

Effects of catalase and endothelin on anoxia-induced vasoconstriction of porcine basilar artery *in vitro*¹

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KEY WORDS anoxia; vasoconstriction; basilar artery; endothelins; catalase

ABSTRACT

AIM: To study whether anoxia-induced vasoconstriction was related to the release of endothelin (ET). **METHODS:** Acute anoxia was induced by gassing the organ chamber with 95 % N₂ + 5 % CO₂. Changes in tension of porcine basilar arterial ring was recorded. **RESULTS:** Anoxia-induced increases in tension were 0.21 g ± 0.08 g and 0.24 g ± 0.09 g under basal tension and during ET 3 nmol · L⁻¹-induced contractions, respectively. In the rings tension did not further augment following the increase of ET from 100 to 300 nmol · L⁻¹, acute anoxia did cause further increase in tension of 0.16 g ± 0.10 g (n = 4). Catalase 800 and 2400 kU · L⁻¹ decreased the anoxia-induced contraction, with inhibitory rate of 33 % ± 7 % and 47 % ± 9 %, respectively. **CONCLUSION:** Anoxia-induced vasoconstriction was related to release of hydrogen peroxide from endothelial cells.

INTRODUCTION

Acute anoxia augmented the contractile responses of some arteries to norepinephrine, KCl, and prostaglandin F_{2α}, and anoxic augmentation was related to the vascular endothelial cell^[1-5]. Endothelium-mediated anoxic faci-

litation was due to the interruption of the production of vasodilator signal by the endothelial cells^[1]. Either inhibitor of the production (quinacrine) or inactivator (phenidone) of endothelium-derived relaxing factors completely and rapidly reversed the relaxations induced by acetylcholine in the preparations with endothelium, but did not affect the anoxic augmentation, hence it was concluded that hypoxia and anoxia caused the release of a vasoconstrictor substance from endothelial cell which then diffused to the adjacent smooth muscle to activate or potentiate the contractile process^[4]. The experiments with cultured endothelial cells suggested that the hypoxic endothelial cells released peptide (s) with constrictor properties^[6,7], endothelium-derived constrictor factor (EDCF)^[7]. Yanagisawa *et al* isolated the vasoconstrictor peptide from the culture supernatant of porcine aortic endothelial cells, determined its amino-acid sequence, and named this peptide as endothelin (ET)^[8].

This experiment was designed to investigate whether or not the anoxic augmentation was due to the release of endothelin from vascular endothelial cells.

MATERIALS AND METHODS

The basilar arteries were isolated from fresh adult porcine brain, 20 - 30 min after death and kept in 4 °C Krebs-Ringer solution gassed with 95 % O₂ + 5 % CO₂. Arterial segments were cut into 4-5 mm rings.

Isometric tension recording Each ring was attached to an isometric force transducer and

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suspended in a glass organ chamber filled with 4 mL Krebs-Ringer solution at $(37.0 \pm 0.5) ^\circ\text{C}$ and gassed with 95 % $\text{O}_2 + 5\% \text{CO}_2$. Isometric tension was continuously recorded by a recorder (type XWT-204, Dahua Instrumental Factory, Shanghai). Basilar arterial rings were equilibrated at a resting tension of 1 g for 2 h before the test.

Acute anoxia was induced by switching the gas to 95 % $\text{N}_2 + 5\% \text{CO}_2$. Changes in tension of basilar arterial rings evoked by acute anoxia under basal tension and during contractile response to ET 3 and 300 $\text{nmol} \cdot \text{L}^{-1}$ were recorded. In some arterial rings, the anoxia-induced contractions during the contractile response to ET 3 $\text{nmol} \cdot \text{L}^{-1}$ were measured before and after giving catalase (800 or 2400 $\text{kU} \cdot \text{L}^{-1}$). The inhibitory rate of catalase on anoxia-induced contraction was calculated: $\text{Inhibitory rate} = (Y_1 - Y_2) / Y_1 \times 100\%$. Y_1 and Y_2 were the anoxia-induced contractions before and after giving catalase, respectively.

Drugs ET (Peninsula Lab Inc, Belmont CA 94002, USA), catalase (Shanghai Institute of Biochemistry, Chinese Academy of Sciences). Stock solution were prepared in distilled water for endothelin or in phosphate buffer solution (pH = 7.0) for catalase (200 $\text{MU} \cdot \text{L}^{-1}$) before use. All concentrations were expressed as final concentration in the bath.

Statistical analysis Data were shown as $\bar{x} \pm s$. Paired or unpaired t test were used.

RESULTS

Response of basilar arteries to anoxia during basal tension and during the contraction induced by ET 3 $\text{nmol} \cdot \text{L}^{-1}$

Acute anoxia caused a reversible increase in basal tension of basilar arterial rings. The tension of arterial rings decreased rapidly below the initial tension when bath solution was oxygenated with 95 % O_2 , and then restored

slowly to base line. Thirty min later, ET 3 $\text{nmol} \cdot \text{L}^{-1}$ was added, and the tension of arterial rings increased slowly. When the contraction induced by ET reached stable (increase in tension of $0.11 \text{ g} \pm 0.05 \text{ g}$, acute anoxia caused a reversible further increase in tension called "anoxic potentiation". By basal tension and during ET-induced contractions, anoxia-induced increase in tension were $0.21 \text{ g} \pm 0.08 \text{ g}$ and $0.24 \text{ g} \pm 0.09 \text{ g}$ ($n = 10$) respectively. (Fig 1)

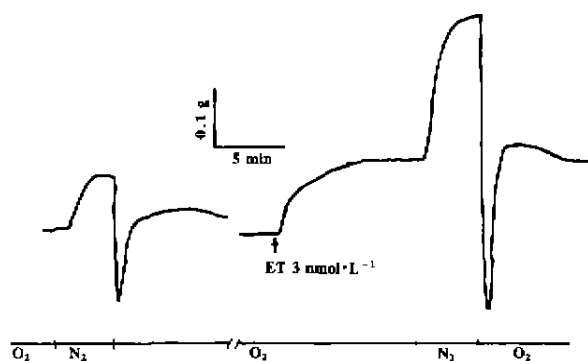


Fig 1. Anoxia-induced change in tension of porcine basilar arterial ring under basal tension and during the contraction induced by endothelin 3 $\text{nmol} \cdot \text{L}^{-1}$.

Response of basilar arteries to anoxia during the contraction induced by ET 300 $\text{nmol} \cdot \text{L}^{-1}$ ET 1 – 100 $\text{nmol} \cdot \text{L}^{-1}$ caused concentration-dependent constrictions of basilar arterial rings with maximal increase in tension of $0.52 \text{ g} \pm 0.13 \text{ g}$. (Tab 1)

When the concentration of ET increased to

Tab 1. Endothelin induced vasoconstriction of porcine basilar arterial rings. $n = 6$ pigs. $\bar{x} \pm s$.

Endothelin/ $\text{nmol} \cdot \text{L}^{-1}$	Increase in tension/mg
1	0.09 ± 0.03
3	0.19 ± 0.04
10	0.29 ± 0.09
30	0.46 ± 0.14
100	0.52 ± 0.13

300 nmol·L⁻¹, the tension of arterial rings did not increase further, sometimes decreased slightly. In the rings which were contracted by ET 300 nmol·L⁻¹, acute anoxia did cause further increase in the tension of 0.16 g ± 0.10 g.

Effect of catalase on anoxic potentiation

Anoxia caused a reversible further increase in tension in rings contracted with ET 3 nmol·L⁻¹. On return to 95 % O₂, the rings transiently relaxed below the initial tension, followed by rising to the level before anoxia. Thirty min later, second anoxia was performed in certain rings. The increase in tension caused by the second anoxia was not different from that in the first time (0.18 g ± 0.04 g vs 0.17 g ± 0.05 g, *n* = 7, *P* > 0.05). In remained 8 arterial rings, catalase 800 or 2400 kU·L⁻¹ was added. The drug concentration-dependently decreased the anoxia-induced contraction. The inhibitory rates caused by catalase 800 and 2400 kU·L⁻¹ were 33 % ± 7 % (*n* = 4) and 47 % ± 9 % (*n* = 4), respectively.

DISCUSSION

The present experiments showed that acute anoxia caused a reversible increase in the tension of porcine basilar arterial rings with endothelium, either under the basal condition or during ET-induced contraction. This was different from that in the femoral artery of dog, in which anoxia only augmented contractile response to norepinephrine, KCl, and BaCl₂, but did not affect basal tension³⁾. The reason that caused the above difference might be due to that the response of porcine arteries to anoxia was different from canine arteries. The fact that anoxia evoked the increase in the basal tension of canine basilar arteries⁵⁾, rejected the above possibility. It was speculated that the response of cerebral arteries to anoxia under basal condition might be different from peripheral arteries.

The tension of arterial rings did not further

augment, following the increase in ET 100 to 300 nmol·L⁻¹, which indicated that the ET receptors were saturated. The fact that acute anoxia did cause further increase in tension of arterial rings precontracted with ET 300 nmol·L⁻¹ suggested that the anoxic potentiation was not related to endothelin. In addition, a major difference between anoxic contraction and endothelin-evoked contraction related to their time course. Anoxic contractions were very rapid and readily reversible upon return to 95 % O₂, whereas the contractions induced by endothelin were long-lasting and more difficult to wash out⁸. Douglas found that BQ 123 (10 μmol·L⁻¹), an ETA receptor selective blocker, antagonized the vasoconstriction induced by ET (0.1 – 300 nmol·L⁻¹), but did not affect the hypoxic contraction in canine pulmonary, coronary, and femoral arteries⁹⁾. These observations were consistent with the suggestion that the anoxic potentiation was not due to endothelin release from anoxic endothelial cells.

Catalase antagonized partially the anoxic augmentation during ET-induced contraction in concentration-dependent fashion. This observation suggested that anoxic potentiation might be related to release of hydrogen peroxide from endothelial cells.

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过氧化氢酶和内皮素对缺氧引起的猪基底动脉收缩的体外影响¹ R

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关键词 缺氧症; 血管收缩; 基底动脉; 内皮素类; 过氧化氢酶

目的: 研究缺氧引起的血管收缩是否与血管内皮细胞释放内皮素(ET)有关. 方法: 95 % O₂ + 5 % CO₂混合气体换成 95 % N₂ + 5 % CO₂引起急性缺氧, 描记猪基底动脉环的张力变化. 结果: 在基础张力条件下和由 ET 3 nmol·L⁻¹引起收缩时, 缺氧分别使基底动脉张力增加 0.21 g ± 0.08 g 和 0.24 g ± 0.09 g. 当 ET 浓度从 100 nmol·L⁻¹增加到 300 nmol·L⁻¹时, 动脉的张力不进一步增加, 此时急性缺氧仍使张力进一步增加 0.16 g ± 0.10 g. 过氧化氢酶 800, 2400 kU·L⁻¹明显降低缺氧引起的收缩, 抑制率分别为 33 % ± 7 % 和 47 % ± 9 %. 结论: 缺氧引起的血管收缩与内皮细胞释放过氧化氢有关.

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