The Impaired Sleep Architecture That Occurs with Myocardial Infarction Is Not Associated with Altered Peripheral Cytokine Levels in C57BL/6J Mice

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Introduction: We have recently demonstrated that experimentally-induced myocardial infarction (MI) in mice increases the propensity to sleep, as well as the total NREM sleep time during the dark/active phase. An increase in circulating cytokines associated with MI is one potential mechanism that could increase NREM sleep in our murine model.

Hypothesis: Acute MI alters circulating levels of cytokines known to cause increased NREM sleep.

Methods: Experiments were conducted in 72 male C57BL/6J mice (10-14 weeks). All animals underwent isoflurane anesthesia and mechanical ventilation. Thoracotomy was performed in 48 mice and a ligature placed around the left coronary artery 1mm below the left auricular margin. This ligature was either tied tightly (CAL, n=24) or loosely (SHAM, n=24). The chest was closed and the animals were allowed to recover. The Control group (n=24) were subjected to comparable anesthesia and mechanical ventilation as the SHAM and CAL groups, but without surgery. Six animals from each of the three groups were sacrificed on post-thoracotomy days 1, 3, 5, and 8. Serum was immunoassayed for 23 cytokine profiles by multiplex analysis (Bioplex Mouse 23plex; Bio-Rad, CA).

Results: There was no statistical difference in the 23 cytokine levels between the SHAM and CAL groups, even for the NREM-promoting cytokines IL-1beta and TNF-alpha. IL-6 levels were elevated ($P < 0.05$) in CAL animals compared to Control, but again there was no difference between SHAM and CAL animals. **Conclusion:** These data suggest that the increased NREM sleep that occurs in experimentally-induced MI is not associated with alterations in peripheral cytokines.

	IL-1beta (pg/ml)	TNF-alpha (pg/ml)	IL-6 (pg/ml)	IL-10 (pg/ml)
Control	51 ± 8	639 ± 95	11 ± 3	118 ± 26
SHAM	83 ± 40	424 ± 73	23 ± 4	67 ± 8
CAL	45 ± 12	382 ± 74	28 ± 6*	76 ± 12

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Post-Sigh Breathing Behavior and Spontaneous Pauses in the C57BL/6J (B6) Mouse

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Rationale: Genetic background affects post-hypoxic ventilatory behavior (Han et al, 2001), but does it affect breathing behavior at rest? **Objectives:** The purpose was to compare B6 and A/J strains, already known to differ in post-hypoxic breathing behavior, in terms of post-sigh breathing behavior and spontaneous pauses at rest, and then attempt to alter B6 behavior using buspirone, a 5-HT$_{1A}$ receptor agonist, and the use of a chromosomal substitution strain, B6a1 (the A/J Chromosome 1 on a B6 background). **Methods:** Measurements of ventilatory behavior in the room air were collected for 10 minutes from unanaesthetized adult male B6, A/J mice, and B6a1 mice (n = 6 in each group) using flow-through body plethysmography. On the separate days, B6 mice were given an intraperitoneal injection of saline or buspirone (5 mg/kg), and 25-minutes later breathing data was collected. Comparative analyses included linear as well as non-linear (Shannon entropy) metrics for expiratory time (Te) over time. **Results:** No difference in sigh number was observed among strains. Compared to the AJ and B6a1, post-sigh apneas were seen significantly more often and coefficients of variation (CV) for Te after sigh were significantly higher in B6, and Shannon Entropy values for Te after sigh was lower in B6 than that in A/J and B6a1. Spontaneous pauses were seen significantly more often in the B6 than in the other strains. Buspirone decreased the number of post-sigh apneas and spontaneous pauses in B6. **Conclusions:** Post-sigh apnea as well as spontaneous pauses was consistently present in the B6, compared to A/J and B6a1 strains. The irregularity and the lower complexity of post-sigh breathing in the B6 are reversed by buspirone or by elements on A/J Chromosome 1.

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The Acute Hypoxic Ventilatory Response Is Augmented in PHD2-Deficient Mice

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Aim: To determine the ventilatory effects of targeted inactivation of the prolyl hydroxylase-domain enzymes (PHDs) that regulate hypoxia-inducible factor (HIF).

Background: The HIF family of transcription factors controls intracellular responses to hypoxia throughout the body. HIF is primarily regulated by three PHDs that initiate its degradation. Mice that are heterozygously deficient for functional HIF genes have abnormal ventilatory acclimatisation to hypoxia, and our recent work strongly implicates HIF in regulating human cardiopulmonary physiology, including the acute hypoxic ventilatory response (AHVR) (Smith TG et al. *PLoS Med* 2006;3:1178-85).

Hypothesis: That inactivation of the three respective PHDs alters ventilatory responses to acute and sustained hypoxia.

Methods: Genetically engineered mice of genotypes PHD1$^{-/-}$, PHD2$^{+/-}$ and PHD3$^{-/-}$ were compared with wild-type controls (all groups n=8). Heterozygotes were used for PHD2 experiments as germline homozygous inactivation of PHD2 is embryonic lethal. Awake, unrestrained mice underwent whole body plethysmography to determine basal minute ventilation and the AHVR to 5 minutes of 12% O_2. Mice then underwent 24 hours of sustained hypoxia (10% O_2) in a hypoxia chamber, after which the initial ventilatory measurements were repeated. Ventilation was normalised to body weight.

Results: The most striking finding was that AHVR was 60% greater in PHD2$^{+/-}$ mice than in littermate controls (hypoxia-induced increase in ventilation of 2.2 [SEM 0.2] vs 1.4 [0.1] ml.min^{-1}g^{-1}; $p<0.01$). Wild-type mice did not acclimatise to 24 hours of hypoxia in a reliably detectable manner.

Conclusions: PHD2 may be specifically responsible for regulating the function of HIF in respiratory control.

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The Coexistence of Mimic Nocturnal Sustained Hypoxia and Obesity Additively Increases Cardiac Apoptosis

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Background. Nocturnal sustained hypoxia during sleeping time was reported in severe obesity, but no information regarding the cardiac molecular mechanism in the coexistence of mimic nocturnal sustained hypoxia and obesity is available. This study evaluates whether the coexistence of mimic nocturnal sustained hypoxia and obesity will increase cardiac Fas death receptor and mitochondrial dependent apoptotic pathway. ***Methods.*** 32 lean and 32 obese rats with or without nocturnal sustained hypoxia were studied at the age of 5-6 months and assigned as four subgroups: normoxia lean (NL), normoxia obese (NO), hypoxia lean (HL, 12% O_2 for 8hr and 21% O_2 16 hr/day, 1 week), and hypoxia obese (HO). The heart weight index, tail cuff plethysmography, echocardiography, HE staining, TUNEL assays, Western blotting and RT-PCR were performed. ***Results.*** SBP and DBP in HO were higher than NL. Fractional shortening in HO is reduced, compared to others. The whole heart weight, the left ventricular weight, the abnormal myocardial architecture, and TUNEL-positive apoptotic cells, as well as the activity of cardiac Fas receptor- and mitochondrial-dependent apoptotic pathway, were significantly increased in obese group or nocturnal sustained hypoxia group, and were further increased in the coexistence of obesity and nocturnal sustained hypoxia, the evidence for which is based on decreases in an anti-apoptotic protein Bcl2 level and Bid and increases in Fas, FADD, Bad, BNIP3, cytosolic cytochrome *c*, activated caspase-8, activated caspase-9 and activated caspase-3. ***Conclusions.*** The cardiac Fas receptor- and mitochondrial-dependent apoptotic pathways were more activated in the coexistence of obesity and nocturnal sustained hypoxia, which may provide one possible apoptotic mechanism for developing heart failure in obesity with nocturnal sustained hypoxia.

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Apneas Occur More Frequently in Orexin Neuron-Ablated Mice Than in Orexin-Knockout Mice

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We have previously reported suppressive effect of orexin (hypocretin) pathway on the genesis of central sleep apneas by showing higher frequency of sleep apneas in prepro-orexin knockout mice (ORX-KO) than in wild type mice (Nakamura et al., 2007). To better understand the mechanism of apnea genesis, we adopted orexin neuron-ablated mice (ORX/ATX-Tg) in this study, in which orexin-containing neurons were specifically ablated by transgenic technique (Hara et al., 2001) to clarify the role of co-localizing neurotransmitters contained in orexinergic neurons, such as dynorphin. [Methods] Five ORX/ATX-Tg mice were implanted electrodes for electroencephalogram (EEG) and electromyogram (EMG) more than 7 days before experiments. On the day of experiment, each animal was left in a quiet chamber during the daytime for 6 hours, where ventilation was assessed with whole body plethysmography and vigilance state was rated as wakefulness, slow-wave sleep (SWS), and rapid eye-movement sleep (REM) based on EEG and EMG signals. Apneas were defined as cessation of plethysmographic signals for at least two respiratory cycles, and classified into the following two categories. Post-sigh apneas (PSA) were the ones seen within 10 seconds after a sigh, a breath whose amplitude exceeds 100% of average. Spontaneous apneas (SA) were the ones irrelevant to any sighs. Frequency of episodes was expressed as occurrence index (counts/ sleep stage hr). [Results] Sleep architecture of these two genetically engineered mice were almost similar. Occurrence index of spontaneous apneas were greater in ORX/ATX-Tg mice than in ORX-KO mice especially during REM sleep. [Conclusions] The result possibly suggests suppressive effect of co-localizing factors on central apnea genesis.

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