponed after stress for 8 h (Tab 1). These results reflected biphasic changes of VES, a decrease followed by an increase.

In conscious rats, ventricular arrhythmias induced by ip aconitine were influenced similarly by stress (Tab 2).

In conscious rabbits, ventricular arrhythmias induced by iv $BaCl_2$ or adrenaline also showed a biphasical change, but to a less degree, particularly the changes of arrhythmogenic response to iv adrenaline after stress for 2 h were not statistically different (Tab 3).

Tab 1. Arrhythmogenic responses to icv aconitine (17 μg) after restraint stress for 1—12 h in rats. $n=6$, $\bar{x}\pm s$. $^aP>0.05$, $^bP<0.05$, $^cP<0.01$ *vs* control. VP = Ventricular premature; VT = Ventricular tachycardia; VF = Ventricular fibrillation.

Stress/h	Latency (min) of VP	Latency (min) of VT	Time (min) from VP to VT	Incidences of VF
0	4.1±0.9	6.9±0.8	2.8±0.8	1/6
1	3.1±0.7^b	5.0±1.5^b	2.0±1.0^a	3/6^a
2	2.9±0.9^b	3.9±1.0^c	1.2±0.3^c	5/6^b
4	4.3±0.9^a	7.1±1.1^a	2.8±0.7^a	2/6^a
6	6.6±1.9^b	9.8±2.5^b	3.0±0.7^a	2/6^a
8	9.3±3.8^b	12.0±4.7^b	4.1±1.2^b	1/6^a
12	10.5±3.7^c	14.4±4.4^b	3.8±1.6^a	0/6^a

Tab 2. Arrhythmogenic responses to ip aconitine (200 μg kg^{-1}) after restraint stress for 2 or 8 h in conscious rats. $n=6$, $\bar{x}\pm s$. $^aP>0.05$, $^bP<0.05$, $^cP<0.01$ *vs* control.

Stress/h	Latency (min) of VP	Latency (min) of VT	Latency (min) of VF	Death time/min
0	7.7±0.9	9.4±1.2	16.1±1.3	19.2±2.1
2	6.4±0.7^b	7.7±0.8^b	11.7±1.9^c	13.4±2.2^c
8	9.6±1.9^b	13.3±2.2^c	21.8±3.5^b	24.6±5.5^b

Tab 3. Changes of arrhythmogenic responses to iv $BaCl_2$ 4 mg kg^{-1} or adrenaline (Adr) 60 μg kg^{-1} after restraint stress for 2 or 8 h in conscious rabbits. $n=6$, $\bar{x}\pm s$. $^aP>0.05$, $^bP<0.05$, $^cP<0.01$ *vs* control.

Drugs	Stress/h	Latency (s) of VP	Duration (min) of arrhythmia	Incidences of VF
$BaCl_2$	0	24±3	8.5±2.8	0/6
	2	17±5^b	10.4±2.9^a	5/6^c
	8	40±8^c	2.5±2.0^c	0/6^a
Adr	0	24±9	3.4±0.7	0/6
	2	21±8^a	3.5±1.1^a	2/6^a
	8	60±17^c	0.7±0.4^c	0/6^a

Effects of stress on arrhythmogenesis to aconitine *in vitro* In Langendorff heart, local injection of aconitine stably evoked ventricular arrhythmias. After stress for 2 or 8 h, the changes of VES *in vitro* showed a tendency similar to that *in vivo*, though the statistical differences were not significant (Tab 4).

Tab 4. Stress-induced changes of aconitine arrhythmias in Langendorff heart from rats restrained for 2 or 8 h. $n=6$, $\bar{x}\pm s$. $^aP>0.05$, $^bP<0.05$ *vs* **control.**

Stress/h	Latency of VT/min	Duration of VT/min	Incidences of VF
0	4.1±0.9	14.8±6.7	1/6
2	2.7±1.2[b]	23.2±6.1[a]	3/6[a]
8	5.4±1.6[a]	12.3±5.5[a]	0/6[a]

Influences of pretreatments on stress-induced changes of VES Pretreatment with adrenalectomy attenuated stress-induced decrease of VES at 2 h, with a less inhibitory effect on increase of VES at 8 h. Pretreatment with both vagotomy and aminophylline depressed stress-induced increase of VES at 8 h (Tab 5).

Tab 5. Influences of pretreatment with adrenalectomy (AE) or both vagotomy (Vag) and ip aminophylline (Ami) on stress-induced changes of arrhythmogenic responses to icv aconitine in rats. $n=6-7$, $\bar{x}\pm s$. $^aP>0.05$, $^bP<0.05$, $^cP<0.01$ *vs* **S2 (Stress for 2 h).** $^dP>0.05$, $^eP<0.05$ *vs* **S8 (Stress for 8 h).**

	Latency/min VP	Latency/min VT	Incidences of VF
Control	4.1±0.9	6.9±0.8	1/6
S2	2.9±0.9	3.9±1.0	5/6
AE+S2	4.5±0.9[b]	7.6±1.3[c]	3/6[a]
S8	9.3±3.8	12.0±4.7	1/6
AE+S8	4.8±1.3[d]	8.4±2.0[d]	4/6[d]
Vag+Ami+S8	4.1±0.5[e]	6.8±1.8[e]	6/7[e]

Enzymes in serum after stress The activities of LDH, CK, AST, and their heart-specific isozymes (LDH_1 and CK-MB) were elevated after stress for 2 h, and rose further after stress for 8 h (Tab 6).

Tab 6. Changes of serum activities (IU L^{-1}) of lactate dehydrogenase (LDH), creatine kinase (CK), aspartate aminotransferase (AST), LDH_1, and CK-MB after restraint stress in rats. $n=6$, $\bar{x}\pm s$. $^cP<0.01$ *vs* **control.**

	Control	Stressed time 2 h	Stressed time 8 h
LDH	82±18	760±120[c]	1 726±463[c]
CK	80±12	587±134[c]	1 371±399[c]
AST	77±6	185±13[c]	596±100[c]
LDH_1	19±5	160±26[c]	390±98[c]
CK-MB	53±11	262±45[c]	335±66[c]

DISCUSSION

Our results indicate that acute restraint stress produces biphasic effect on VES. Short-term stress increases the susceptibility of heart to arrhythmia (first phase), but when animal was continuously exposed to stress, the heart would develop high resistance to arrhythmia (second phase). One of our important findings was that the development of adaptive cardioprotection was not limited to repeated intermittent stress[5-7]. The biphasic changes of VES after stress were partially related to catecholamine release, and adaptive decrease of cAMP and vagal activation respectively. The results of cAMP for cold swim stress[8] and reperfusion arrhythmias for heat stress[9] are consistent with our finding.

In the first phase of acute stress, catecholamine hypersecretion and sympathetic activation[4,10] play a key role in the decrease of VES as adrenalectomy indicated. High level of catecholamines stimulates the production of cAMP in myocardium and enhances ventricular arrhythmias. In the arrhythmia model of

iv adrenaline, the changes of VES for 2 h stress were not statistically different, this discrepancy might be due to the overlapping of exogenous and endogenous adrenaline. As high level of catecholamines already existed in the body after 2 h stress, exogenous adrenaline could not enhance ventricular arrhythmias to the same degree as that in normal rats. The protective mechanisms was activated as a defence reaction secondary to harmful factors such as catecholamine hypersecretion in the first phase of acute stress, since removal of the latter by adrenalectomy also attenuated the second phase of acute stress. By comparing the results *in vivo* and *in vitro*, we found that stress-induced resistance to arrhythmia was due not only to the activation of central stress-limiting system (eg. vagal activation), but also to protective mechanisms formed at heart level (eg. adaptive decrease of cAMP). It should be determined next whether heat shock protein expression[7,11] and activation of antioxidants[12] also contribute to the protective mechanisms in the second phase of acute stress.

According to cardiac enzyme assay, the injury of heart was aggravated along with stress, and did not parallel the changes of VES. This result suggested that the injury degree of heart was not always related close to VES. The mechanism of this phenomena remained unclear at present. Our results also suggest that if conscious restrained animals were used to study the actions of drugs on arrhythmias, stress would become an interfering factor.

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制动应激改变心脏对致心律失常药物的敏感性[1]

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目的：研究急性制动应激对心脏电稳定性的作用及机制。**方法**：应激后用icv或ip乌头碱及iv氯化钡或肾上腺素致心律失常反应的改变来评价在体及离体心脏电稳定性，施予预处理初步分析机制。**结果**：应激2 h促进药物性心律

失常：应激8 h 反而抑制之. icv 乌头碱致心律失常的潜伏期在正常大鼠为4.1±0.9 min，但应激2 h 后缩短至2.9±0.9 min，应激8 h 后延长至9.3±3.8 min. 该双相反应可分别被预先肾上腺切除和迷走切断加 ip 氨茶碱削弱. 血中心肌特异酶活性随应激持续而上升. **结论**：急性制动应激使心脏电稳定性先降后升. 前者与儿茶酚胺释放有关，后者与 cAMP 适应性降低及迷走激动有关. 应激后心肌损伤与心脏电稳定性改变并不平行.

关键词 身体的约束；心律失常；乌头碱；氨茶碱；肾上腺切除术；迷走神经切断术；乳酸脱氢酶；肌酸激酶

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Effects of *Panax notoginseng* saponin Rg_1 on cardiac electrophysiological properties and ventricular fibrillation threshold in dogs

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AIM: To study the effects of Rg_1 isolated from saponins of *Panax notoginseng* on cardiac electrophysiological properties and ventricular fibrillation threshold (VFT). **METHODS**: Seventeen open-chest dogs were randomly allocated into a Rg_1 group (20 mg kg^{-1}, iv) and a control group. The electrophysiological variables and VFT were evaluated by standard electric stimuli and monophasic action potential (MAP) recording. **RESULTS**: Rg_1 prolonged sinus node recovery time (SNRT) by 19.1 %, AV conduction Wenckebach cycle length (AVWCL) by 7.1 %, and ventricular effective refractory period (VERP) by 7.9 %. It prolonged ventricular $MAPD_{30}$, $MAPD_{50}$, and $MAPD_{90}$ by 25.5 %, 24.2 %, and 13.5 %, respectively. VFT was increased by 19.2 %. **CONCLUSION**: Rg_1 prolonged ventricular refractoriness and repolarization, and increased VFT. It was indicated that cardiac electrophysiological effects of Rg_1 were similar to those of amiodarone.

KEY WORDS *Panax notoginseng*; panaxatriol saponins; anti-arrhythmia agents; electrophysiology; action potentials; ventricular fibrillation

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Extract of the root of *Panax notoginseng* (Burk) F H Chen mainly contains saponins (PNS). Panaxatriol saponins (PTS) are isolated from total saponins of PNS and PNS saponin Rg_1 is the product of further purification. The cardiac electrophysiological effects of PNS or PTS have been studied in mouse, rat, rabbit, or isolated guinea pig papillary muscles or sheep cardiac Purkinje fibers[1-6], as well as the *in vitro* studies on Rg_1[7]. In this paper, the effects of saponin Rg_1 on cardiac electrophysiological properties and ventricular fibrillation threshold (VFT) in dogs were evaluated by the technic of standard electric stimuli and monophasic action potential (MAP) recording.