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四甲基吡嗪对犬体外循环中血小板及动脉血栓形成的抑制作用

R963

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提要 犬体外转流结束，四甲基吡嗪(川芎嗪，TMP，iv 10 mg · kg^{-1})使血小板计数为转流前的82.8%，对照组为56.7%，血小板表面α颗粒膜蛋白分子数显著低于对照组($P<0.01$)。制备犬股动脉血栓模型4 h，TMP处理组离体血栓的重量为对照组的23%，而血栓与血液的放射活性比值仅为对照组的29%。提示TMP能抑制体外循环中血小板的活化及动脉血栓的形成。

关键词 四甲基吡嗪；血小板活化；心肺转流术；血栓形成；单克隆抗体；膜蛋白

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Global depletion of myocardial norepinephrine and ATP after left coronary artery occlusion in rats[1]

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ABSTRACT After ligation of the left coronary artery in rats, myocardial norepinephrine (NE) and ATP depletions in both infarcted (IZ) and non–infarcted zone (NIZ) were studied. In IZ, the depletions of NE and ATP were biphasic and the depleting rate constants were found to be $K_1 = 0.71$ h^{-1} and $K_2 = 0.015$ h^{-1} for NE, and $K_1' = 0.52$ h^{-1} and $K_2' = 0.016$ h^{-1} for ATP. In NIZ, the depletion of NE was monophasic, slowly progressive, and quite durable with rate constant $K_3 = 0.018$ h^{-1}; The depletion of ATP was transient. Propranolol (Pro) and verapamil (Ver) were beneficial but only partly effective against NE and ATP depletions.

KEY WORDS myocardial infarction; propranolol; verapamil; norepinephrine; epinephrine; adenosine monophosphate; adenosine biphosphate; adenosine triphosphate

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The release of norepinephrine (NE) would

be out of control in myocardium suffering from severe ischemia[1]. We found that the depletion of NE occurred not only in infarcted zone (IZ) but also in non-infarcted zone (NIZ)[2] where the changes in bioactive substances seemed to have been scarcely investigated. The depletion of myocardial ATP is a sensitive index reflecting the extent of ischemia and the efficacy of anti-ischemic drugs[3]. Here we compared the time course of changes in NE with ATP in both IZ and NIZ, focusing on the NIZ and responses to Pro and Ver.

MATERIALS AND METHODS

Reagents and rats NE, epinephrine (E), ATP, ADP, and AMP were products of Sigma. Isoprenaline and the chemical reagents used were of AR grade. Wistar rats weighing 224 ± *s* 19 g were supplied by Shanghai Experimental Animal Center, Chinese Academy of Sciences.

Infarcted rat[2] Rats of either sex were subjected to surgical ligation of the left coronary artery for developing myocardial infarction . After closing the chest, 0.2 ml of gentamicin were injected sc. The rats were randomly grouped for ip Pro (8 or 16 mg · kg^{-1}), Ver (2 or 8 mg · kg^{-1}), and saline 0.4 ml at 30 min prior to operation. Measurements were carried out at 3, 6, 12, 24, and 96 h after myocardial infarction. The influence on NE by infarction was compared with beta-receptor activation by iv isoprenaline 100 $\mu g \cdot kg^{-1}$. Myocardial NE was measured at 3 h after iv isoprenaline.

Measuring myocardial NE and E The heart was excised and washed with icy saline. The ventricle was cut into the left and right portion corresponding to IZ and NIZ, respectively. A sample of myocardium about 300 mg was homogenized in 5 ml icy $HClO_4$ 0.4 mol · L^{-1} and an aliquot of 4.5 ml supernatant was taken for measuring NE and E. The fluorophore was formed by oxidation reaction with iodine and measured at wave-lengths of 387 / 470 and 450 / 520 nm[2].

Measuring myocardial ATP, ADP, and AMP Myocardial ATP, ADP, and AMP were extracted with $HClO_4$. The pH of supernatant was adjusted to 8. Supernatant (10 μl) was injected into the HPLC system[3].

Data derived (per g wet tissue) were analyzed by 3P87 program using IBM computer.

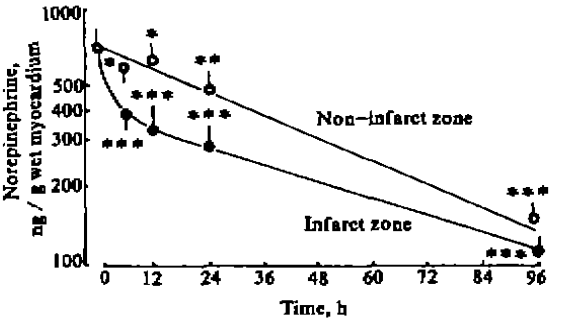

Fig 1. NE in myocardium after left coronary artery ligation. $n = 6$–12, $\bar{x} \pm s$. $^{*}P > 0.05$, $^{**}P < 0.05$, $^{***}P < 0.01$ *vs* **time 0.**

RESULTS

Depletion of NE In both IZ and NIZ the myocardial NE was markedly depleted. The first decline rate during 6 h was more prominent in the IZ than in NIZ with a depletion rate constant K_1 0.71 h^{-1} and the second phase of decline was slow with K_2 0.015 h^{-1} (Fig 1). The course of NE depletion in NIZ was gradual but progressive and durable in a monophasic manner with a rate constant K_3 0.018 h^{-1} (Fig 1). There was no significant changes in myocardial E (Tab 1).

Isoprenaline (iv 100 $\mu g \cdot kg^{-1}$) caused only a reduction of NE from 820 ± 170 ng · g^{-1}

Tab 1. Influence of propranolol (8 mg · kg^{-1}, ip) and verapamil (8 mg · kg^{-1}, ip) on epinephrine (ng / g wet myocardium) in infarcted zone (IZ) and non-infarcted zone (NIZ) of rat heart. $\bar{x} \pm s$.

Group	*n*	Epinephrine, ng / g wet myocardium	
		Infarcted zone	Non-infarcted zone
Control	11	75 ± 39	80 ± 56
After acute coronary ligation for 24 h			
Saline	6	58 ± 28	88 ± 31
Pro	12	88 ± 31	84 ± 54
Ver	6	41 ± 20	52 ± 26

wt to 720 ± 140 ng · g^{-1} in normal rats in contrast to the more significant reduction to 175 ± 140 ng · g^{-1} ($P<0.01$) in a heart infarcted for 9 d plus isoprenaline ($n=6$).

Rats were pretreated with Pro or Ver ip and killed 24 h after myocardial infarction. The prevention from NE-depleting response by drugs was within a range from 20 − 34% in IZ and 0 − 46% in NIZ (Tab 2).

Tab 2. Influence of propranolol (8 and 16 mg · kg^{-1}, ip) and verapamil (2 and 8 mg · kg^{-1}, ip) on norepinephrine (ng / g wet myocardium) in infarcted zone (IZ) and non-infarcted zone (NIZ) of rat heart. $\bar{x} \pm s$. $^{*}P>0.05$, $^{**}P<0.05$ vs saline group.

Group (mg · kg^{-1})	n	Norepinephrine, ng / g wet myocardium	
		Infarcted zone	Non-infarcted zone
Control	11	680 ± 120	760 ± 210
Infarcted for 24 h			
Saline	6	260 ± 80	410 ± 80
Pro 8	12	370 ± 80**	520 ± 100**
Pro 16	6	370 ± 60**	580 ± 90**
Ver 2	6	300 ± 100*	360 ± 110*
Ver 8	6	310 ± 130*	410 ± 90**

Depletion of ATP, ADP and AMP

High energy phosphates were measured at 0, 3, 6, 12, and 24 h after acute myocardial infarction (AMI) in both IZ and NIZ. The depletion of ATP in IZ followed the same pattern as that of NE. It was rapid and prominant with a depletion rate constant K_1' 0.52 h^{-1} during the first 6 h and then the succeeding decline was less steep, its rate constant K_2' being 0.016 h^{-1} (Fig 2). The decline of myocardial ATP in NIZ returned to normal at about 24 h (Fig 2).

ATP depletion was accompanied by a transient rise of myocardial ADP and AMP resulting from degradation of ATP during the first 3 h following AMI (Fig 3). ADP and AMP, then, decreased, showing a time lag between the ligation and depletion. If taking the sum of high energy phosphates in myocardium into account, the depletion induced by ischemia was notable and more even. Both Pro and Ver exerted their protective effects in both IZ and NIZ after having been ligated for 24 h (Tab 3).

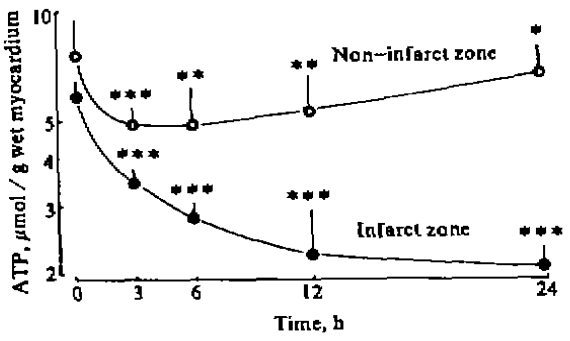

Fig 2. ATP level in myocardium after left coronary artery ligation. $n=9-10$, $\bar{x} \pm s$. $^{*}P>0.05$, $^{**}P<0.05$, $^{***}P<0.01$ vs time 0.

The protective effect of Pro or Ver on myocardial ATP was not substantial until 24 h after ligation. However, the prevention of AMP from depletion occurred as early as 6 h in NIZ.

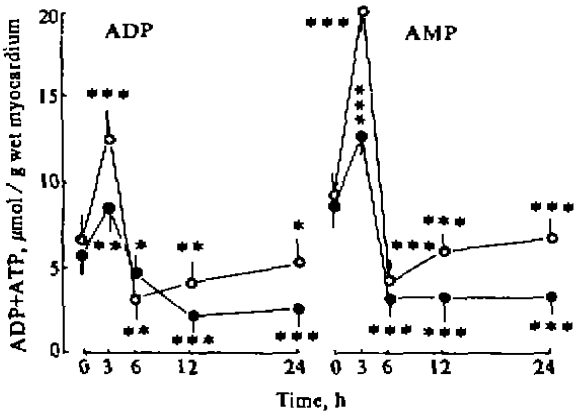

Fig 3. Myocardial ADP and AMP after left coronary artery ligation. $n=6-10$, $\bar{x} \pm s$. NIZ (○): non-infarct zone, IZ (●): infarct zone. $^{*}P>0.05$, $^{**}P<0.05$, $^{***}P<0.01$ vs time 0.

Tab 3. Effects of propranolol (Pro 8 mg · kg^{-1}, ip) and verapamil (Ver 8 mg · kg^{-1}, ip) on ATP, ADP, and AMP in infarcted zone (IZ) and non-infarcted zone (NIZ) of rat heart. $\bar{x} \pm s$. $^{*}P>0.05$, $^{**}P<0.05$, $^{***}P<0.01$ *vs* **saline group.**

Group	*n*		ATP	ADP (μmol / g wet myocardium)	AMP
Normal	10	IZ	5.8 ± 1.6	5.7 ± 2.0	10 ± 4
		NIZ	7.5 ± 2.0	6.6 ± 2.0	9.7 ± 4.4
6 h following myocardial infarction					
Saline	6	IZ	2.7 ± 1.2	4.2 ± 2.7	3.2 ± 2.1
		NIZ	5.0 ± 1.2	3.3 ± 0.8	3.9 ± 0.5**
Pro	6	IZ	3.5 ± 0.7*	4.2 ± 1.7*	6.4 ± 1.2**
		NIZ	6.9 ± 1.7**	5.6 ± 1.3*	9.5 ± 3.0***
Ver	6	IZ	3.6 ± 1.2*	4.8 ± 2.1*	5.6 ± 3.0*
		NIZ	5.8 ± 2.1*	7.5 ± 4.0**	11 ± 5***
12 h following myocardial infarction					
Saline	6	IZ	2.5 ± 1.0	2.1 ± 0.9	3.0 ± 1.8
		NIZ	5.2 ± 1.4	4.3 ± 2.1	6 ± 3
Pro	8	IZ	4.4 ± 1.9*	5.9 ± 2.9***	6 ± 3**
		NIZ	4.0 ± 1.4*	3.9 ± 0.8*	4.4 ± 2.2*
Ver	6	IZ	3.2 ± 1.6*	3.6 ± 2.7*	7 ± 3**
		NIZ	7.4 ± 1.2**	9.0 ± 2.9***	18 ± 7***
24 h following myocardial infarction					
Saline	6	IZ	2.0 ± 0.4	2.4 ± 0.7	3.4 ± 1.5
		NIZ	6.0 ± 1.8	5.4 ± 1.5	5.6 ± 1.4
Pro	6	IZ	4.1 ± 1.8**	3.6 ± 2.6*	7 ± 3**
		NIZ	6.0 ± 2.0*	4.6 ± 1.2*	8.5 ± 2.8***
Ver	6	IZ	3.6 ± 0.9**	3.1 ± 0.7**	8 ± 4**
		NIZ	5.8 ± 1.8*	5.2 ± 1.1*	11 ± 4***

DISCUSSION

The NE depletion phenomena at the IZ[4-6] may exert its effect on electric homogeneity which is closely related to arrhythmogenesis and myocardial necrosis[7,8]. The depletion of NE and ATP was not limited to the area where the blood supply was cut off by ligation. We confirmed that NE depletion also took place in the NIZ[9] where myocardial NE was maintained at a low level in the chronically infarcted model[1,9]. It seemed that the depletion of bioactive substances like ATP and NE may be a global response to an infarcted lesion in the rat heart. A scar formed in IZ may turn the surrounding myocardium and even those at a distance into a depleted state. The findings suggested that the dysfunctional myocardium in an infarcted heart could be extended to the normally perfused area where the level of bioactive substances was found also abnormal.

The NE depletion by iv isoprenaline in normal rats was much less than those caused by infarction and Pro could partially prevent the myocardium from NE-depletion caused by infarction indicating that beta-receptor activation might be involved to some extent in the mechanism responsible for NE depletion. This was compatible with the increased NE-release by the partial agonist pindolol[10]. Ver was also able to reduce the extent of depleted state in NIZ suggesting that this phenomenon could be related to an inflow of calcium ion into cytosol.

The ATP, ADP and AMP are the chief source of energy supply which can be pre-

served in an infarcted heart by interventions with Pro and Ver. A more appropriate view of myocardial energy supply can be offered by the determination of the 3 high energy nucleotides than by that of ATP alone. The rapid disappearance of myocardial ATP during the early period after coronary occlusion accelerated the accumulation of ADP and AMP to form a peak which disappeared 6 h after infarction.

The difference in depletion of ATP and NE in NIZ may reflect the fact that the energy supply of myocardium is normal. However, the response to sympathetic neurotransmitter could be altered by depletion of myocardial NE.

The increased release of NE in an infarcted heart is considered to be arrhythmogenic but still controversial[11]. Prevention of the NE-depleting phenomena by lidocaine is responsible for its anti-arrhythmic effect on the infarcted rat heart[12]. The sustained reduction in NE but not in ATP could be one of the biochemical bases contributing to the vulnerability[13] of a chronically infarcted heart to fibrillation.

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大鼠结扎左冠脉后全心性去甲肾上腺素和腺苷三磷酸的耗竭

R963

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提要 结扎大鼠左冠脉的心梗模型，观察心肌内 NE 和 ATP 的排空，梗死区中二者的排空均呈双相性. NE 的排空速率常数为 $K_1=0.71\ h^{-1}$ 和 $K_2=0.015\ h^{-1}$; ATP 的排空速率常数为 $K_1'=0.52\ h^{-1}$ 和 $K_2'=0.016\ h^{-1}$. 非梗死区 NE 的排空呈单相而持久，速率常数为 $K_3=0.018\ h^{-1}$; ATP 的排空呈一过性. 普萘洛尔及维拉帕米均可改善 NE 和 ATP 的耗竭.

关键词 心肌梗死; 普萘洛尔; 维拉帕米; 去甲肾上腺素; 肾上腺素; 腺苷一磷酸; 腺苷二磷酸; 腺苷三磷酸