

Research Article

## Effect of Chronic hypoxia on Carotid vascular responses to noradrenaline in rats

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**Keywords:**

Chronic hypoxia, carotid circulation, noradrenalin, nitric oxide

**ABSTRACT**

**Aim:** The aim of the present study was to examine whether chronic hypoxia would alter the noradrenaline (NA)-evoked vascular responses in carotid circulation in rats. Furthermore, whether the carotid autoregulatory response to NA-evoked rise in arterial blood pressure (ABP) is compromised by chronic hypoxia or not. Also, the role of tonically synthesised nitric oxide (NO) in these responses was investigated.

**Methods:** the study was done using two comparable age groups of adult Wistar rats; the first were breathing normal 21% O<sub>2</sub> (normoxic; N), whereas the second were made chronically hypoxic (CH) by breathing 12% O<sub>2</sub> for 3 weeks, while they were growing from 7 to 10 weeks. In anaesthetised rats, the carotid blood flow (CBF) and carotid vascular conductance (CVC) were recorded during a 3 min infusion of NA at a dose 2.5 µg kg<sup>-1</sup> to induce an acute rise in ABP to 150 mm Hg, the upper limit of autoregulatory range, before and after a bolus dose of the nitric oxide synthase inhibitor, L-NAME (10mg kg<sup>-1</sup>).

**Results:** the NA-evoked rise in ABP was comparable in N and CH rats, but the increase in heart rate was more significant in CH rats. In CH rats, the NA-evoked rise in ABP was associated with a significant reduction in CVC but with no change in CBF, whereas in N rats the reduction in CVC was accompanied with a significant reduction in CBF (p<0.05). In both groups, L-NAME did not accentuate the rise in ABP induced by NA. However, in CH rats the reduction in CBF became more significant after L-NAME.

**Conclusion:** the carotid vasculature in CH rats is less sensitive to vasopressor effect of NA, when compared to N rats. However, the carotid autoregulatory response to NA-induced rise in ABP was not compromised by chronic hypoxia. Further, it seems that NO does not play a role in the blunted carotid vasoconstrictor responses to NA in CH rats.

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### INTRODUCTION

<sup>1</sup>In response to prolonged reduction in O<sub>2</sub> availability, vascular adaptations occur in the systemic circulation that may help to ensure redistribution and increase

delivery of O<sub>2</sub> to vital organs, including the brain. In the pulmonary circulation, vascular remodelling occurs with an increase in the thickness of vascular smooth muscle along the arterial tree such that pulmonary vascular resistance is increased (Ward & Aaronson., 1999). Similar results obtained in studies on rat cerebral vasculature that have found that exposure to chronic hypoxia (~10% O<sub>2</sub>) for three weeks was associated with a significant increase in the microvessels density in the cortex, hippocampus, striatum and cerebellum (Xu & LaManna., 2006). However, these adaptations may not be great enough to ensure adequate tissue oxygenation and when they are very pronounced they may lead to further pathology. Regarding whether chronic hypoxia affects vascular responses to pressor agonists has attracted attention.

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There is agreement that the vasoconstrictor responses are attenuated or blunted in chronic hypoxia. Previous *in vitro* studies showed reduced vasoreactivity to phenylephrine (PE) in rat isolated aortic segments exposed to hypoxia for 2 days (Auer & Ward., 1998; Gonzales & Walker., 2002 & Zacour et al., 2002), or for 4 weeks compared to normoxic (N) rats (Doyle & Walker., 1991; Caudill et al., 1998). Also, in iliac arteries, the contractile response to noradrenalin (NA) was attenuated in chronically hypoxic (CH) rats adapted to chronic hypoxia for 4 weeks relative to N rats (Bartlett & Marshall., 2003). Further, the responses were accentuated by L-NAME or by endothelium removal in the arteries from CH rats suggesting a role of nitric oxide (NO) in this blunted response (Bartlett & Marshall., 2003). Similarly, previous *in vivo* studies performed on rats have demonstrated this depressed response to different pressor stimuli. An early study by Jin et al. (1989) demonstrated a blunted pressor response to arginine vasopressin in both conscious and anaesthetised rats that breathed 10% O<sub>2</sub> for 4 weeks, relative to the N rats. Doyle & Walker (1991) showed that the pressor responses to systemic phenylephrine (PE) and angiotensin II (ANG II) were attenuated in conscious CH rats breathed 12% O<sub>2</sub> for 4 weeks, relative to N rats. Another study showed that the renal vasoconstrictor responses evoked in CH rats, adapted to 12% O<sub>2</sub> for 4 weeks, by intravenous infusion of PE were significantly attenuated relative to N rats (O'Donoghue & Walker., 2000). Consistent with these results, *in vivo* studies on mesenteric and muscle arterioles showed that the constrictor responses to NA were reduced in CH rats relative to N rats and in CH rats these responses were accentuated by L-NAME implicating NO in blunting responses (Mian & Marshall., 1994). However, to our knowledge, no comparable study has tested whether chronic hypoxia alters NA-evoked vasoconstrictor responses of carotid circulation, or tested the role of NO in these responses.

Therefore, the main aim of the present study was to test the carotid vascular responses of CH rats to NA and to test the role of NO in these responses. Noradrenalin infusion was aimed at inducing a rise in ABP to 150 mm Hg, the upper end of autoregulatory range for cerebral circulation (Paulson., 2002), giving the chance to further investigate whether prolonged exposure to hypoxia would compromise the carotid autoregulatory response to an acute rise in ABP.

## **MATERIALS AND METHODS**

Experiments were performed using 16 male Wistar rats maintained at the age of ~ 7 weeks with body weights of ~ 170 gm in the animal unit (Medical Experimental Research Centre, Mansoura Medical School, Mansoura University). All procedures were performed according to the guidelines of animal care in the field. The rats were kept for 3-4 weeks till the age of 10-11 weeks, the age of performing the acute experimental protocol. They were given standard rat chow and water *ad libitum*. At the age of 7 weeks the rats were divided into 2 groups; the first 8 rats were housed in usual cages and breathed room air as they served control normoxic (N) rats. The second 8 rats were placed in 2 cages prepared to be supplied with 12% O<sub>2</sub> instead of the atmospheric air with O<sub>2</sub> of 21% and this group served as chronically hypoxic (CH) rats. The temperature was kept around 22-23 °C, and the CH rats were fed similarly to the N rats. The rats breathed the hypoxic gas mixture throughout the 3-4 weeks except for 20 min daily, when the cages were cleaned.

On the day of experiment, the rat was weighed and transferred to the laboratory. The weight of CH rats was 319 ± 10.6 gm, comparable to that of N rats (321 ± 4.5 gm). During surgery the CH rats breathed a hypoxic gas mixture of 12% O<sub>2</sub>, the level of O<sub>2</sub> to which they were acclimated. This was delivered across the main arm of a T-tube inserted into the trachea through a tracheotomy, while the other arm of the tube was sealed by a removable screw. Before the start of experimental protocol, arterial blood samples were taken via the femoral cannula for analysis of arterial blood PaO<sub>2</sub>, PaCO<sub>2</sub> and pH by using laboratory blood gas analyser (Instrumentation Laboratory, MA, USA). In CH rats, the mean values of the arterial blood PaO<sub>2</sub>, PaCO<sub>2</sub> and pH were; 51.5 ± 2.4 mm Hg, 26.95 ± 1.01 mm Hg, and 7.4 ± 0.02 respectively. In all experiments, anaesthesia was initially induced with cotton soaked with 5-10ml of ether delivered into a box containing the rat, then maintained by sodium thiopental (40mg/kg; IP; (Waynforth & Flecknell., 1998). The right femoral artery and vein were exposed and cannulated with polythene tubing filled with heparinized saline (25 U ml<sup>-1</sup> physiological saline). Femoral artery cannula was used to directly measure the arterial blood pressure by connecting it to a pressure transducer, whereas the femoral vein cannula used for administration of the NO synthase (NOS) inhibitor L-nitro methyl arginine (L-NAME). Another cannula in left femoral artery was used for infusion of NE. A midline incision in the neck was done to locate and to

allow exposure of the left common carotid artery that was carefully freed from the surrounding tissues, with care to avoid damaging the accompanying nerve trunks. Carotid blood flow (CBF) was recorded by means of a transonic flow probe (0.7 V) connected to a flow meter. The end of the flow probe was filled with acoustic coupling gel to facilitate good ultrasonic conduction. Arterial pressure and CBF were sampled by a PowerLab/8S at 100 Hz and connected to a computer by Chart (AD Instruments Pty Ltd., Australia). Mean arterial pressure (ABP) and heart rate (HR) were derived from the pressure signal and carotid vascular conductance (CVC) was computed as CBF/ABP.

**Protocol.**

An equilibration period of at least 30 min was allowed following surgery so that all baselines stabilised. Then, the cardiovascular variables were recorded in response to a 3 min of NA infusion at a dose of 2.5µg Kg<sup>-1</sup>. This infusion rate was chosen as the lowest to induce the rise in ABP to 150 mm Hg without being fatal to the rats (pilot experiments have done to choose the dose. After the cardiovascular variables had stabilised again, a bolus dose of N<sup>G</sup>-nitro-L-arginine methyl ester (L-NAME; 10 mg kg<sup>-1</sup>, i.v; (Ray & Marshall., 2005) was given. After a period of ~ 15 min, the protocol was repeated as described above.

**Chemicals**

All chemicals were obtained from (Sigma-Egypt). Noradrenaline and L-NAME were dissolved in physiological saline (0.9%) and were freshly prepared on the day of the experiment.

**Statistical Analysis**

All results are expressed as mean ± SEM. In each group the baseline value of each variable before NA infusion was compared with the mean value over the 3 min of the NA infusion by Students' paired *t*-test before and after L-NAME. Students' paired *t*-test was also used to compare baseline values before and 15 min after L-NAME. Comparison between N rats and CH rats was done using Factorial ANOVA with Scheffe's *post-hoc* test. P < 0.05 was considered significant.

**RESULTS**

***Effect of chronic hypoxia on baselines***

Table 1 shows the resting values of recorded cardiovascular variables in N rats and CH rats. The resting ABP and HR values of CH rats breathing 12%

O<sub>2</sub> were comparable to those of N rats breathing air. Although the resting baseline values of CBF and CVC tended to be smaller in CH rats than in N rats, when compared statistically, this difference was not significant (p= 0.1).

**Table 1.** Baseline values of cardiovascular variables recorded in chronic hypoxic (CH) rats breathing 12% O<sub>2</sub> (n = 8, weight: 319 ± 10.6 gm) and in normoxic (N) rats breathing air (n = 8, weight: 321 ± 4.5 gm). Values are mean ± SEM. There was no significant difference between the two groups. Abbreviations: ABP; arterial pressure, HR; heart rate, CBF; carotid blood flow, CVC; carotid vascular conductance

	N rats	CH rats
ABP (mm Hg)	119.66 ± 6.08	120.80 ± 3.17
HR (b.p.m)	390.46 ± 17.63	369.84 ± 2.15
CBF (ml min <sup>-1</sup> )	2.32 ± 0.44	1.70 ± 0.11
CVC (ml min <sup>-1</sup> mm Hg <sup>-1</sup> )	0.019 ± 0.003	0.014 ± 0.001

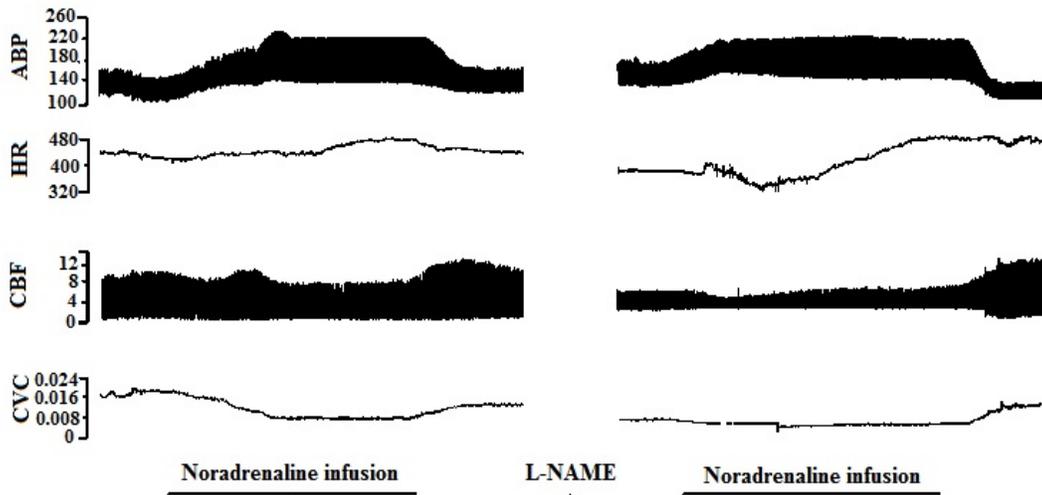
***Responses evoked by NA***

Original traces showing the cardiovascular responses evoked by a 3 min infusion of NA at 2.5 µg kg<sup>-1</sup> in N and CH rat are shown in **Figures 1 & 2** respectively. The mean results showed that NA caused a significant increase in ABP in CH rats as in N rats (**Fig. 3**). This rise was accompanied by a significant reduction in CVC but with no significant change in CBF (p= 0.4) in CH rats, whereas in N rats there was a significant fall in CBF (p<0.05) with the reduction in CVC. Unlike N rats, NA caused a significant increase in HR in CH rats (p<0.01).

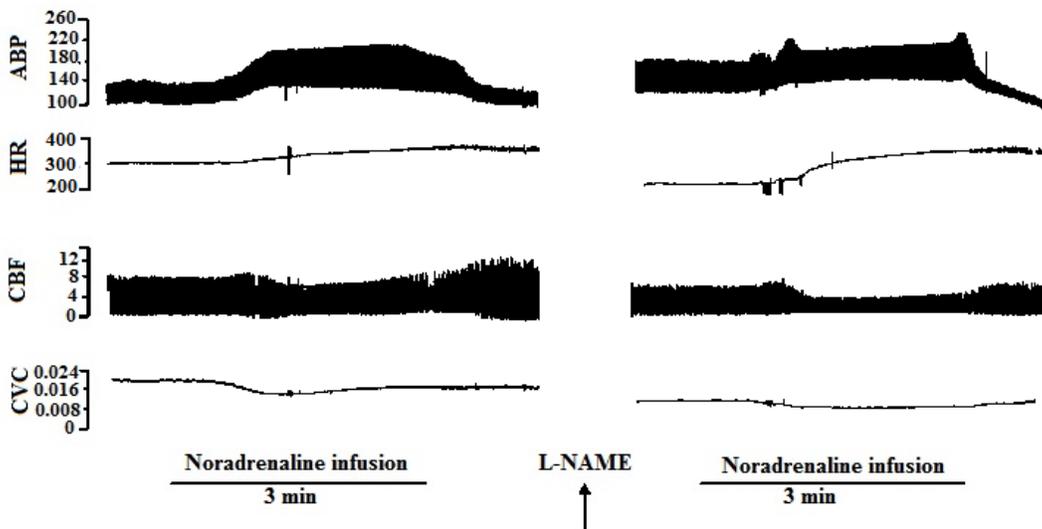
***Effect of L-NAME on NA-evoked responses***

In CH rats, NA infusion did not cause any greater increase in ABP after L-NAME as in N rats (see **Figs. 4 & 5**). However, in CH rats, the reduction in CVC was associated with a significant reduction in CBF (**Fig. 5**). This in contrast to the findings in N rats: in N rats, NA infusion after L-NAME caused a reduction in CVC but any change in CBF did not reach significance (see Fig. 4).

*Hypoxia and carotid responses to noradrenaline*



**Fig.1** Original traces of cardiovascular variables evoked by NA infusion before & after L-NAME in normoxic rat. Abbreviations: ABP; arterial blood pressure (mm Hg), HR; heart rate (bpm), CBF; carotid blood flow (ml /min), CVC; carotid vascular conductance (ml min mm Hg<sup>-1</sup>)

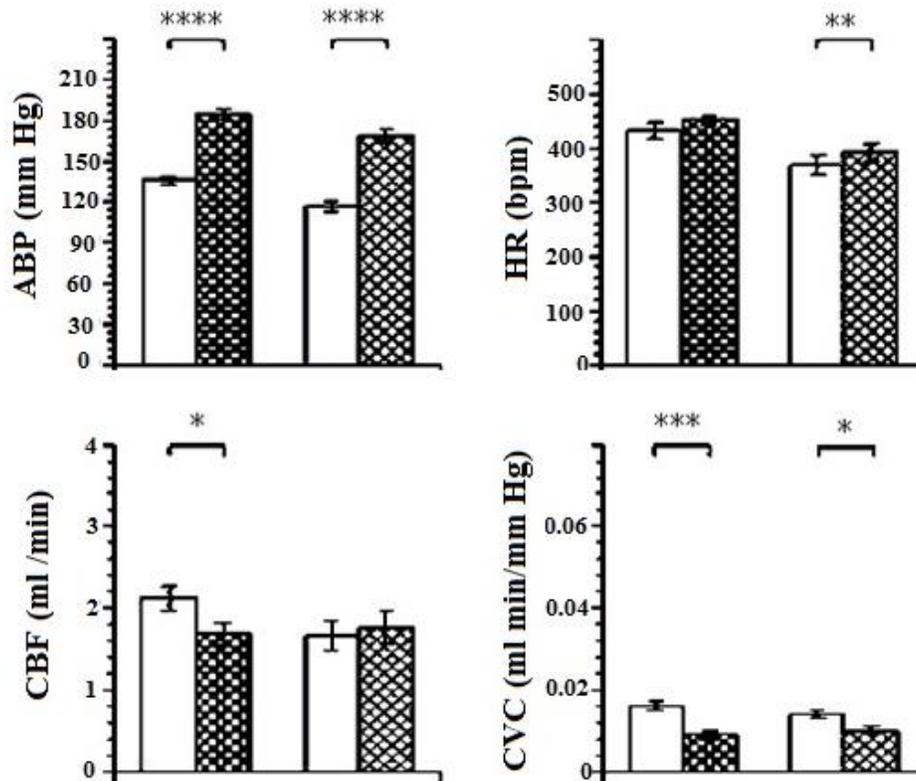


**Fig. 2.** Original traces of cardiovascular variables evoked by NA infusion before & after L-NAME in chronically hypoxic rat. Abbreviations: ABP; arterial blood pressure (mm Hg), HR; heart rate (bpm), CBF; carotid blood flow (ml /min), CVC; carotid vascular conductance (ml min /mm Hg).

**Effect of L-NAME (10 mg kg<sup>-1</sup>) on baselines**

The effects of L-NAME at a dose of 10 mg kg<sup>-1</sup> on baseline cardiovascular variables are shown in **Table 2**. These results are presented in the companion paper, submitted with this. Thus, in CH rats, L-NAME administration caused a significant increase in ABP that was associated with significant reduction in CVC and

CBF comparable to the effects seen in N rats. L-NAME also induced a significant reduction in HR in CH rats as in N rats. Comparison between mature N and CH rats for the effect of L-NAME did not reveal any significant differences between the two groups.



**Fig. 3.**

Mean cardiovascular responses evoked by a 3 min of noradrenaline infusion (NA, 2.5  $\mu\text{g kg}^{-1}$ ) in normoxic (N) rats and in chronically hypoxic (CH) rats ( $n=8$  &  $8$  respectively). Values are mean  $\pm$  SEM. \*, \*\*, \*\*\*, \*\*\*\*:  $P < 0.05, 0.01, 0.001, 0.0001$  respectively; indicating significant difference from values recorded before NA (Student's paired t-test).

- Baseline values before NA.
- ▣ NA-evoked responses in N rats
- ▤ NA-evoked responses in CH rats.

Abbreviations: ABP; arterial blood pressure (mm Hg), HR; heart rate (bpm), CBF; carotid blood flow (ml/min), CVC; carotid vascular conductance (ml min mm Hg $^{-1}$ )

## DISCUSSION

The adrenergic-mediated regulation of systemic arterial tone represents an important defence mechanism for oxygen delivery to vital organs. The results from previous *in vitro* and *in vivo* studies demonstrated that chronic hypoxia is associated with a generalised reduction in the pressor responses to NA and in vasoconstrictor responses evoked in systemic arteries and arterioles by NA (Doyle & Walker., 1991; Mian & Marshall., 1994; Gonzales & Walker., 2002 & Bartlett & Marshall., 2003). On the other hand, some studies have indicated that the function of sympathetic nerves in cerebral arteries from adult sheep was not altered by chronic exposure to hypoxia, as the stimulated NA

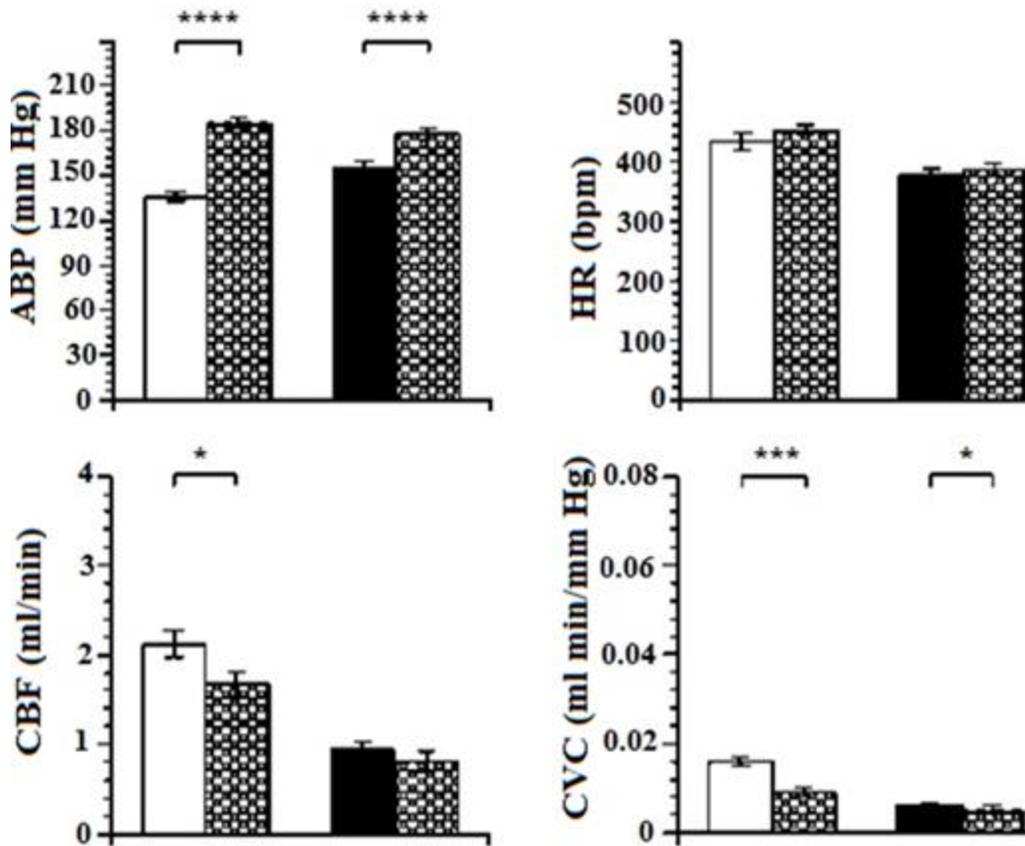
release was maintained (Buchholz et al., 1999, Buchholz & Duckles., 2001). In spite of this cumulative data about the effect of chronic hypoxia on the pressor response to NA, there was no available data whether chronic hypoxia alter NA-evoked vascular responses in carotid circulation in rat. Therefore, the primary aim of the present study was to test whether or not CH rats show compromised autoregulatory and vasoconstrictor responses in the carotid vasculature during NA-induced hypertension and to test the role of NO in these responses.

*Hypoxia and carotid responses to noradrenaline*

**Table 2.** Baseline values of cardiovascular variables recorded before and ~ 15 min after L-NAME (10 mg kg<sup>-1</sup>, i.v.) in CH rats breathing 12% O<sub>2</sub> and in N rats breathing air (n =8 & 8).

	N rats		CH rats	
	Before L-NAME	After L-NAME	Before L-NAME	After L-NAME
ABP (mm Hg)	114.31± 3.56	131.29 ± 4.83**	124.33± 2.10	149.01 ± 3.73 ***
HR (b.p.m)	403.00± 9.91	338.00± 10.55***	363.02± 17.89	308.10 ± 16.35***
CBF (ml min <sup>-1</sup> )	1.78± 0.11	0.93± 0.06**	1.39± 0.08	0.88 ± 0.05**
CVC (ml min <sup>-1</sup> mm Hg <sup>-1</sup> )	0.015± 0.002	0.007 ± 0.001**	0.011± 0.001	0.006 ± 0.001***

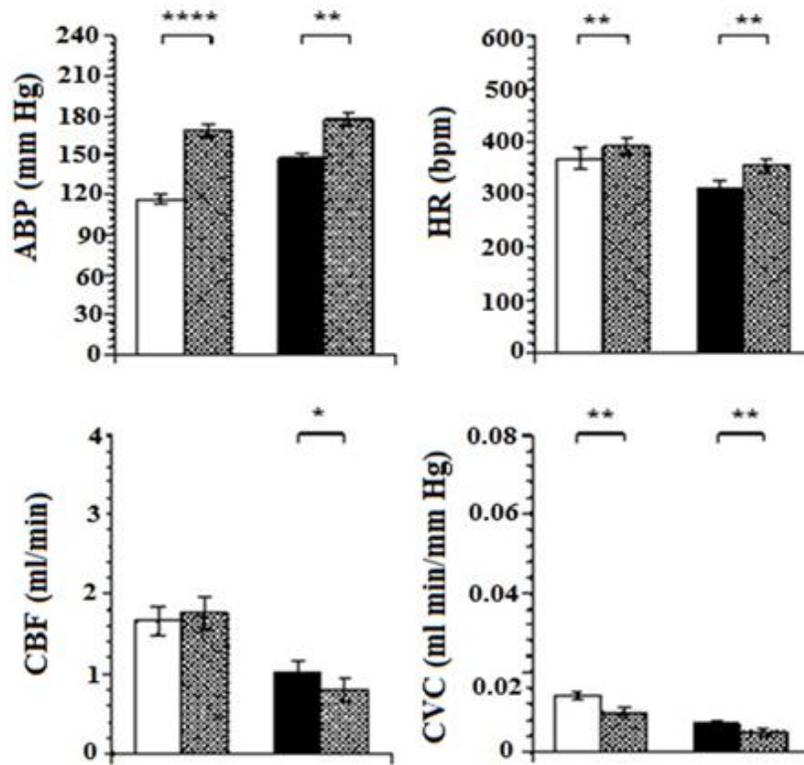
Values are mean ± SEM. \*\*, \*\*\*: P < 0.01, 0.001; significantly different from value before L-NAME (Student's paired t-test).



**Fig. 4.** Effect of L-NAME (10 mg kg<sup>-1</sup>, i.v.) on Noradrenaline-evoked responses (NA, 2.5 µg kg<sup>-1</sup>) in normoxic rats (n= 8). Values are mean ± SEM. \*, \*\*\*, \*\*\*\*: P < 0.05, 0.001, 0.0001 respectively, indicating significant difference from baseline value before NA infusion (Student's paired t-test).

- Baseline value before 1<sup>st</sup> NA infusion
- Baseline value after L-NAME and before 2<sup>nd</sup> NA infusion
- ▨ NA-evoked responses.

*Hypoxia and carotid responses to noradrenaline*



**Fig. 5.**

Effect of L-NAME (10 mg kg<sup>-1</sup>, i.v.) on noradrenaline (NA, 2.5µg kg<sup>-1</sup>)-evoked responses in chronically hypoxic rats (n= 8). Values are mean ± SEM.

\*, \*\*, \*\*\*\*: P < 0.05, 0.01, 0.0001 respectively indicating significant difference from value recorded before NA infusion (Student's paired t-test).

- Baseline value before 1<sup>st</sup> NA infusion
- Baseline value after L-NAME and before 2<sup>nd</sup> NA infusion
- ▨ NA-evoked responses.

Abbreviations: ABP; arterial blood pressure (mm Hg), HR; heart rate (bpm), CBF; carotid blood flow (ml /min), CVC; carotid vascular conductance (ml min mm Hg<sup>-1</sup>).

Comparing the baseline values of cardiovascular variables of CH rats revealed that they were not significantly different from N rats, although CVC and CBF of CH rats tended to be smaller. Such finding is in agreement with that of a previous study in which CBF is recorded as an index for cerebral blood flow (Thomas & Marshall., 1997).

In this study, NA infusion at 2.5 µg kg<sup>-1</sup> caused a comparable rise in ABP in the CH rats to that seen in the N rats, indicating that both groups showed a similar sensitivity to the systemic pressor effect of NA. The vascular remodelling and angiogenesis that occur as adaptation to chronic hypoxia and consequently the increase in muscle vascular tree would be expected to lead to greater rise in ABP in CH rats. Thus, the increase in total peripheral resistance evoked by NA was smaller in CH rats than in N rats for the same dose of NA. Moreover, the increase in ABP may have been

comparable because the cardiac output increased more in the CH rats: HR increased significantly in CH rats but not in N rats.

Certainly, the carotid vascular responses to NA were different between the two groups: in CH rats the rise in ABP induced by NA was associated with a significant reduction in CVC but with no change in CBF. On the other hand, in N rats the reduction in CVC was accompanied by a significant reduction in CBF indicating that the carotid circulation showed an active vasoconstrictor response to NA. Thus, the carotid circulation of CH rats apparently showed less vasoconstriction or blunted vasoconstrictor response for a given rise in ABP than that of the N rats. This finding is novel regarding the carotid circulation and it is in agreement with observation of previous studies that tested vascular reactivity of other vascular beds to vasopressor agents in chronic hypoxia. Thus, an early

study showed blunted vascular reactivity in the forearm of chronically hypoxic human subjects compared with controls (Heistad et al., 1972) and in aortic sections taken from CH rats compared with N rats (Doyle & Walker., 1991). Moreover, the contraction evoked by NA in rat mesenteric and muscle arterioles and in iliac arteries from CH rats acclimated to 12% O<sub>2</sub> for 3-4 weeks was smaller than in N rats [(Mian & Marshall., 1994; Bartlett & Marshall., 2003). On the other hand, in CH rats the fact that the rise in ABP was associated no significant change in CBF indicates that the carotid circulation showed good autoregulatory response that was not compromised by chronic hypoxia. In N rats, the significant reduction in CBF in the face of NA-induced rise in ABP does not mean that their autoregulation fails because CBF would increase not decrease in such condition.

In both groups of rats, after L-NAME, NA did not cause a greater increase in ABP and the decrease in CVC was comparable to that evoked before L-NAME. However, in CH rats the carotid vasoconstrictor effect of NA was accompanied by a significant reduction in CBF (Fig. 5). Looking at this, it seems that when the baseline ABP was raised by L-NAME, the further increase in ABP evoked by NA only increased ABP to about the same level as was achieved with NA before L-NAME. In other words, the magnitude of the NA-evoked increase in ABP was smaller after L-NAME. This may explain why CBF decreased in response to NA after L-NAME but not before: the same reduction in CVC was more effective in reducing CBF in the face of a smaller increase in ABP. Thus, from this result there is no obvious reason to suggest that tonic, or NA-stimulated synthesis of NO blunted the carotid vasoconstrictor responses to NA. In previous studies on CH rats, a modulatory role of NO over vasoconstrictor responses to NA has been reported. Thus, Early & Walker (2003) demonstrated that NO contributed to the blunted reactivity of mesenteric arteries taken from CH rats exposed to chronic hypoxia for only two days. They attributed this to an increased production of NO caused by an enhanced nitric oxide synthase (NOS) activity resulting from increased endothelial cell [Ca<sup>2+</sup>], rather than to increased expression of endothelial NOS. Further, in iliac arteries from CH rats that had acclimated to 12% O<sub>2</sub> for 3-4 weeks, the contractile response to NA was depressed in endothelium intact rings, but not in the presence of L-NAME and not when the endothelium was removed, indicating a role for endothelial NO in the blunted responsiveness to NA (Bartlett & Marshall., 2003). In the present experiments it is difficult to suggest that NO

contributed to the blunted response to NA in carotid vasculature of CH rats. However, it would be interesting to further explore the contribution of NO to the constrictor responses evoked in carotid circulation by NA in CH rats, by using higher doses of L-NAME.

The results of the present experiments indicated that the dilator role of tonically synthesised NO was not different between the N rats that breathed air and CH rats that had acclimated to breathing 12% O<sub>2</sub>. This conclusion can be drawn from the finding that L-NAME had comparable effects on the different cardiovascular variables in the two groups, including CVC and CBF. This finding was previously made in rat hind limb vasculature too: L-NAME at an equivalent dose had similar effects on baseline cardiovascular variable (ABP, HR, femoral blood flow, and femoral vascular conductance) in N and CH rats (Coney et al., 2004). In previous studies performed on adult rats breathing 10% O<sub>2</sub> for 7 days and for 3 weeks, there was increased eNOS expression in the pulmonary circulation [Shaul et al., 1995; Le Cras et al., 1998]. However, an increased eNOS expression was found in rat lung after 7 days of hypoxia, whereas the eNOS expression in the aorta was decreased after the same period (Toporsian et al., 2000). Thus, it seems that chronic hypoxia may alter the expression of eNOS differently in different vascular beds. Another study has shown that during early exposure to chronic hypoxia there is greater role for tonically synthesised NO, but this had diminished by 3-4 weeks of hypoxia (Walsh & Marshall., 2006). Therefore, in future studies it would be useful to investigate the tonic influences of NO in the carotid circulation in early chronic hypoxia, by using L-NAME.

Thus, in conclusion, the main aim of the present experiments was to test whether prolonged exposure to chronic hypoxia as rats grow from 7 to 10-11 weeks would affect the constrictor responses of carotid vasculature to the sympathetic neurotransmitter NA as well as their autoregulatory response to NA-evoked rise in ABP. It seems that the sensitivity of the CH rats to the systemic pressor effect of NA at 2.5 µg kg<sup>-1</sup> was blunted. Further, the carotid circulation showed less vasoconstrictor response to NA in the CH rats in contrast to the active carotid vasoconstrictor response in the N rats. However, the carotid autoregulatory response to the upper end of autoregulatory range was not compromised by chronic hypoxia. Further, L-NAME administration did not accentuate the pressor response to NA in the CH rats suggesting that NO is not implicated in the blunted response to NA.

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