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Effects of inhibitor of endothelium-derived relaxing factor on hypoxic contraction of isolated pig coronary artery[1]

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ABSTRACT Exposure of isolated pig coronary artery with endothelium intact to hypoxia Krebs-Henseleit solution aerated with 95 % N_2 + 5 % CO_2 caused a transient contractile response, and the coronary artery without endothelium exhibited a gradual decrease in basal tension. The endothelium-dependent contractile response to hypoxia was almost completely blocked by nitro-*L*-arginine (0.2 mmol·L^{-1}), and inhibited by methylthioninium chloride (10 μmol·L^{-1}). The inhibitory effect of the NLA was partially reversed by *L*-arginine (2 mmol·L^{-1}). Sodium nitroprusside (10 μmol·L^{-1}) was also completely antagonized and nicorandil (0.3 mol·L^{-1}) remarkably reduced the hypoxic contractile response. Tetraethylammonium (10 mmol·L^{-1}) and glibenclamide (1 μmol·L^{-1}) had little effect on hypoxia-induced vascular constraction, whereas cromakalim (1 μmol·L^{-1}) produced obvious relaxing effect on hypoxic response. These results suggest that suppression of basally released nitric oxide (NO) is an important mechanism of coronary vasoconstriction induced by hypoxia.

KEY WORDS nitro-*L*-arginine; tetraethylammonium compounds; coronary vessels; endothelium; anoxia

Hypoxia induces endothelium-dependent contractile response of the isolated pig coronary artery[1,2]. Which might be related to the production of postanoids by the endothelium[1]. However, endothelium-dependent hypoxic contraction in quiescent dog coronary artery might be due to decreased production of NO[3]. It remains unclear whether suppression of the endothelium-derived relaxing factor (EDRF) can affect the pig coronary artery contraction in response to hypoxia.

Pig coronary artery endothelium releases both EDFR and an unknown endothelium-derived hyperpolarizing factor (EDHF)[4-6]. EDHF causes vascular smooth muscle relaxation by hyperpolarization of the cell membrane[4,5], is due to the activation of membrane K^+ channels and increase in K^+ efflux[7].

The purpose of this study was to determine the role of *L*-arginine (*L*-Arg) metabolism and K^+ channel in the hypoxic response of the pig coronary artery.

MATERIALS AND METHODS

Coronary artery ring The right coronary arteries were taken from freshly slaughtered pig hearts. The blood vessel rings (5—6 mm long) were used. In some preparations the endothelium was removed mechanically. The presence of a functional endothelium was confirmed by the presence of calcimycin (0.1 μmol·L^{-1})-induced relaxation in coronary artery precontracted with 5-HT (5 μmol·L^{-1}). The rings were equilibrated in Krebs-Henseleit (K-H) solution (35±0.5 ℃, pH 7.3—7.4) gassed with 95 % O_2 + 5 % CO_2 for 2 h at resting tension of 5—7 g. The tension of the rings was recorded isometrically by transducer and XWT-204 model potentiometric recorder.

Protocol Hypoxia was induced by replacing by 95% N_2+5% CO_2. After the hypoxia-induced tension

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changes had returned to baseline tension, or hypoxia remained for 20 min, oxygenation was restored by washing with K-H solution aerated with 95% O_2+5% CO_2. The hypoxic challenge was repeated twice at a 60-min interval of controlled oxygenation.

Each rings was pretreated with nitro-*L*-arginine (NLA), methylthioninium chloride (Met), sodium nitroprusside (SNP), nicorandil (Nic), cromakalim (Cro), tetraethylammonium (TEA), glibenclamide (Gli) for 30 min. In order to investigate the protective effects of *L*-Arg on NLA action, after rings were pretreated with *L*-Arg for 20 min, NLA was added to the bath for equilibrating another 30 min. The hypoxic challenge was replicated in the presence of the antagonist. The contraction induced by hypoxia in the presence of the antagonist or its solvent was expressed as developed isometric tension. Statistical significance between responses was evaluated by *t*-test.

Drugs We bought NLA, *L*-Arg, Cro, Met, calcimycin from Sigma, Nic from Shanxi Institute of Medical and Pharmaceutical Industry, Gli from Tianjin Institute of Medical and Pharmaceutical industry, TEA from Beijing Chemical Factory, SNP from Beijing Institute of Pharmaceutical Industry. All drugs were prepared with freshly distilled water except that Cro and Gli were dissolved in 0.5 % Me_2SO.

RESULTS

Effect of hypoxia Hypoxia caused a transient contraction of isolated pig coronary artery with endothelium intact, and a gradual decrease in basal tension of the coronary artery without endothelium (Fig 1).

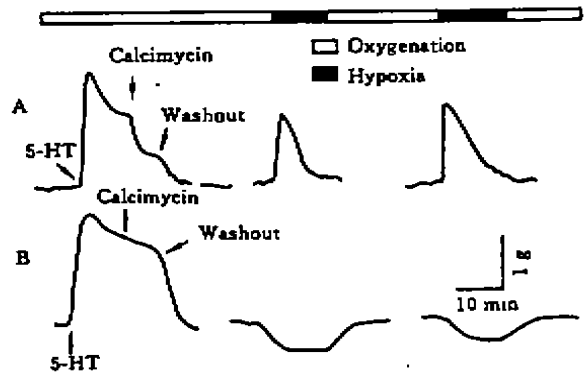

Fig 1. Effect of endothelium on hypoxic contraction in isolated pig coronary artery. A: endothelium intact; B: endothelium denuded; 5-HT 5 μmol·L^{-1}; Calcimycin 0.1 μmol·L^{-1}.

Effects of NLA and Met NLA (0.2 mmol·L^{-1}) and Met (10 μmol·L^{-1}) caused an increase in basal tention, and an inhibition of the hypoxic contraction (Tab 1, Fig 2). These effects of NLA were more potent than that of Met. NLA almost completely blocked the vasoconstriction induced by hypoxia. Pretreatment with *L*-Arg attenuated the effects of NLA on basal tension and hypoxic

Tab 1. Effects of different pharmacological antagonists on hypoxic contractile response in isolated pig coronary arteries. $n=6-8$, $\bar{x}\pm s$. $^aP>0.05$, $^cP<0.01$ *vs* **control**; $^fP<0.01$ *vs* **Nitro-*L*-arginine.**

Drug/ μmol·L^{-1}	Developed isometric tension/g		
	Oxygenation +Antagonist	Hypoxia Control	Hypoxia +Antagonist
Nitro-*L*-arginine (200)	1.10±0.17	1.40±0.10	0.10±0.09^c
Nitro-*L*-arginine (200) + *L*-arginine (2000)	0.77±0.16^f	1.44±0.20	0.47±0.13^{cf}
Methylthioninium chloride (10)	0.28±0.08	1.55±0.21	0.96±0.16^c
Sodium nitroprusside (10)	0	1.31±0.14	0
Nicorandil (300)	0	1.42±0.23	0.22±0.11^c
Cromakalim (1)	−0.45±0.06	1.34±0.18	0.13±0.13^c
Tetraethylammonium (10 mmol·L^{-1})	0.52±0.09	1.40±0.20	1.30±0.12^a
Glibenclamide (1)	0	1.30±0.24	1.28±0.22^a

contraction, but did not reverse it to normal level (Tab 1, Fig 2). The inhibitory effect of NLA on hypoxic vascular responsiveness lasted at least 8 h after repeated washouts. Incubation of rings with *L*-Arg (2 mmol·L^{-1}) caused a decrease in the basal tension, and partially restored the hypoxic contraction. The magnitude of the control response was 0.17±0.10 g, whereas that undergoing pretreatment with *L*-Arg was 0.91±0.30 g (n=8, P<0.01) (Fig 3).

Effects of vasodilators Vasoconstriction induced by hypoxia was completely abolished by SNP 10 μmol·L^{-1}, and markedly inhibited by Nic 0.3 mol·L^{-1} (Tab 1). Cro 1 μmol·L^{-1} caused an attenuation of the basal tension (Tab 1, "—" indicates relaxation below baseline) and almost completely antagonized the contractile response to hypoxia (Tab 1).

Effects of K^+ channel antagonists TEA 10 mmol·L^{-1} caused an increase in baseline tention, but Gli 1 μmol·L^{-1} had no influence on it. Neither TEA nor Gli affected the coronary artery vasoconstriction induced by hypoxia significantly (Tab 1, Fig 2).

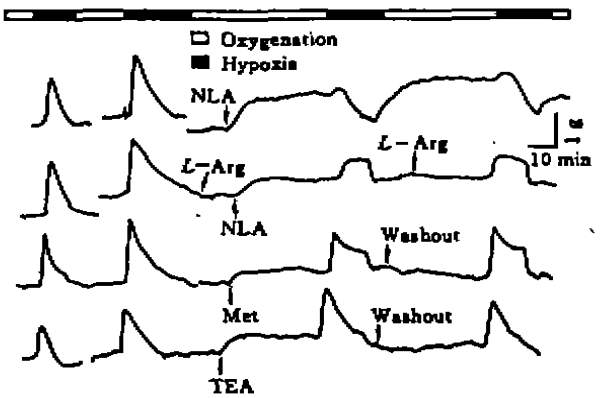

Fig 2. Effects of nitro-*L*-arginine (NLA, 0.2 mmol·L^{-1}), methylthioninium chloride (Met, 10 μmol·L^{-1}), and tetraethylammonium (TEA, 10 mmol·L^{-1}) on hypoxic contraction in isolated pig coronary artery. *L*-arginine (*L*-Arg, 2 mmol·L^{-1}).

DISCUSSION

In the present experiments, increasing

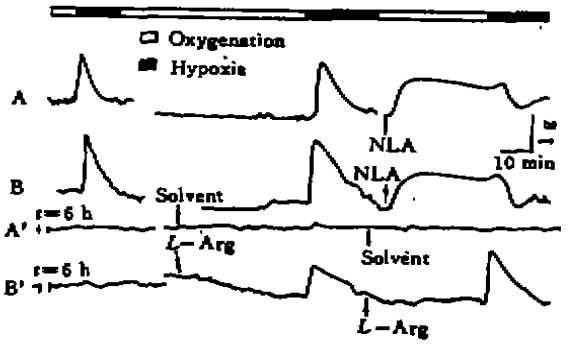

Fig 3. Effects of *L*-arginine (*L*-Arg, 2 mmol·L^{-1}) on nitro-*L*-arginine (NLA, 0.2 mmol·L^{-1}) inhibition of hypoxic contraction in isolated pig coronary. A': continued from A; B': continued from B.

the basal tension and inhibiting the hypoxic contractile response by NLA, NO synthase inhibitor or Met, soluble guanylate cyclase inhibitor were consistent with the hypothesis that the endothelium-dependent contraction in response to hypoxia is due to the inhibition of the basal release of EDRF, a mechanism that has been proposed to account for the hypoxic constriction in dog coronary artery[3].

Our results indicate that hypoxic contractile response was completely blocked by SNP or significantly inhibited by Nic. It suggested that SNP by supplement of NO[8] and Nic by activation of the guanylate cyclase[9,10] could prevent the coronary vasospasm evoked by hypoxia.

Hasunuma *et al*[11] reported that TEA, a nonselective blocker of K^+ channal, increased the baseline pressure and potentiated the peak of the hypoxic contractile response in the perfused rat lung. Our observations that K^+ channel blockers TEA and Gli did not affect the hypoxic contractile response raised the possibility that EDHF would not modulate the hypoxic pig coronary artery contraction.

However, Cro, by opening ATP-sensitive K^+ channels, can effectively antagonize the hypoxic contractile response.

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内皮舒张因子抑制剂对低氧收缩离体猪冠脉的影响

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摘要 左旋硝基精氨酸，NLA (0.2 mmol·L^{-1})可阻断离体猪冠脉依内皮性缺氧收缩反应，用左旋精氨酸，*L*-Arg (2 mmol·L^{-1})预处理可显著降低 NLA 的抑制作用。四乙胺，TEA (10 mmol·L^{-1})和格列本脲，Gli (1 μmol·L^{-1})对缺氧收缩反应无明显影响，而 Cromakalim，Cro (1 μmol·L^{-1})则可抑制缺氧冠脉收缩。

关键词 左旋硝基精氨酸；四乙铵化合物；内皮；冠状血管；缺氧症