



# CHRONIC INTERMITTENT HYPOXIA PROMOTES DIFFERENTIAL EXPRESSION OF STRESS PROTEINS FOLLOWING *in vitro* HYPOXIA IN THE GUINEA PIG HEART

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## INTRODUCTION

The sino-atrial node/atria are markedly resistant to the deleterious effects of hypoxia and ischaemia, continuing to beat long after the ventricular contraction has ceased.

Heat Shock Proteins (HSP) are a distinct family of proteins that are synthesised in response to physiological stressors (e.g. exercise, heat, myocardial ischaemia, acute hypoxia).

The expression of HSPs in the heart following either acute, chronic or intermittent hypoxia is well established, however, contrasting studies have shown that acute hypoxia failed to induce HSP expression in endothelial cells and ventricular tissue.

## AIMS OF THIS STUDY

1. To investigate whether 21 days of intermittent hypoxia training (IHT) increases the tolerance of atria to subsequent severe *in vitro* hypoxia.
2. To determine whether changes in HSP expression are altered by either IHT alone or IHT in combination with *in vitro* hypoxia.

## MATERIALS & METHODS

**Guinea pigs:** (200-250g) were divided into 4 groups:

- IHT: tissues removed immediately after IHT (n=4).
- IHT-IV: animals undergoing IHT+ *in vitro* hypoxia (n=10).
- CON: control (n=4).
- CON-IV: control animals undergoing *in vitro* hypoxia (n=10).

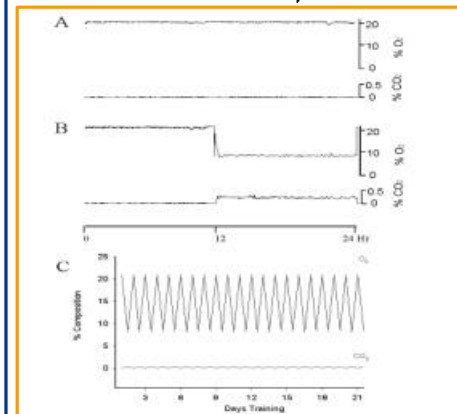
**IHT:** 12 hr periods during the dark phase of the animals circadian cycle. IHT  $F_{CO_2} = 8-8.5\%$ ,  $F_{CO_2} = 0.3-0.35\%$ . CON  $F_{CO_2}=21\%$  and  $F_{CO_2}=0.04\%$ . After 21 days IHT, atria were mounted vertically with the right atrium attached to an isometric force transducer to measure heart rate.

***In vitro* Hypoxia:** Atria were equilibrated in an organ bath for 90 minutes before exposure to 20%  $O_2/5\% CO_2$ , 10%  $O_2/5\% CO_2$ , 5%  $O_2/5\% CO_2$  or 0%  $O_2/5\% CO_2$  for 3-4 minutes. Nitrogen was also passed across the surface of the bath.

**Western Blotting:** Tissues were lysed and 50µg of protein separated by SDS-PAGE and electroblotted onto PVDF. After incubation with specific monoclonal antibodies, proteins were detected by chemiluminescent autoradiography. Digitised images were analysed using GeneTools gel analysis software (Syngene, UK).

## RESULTS

**FIGURE 1. REPRESENTATIVE RAW DATA TRACE SHOWING THE IHT PROTOCOL**



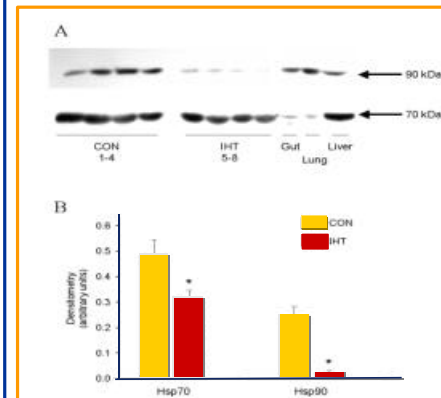
Daily exposure to oxygen ( $O_2$ ) and carbon dioxide ( $CO_2$ ) in Control (A) and IHT (B) groups. During the 21-day IHT, concentrations of  $O_2$  and  $CO_2$  were maintained at  $8.22 \pm 0.03\%$  and  $0.29 \pm 0.01\%$  respectively during the hypoxic periods (C).

**TABLE 1. EFFECT OF IHT ON PHYSICAL CHARACTERISTICS**

	Body Weight, (g)	Growth Rate, (g/day)	<sup>1</sup> Whole Heart Weight (mg)	Ventricular Weight/Body Weight, (mg/g)
Control	394 ± 18	10.8 ± 0.4	1.3 ± 0.1	2.8 ± 0.2
IHT	277 ± 10 *	4.4 ± 0.4 *	1.6 ± 0.1 *	4.4 ± 0.2 *

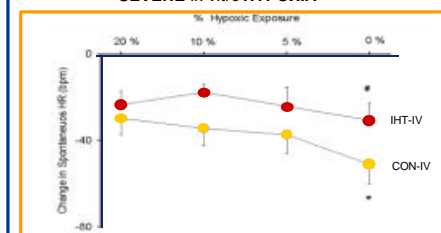
IHT induced the typical adaptations of increased whole heart weight, higher ventricular weight/body weight ratios and reduced body mass relative to controls (n=14 (†n=4); mean ± SEM; \* $p \leq 0.01$  CON vs IHT). A 23% increase in heart mass was found in the IHT group when body weight-matched animals were compared.

**FIGURE 2. EFFECT OF IHT ON HSP PROTEIN EXPRESSION**



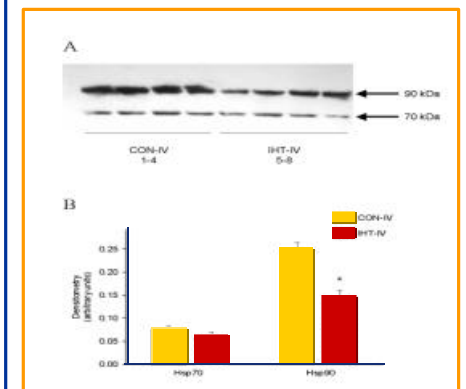
(A) Western blot showing Hsp70 and Hsp90 protein levels. Densitometry (B) revealed that IHT significantly decreased atrial Hsp70 and Hsp90 expression (\* $p < 0.05$ ). Tissue controls were used to confirm antibody specificity.

**FIGURE 3. EFFECT OF PRIOR IHT ON HEART RATE RESPONSES FOLLOWING EXPOSURE TO SEVERE *in vitro* HYPOXIA**



The heart rate (HR) response to 0%  $O_2$  was significantly lower than that at 20% for CON-IV (\* $p < 0.05$ ). This effect was reduced by prior IHT (IHT-IV). The HR responses in the IHT-IV were lower for all oxygen tensions, only reaching significance at 0% (\* $p < 0.05$ ).

**FIGURE 4. EFFECT OF *in vitro* HYPOXIA ON HSP PROTEIN EXPRESSION FOLLOWING IHT**



(A) Western blot showing Hsp70 and Hsp90 protein levels. Densitometry (B) revealed that Hsp90 expression was significantly decreased in the IHT-IV group compared to the CON-IV group (\* $p < 0.05$ ). A similar trend was also observed for Hsp70, although this did not reach statistical significance.

## CONCLUSION

- We have shown that IHT reduces the level of expression of Hsp 70 and Hsp90 protein in guinea pig atria.
- IHT minimized the depression in HR caused by acute *in vitro* hypoxia.
- Hsp90 (and to a lesser extent Hsp70) levels remain suppressed during subsequent severe *in vitro* hypoxia, suggesting that this tissue has become pre-conditioned to resist this stress.

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