目的：检测 ACE 抑制剂对主动脉平滑肌细胞内 Ca^{2+} 的影响. **方法**：用荧光标计和图象处理技术. **结果**：SHR 细胞内 Ca^{2+} 以及 KCl, NE 和 Ang 在 SHR 细胞引起的 Ca^{2+} 增加多于 WKY 细胞. Cap 和 Ena 不影响 KCl 和 Ang 在 WKY 细胞的作用，但 Cap, Ena 和 Nif 抑制 KCl, NE 和 Ang 在 SHR 细胞的作用. **结论**：Cap 和 Ena 阻断功能和特异性已改变的电压依赖性钙通道.

BIBLID: ISSN 0253-9756 Acta Pharmacologica Sinica 中国药理学报 1996 Mar; 17 (2): 145 - 148

Orthogonal analysis of aggravating effects of α-, β-agonists and leukocytes on reperfusion-induced arrhythmias and ventricular fibrillation in Langendorff's rat heart

DAI De-Zai, AN Lu-Fan, HUANG Dan-Yang, SUN Rui-Yuan[1]
(*Research Division of Pharmacology, China Pharmaceutical University, Nanjing 210009, China*; [1]*Department of Pharmacology, Wannan Medical College, Wuhu 241001, China*)

KEY WORDS myocardial reperfusion injury; arrhythmia; ventricular fibrillation; phenylephrine; isoproterenol; cyclophosphamide; leukocyte

AIM: To study the effects of leukocyte (Leu), α-agonist (α-Ago), and β-agonist (β-Ago) on the arrhythmias induced by ischemia and reperfusion to determine which of the 3 factors was the most important one in exacerbating arrhythmias. **METHODS**: Arrhythmias were induced by the reduction and subsequent resumption of perfused flow in Langendorff's perfused rat hearts. Ventricular tachycardia (VT) and ventricular fibrillation (VF) were recorded on ECG, and the results were orthogonally analyzed. **RESULTS**: When Leu was present, the incidence of VF induced by ischemia-reperfusion was 80 %. The incidence in Leu-depleted hearts was 20 %, α-Ago and β-Ago elevated it to 60 % and 100 %, respectively. The results by orthogonal analysis demonstrated Leu or α-Ago + β-Ago increased VF incidence. With regard to arrhythmias, arrhythmia score was remarkedly increased by all of 3 factors and various combinations except β-Ago + Leu. **CONCLUSION**: Among these 3 factors, Leu was the most important one in facilitating reperfusion-induced arrhythmias.

Ischemia-reperfusion arrhythmias are related to the elevation of cAMP, the formation of lysophosphatides, the genesis of free oxygen radicals, disturbances of ionic homeostasis, leukocyte activation, and the stimulation of α- and β-adrenoreceptors[1-6]. Depletion of leukocyte in reflow blood produced a reduced incidence of arrhythmias induced by ischemia-reperfusion[5], which induced a significant release of endogenous catecholamines that intensify arrhythmias by the stimulation of α- and β-adrenoreceptors.

Isolated rat heart was used to study the effects of leukocyte, α-Ago and β-Ago on the arrhythmias induced by ischemia and reperfusion in the present experiment, and the results were orthogonally analyzed to determine which of the 3 factors was the most important in terms of exacerbating arrhythmias.

MATERIALS AND METHODS

Experimental protocol Sprague-Dawley rats (230 ± 20 g) were used to prepare the Langendorff's heart at a perfusion pressure of 9.3 kPa. The perfusate was composed (mmol · L^{-1}): NaCl 128, KCl 4.7, $CaCl_2$ 2.5, $MgCl_2$ 1.2, NaH_2PO_4 0.8, $NaCO_3$ 12.5, glucose 11.0, gassed with O_2 (pH 7.4 ± 0.5, 38 ℃). The heart was initially given 15-min aerobic perfusion, and global ischemia was produced by reducing the perfusion flow to one tenth for 10 min. Then the perfusion flow was resumed for 10 min (reperfusion).

Throughout the experimental period epicardial ECG was recorded to analyze the arrhythmia score[7] and the incidences

Received 1994-02-01 Accepted 1995-05-12

and durations of ventricular fibrillation (VF) and ventricular tachycardia (VT)[8]. Phenylephrine (Phe, 10 μmol·L^{-1}) and isoproterenol (Iso, 10 μmol·L^{-1}) were added into perfusate 5 min before the onset of ischemia. Leu depletion was achieved by cyclophosphamide (Cyc) 100 mg·kg^{-1} ip for 3 d prior to experiment.

Design of orthonal analysis Reperfusion arrhythmias induced by low perfusion of Langendorff's rat heart were assayed orthogonally for 3 factors (Leu, α-Ago, β-Ago) and the interactions at 2 levels (1-in its absence and 2-in its presence), arranged in 8 groups as Tab 1.

The less Ischemia-reperfusion injury model A 7-min-low perfusion protocol was adopted for assaying effect of α-Ago and β-Ago in the presence of normal Leu count under less ischemia-reperfusion injury which was assessed by the reduced arrhythmias and VF.

Assay of SOD activity The SOD activity in myocardium was assayed by the 'adrenaline autooxidation method'[9].

Statistics All values were expressed as $\bar{x} \pm s$. The significance of differences was assessed using t-test or Mann-Whitney u-test. Changes in the incidence of events were analyzed by χ^2 test.

RESULTS

Effect of Phe and Iso on arrhythmias After Cyc 100 mg·kg^{-1} for 3 d, the leukocyte count was decreased by 90 %. The incidence of VF and VT were 20 % and 30 %, repectively, after 10 min of global ischemia. Phe 10 μmol·L^{-1} resulted in a significant elevation of VF incidence (60 %, $P<0.05$), without significant change in VF duration and arrhythmia score. Iso 10 μmol·L^{-1} obviously exacerbated the reperfusion induced arrhythmia scores (from 2.6 ± 2.5 to 6.0 ± 0, $P<0.05$) and VF incidence and duration. The VF incidence increased from 20 % to 100 % ($P<0.05$), and the duration from 1.1 ± 2.5 to 5.0 ± 4.0 min ($P<0.05$). However, they were not significantly influenced by a combination of Phe and Iso.

Orthogonal analysis of exaggerating effect of α-Ago, β-Ago, and Leu on 10-min ischemia-reperfusion arrhythmias Reperfusion-induced arrhythmias could be aggravated by all of the 3 factors and various combinations except β-Ago + Leu (Tab 1).

VF incidence data were listed and calculated by 2 choices in Tab 2.

(1) If two minima were chosen, $nd = 2$, $\Sigma d^2 = 20^2 + 20^2 = 800$, $Se' = (\Sigma d^2/n)^{0.5} = 20$, $t_{0.05} = 4.303$, $t_{0.01} = 9.925$. β-Ago, Leu or β-Ago + α-Ago increased VF incidence ($P<0.05$).

(2) If four minima were chosen, $nd = 4$, $\Sigma d^2 = 60^2 + (-60)^2 + 20^2 + 20^2 = 8000$, $Se' = (\Sigma d^2/n)^{0.5} = 44.72$, $f = 4$, $t_{0.05} = 2.775$, $t_{0.01} = 4.604$. Among the 3 factors Leu was only one increasing the VF incidence.

Influence of Phe and Iso on arrhythmias by less severe ischemia-reperfusion injury In the control-2 group with normal Leu count, the arrhythmia

Tab 1. Orthogonal analysis of influence of α-agonist (A), β-agonist (B), and leukocyte (C) on the arrhythmia scores of reperfusion arrhythmias in isolated rat heart. $^aP>0.05$, $^bP<0.05$ by F test.

No	A	B	C	AB	AC	BC		Arrhythmia score	Total	$\bar{x}$	s
1	1	1	1	1	1	1	1	6 6 4 1 1 2 1 2 1 2	26	2.6	2.5
2	1	1	2	1	2	2	2	6 6 6 6 6 6 6 6 2 2	52	5.2	1.7
3	1	2	1	2	1	2	2	6 6 6 6 6 6 6 6 6 6	60	6.0	0
4	1	2	2	2	2	1	1	6 6 6 6 6 6 6 6 6 6	60	6.0	0
5	2	1	1	2	2	1	2	6 6 6 6 6 6 4 3 3 1	47	4.7	1.8
6	2	1	2	2	1	2	1	6 6 6 6 6 6 6 6 6 6	60	6.0	0
7	2	2	1	1	2	2	1	6 6 6 4 1 6 6 4 6 1	46	4.6	2.1
8	2	2	2	1	1	1	2	6 6 6 6 6 6 6 6 6 6	60	6.0	0
T_1	198	185	179	184	206	193	192				
T_2	213	226	208	227	205	213	219				
D	15	41	53	43	1	25	27				
t	12	32	41	33	0.8	19	21				
P	b	b	b	b	a	b	b				

$D = |T_1 - T_2|$

$Se = (\Sigma d^2/n)^{0.5}$, $t = D/Se$

Tab 2. Orthogonal analysis of influence of α-agonist (A), β-agonist (B), and leukocyte (C) on VF incidence after reperfusion in isolated rat heart. $^{a}P>0.05$, $^{b}P<0.05$ by F test.

No	A	B	C	AB	AC	BC		VF incidence/ %
1	1	1	1	1	1	1	1	20
2	1	1	2	1	2	2	2	80
3	1	2	1	2	1	2	2	100
4	1	2	2	2	2	1	1	100
5	2	1	1	2	2	1	2	60
6	2	1	2	2	1	2	1	100
7	2	2	1	1	2	2	1	60
8	2	2	2	1	1	1	2	100
T_1	300	260	240	260	320	280	280	
T_2	320	360	380	360	300	340	340	
D	20	100	140	100	20	60	260	
t	1	5	7	5	1	3	3	
P	a	b	b	b	a	b	b	
t'	0.45	2.24	3.13	2.24	0.45	1.34	1.34	
P	a	a	b	a	a	a	a	

score resulting from 7-min ischemia-reperfusion was still higher than the control-1 group (Leu depleted). Phe was effective to increase in arrhythmia score and VF duration, but no increase by iso or Phe + Iso was observed (Tab 3).

Tab 3. Influence of phenylephrine (Phe, 10 μmol·L^{-1}) and isoprenaline (Iso, 10 μmol·L^{-1}) on reperfusion-induced arrhythmias after 7-min ischemia in Langendorff's rat hearts. $\bar{x}\pm s$. $^{a}P>0.05$, $^{b}P<0.05$ *vs* control-1; $^{d}P>0.05$, $^{e}P<0.05$ *vs* control-2. Control-1: Leu depleted heart was subjected to 10-min ischemia; Control-2: Normal Leu heart was subjected to 7-min ischemia.

Group	Incidence of VF/ %	Duration of VF/ min	Incidence of VT/ %	Duration of VT/ min	Arrhythmia score
Control-1	20	1.1±2.5	30	0.4±0.9	2.6±2.5
Control-2	40^{a}	1.2±2.7^{a}	90^{b}	1.3±1.4^{a}	4.9±1.0^{b}
Phe	80^{d}	6±3^{e}	80^{d}	1.3±0.9^{d}	5.4±1.5^{e}
Iso	40^{d}	2±4^{d}	60^{d}	3±3^{d}	3.9±2.1^{d}
Phe + Iso	60^{d}	1.4±2.2^{d}	70^{d}	4±4^{d}	4.1±2.7^{d}

Influence of ISO, Phe, and Leu depletion on myocardial SOD activity SOD activity (U/mg wet wt) in normally perfused isolated heart was 2.6 ± 0.4, and significantly reduced to 1.34 ± 0.18 ($P<0.01$) after 10 min of ischemia and reperfusion. The reduction in SOD activity was not changed by Phe (1.24 ± 0.16) and Iso (1.32 ± 0.26), however being worse by Phe + Iso (1.00 ± 0.13), compared with that of heart without treatment (1.34 ± 0.18).

In 3 Leu depleted groups: Leu depleted only (2.12 ± 0.27), Leu depleted + Iso (1.9 ± 0.4), and Leu + Phe (1.9 ± 0.5), the SOD activity was increased after ischemia-reperfusion ($P<0.01$).

DISCUSSION

Endogenous catecholamines stimulating the adrenergic receptors may be involved in the genesis of arrhythmias induced by ischemia-reperfusion[1,10-13]. By orthogonal analysis it is also true that arrhythmias in Langendorff's rat heart were aggravated by α-Ago, β-Ago and Leu as well. In Leu depleted heart, the intensified arrhythmias could be caused by α-Ago, β-Ago, and α-Ago + β-Ago. Under this condition β-Ago might be a more important factor than α-Ago participating in the reperfusion arrhythmias. When Leu was present, and arrhythmia-facilitating effect of both β-Ago and its combination with α-Ago were obvious, but no effect with α-Ago alone. With regard to VF incidence, β-Ago, Leu or combination of the 2 exerted a facilitating effect, furthermore, Leu was a more important factor than β-Ago.

To observe the influence of these factors on arrhythmias and VF in normal Leu heart suffering from less severe ischemia injury (7 min of ischemia), it was further emphasized by an increased arrhythmia score versus 10 min of ischemia of Leu depleted heart that the presence of normal Leu count was an important factor risk for development of cardiac dysarrhythmias. β-Ago was not as significant as α-Ago in terms of initiating arrhythmias in less severe ischemia injury, implying that α-antagonist might be more useful than β-antagonist in the treatment of arrhythmias caused by mild ischemia insult in clinical pratice.

Free radicals released through antooxidation of catecholamines may be responsible for its arrhythmogenic potential of catecholamine[1,14]. It is interesting that Phe and Iso exacerbated the arrhythmia,

but did not exerted any detectable influence on myocardial SOD activity in the meantime. These observations suggested that stimulation of adrenergic receptors may be a direct factor involved in the genesis of arrhythmia and VF.

Leu played an important role in the genesis of arrhythmias and VF, but its exacerbating effect on VF might be attenuated in the presence of α-Ago or β-Ago. This indicated that Leu exerted its effect through some different route.

Implying from data assayed by orthogonal analysis, it might be a beneficial intervention to reduce Leu count temporarily in patients suffering from severe myocardial ischemia injury, who are at risk of developing life-threatening arrhythmias.

REFERENCES

1 Tosaki A, Woodward B, Yamamoto F, Hearse DJ. Isoproterenol and the genesis of reperfusion-induced arrhythmias in isolated rat heart: adrenoceptor or free radical-mediated mechanism? J Cardiovasc Pharmacol 1990; **15**: 398 - 407.

2 Frey MJ, Molinoff PB. Mechanisms of downregulation of β-adrenergic receptors: perspective on the role of β-adrenergic receptors in congestive heart failure. J Cardiovasc Pharmacol 1989; **14** Suppl 5: S13 - S18.

3 Chess-Williams R, Coker SJ. Ventricular fibrillation is reduced in hypothyroid rats with enhanced myocardial α-adrenoceptor responsiveness. Br J Pharmacol 1989; **98**: 95 - 100.

4 Ohyanagi M, Matsumori Y, Iwasaki T. β-Adrenergic receptors in ischemic and non-ischemic canine myocardium: relation to ventricular fibrillation and effects of pretreatment with propranolol and hexamethonium. J Cardiovasc Pharmacol 1988; **11**: 107 - 14.

5 Engler RL, Dahlgren MD, Morris DD, Peterson MA, Schmid-Schonbein GW. Role of leukocyte in response to acute myocardial ischemia and reflow in dogs. Am J Physiol 1986; **251**: H314 - H322

6 Werns W, Lucchesi BR. Leukocytes, oxygen radicals, and myocardial injury due to ischemia and reperfusion. Free Radical Biol Med 1988; **4**: 31 - 7.

7 Dai DZ, Rong P, Huang J, Liu J, Cheng JH, Chen YH, *et al*. Anti-arrhythmic activities of six indole derivatives of changrolin. Acta Pharmacol Sin 1991; **12**: 411 - 5.

8 Simpson PJ, Mickelson J, Fantone JC, Gallagher KP, Lucchesi BR. Iloprost inhibits neutrophil function *in vitro* and *in vivo* and limits experimental infarct size in canine heart. Circ Res 1987; **60**: 666 - 73.

9 Yuan QS, Wang ZY, Weng QQ. Determination of superoxide dismutase — a method epinephrine auto-oxidation. Biochem Pharm Manufac from Organs 1983; **3**: 4 - 7.

10 Carlsson L. Mechanisms of local noradrenaline release in acute myocardial ischemia. Acta Physiol Scand 1987; **129** Suppl 559: 1 - 85

11 Wilde AAM, Peters RJG, Janse MJ. Catecholamine release and potassium accumulation in the isolated globally ischemic rabbit heart. J Mol Cell Cardiol 1988; **20**: 887 - 96.

12 Rong P, Dai DZ, Zhang JE. Global depletion of myocardial norepinephrine and ATP after left coronary artery occlusion in rats. Acta Pharmacol Sin 1992; **13**: 333 - 7.

13 Paletta MJ, Abraham S, Beatch GN, Walker MJA. Mechanisms underlying the antiarrhythmic properties of β-adrenoceptor blockade against ischemia-induced arrhythmias in acutely prepared rats. Br J Pharmacol 1989; **98**: 87 - 94

14 Werns SW, Shea MJ, Lucchesi BR. Free radicals and myocardial injury: pharmacologic implications. Circulation 1986; **74**: 1 - 5.

α,β-受体激动剂及白细胞对再灌注心律失常及室颤影响的正交分析

戴德哉，安鲁凡，黄丹阳，孙瑞元[1]
(中国药科大学药理研究室，南京 210009；
[1]皖南医学院药理教研室，芜湖 241000，中国)

关键词 心肌再灌注损伤；心律失常，心室纤颤；去氧肾上腺素；异丙肾上腺素；环磷酰胺；白细胞

目的：比较白细胞(Leu)，α-受体激动(α-Ago)和β-受体激动(β-Ago)三因素对缺血-再灌诱发的心律失常的影响. **方法**：采用 Langendorff 离体心脏模型，以降低灌流量继之恢复灌流量(模拟再灌注)诱发心律失常，对三因素进行正交分析. **结果**：对 Leu 未耗竭及已耗竭的心脏，再灌注诱发的 VF 发生率分别为 80 % 和 20 %，α-及 β-Ago 明显升高 VF 发生率. 正交分析表明 Leu 明显提高 VF 发生率，三因素组合均增加心律失常分数. **结论**：三因素中 Leu 是最为重要的促进恶性心律失常发生的因素.