INTERTMITTENT HYPOXIA REDUCES nNOS EXPRESSION AND INCREASES THE HEART RATE RESPONSE TO CARDIAC SYMPATHETIC NERVE STIMULATION *in-vitro*

R.M. Mohan, S. Golding and D.J. Paterson
University Laboratory of Physiology, Oxford, OX1 3PT, UK

INTRODUCTION

Long term exposure to intermittent hypoxia (eg. obstructive sleep apnoea) elevates urinary and plasma catecholamines, muscle sympathetic nerve activity and systemic arterial blood pressure (Fletcher 2000).

Functionally, enhanced sympathetic activity may be due to reduced bioavailability of nitric oxide (NO) since activation of the NO-cGMP pathway decreases central sympathetic activity (Zanzinger et al. 1997), the peripheral pre-synaptic release of noradrenaline (NA) (Schwarz et al. 1995) and the heart rate (HR) response to sympathetic nerve stimulation (SNS) (Choate and Paterson 1999).

AIMS

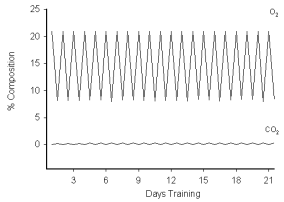
To investigate whether:

a) Intermittent hypoxia (IH) enhances the heart rate (HR) response to peripheral sympathetic nerve stimulation (SNS) in the guinea-pig atria *in-vitro*.
b) NO plays a role in the HR response to SNS following IH.

METHODS

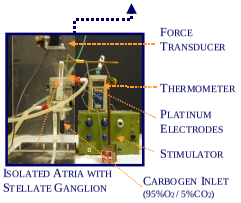
TRAINING PROTOCOL

- Animals undergoing IH were placed inside a purpose-built hypoxic chamber.
- Manual adjustments to the flow of nitrogen and air maintained Fc_{O2} at 8-8.5% and Fc_{CO2} at 0.3-0.35%.
- Animals underwent IH for 21 consecutive days. Control animals remained in an ambient atmosphere (Fc_{O2} :21%, Fc_{CO2} :0.04%) for the same duration.



IN-VITRO GUINEA PIG ATRIAL PREPARATION AND WESTERN BLOTTING

The atria and right stellate ganglion were dissected free, sutures placed on the lateral edges of both atria and attached to an isometric force transducer. Heart Rate (bpm) was triggered from contraction. The right stellate ganglion was placed through a pair of platinum ring electrodes connected to a stimulator. Western blot analysis for nNOS was performed on atria from both groups.



RESULTS : *PHYSICAL CHARACTERISTICS*

Intermittent hypoxia groups demonstrated typical responses to hypoxic exposure, characterised by significantly ($p<0.05$) lower body weights, reduced growth rates and increased heart weight / body weight ratios.

RESULTS: *HR RESPONSE TO PERIPHERAL SNS AND BATH APPLIED NORADRENALINE*

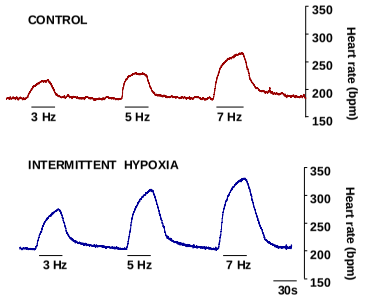


Figure I

- Raw data traces showing the effects of cardiac SNS (3, 5 and 7Hz, 10V, 1ms pulse width) on heart rate (bpm) in a double atrial / right stellate ganglion preparation from a Control (Top trace) and an IH trained (Lower trace) animal. Note the enhanced HR response in IH group.

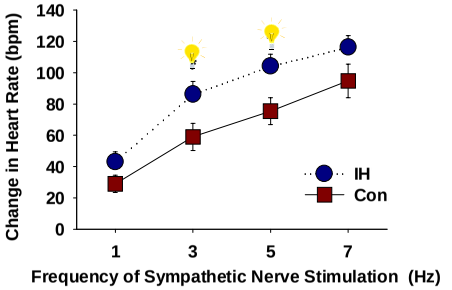


Figure II

- The positive chronotropic response to SNS at 3 and 5Hz was significantly enhanced in IH atria (*$p<0.05$, n=13-14).

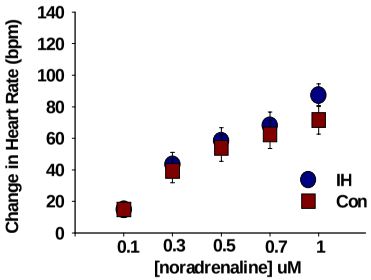


Figure III

- Dose response curves for the increase in heart rate (bpm) with bath-applied noradrenaline (NA, 0.1-1µM) in atria from Control (n=7) and IH (n=10). Note that there was no difference in the response suggesting a pre-synaptic action of training as shown in Figure II.

RESULTS: *ROLE OF NITRIC OXIDE*

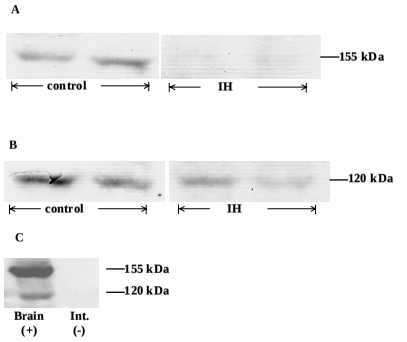


Figure IV

- Western blot analysis of nNOS protein expression, using a specific monoclonal antibody in the atria from control (n=4) and IH (n=4) animals. A 155 kDa protein band compatible with the nNOS was absent in IH samples, but was identifiable in control samples (Panel A).
- A 120 kDa protein band compatible with the nNOS variant was lower (by 52%) in the IH group compared to the control group (Panel B). Isolated guinea pig small intestine (-ve control) and guinea pig fore-brain (+ve control) were used to verify nNOS antibody specficity (Panel C).

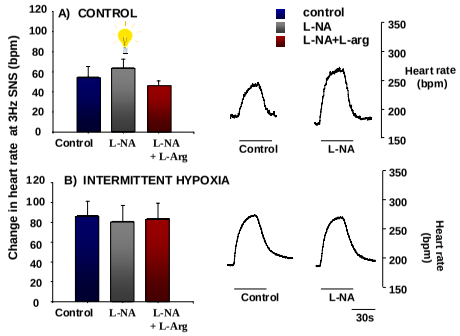


Figure V

- The effect NOS inhibition with L-NA (100µM) and its reversal with L-arginine (1mM) on the increase in heart rate with SNS at 3 Hz (n=6 control, n=7 IH).
- L-NA significantly enhanced the magnitude of the positive chronotropic response to SNS in control atria at 3 Hz stimulation and this effect was attenuated with L-arginine (*$p< 0.05$).
- The heart rate response to sympathetic stimulation was unaltered by NOS inhibition in the IH group.

CONCLUSION

The new findings in this study are that:

1) There is a significant peripheral pre-synaptic component underlying the enhanced heart rate response to cardiac SNS with intermittent hypoxia.

2) The enhanced HR response to SNS following intermittent hypoxia may be related to a decreased inhibitory action of NO on pre-synaptic NA release.

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