以及 ADP、凝血酶和胶原引起血小板聚集和 NPY 释放的差别. 方法: 应用放射免疫分析法测定血小板及血浆中 NPY 的含量. 结果: SHR 和 WKR 大鼠血浆 NPY 含量无明显差别, 分别为 2.1±1.0和1.8±1.0 $\mu g \cdot L^{-1}$, 而 SHR 血小板中 NPY 含量(32±6 $\mu g \cdot L^{-1}$)显著高于 WKY 大鼠(22±9 $\mu g \cdot L^{-1}$). 凝血酶和胶原可引起血小板聚集和 NPY 释放, SHR 显著高于 WKR 大鼠; 而 ADP 虽可引起血小板聚集, 却不引起 NPY 释放. 结论: SHR 血小板中 NPY 含量增高、释放增多.

关键词 血小板; 血小板聚集; 神经肽 Y; 近交 SHR 大鼠; 近交 WKY 大鼠; 凝血酶; 胶原

BIBLID: ISSN 0253-9756 Acta Pharmacologica Sinica 中国药理学报 1995 Mar; 16 (2): 152—155

Effects of anisodamine against myocardial ischemia-reperfused injuries and antilipid peroxidation

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AIM: Anisodamine (Ani), an alkaloid first isolated in China. To study the relationship between the protective effects of Ani on myocardial cells of reperfused injuries and the antilipid peroxidation. **METHODS**: Coronary ligation for 15-min followed by 10 min reperfusion was performed in anesthetized rats. **RESULTS**: Ani 1, 3, 5 $mg \cdot kg^{-1}$ iv 1 min prior to reperfusion could dose-dependently lower the release of creatine kinase (282±29, 252±53, 226±50), counteract the increase of malondialdehyde content (3.3±1.3, 3.2±1.6, 3.1±1.2) in the reperfused myocardium and preserve the SOD activity (41±7, 46±8, 55±8). Ani completely abolished the drop in the contents of principal unsaturated fatty acids (oleic, linoleic, and arachidonic acids) of the membrane lipids in the reperfused myocardium. SOD 75 $U \cdot kg^{-1}$ iv 1 min prior to reperfusion exerted similar effects like Ani 3 $mg \cdot kg^{-1}$. **CONCLUSION**: It is suggested that the antilipid peroxidative effect of Ani may contribute to its protection against reperfusion-induced myocardial injuries.

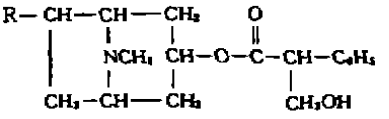
KEY WORDS anisodamine; superoxide dismutase; myocardial reperfusion injury; creatine kinase; malondialdehyde; unsaturated fatty acids; lipid peroxidation; atropine derivatives

Received 1992-10-15 Accepted 1994-06-25

Anisodamine (Ani), an alkaloid first extracted from *Hyoscyamuse niger* L in China, is very similar to atropine in chemical structure. It showed protective effects against certain experimental arrhythmias[1] and of ischemia-reperfusion arrhythmias[2, 3] and improved the cardiac performance during reperfusion[3]. We observed that the protective effects of Ani against reperfusion induced arrhythmia may have something to do with reduced myocardial lipid peroxidation[4].

In order to ascertain the relationship between the protective effects of Ani on myocar-

dial cells of reperfused injuries and the anti-lipid peroxidation, the present study on anesthetized rats, determined creatine kinase (CK) release, mycardial malondialdehyde (MDA) contents, superoxide dismatase (SOD) activity, and unsaturated fatty acids (UFA) of the membrane lipid in myocardial reperfused injuries.



R= − OH Anisodamine

R= − H Atropine

MATERIALS AND METHODS

Agents Ani (Beijing Pharmaceutical Factory), SOD (Changsha Biochemical Pharmaceutical Factory), arachidonic acid (Shanghai Biochemical Reagent Plant), 1, 1, 3, 3-tetraethoxypropane, creatine phosphate sodium salt (Merck), oleic acid, linoleic acid, linolenic acid (Sigma). All the reagents were of AR grade.

Instruments Ultraviolet spectrophotometer, and MDF-4 fluorescent spectrophotometer (Japan). 106 G gas chromatography and hydrogen flame ionization detector (Shanghai Analysis Instrument Plant) gas chromatograph-mass spectrometer-QP 1000 (Shimadzu GC-MS-QP 1000).

Ischemic-reperfusion injuries and grouping Sprague-Dawley ♂ rats, weighing 228 ± s 39 g, were anesthetized with pentobarbital sodium 45 $mg \cdot kg^{-1}$ ip. The right carotid artery was cannulated for recording the blood pressure. Thereafter, the rats were carried out artificial respiration. Then the chest was opened, the heart was gently supported, and the coronary artery was ligated as described previously[4]. A 15-min regional ischemia was established, then reperfusion was maintained for 10 min. The ventricular blood was taken at the end of the reperfusion, and serum CK contents were determined with creatine coloration technique[5]. The heart was quickly cut open and part of the ischemic reperfused myocardium was excised and cut into pieces. MDA, SOD and UFA were assayed. The above described processes were finished within 48 h. Ischemic myocardium was proved with nitroblue tetrazolium stain.

Seventy rats were randomly assigned into 7 groups the sham operation group underwent all the procedures processes except ligation of coronary artery. In the coronary artery occlusion (CO) group, blood, and heart were taken out at the end of 15 min ligation. The rest 5 groups were reperfused after ischemia. These 5 groups were given iv Ani 1, 3, 5 $mg \cdot kg^{-1}$, SOD 75 $U \cdot kg^{-1}$ and normal saline (NS), respectively, 1 min prior to reperfusion.

Measurements of MDA contents and SOD activity[4, 6] Myocardial protein content was determined colorimetrically[7].

Measurement of UFA in myocardial membrane lipids Myocardial fluidity sample was mixed with $(CH_3)_2CHOH$, $CHCl_3$, and H_2O (11:7:1) and was extracted, replicated twice, and dried with nitrogen[8, 9]. CH_3OH, C_6H_6, and NaOH (30 mL: 20 mL: 1 g) were added to extract again. Fatty acid methyl ester[10] was thus formed. Oleic acid ($C_{18:1}$) 1 g, linoleic acid ($C_{18:2}$) 1 g, linolenic acid ($C_{18:3}$) 1 g, arachidonic acid ($C_{20:4}$) 1 g, methanol 4 mL, and 50 % H_2SO_4 1 mL were added and warmed at 56 C for 30 min. Hexane was added, and standard fatty acid methyl ester was extracted twice and replicated to remove the solvent. Hexane (0.2 mL) was added again and the sample (3.0 μL) was analyzed in a gas chromatographer.

The reserved time of standard fatty acid methyl ester was compared with that of myocardial membrane. The results showed that in myocardial membrane lipids of SD rats the principal UFA were ($C_{18:1}$), ($C_{18:2}$), and ($C_{20:4}$). These were further verified by GC-MS-QP 1000.

Coefficients of variation of $C_{18:1}$, $C_{18:2}$, and $C_{20:4}$ were 5.0 %, 3 %, and 1.1 %, respectively, in the myocardial membrane lipids.

RESULTS

Effects of Ani and SOD on CK release in reperfused myocardium The CK release of ischemic myocardium increased significantly in comparison with that of the sham operation group ($P < 0.05$). The CK release of NS

increased too (*vs* the ischemic group, $P<0.01$). The values in groups of Ani 1, 3, 5 mg·kg^{-1} and SOD 75 U·kg^{-1} decreased markedly in reperfused myocardium (*vs* that of NS, $P<0.01$) and the lowering of the release of CK ($r=-0.999$, $P<0.05$) seemed to be dose-dependent. The Ani groups showed no difference comparing with SOD group (Tab 1).

Tab 1. Effects of Ani and SOD injected iv 1 min prior to reperfusion (R) on myocardial creatine kinase (CK) release, MDA content, and SOD activity in anesthetized rats subjected to 15-min coronary occlusion (CO) followed by reperfusion. $n=10$, $\bar{x}\pm s$. $^bP<0.05$, $^cP<0.01$ *vs* **normal saline (NS).** $^eP<0.05$, $^fP<0.01$ *vs* **CO.**

	CK activity U·L^{-1}	MDA content nmol/mg protein	SOD activity U/mg protein
Sham operation	249±26^e	2.9±1.2^e	32±5^e
CO	281±32	4.0±0.8	26±6
CO+R; NS	347±53^f	5.0±1.2^e	20±6^e
Ani 1 mg·kg^{-1}	282±29^c	3.3±1.3^b	41±7^c
3 mg·kg^{-1}	252±53^e	3.2±1.6^b	46±8^c
5 mg·kg^{-1}	226±50^e	3.1±1.2^c	55±8^f
SOD 75 U·kg^{-1}	254±46^e	3.3±1.5^b	51±8^e

Effects of Ani and SOD on the MDA in reperfused myocardium The content of MDA in ischemic myocardium was increased (*vs* the sham operation group, $P<0.05$) and the content of NS increased too, *vs* the ischemic myocardium group ($P<0.05$). But the MDA values of Ani groups were depressed markedly ($P<0.05$ or $P<0.01$) with increasing dosage ($r=-0.99$). The MDA values in the SOD group with the same dose decreased too ($P<0.05$). There were no marked differences in MDA values of SOD group *vs* the Ani groups (Tab 1).

Effects of Ani and SOD on internal SOD activity in reperfused myocardium When the ischemic myocardium groups were subjected to coronary occlusion, their SOD activity decreased obviously *vs* sham operation group ($P<0.05$). The values of NS group decreased even more marked (*vs* the ischemia group $P<0.05$). However, the SOD activity of Ani groups was higher (*vs* NS group $P<0.01$) and was dose-dependent ($r=0.992$). The values in the SOD group increased markedly *vs* NS ($P<0.01$) and Ani 1 mg·kg^{-1} ($P<0.05$), and showed no sharp difference *vs* Ani 3 mg·kg^{-1} (Tab 1).

Effects of Ani and SOD on UFA in membrane lipids of reperfused myocardium The contents of principal UFA-$C_{18:1}$, $C_{18:2}$, $C_{20:4}$ decreased markedly in membrane lipids of ischemic myocardium group (*vs* sham operation group $P<0.05$ or $P<0.01$). These values of NS decreased further, with $C_{18:2}$ and $C_{20:4}$ reducing in comparison with the ischemia group in particular ($P<0.01$). Ani 1, 3 and 5 mg·kg^{-1} completely nullified the decrease of the UFA contents in reperfused myocardium. SOD 75 U·kg^{-1} had the same effect as that of Ani (Tab 2).

Tab 2. Effects of Ani and SOD injected iv 1 min prior to reperfusion (R) on unsaturated fatty acid contents (μg·mg^{-1}) of membrane lipids from anesthetized rat hearts subjected to 15-min coronary occlusion (CO) followed by R. $n=9$, $\bar{x}\pm s$. $^bP<0.05$, $^cP<0.01$ *vs* **normal saline (NS).** $^eP<0.05$, $^fP<0.01$ *vs* **sham operation.** $^aP>0.05$, $^fP<0.01$ *vs* **CO.**

	Oleic acid methyl ester	Linoleic acid methyl ester	Arachidonic acid methyl ester
Sham operation	1.1±0.2	2.6±0.3	5.0±0.3
CO	0.9±0.1^f	2.3±0.1^e	4.2±0.2^f
CO+R; NS	0.8±0.2^a	2.1±0.2^f	3.9±0.2^f
Ani 1 mg·kg^{-1}	1.1±0.1^c	3.1±0.2^c	5.1±0.3^c
3 mg·kg^{-1}	1.1±0.1^c	3.1±0.2^c	5.1±0.2^c
5 mg·kg^{-1}	1.3±0.1^c	3.6±0.1^c	6.3±0.2^c
SOD 75 U·kg^{-1}	1.0±0.2^b	2.7±0.2^c	4.8±0.2^c

DISCUSSION

Transient (15 min) regional ischemia induced by coronary artery ligation increased the myocardial CK release, elevated the MDA content and reduced the SOD activity in anesthetized rats. All the changes were exaggerated abruptly after reperfusion. These results supported the viewpoint[1] that myocardial reperfused injuries were closely related to oxygen free radicals. Ani (1-5 mg·kg^{-1}) administered iv 1 min prior to reperfusion dose-dependently counteracted all the above changes occurring during reperfusion. The effects revealed by SOD on reperfusion-induced injuries were similar to those by Ani. This suggested that Ani exerted protective effects against reperfusion-induced myocardial injuries which may be related to the inhibition of membrane lipid peroxidation. Thus alleviated the reperfused myocardial injuries.

The experiments also determined simultaneously the contents of the principal UFA $C_{18:1}$, $C_{18:2}$, $C_{20:4}$ in membrane lipids of myocardium. The results proved that Ani and SOD can abolish the decrease of the UFA contents in reperfused myocardium. It was apparent that Ani has protective effects on the regular structure of membrane lipids due to its antilipid peroxidation.

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山莨菪碱对心肌缺血再灌注损伤的作用与抗脂质过氧化

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目的: 研究山莨菪碱对心肌缺血再灌注损伤的作用与抗脂质过氧化的关系. **方法**: 在麻醉大鼠心肌缺血15 min 再灌注10 min 模型上, 于再灌注前1 min iv 山莨菪碱(Ani) 1, 3, 5 mg·kg^{-1}. **结果**: 减少心肌 CK 的释放和 MDA 含量的升高, 保持SOD 活性, 并完全阻止再灌注心肌膜中油酸, 亚油酸和花生四烯酸的减少. SOD 75 U·kg^{-1}的作用与 Ani 3 mg·kg^{-1}相当. **结论**: Ani 的抗脂质过氧化可能是其保护再灌注心肌的机制.

关键词 山莨菪碱; 超氧化物歧化酶; 心肌再灌注损伤; 肌酸激酶; 丙二醛; 不饱和脂肪酸; 脂质过氧化; 阿托品衍生物