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## 多培沙明、非诺多泮和丙卡特罗对大鼠心肌营养血流影响的比较

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**摘要** 用放射核素技术比较了多培沙明、非诺多泮和丙卡特罗对大鼠心肌营养血流的影响. 结果表明，此三种药(25 nmol·kg$^{-1}$)均能增加心肌营养血流，但程度明显不同. 多培沙明使每克心肌$^{99m}$Tc-MIBI 的摄取量较对照增加 80.8±10.2 % ($P<0.01$)，而非诺多泮和丙卡特罗则分别增加 44.9±6.3 % ($P<0.05$) 和 30.2±5.4 % ($P<0.05$). 提示多培沙明用于缺血性心脏病治疗可能优于其它多巴胺受体激动剂.

**关键词** 多巴胺剂；心肌；血流速度；锝 Tc 99m 甲氧异晴

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## Protection of bradykinin on neonatal rat myocytes subjected to anoxia/reoxygenation injury

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**ABSTRACT** This study was to investigate the effects of bradykinin (BK) on myocyte cultures. The effects of BK against lipid peroxidation and oxygen free radicals were estimated on an anoxia/reoxygenation injured model. A salicylate hydroxylation product dihydroxybenzoic acids (DHBA) was detected using HPLC with electrochemical detection, a sensitive device for assaying the hydroxyl free radical. BK (10 nmol·L$^{-1}$) reduced thiobarbituric acid reactive substances (TBARS, from 1.94±$s$ 0.28 to 0.25±0.03 nmol/10$^6$ cells, $n=5$, $P<0.01$), lactate dehydrogenase (LDH) release (1.28±0.23 to 0.63±0.12 u·ml$^{-1}$, $n=8$, $P<0.01$) and DHBA (60±5 to 44±12 nmol·L$^{-1}$, $n=6$, $P<0.01$). These improvements were nullified by pretreatment with $K_{86/4321}$ (1 μmol·L$^{-1}$), a kind of BK receptor antagonist. Indometacin (Ind, 1 μmol·L$^{-1}$) had a synergic action with BK to decrease the LDH release, but Ind (30 μmol·L$^{-1}$) attenuated the protection of BK, while both LDH and TBARS were increased. The mechanism for protection of BK was ascribable to the activation of BK receptor, the inhibition of lipid peroxidation, and the decreased hydroxyl free radical formation.

**KEY WORDS** bradykinin; myocardium; cultured cells; lactate dehydrogenase; lipid peroxidation; free radicals; indometacin; hydroxybenzoic acids

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Bradykinin (BK), a vasoactive peptide in plasma, increases during many diseases. BK influences the vascular tone and permeability, decreases the blood pressure, and initiates or enhances the release of some mediators from endothelium and leukocytes[1]. BK showed cardioprotective effects on ischemic hearts of anesthetized dogs and isolated ischemic rat hearts[2,3]. The mechanisms may involve: 1, the increase of epoprostenol (prostaglandin $I_2$) synthesis; 2, the improvement of glucose uptake; 3, the release of endothelium-derived relaxing factor; 4, the attenuation of catecholamine release; and 5, the reduction of oxygen free radical (OFR) formation, the levels of hydroxyl free radical was increased during ischemia/reperfusion[4].

The present experiment was designed to investigate the correlation between anti-anoxia/reoxygenation injury and anti-lipid peroxidation in cultured myocytes, as well as studied the possible influences of BK on hydroxyl free radical formation.

## MATERIALS AND METHODS

**Drugs and instrument** Bradykinin (BK), indometacin (Ind), sodium salicylate (SA) were purchased from Sigma Chemical Co. Culture medium 199 was obtained from Gib Co. $K_{86/4321}$ was kindly provided by Dr Wolfgang Linz. BK and $K_{86/4321}$ were prepared as stock solutions (1 mmol·$L^{-1}$) in distilled water and kept at −70 ℃ in small aliquots. Ind was prepared daily in equimolar solution.

**Myocyte cultures** Hearts dissected from 2—4 d old Wistar rats were transferred to Hank's solution. Ventricular myocytes were prepared. The cells were plated in culture dishes (Falcon, 60 mm × 15 mm) at a density of $3\times10^6$ cells/dish. Myocytes were separated from other cells by a selective adhesion technique[5]. The myocytes were kept in a humidified incubator (37 ℃, Hitec IT-61, Japan) with 95 % air + 5 % $CO_2$. Medium 199 was changed after 12 h and 48 h. Three days after plating a confluent monolayer was formed which beat spontaneously and synchronously. This culture was used for the experiment.

**Anoxia/reoxygenation (A/R) injury** Control group myocytes were cultured with normal Hank's solution. Anoxia was instituted by changing the culture medium with Hank's solution deprived of glucose and $O_2$ (saturated with $N_2$). Reoxygenation was performed 5 h later by replacing with normal Hank's solution. The reoxygenation period was 1 h. Drugs were added to the culture 1 h before anoxia.

**Biochemical analysis** LDH activity in culture supernatant was assayed spectrophotometrically[6]. TBARS were measured by fluorescence method[7].

Hydroxyl free radical formation was assayed utilizing HPLC-ED of salicylate hydroxylation products, dihydroxybenzoic acids (DHBA)[3,8]. Trichloracetic acid 20 % 1 ml was added to the culture medium (2 ml) before centrifugation at 1200 × *g* *at* 4 ℃ for 15 min. The supernatants were retained and 20 μl aliquots were injected into HPLC for detecting the DHBA contents.

**Statistical analysis** Data were presented as $\bar{x}\pm s$. For differences between several values analysis of variance was performed by using *q*-test (Newman-Keuls).

## RESULTS

**LDH release and TBARS contents of A/R myocytes** A/R enhanced the LDH release in myocyte culture supernatant and increased the TBARS level in myocytes. BK reduced the LDH release and attenuated the TBARS contents of myocytes injured by A/R ($P<0.01$). This protective effect was blocked by $K_{86/4321}$ (1 μmol·$L^{-1}$), a kind of BK receptor antagonist (Fig1).

Ind (1 μmol·$L^{-1}$) reduced the LDH release synergically with BK, but Ind (30 μmol·$L^{-1}$) showed an antagonistic effect against BK (both the LDH release and TBARS contents were increased, $P<0.01$).

**Hydroxyl free radical formation** Few detectable DHBA was produced in the control group (1.0 ± 0.5 nmol·$L^{-1}$). There were much higher DHBA levels in A/R group (60 ± 5 nmol·$L^{-1}$, $n=5$, $P<0.05$ *vs* control).

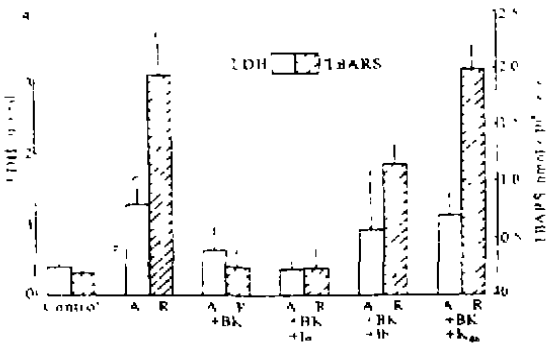


**Fig 1. Effects of bradykinin (BK, 10 nmol·L$^{-1}$) on LDH release and TBARS contents of myocyte injured by anoxia/reoxygenation (A/R). Ia: Indometacin 1 μmol·L$^{-1}$; Ib: Indometacin 30 μmol·L$^{-1}$; $K_{86}$: $K_{86/4321}$ 1 μmol·L$^{-1}$. $^{a}P<0.01$ *vs* control; $^{b}P<0.01$ *vs* A/R; $^{c}P<0.01$ *vs* A/R+BK.**

Adding $FeCl_3$ (100 nmol·L$^{-1}$) did not increase the DHBA formation (55±17 nmol·L$^{-1}$ *vs* 60 ±5 nmol·L$^{-1}$, $n=5$, $P>0.05$). BK (10 nmol·L$^{-1}$) reduced the DHBA level (60±5 nmol·L$^{-1}$ *vs* 44±12 nmol·L$^{-1}$, $n=5$, $P<0.05$).

## DISCUSSION

Our results demonstrated that treatment with BK prevented the myocyte from anoxia/reoxygenation impairment, supported Linz's view[2]. This research has shown that the myocytic protection of BK against A/R injury can be abolished by $K_{86/4321}$ (30 μmol·L$^{-1}$), indicating that the protective action of BK is receptor mediated.

Recently, investigations have demonstrated that myocardium can synthesize epoprostenol and $TXA_2$. Ind (1 μmol·L$^{-1}$), an inhibitor of cyclooxygenase (CO) reduces the LDH release in the supernatant, showing a synergic effect with BK. We considered that $TXA_2$ increased during A/R impairment[10], while the epoprostenol/$TXA_2$ ratio decreased. Lowering the $TXA_2$, Ind increased the epoprostenol/$TXA_2$ ratio, indicating its protective effects.

But Ind (30 μmol·L$^{-1}$) showed an antagonistic action on BK. We hypothesized that Ind inhibited the CO activity and then altered the arachidonic metabolism pathway. Inhibition of CO may lead to: 1) reduction of the synthesis of epoprostenol; 2) lipooxygenase pathway up-regulation, bringing about the release of a series of substances such as leukotrienes, and causing impairment. All these alterations may aggravate the impairment of myocytes. This biphasic effect of Ind has also been identified in brain[11,12]. The underlying mechanisms remain to be further investigated.

A number of studies have suggested that OFRs played an important role in myocardial ischemia/reperfusion impairment[13]. Further studies suggested that hydroxyl free radical was the most reactive OFR in myocardial ischemia/reperfusion[14]. A/R activated the HX/XO system, and thus OFRs formed, which elicited the lipoperoxydation and subsequent cell membrane impairment. Our study has found the OH· formation of myocytes during A/R impairment, showing that the Haber-Weiss reaction can go on under myocyte culture condition without exogenous $Fe^{3+}$ added. BK attenuate the OH· formation (60.2±15.2 to 43.5±12.6 nmol·L$^{-1}$).

In conclusion, this work showed that BK had an effect of anti-A/R induced myocyte injury, paralleled with its antilipoperoxidation action and this effect was BK receptor mediated. BK also attenuated the OH· formation during A/R injury, which may be related to its protective effect.

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## 缓激肽对新生大鼠心肌细胞缺氧/再给氧损伤的保护作用

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**摘要** 缓激肽(BK，10 nmol·L$^{-1}$)能使培养乳鼠心肌细胞缺氧再给氧(A/R)损伤时，LDH释放减少和TBARS含量降低，此作用能被缓激肽拮抗剂$K_{86-1521}$(1 μmol·L$^{-1}$)所取消；心肌细胞A/R损伤后，HPLC-ED法测定培养液中有羟自由基生成，BK能减少其产生。BK保护作用的机理可能与其抗脂质过氧化和减少羟自由基生成有关。

**关键词** 缓激肽；心肌；培养的细胞；乳酸脱氢酶；脂质过氧化；自由基；吲哚美辛；羟基苯甲酸