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Cardioprotective Effect of Chronic Hypoxia is Blunted by Concomitant Hypercapnia

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Summary

The effect of chronic hypercapnia on cardioprotection induced by chronic hypoxia was investigated in adult male Wistar rats exposed to isobaric hypoxia (10 % O_2) for three weeks. In the first experimental group, CO_2 in the chamber was fully absorbed; in the second group, its level was increased to 4.1 %. Normoxic controls were kept in atmospheric air. Anesthetized open-chest animals were subjected to 20-min LAD coronary artery occlusion and 3-h reperfusion for infarct size determination (TTC staining). Chronic hypoxia alone reduced body weight and increased hematocrit; these effects were significantly attenuated by hypercapnia. The infarct size was reduced from 61.9 ± 2.2 % of the area at risk in the normoxic controls to 44.5±3.3 % in the hypoxic group (P<0.05). Hypercapnia blunted the infarct size-limiting effect of hypoxia (54.8±2.4 %; P<0.05). It is concluded that increased CO_2 levels in the inspired air suppress the development of the chronic hypoxia-induced cardioprotective mechanism, possibly by interacting with ROS signalling pathways.

Key words
Rat heart • Chronic hypoxia • Hypercapnia • Infarction • Protection

Introduction

Chronic hypoxia is associated with increased oxidative stress as evidenced by marked lipid peroxidation and the induction of antioxidant enzyme response in various tissues and organs (Yoshikawa *et al.* 1982, Nakanishi *et al.* 1995). Reactive oxygen species (ROS) contribute to the development of tissue injury, which can be reduced by concomitant hypercapnia (Ooi *et al.* 2000, Herget *et al.* 2001) or by administration of antioxidants (Lai *et al.* 1998, Herget *et al.* 2000). On the other hand, chronic hypoxia increases cardiac tolerance to subsequent acute ischemia: it reduces myocardial cell injury (Turek *et al.* 1980, Neckář *et al.* 2002a), suppresses ischemic and reperfusion ventricular arrhythmias (Asemu *et al.* 1999) and enhances the recovery of contractile functions during reperfusion (Baker *et al.* 1997, Neckář *et al.* 2002b). The mechanism of protection by chronic hypoxia is not understood (for review see Kolář 1996) but ROS signalling may be implicated in the process. Our aim was to examine whether hypercapnia modulates the protective effect of chronic hypoxia on ischemia-induced myocardial injury.

Methods

Adult male Wistar rats (weighing 220-250 g) were exposed to chronic isobaric hypoxia (10 % O_2) for

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three weeks. In the first experimental group, CO_2 in the chamber was fully absorbed; in the second group, its level was increased to 4.1 % (range 3.8-4.3) and continuously monitored by a capnometer (Capnocheck, BCI International, USA). Normoxic controls were kept in atmospheric air. All animals had free access to water and a standard laboratory diet. The study was conducted in accordance with the Guide for the Care and Use of Laboratory Animals published by US National Institutes of Health (NIH Publication No. 85-23, revized 1996).

Rats were anesthetized with pentobarbital (Sanofi, France; 60 mg/kg body weight, intraperitoneally) on the day after the last hypoxic exposure. Thoracotomy and occlusion of the left anterior descending (LAD) coronary artery were performed in pump-ventilated animals as described earlier (Neckář *et al.* 2002a). Blood pressure was monitored in the left carotid artery and the heart rate was derived from the blood pressure curve. A sample of blood was taken from the tail to measure hematocrit by the capillary micromethod. After the surgical preparation, the rats were allowed to stabilize for 10 min before the 20-min ischemic intervention followed by 3-h reperfusion. At the end of reperfusion, the hearts were arrested in diastole with 0.25 mg verapamil (Isoptin, Knoll, Germany) injected into the jugular vein. The area at risk and the infarct area were delineated by 5 % potassium permanganate and by 1 % 2,3,5-triphenyltetrazolium chloride (Sigma, MO, USA) as described earlier (Neckář *et al.* 2002a). The hearts were cut perpendicularly to the long axis of the left ventricle into slices 1 mm thick and stored overnight in 10 % neutral formaldehyde solution. The infarct size (IS), the size of the area at risk (AR) and the size of the left ventricle were determined by a planimetric method. The IS was normalized to the AR (IS/AR) and the size of AR was normalized to the LV (AR/LV).

The results are expressed as means ± S.E.M. One-way ANOVA or ANOVA for repeated measures and subsequent Student-Newman-Keuls test were used for comparison of differences in parametric variables between the groups. Differences were assumed as statistically significant when $P<0.05$.

Results and Discussion

Adaptation of rats to chronic hypoxia alone significantly decreased body weight (230±2.7 g) and increased hematocrit (61.8±0.74 %) as compared with age-matched normoxic controls (329±9.5 g and 47.9±0.40 %, respectively). Hypercapnia significantly attenuated the effects of hypoxia on body weight (247±2.6 g) and the hematocrit (55.8±0.43 %). Table 1 summarizes the values of heart rate (HR) and mean arterial blood pressure (MAP) in all groups, determined at baseline (before ischemia), at the end of test ischemia (20 min) and at the end of reperfusion (3 h).

Table 1. Heart rate and mean arterial blood pressure after stabilization (baseline), at the end of 20-min ischemia and at the end of the 3-h reperfusion.

	Normoxic	Hypoxic	Hypoxic/ Hypercapnic
n	12	11	12
Heart rate (beats/min)			
Baseline	432±7.7	397±12.2*	451±10.1**
Ischemia	450±5.7	414±9.3*	443±6.1**
Reperfusion	404±7.8†‡	406±12.0	399±11.4†‡
Blood pressure (mmHg)			
Baseline	126±3.2	136±5.7	137±3.1
Ischemia	130±4.9	135±3.3	144±2.9
Reperfusion	105±4.7†‡	121±8.0	115±3.6†‡

*Values are means ± S.E.M.; n, number of animals; * P<0.05 vs. normoxic; ** P<0.05 vs. hypoxic; † P<0.05 vs. baseline; ‡ P<0.05 vs. ischemia*

Adaptation to chronic hypoxia decreased baseline and ischemic values of HR as compared with those of normoxic controls. This effect was absent in the hypoxic/hypercapnic group. No significant differences in MAP were found at baseline and during ischemia between the groups. At the end of reperfusion, HR and MAP were significantly lower in normoxic and hypoxic/hypercapnic groups as compared with respective baseline and ischemic values. Figure 1 presents the size of the area at risk (AR/LV) and the relative infarct size (IS/AR). The AR/LV was significantly larger in the hypoxic group than in the normoxic one (49.3±2.8 % and 34.1±1.7 %, respectively), but it did not differ in the hypoxic/hypercapnic group (42.2±3.4 %). The infarct size reached 61.9±2.2 % of the AR in normoxic controls and adaptation to chronic hypoxia significantly decreased IS/AR to 44.5±3.3 %. Hypercapnia blunted the infarct size-limiting effect of hypoxia (54.8±2.4 %).

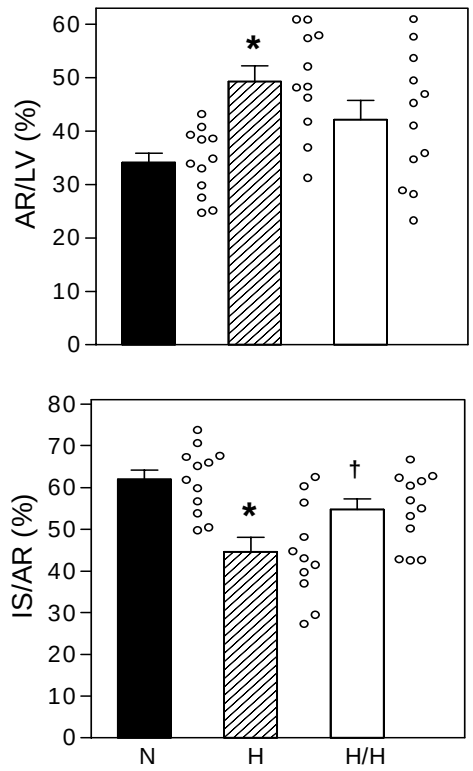

Fig. 1. *The relative size of the area at risk (AR/LV) and the relative infarct size (IS/AR) in normoxic (N), hypoxic (H) and hypoxic/hypercapnic (H/H) groups. Circles indicate individual experiments. Values are means ± S.E.M.; * P<0.05 vs. normoxic; † P<0.05 vs. hypoxic.*

The main result of the present study concerns the finding that concomitant hypercapnia markedly suppresses the cardioprotective effect of chronic hypoxia. This is in line with previous studies describing the inhibitory influence of hypercapnia on hypoxia-induced changes, such as pulmonary hypertension, right ventricle hypertrophy, enlargement of carotid bodies and the rise of hematocrit values(Dhillon *et al.* 1984, Ooi *et al.* 2000, Herget *et al.* 2001). Hence, both harmful and beneficial effects of chronic hypoxia are counteracted by increased concentration of CO_2 during the period of adaptation. Hypercapnia alone has no appreciable effect on the cardiopulmonary system in normoxic animals (Ooi *et al.* 2000).

Increase of ROS production and oxidative damage of tissue appear to be involved in the pathogeny of pulmonary hypertension in chronic hypoxia (for review see Herget *et al.* 2000). The presence of increased CO_2 level attenuates hypoxic pulmonary hypertension most probably by a reduction of oxidative stress. It has been reported that CO_2 interacts with peroxynitrate (Lymar and Hurst 1996), the radical product of superoxide and nitric oxide reaction. Hypercapnia reduces the hypoxia-induced increase in the serum concentration of nitrotyrosine, the marker of peroxynitrite production (Herget *et al.* 2001), eliminates lipid peroxidation in erythrocytes (Veselá *et al.* 2001) and prevents hypoxia-induced cleavage of collagen type I in the lung vessels (Herget *et al.* 2002).

Tissue oxidative stress plays an important role in the development of ischemia/reperfusion injury of the heart and the increase in endogenous or exogenous antioxidants may have beneficial effects (Šochman *et al.* 1990, Kukreja and Hess 1992, Dhalla *et al.* 2000). Experimental studies have suggested, however, that ROS generated during brief periods of ischemia/reperfusion activate an endogenous protective mechanism which increases cardiac tolerance to subsequent ischemia-induced injury (Baines *et al.* 1997, Vanden Hoek *et al.* 1998). This view is supported by studies that have shown that administration of ROS scavengers before the test ischemia abolished protection by preconditioning (Yao *et al.* 1999, Pain *et al.* 2000). Recently, increased production of ROS has been associated with the activation of mitochodrial ATP-sensitive potassium channels by ischemic or pharmacological preconditioning (Pain *et al.* 2000, McPherson and Yao 2001). As the activation of these channels appears to have an important role in cardioprotection against chronic hypoxia (Asemu *et al.* 1999, Eells *et al.* 2000, Neckář *et al.* 2001b), the ROS signalling may also be implicated in this process. We can therefore speculate that increased production of ROS during long-term exposure of rats to hypoxia contributes to the development of increased ischemic tolerance of their hearts, which manifests itself as a reduction of myocardial infarction. Hypercapnia then blunts this protective effect by attenuating the oxidative stress induced by chronic hypoxia.

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