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半胱胺对刺激猫 C 传入纤维引起的屈反射易化的阻抑作用

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提要 电刺激麻醉猫的膝肠或腓肠肌神经 C 纤维，可易化皮肤刺激引起的后二头半腱肌神经细束的反射性放电。生长抑素静空剂半胱胺(50 mg·kg^{-1}, iv)明显减小易化效应，而对多突触反射的振幅没有影响。提示生长抑素可能参与脊髓伤害性信息的传递和调制。

关键词 伤害性感受器；脊髓；半胱胺；生长抑素

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Effect of ubiquinone on ischemic arrhythmia in conscious rats[1]

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ABSTRACT Ubiquinone 6.2, 12.5 or 2.5 mg·kg^{-1} respectively twice iv 24 h and 30 min before coronary artery ligation, ameliorated the ischemic arrhythmia in conscious rats, and there was a close positive correlation between the ubiquinone concentration in myocardium and plasma and its anti–arrhythmic effect. Ubiquinone iv 3.1, 6.2, and 12.5 mg·kg^{-1} increased, while 25 mg·kg^{-1}

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decreased 6-keto-$PGF_{1\alpha}$, and 12.5 and 25 mg · kg^{-1} decreased TXB_2, which was in accordance with inhibitory effects on the synthesis of 6-keto-$PGF_{1\alpha}$ and TXB_2 *in vitro*. But the ratio of metabolites of PGI_2/TXA_2 *in vivo* was increased in all ubiquinone groups. These results indicated that ubiquinone possesses protective effects on ischemic arrhythmia of conscious rats and the beneficial effects on myocardial ubiquinone content and PGI_2/TXA_2 seem to contribute to its myocardial protective action.

KEY WORDS ubiquinone; myocardial infarction; arrhythmia; thromboxane B_2; 6-keto-prostaglandin F_1 alpha

A close correlation existed between the myocardial ubiquinone deficiency and myocardial dysfunction[1]. That the protective effects of ubiquinone on the acute myocardial ischemia may be related to the myocardial ubiquinone level was reported in a previous paper[2]. The present study was designed to investigate the relationship between the anti-arrhythmic action of ubiquinone and the myocardial ubiquinone content in coronary artery ligated consious rats. Indomethacin abolished the restorative action of ubiquinone in the ischemic myocardium, and did not attenuate this restorative action of exogenous PGI_2[3]. These findings suggested that prostaglandins may participate in the action of ubiquinone, but the report on the direct demonstration of the prostaglandins to take part in the action of ubiquinone has not been seen yet. The present study was aimed to observe the relationship between the anti-arrhythmic action of ubiquinone and the changes of plasma 6-keto-$PGF_{1\alpha}$ and TXB_2 levels.

MATERIALS AND METHODS

Chemicals Ubiquinone used was the product from Mitsubishi Gas Chemicals Company, Japan. The radioimmunoassay (RIA) kits for 6-keto-$PGF_{1\alpha}$ and TXB_2, pig lung microsomes, arachidonic acid (AA) and isoprenaline were supplied by the Institute of Basic Medical Sciences, Chinese Academy of Medical Sciences.

Experimental procedures Forty-seven Sprague-Dawley ♂ rats weighing 258 ± SD 32 g were used. Rats were divided into 5 groups. Ubiquinone 3.1, 6.2, 12.5 or 25 mg · kg^{-1} in treated groups or equal volume (2 ml · kg^{-1}) of solvent in control group were twice iv injected 24 h and 30 min before ligating coronary artery. After 2nd iv injection of ubiquinone or solvent, rats placed in a plastic case. Electrodes were attached to the legs for monitoring ECG lead II continuously recorded for 5 min before ligating coronary artery. Then the coronary artery was ligated and ECG was continuously recorded for 20 min[4]. The extra systole (ES) and duration of ventricular tachycardia (VT) and ventricular fibrillation (VF) were enumerated and arrhythmic score was calculated[5]. The differences of arrhythmic scores, the numbers of ES and incidences of VT-VF between control and ubiquinone-treated groups were compared. At the end of experiment, blood was withdrawn from heart and plasma was stored at −30℃ for assay of ubiquinone and prostanoids. The heart was excised and saline was injected via the left coronary orifice. The size of myocardial ischemia was estimated by the ratio of ischemic part (dark red) to total weight of the left ventricle. Only the rats with the ratio of myocardial ischemia within 25% and 50% were included for further analysis in our experiment.

Biochemical assays The myocardium (in the frozen state) 100 mg and plasma 0.2 ml were taken. The myocardial and plasma ubiquinone contents were detected at 275 nm absorbance maximum, by HPLC (Hitachi model-634) with uv detector, after extracting into hexane from a sodium dodecyl sulfate-treated plasma or aqueous myocardial homogenate[6].

One ml of plasma after pretreatment with ether was twice extracted with redistilled ethyl acetate. Supernatants were combined and dried in nitrogen flow. 6-Keto-$PGF_{1\alpha}$ and TXB_2 were measured by RIA[7].

Assay of activity of prostaglandin synthetase in AA metabolism *in vitro*[8] Pig lung microsomes 100 μl and ubiquinone or indomethacin 100 μl were added into the mixed solution of Tris-HCl 670 μl (pH 7.5, 50 mmol · L^{-1}), human hemoglobin 20 μl (3.2 mg · ml^{-1}) and isoprenaline 100 μl (2.5 mg · ml^{-1}). After incubation for 2 min at 37℃, 10 μl AA (1 mg · ml^{-1}) were added and the mixture was incubated for 8 min. The reaction was stopped with HCl 40 μl (1 mol · L^{-1}) and its pH was adjusted to neutral by adding Tris base (1 mol · L^{-1}). The levels of 6-keto-$PGF_{1\alpha}$ and TXB_2 were measured by RIA[7] respectively.

RESULTS

Effect of ubiquinone on arrhythmia Ubiquinone 6.2, 12.5, and 25 mg · kg^{-1} iv reduced dose-dependently ischemic arrhymic score, the number of ES and incidence of VT-VF in coronary artery ligated conscious rats (Tab 1).

Tab 1. Anti-arrhythmic effects of ubiquinone in coronary artery ligated conscious rats ($\bar{x}$ ± SD). $^{**}P<0.05$, $^{***}P<0.01$ *vs* control.

Drug (mg · kg^{-1})	*n*	Arrhythmic score	lg ES	Incidence of VT-VF (%)
Control	12	4.7±2.2	2.1±0.6	83
Ubiquinone				
3.1	10	2.9±3.0	1.6±1.2	50
6.2	10	1.6±2.8***	0.9±1.2**	30**
12.5	9	0.8±2.0***	0.7±0.8***	22***
25.0	6	0.2±0.4***	0.4±0.5**	16**

Effect of ubiquinone on myocardial and plasma ubiquinone Ubiquinone 3.1, 6.2, 12.5, and 25 mg · kg^{-1} iv increased dose-dependently ubiquinone contents in myocardium and plasma of conscious rats after ligation of coronary artery, except the change of myocardial ubiquinone content after 3.1 mg · kg^{-1} was nonsignificant (Tab 2).

Tab 2. Effects of ubiquinone on plasma and myocardial levels of ubiquinone in coronary artery ligated conscious rats ($\bar{x}$ ± SD). $^{**}P<0.05$, $^{***}P<0.01$ *vs* control.

Drug (mg · kg^{-1})	Plasma level (μg · ml^{-1}) (*n*)	Myocardial level (μg · g^{-1}) (*n*)
Control	0.5±0.2 (9)	6.9±1.2 (8)
Ubiquinone		
3.1	1.3±0.4*** (6)	7.5±1.2 (8)
6.2	2.1±1.6*** (6)	8.3±1.1** (8)
12.5	2.6±2.4*** (6)	8.7±0.6** (9)
25.0	4.9±2.3*** (6)	9.6±2.1** (7)

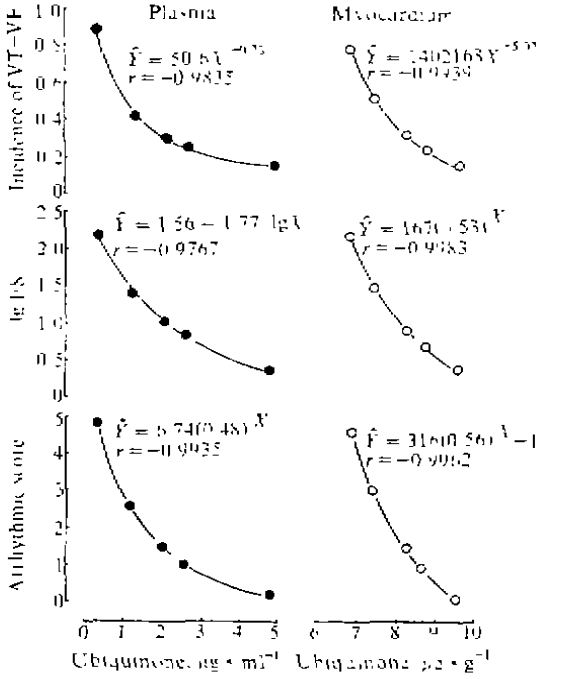

Fig 1. The correlation between the ubiquinone contents of myocardial (○) or plasma (●) and arrhythmic score, lg ES or incidence of VT-VF in coronary artery ligated conscious rats. All the data are $P<0.01$.

Relationship between arrhythmia and plasma or myocardial ubiquinone contents As shown in Fig 1, there was a close negative correlation between myocardial or plasma ubiquinone content and arrhythmic score, the number of ES and incidence of VT–VF ($P<0.05$ or 0.01).

Effect of ubiquinone on plasma levels of 6–keto–$PGF_{1\alpha}$ and TXB_2 in coronary artery ligated conscious rats Plasma 6–keto–$PGF_{1\alpha}$ level was increased after ubiquinone 3.1, 6.2, and 12.5 mg • kg^{-1}, but lowered after 25 mg • kg^{-1}. Ubiquinone 12.5 and 25 mg • kg^{-1} iv decreased plasma TXB_2. The ratios of 6–keto–$PFG_{1\alpha}$ / TXB_2 in all ubiquinone–treated groups were higher than that in control group (Tab 3).

Tab 3. Effects of iv ubiquinone on plasma 6–keto–$PGF_{1\alpha}$ and TXB_2 level (pg • ml^{-1}) in coronary artery ligated conscious rats ($\bar{x}$ ± SD). $^{**}P<0.05$, $^{***}P<0.01$ *vs* control.

Drug (mg • kg^{-1})	*n*	6–keto–$PGF_{1\alpha}$	TXB_2	6–keto–$PGF_{1\alpha}$ / TXB_2
Control	7	105 ± 23	34 ± 5	3.1
Ubiquinone				
3.1	7	139 ± 32**	36 ± 5	3.9
6.2	7	187 ± 57**	32 ± 4	5.8
12.5	6	403 ± 43***	25 ± 3***	16.1
25.0	6	58 ± 11***	9.4 ± 1.6***	6.2

Effects of ubiquinone on synthesis of 6–keto–$PGF_{1\alpha}$ and TXB_2 *in vitro* Ubiquinone 3–100 μg • ml^{-1} inhibited synthesis of 6–keto–$PGF_{1\alpha}$ and TXB_2 in comparison to control group. The concentration–response curves of ubiquinone against 6–keto–$PGF_{1\alpha}$ and TXB_2 synthesis were qualitatively similar to those of indomethacin, a known potent cyclooxygenase inhibitor (Fig 2).

DISCUSSION

Ischemic arrhythmia in anesthetized rats was depressed by ubiquinone[9]. In the present study ubiquinone prevented dose–dependently ischemic arrhythmia in coronary artery ligated conscious rats and this anti–arrhythmic effect of ubiquinone was correlated with plasma and myocardial ubiquinone contents.

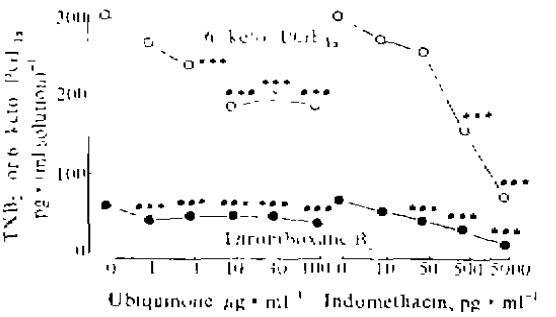

Fig 2. Inhibition by ubiquinone or indomethacin to 6–keto–$PGF_{1\alpha}$ and TXB_2 generating systems in pig lung microsomes incubated with arachidonic acid *in vitro* ($\bar{x}$ ± SD, n=4–5 expts). $^{**}P<0.05$, $^{***}P<0.01$ *vs* control.

Protective effects of ubiquinone on ischemic myocardium may be related to PGI_2[3]. The myocardial PGI_2 may be an endogenous anti–arrhythmic factor[10]. The present study showed that ubiquinone 3.1, 6.2, and 12.5 mg • kg^{-1} iv elevated plasma level of 6–keto–$PGF_{1\alpha}$ (stable metabolite of PGI_2), which is regarded as PGI_2 level indirectly, and the extent of 6–keto–$PGF_{1\alpha}$ level elevated by ubiquinone was parallel to its anti–arrhythmic action (Tab 1 and 3). When the dose of ubiquinone was increased up to 25 mg • kg^{-1} anti–arrhythmic action was potentiated, but plasma levels of 6–keto–$PGF_{1\alpha}$ and TXB_2 (stable metabolite of TXA_2) were lowered. However, the ratio of 6–keto–$PGF_{1\alpha}$ / TXB_2 was in a higher level. These results imply that PGI_2 may play a role in the control of ischemic arrhythmias.

The cause of ubiquinone 3–12.5 mg • kg^{-1} to elevate the plasma PGI_2 level may be that ubiquinone can scavenge the free radicals

produced in the metabolic pathway of arachidonic acid in injured cells and protect PGI_2 synthesis from the inhibition by these free radicals[3]. But in dose of 25 mg · kg^{-1}, ubiquinone lowered the contents of plasma PGI_2 and TXA_2 while the plasma concentration of ubiquinone was 4.9 μg · ml^{-1}, which was similar to that (3 μg · ml^{-1}) of inhibiting the synthesis of PGI_2 and TXA_2 *in vitro*. This fact indicated that this dose of ubiquinone may also possess a direct inhibitory effect on the cyclooxygenase, as indomethacin did. The direct inhibitroy effect of ubiquinone 25 mg · kg^{-1} on the cyclooxygenase may gain dominace over its beneficial action of scavenging free radicals, therefore, PGI_2 was decreased. However, the inhibitory effect may not interfere with its therapeutical action because this dose of ubiquinone can further potentiate its anti-arrhythmic effect and therapeutic doses rarely reach so high level clinically.

In vivo, as ubiquinone in dose of 12.5 mg · kg^{-1}, the plasma level of PGI_2 elevated but that of TXA_2 lowered markedly. *In vitro*, When the final concentration of ubiquinone was 1 μg · ml^{-1}, the synthesis of TXA_2 was depressed but that of PGI_2 did not. These facts indicated that the synthesis of TXA_2 was more sensitive to the inhibiting effect of ubiquinone than that of PGI_2.

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泛醌抗清醒大鼠心律失常作用[1]

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提要 泛醌 6.2，12.5 和 25 mg · kg^{-1} 于结扎冠脉前 24 h 和 30 min iv，可剂量依赖性地升高血浆及心肌泛醌含量，并与其抗清醒大鼠缺血性心律失常作用呈显著正相关。泛醌 3.1，6.2 和 12.5 mg · kg^{-1}，可显著升高血浆 6-keto-$PGF_{1\alpha}$，12.5 和 25 mg · kg^{-1} 则显著降低 TXB_2 水平。但泛醌在体内实验的各剂量组 PGI_2/TXA_2 比值均保持于高水平。提示心肌泛醌和 PGI_2 可能参与泛醌的抗心律失常作用。

关键词 泛醌；心肌梗死；心律失常；血栓素 B_2；6-酮前列腺素 $F_{1\alpha}$