

Effects of Metabolic Inhibition and Acidosis on the Contractility of the Pulmonary and Ear Artery

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= Abstract = Hypoxia causes contraction in pulmonary artery, whereas it causes relaxation in systemic artery. The purpose of this study is to test whether pulmonary artery would respond to metabolic inhibition and acidosis differently from ear artery. Rabbit pulmonary artery and ear artery were precontracted with phenylephrine or KCl, and then exposed to metabolic blockers (dinitrophenol (DNP), Na-cyanide (NaCN)) and acidosis. Contractile forces of ear artery induced by 30mM KCl and 10^{-6} M phenylephrine were 2~3 times ($n=7$) and 5~9 times ($n=7$) larger than that of the pulmonary artery, respectively. DNP and NaCN produced a dose-dependent relaxation in the pulmonary and ear artery, and the relaxation was more profound in the ear artery than in pulmonary artery. This effect was independent of the presence of the endothelium. Extracellular acidosis reduced the tone of the KCl-induced contraction, more in the ear artery than in pulmonary artery. These results indicate that pulmonary artery is more resistant to both of the inhibition of metabolism and acidosis than ear artery.

Key Words: Pulmonary artery, Ear artery, Hypoxic pulmonary vasoconstriction, DNP, Cyanide, acidosis

INTRODUCTION

It is known that a decrease in arterial oxygen tension induces a vasodilation in most systemic arteries, but induces a vasoconstriction in the pulmonary arterial bed (von Euler & Liljestrand, 1946). Hypoxia-induced pulmonary vasoconstriction contributes to the regulation of ventilation/perfusion matching in normal subjects. However, this mechanism is also responsible for the

pulmonary hypertension occurring in patients with hypoxia-related abnormalities, such as chronic obstructive pulmonary disease, chronic bronchitis and emphysema (Cutia & Round, 1990; Voelkel, 1986).

Many results support the hypothesis that hypoxia elicits vasoconstriction by a direct effect on the pulmonary vascular smooth muscle without involvement of endothelial cells or other cells (Bennie et al. 1991; Yuan et al. 1990). Moreover, it was reported that cultured single pulmonary arterial cells were contracted by hypoxia (Madden et al. 1992; Murray et al. 1990). However, the underlying mechanism of hypoxic

pulmonary vasoconstriction is still not clear.

Generally, hypoxia results in the metabolic inhibition and acidosis, which are considered to exert a relaxation effect on smooth muscles. Since the response to hypoxia in pulmonary artery is opposed to that of systemic artery, it could be proposed that contractile properties in pulmonary and systemic artery is regulated in different way. Therefore, in the present study, we investigated the effects of metabolic inhibition and acidosis on the contractility of the pulmonary and ear artery and found that pulmonary artery is more resistant to both of the inhibition of metabolism and acidosis than ear artery.

MATERIALS AND METHODS

Isolated pulmonary and ear arterial preparations: Adult rabbits (1.2~1.5 kg) were anesthetized by phentobarbital sodium (50 mg/kg) and heparinized (1000u/kg). The pulmonary and ear artery were dissected out and placed in cold (4°C) Tyrode solution. After removing the surrounding adventitia, arterial rings were made under dissecting microscope with the dimension of 1.5~3 mm length in both arteries, 500~800 μ m in the ear artery and 3~4 mm diameter in the pulmonary artery.

Solutions: Normal Tyrode solution contained (in mM); 143 NaCl, 5.4 KCl, 0.5 MgCl₂, 5 HEPES, 5.5 glucose, adjusted pH to 7.4 with NaOH. High K solutions were made by equimolar replacement of NaCl with KCl. All solutions were equilibrated with 100% O₂ and maintained at 37°C using a thermocirculator (Havard). DNP and NaCN was added to the perfusate and adjusted pH to 7.4 with NaOH. All chemicals were obtained from Sigma.

Experimental protocol; Each vessel segment was placed in a tissue bath by gently threading the vessel onto a vertically oriented, fixed-position, steel rod (100 μ m in diameter for ear artery, 500 μ m for pulmonary artery). Once anchored, a second wire of the same dimensions, but connected to a force transducer (Glass model FT 03), was introduced into the lumen. Isometric tension was recorded as a function of time on a strip-

chart recorder (Gould model 2400 and Havard). Each vessel segment was stretched to 500~800 mg and allowed the recovery period for 1 hour. Data were compared and presented as means \pm SE (standard error). Student's t-test was used and differences were considered significant at $p < 0.05$.

RESULTS

Effects of KCl and phenylephrine on the pulmonary and ear artery; Dose-response curves to KCl and phenylephrine in the pulmonary and ear artery were shown in Fig. 1. In both arterial segments, arterial tension was increased at 10 mM KCl and showed maximum contraction at 80 mM KCl. When phenylephrine was added to the bathing solution, arterial tension was dose-dependently increased from 10⁻⁸M to 10⁻⁵M. Half maximum dose (EC₅₀) was obtained by fitting the values to the following equation:

$$f(x) = 1 / \{1 + (EC_{50} / x)^n\}$$

f(x) : relative contractile response
x : concentration of KCl or phenylephrine
n : Hill coefficient
EC₅₀ : half maximum dose

When contracted KCl, EC₅₀ of the pulmonary and ear artery were 19.6 (n = 3) and 26.7 mM (n = 3). When phenylephrine was superfused, EC₅₀ of the pulmonary and ear artery were 1.86 \times 10⁻⁷ (n = 7) and 1.20 \times 10⁻⁷M (n = 4), respectively.

To compare the effects of KCl and phenylephrine on the contractility of the pulmonary and ear artery, contractile responses to KCl (30 mM) and phenylephrine (10⁻⁶M) were averaged and corrected by the wet weight of pulmonary (2.4 \pm 0.4, n = 7) and ear artery (0.6 \pm 0.1 mg, n = 7). These data were summarized in Fig. 2. Contractile force of ear artery induced by 30 mM KCl and 10⁻⁶ M phenylephrine were 2~3 times (n = 7) and 5~9 times (n = 7) larger than that of the pulmonary artery, respectively.

The effect of NaCN and DNP; Generally, oxidative metabolism is inhibited in hypoxia. To

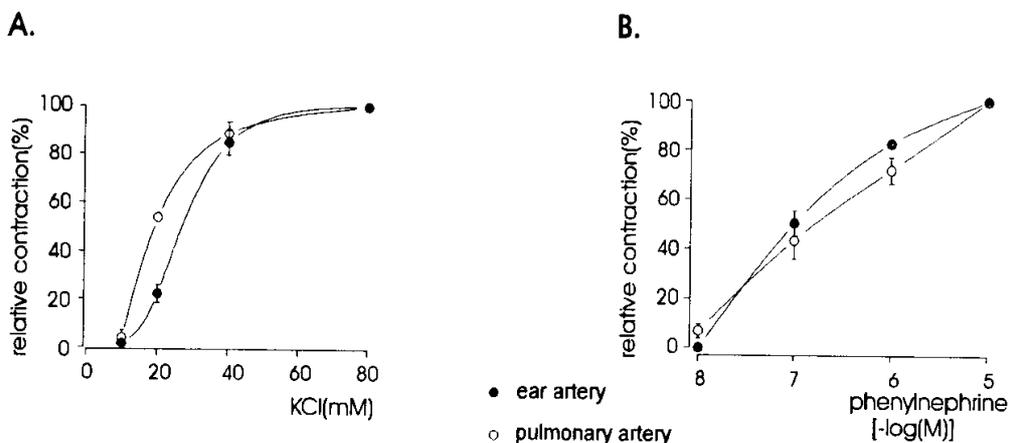


Fig. 1. Dose-response curves to KCl (A. *n* = 3 in pulmonary and ear artery) and phenylephrine (B. *n* = 7 in pulmonary and *n* = 4 in ear artery) in the pulmonary (open circle) and ear artery (filled circle). Responses are expressed as percent change of the maximum contraction to the KCl (80 mM) and phenylephrine (10⁻⁵M).

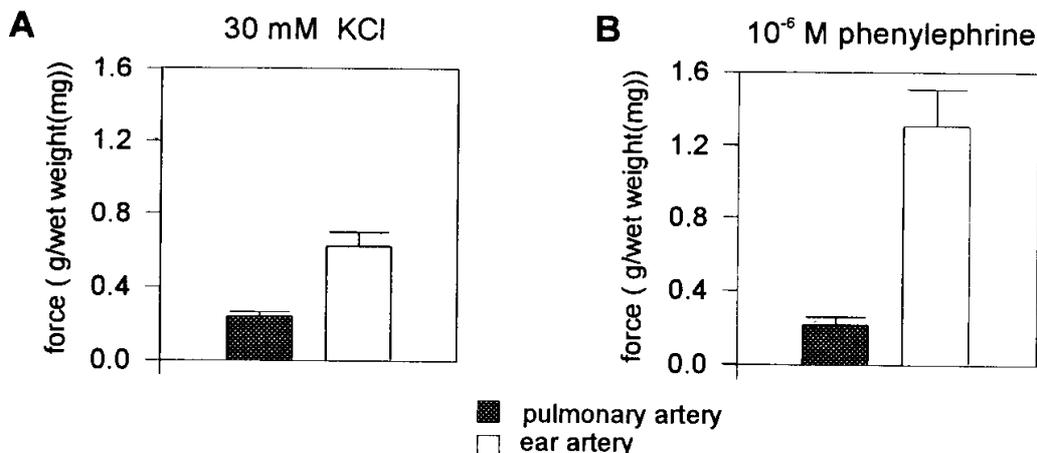


Fig. 2. Effects of KCl (A. 30 mM) and phenylephrine (B. 10⁻⁶M) on the pulmonary and ear artery. Active forces to 30mM KCl and 10⁻⁶M phenylephrine are 0.23 ± 0.02 g (*n* = 6), 0.62 ± 0.07 g (*n* = 6) and 0.21 ± 0.04 g (*n* = 7), 1.30 ± 0.20 g (*n* = 7) in pulmonary and ear artery. Paired t test indicates that pulmonary artery contraction responses to KCl and phenylephrine is different from ear artery responses (*p* < 0.05).

investigate the effect of metabolic inhibition on the precontracted arterial tone, metabolic blockers (DNP and NaCN) were superfused. NaCN induced dose-dependent relaxation in the precon-

tracted arterial rings (Fig. 3A) and the mean responses of the arterial rings to cumulative dose of NaCN were shown in Fig. 3B. DNP had also the dose-dependent relaxation in the phenyle-

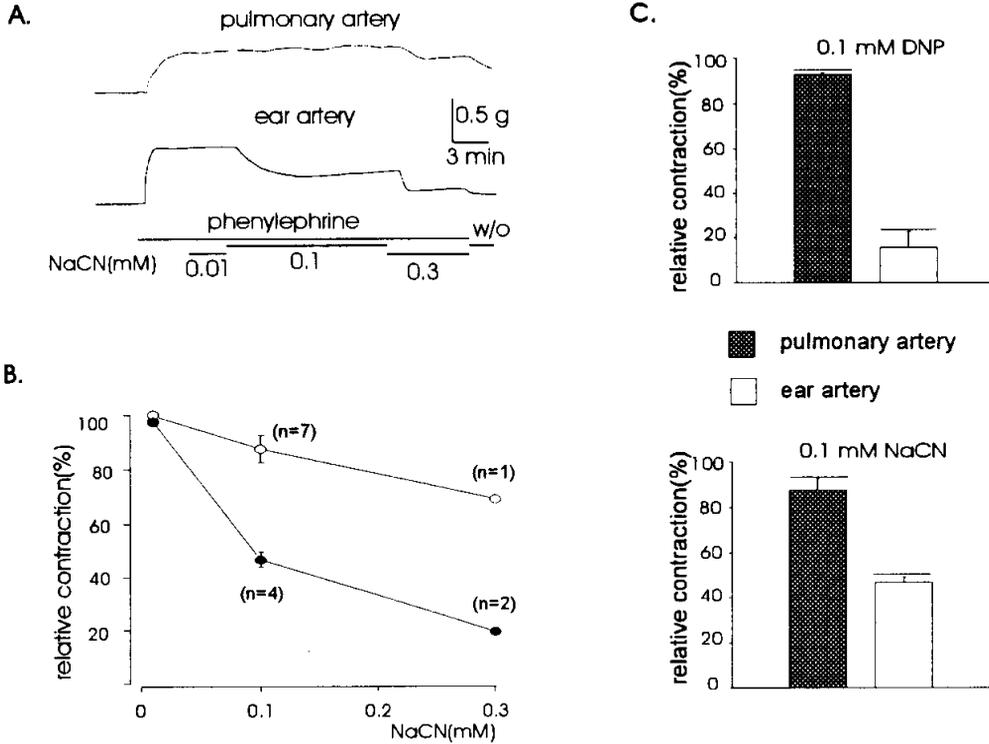


Fig. 3. Effects of DNP and NaCN on the pulmonary and ear artery precontracted with phenylephrine (10^{-6} M). **A.** Effects of the increasing cumulative doses of NaCN on the pulmonary and ear artery were shown. **B.** Relative relaxation responses were obtained (open circle, pulmonary artery; filled circle, ear artery). **C.** DNP (0.1 mM) and NaCN (0.1 mM) decreased phenylephrine-induced tone to 92.7 ± 1.05 ($n = 4$), 87.61 ± 5.11 ($n = 7$) and 15.5 ± 7.24 ($n = 3$), 46.75 ± 2.83 ($n = 4$) in pulmonary and ear artery. Relaxing responses of pulmonary artery to DNP and NaCN are less than that of ear artery ($p < 0.05$).

phrine-precontracted arterial rings (data not shown). Relative relaxation responses to DNP (0.1 mM) and NaCN (0.1 mM) were summarized in Fig. 3C. The degree of the relaxation against metabolic inhibitors was greater in the ear artery than pulmonary artery. Above results suggest that the pulmonary artery was much resistant to the metabolic inhibition than the ear artery.

To test the involvement of endothelium in the metabolic blocker-related relaxation, we removed the endothelium with gentle rubbing the rumen by cotten ball and applied acetylcholine. The ring without the acetylcholine-induced relaxation res-

ponse was regarded as the endothelium-removed arterial ring. In phenylephrine-precontracted rings, the effect of 0.15 mM NaCN had no significant differences between the endothelium-damaged and endothelium-intact pulmonary artery (Fig. 4A, B, $p > 0.05$).

The effect of pH; It is well known that acidosis occurs in hypoxia (Gutierrez, 1991). To evaluate the effect of acidosis on the KCl-precontracted pulmonary and ear artery, the pH of the bathing solution was changed. Acidosis induced the pH-dependent relaxation in the pulmonary and ear artery (Fig. 5A), but the degree of relaxation was

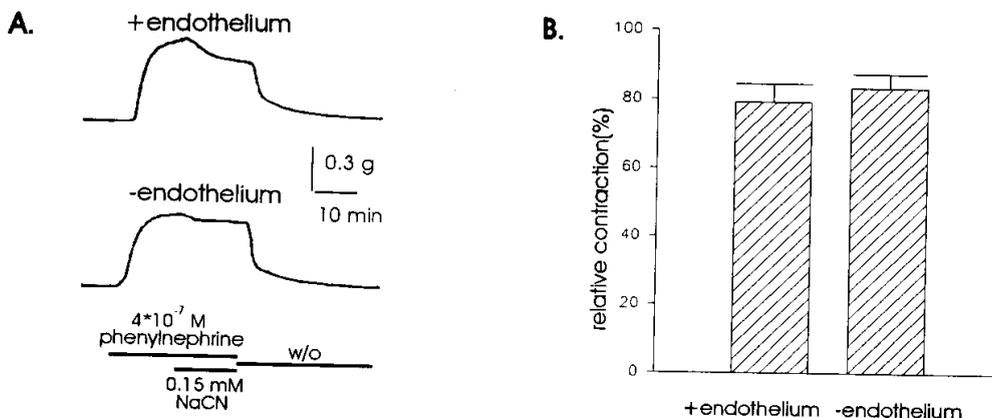


Fig. 4. Effects of NaCN on the precontracted pulmonary artery with or without endothelium. A. Addition of 0.15 mM NaCN induced relaxation in the endothelium-intact or endothelium-damaged pulmonary artery. B. Relative relaxation responses were obtained ($n = 3$) and compared between the endothelium-intact (79.13 ± 5.31) and endothelium-damaged pulmonary artery (83.33 ± 4.17). No significant difference ($p > 0.05$, paired t-test).

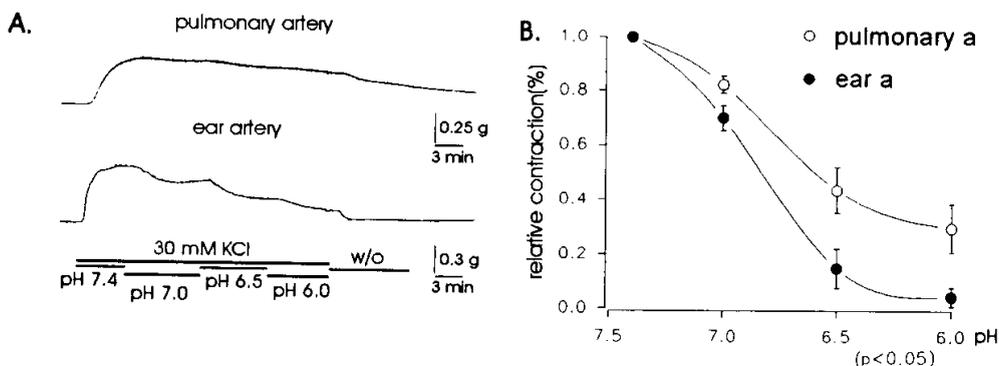


Fig. 5. Effects of pH on the pulmonary and ear artery precontracted with 30 mM KCl. A. Typical traces of responses to the changes of pH were shown. B. Cumulative relaxation responses to pH were obtained ($n = 5$) and compared between pulmonary (open circle) and ear artery (filled circle). p values for pH 6.5 and 6.0 are less than 0.05 (student t-test).

greater in the ear artery than pulmonary artery (Fig. 5A, B, $p < 0.05$ at pH 6.0, 6.5). These result suggests that the pulmonary artery is more resistant to the acidosis than the ear artery.

DISCUSSION

Pulmonary and ear artery showed dose-dependent contraction to KCl and phenylephrine.

Contractile responses of ear artery were larger than that of pulmonary artery in 2~3times to KCl (30 mM) and in 5~9times to phenylephrine (10^{-6} M, α -1 agonist). Therefore, these data suggest that contractile force of pulmonary artery much smaller than that of systemic artery and especially that IP_3 (inositol-triphosphate)-mediated contraction occupied a small fraction of the contraction in the pulmonary artery, since the

phenylephrine-induced contractile tone of the pulmonary artery was significantly less than the tone of the ear artery. Harder et al.(1985) reported that hypoxia-induced action potential of small pulmonary arteries of the cat was dependent on the external [Ca]. Jin et al.(1993) also reported that IP_3 is involved in norepinephrine but not in hypoxia-induced pulmonary arterial contraction. Therefore, it is considered that IP_3 -dependent Ca pool of the pulmonary artery plays a less significant role in the generating of muscle tone in the hypoxic pulmonary vasoconstriction.

The isolated pulmonary arterial hypoxic response was biphasic, displaying an initial rapid contraction of short duration then, before complete relaxation of this first response, a second slow but sustained contraction occurred (Bennie et al. 1991). It has been reported that metabolic blockers produced a rapid transient contraction (Rounds & McMurtry, 1981; Rajamma et al. 1991), and indoacetate or 2-deoxyglucose which inhibit glycolysis potentiated the hypoxic pulmonary vasoconstriction (Stanbrook & McMurtry, 1983). Therefore, hypoxic pulmonary vasoconstriction could be related to the metabolic changes by hypoxia and the hypoxic vasoconstriction response could be regulated within the narrow range of the inhibition of metabolism, since metabolic blockers failed to sustain the transient increased tone (Stanbrook & McMurtry, 1983). Moreover, it is believed that intracellular ATP is necessary to evoke hypoxic pulmonary vasoconstriction, since slow and sustained relaxation by metabolic blockers was attenuated with increase of the bath glucose concentration (Wiener & Sylvester, 1993).

Our results showed a relaxation by DNP and NaCN in the pulmonary and ear artery, but pulmonary artery was much resistant to DNP and NaCN. This result implies that pulmonary artery was easy to maintain the tone in hypoxia and this difference could be related to the hypoxic pulmonary vasoconstriction.

Generally acidosis occurred in hypoxia in consequence of the inhibition of the oxidative phosphorylation (Gutierrez, 1991). Haas and Bergofsky (1968) reported that pulmonary arterial

pressure was increased by the intravenous infusion of HCl and Brimiouille et al.(1990) reported that acidosis enhance hypoxic pulmonary vasoconstriction in dogs. However, Our data showed that acidosis induced pH-dependent relaxation in the pulmonary and ear artery, and the pulmonary artery showed a little relaxation in contrast to the ear artery. Therefore, it could be concluded that pH changes are not a cause of hypoxic pulmonary vasoconstriction, but suggest that acidosis-resistant characteristics of the pulmonary artery could contribute to maintain the hypoxia-induced tone.

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