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EFFECTS OF ACUTE INTERMITTENT HYPOXIA ON ENERGY BALANCE AND HYPOTHALAMIC FEEDING PATHWAYS

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Abstract—This study was done to investigate the effects of acute intermittent hypoxia (IH) on metabolic factors associated with energy balance and body weight, and on hypothalamic satiety-inducing pathways. Adult male Sprague–Dawley rats were exposed to either 8 h IH or normoxic control conditions. Food intake, locomotion and body weights were examined after IH. Additionally, plasma levels of leptin, adiponectin corticosterone, insulin and blood glucose were measured following exposure to IH. Furthermore, adipose tissue was removed and analyzed for leptin and adiponectin content. Finally, the hypothalamic arcuate nucleus (ARC) was assessed for alterations in protein signaling associated with satiety. IH reduced body weight, food intake and active cycle locomotion without altering adipose tissue mass. Leptin protein content was reduced while adiponectin content was elevated in adipose tissue after IH. Plasma concentration of leptin was significantly increased while adiponectin decreased after IH. No changes were found in plasma corticosterone, insulin and blood glucose. In ARC, phosphorylation of signal transducer and activator of transcription-3 and pro-opiomelanocortin (POMC) expression were elevated. In addition, POMC-expressing neurons were activated as determined by immediate early gene FRA-1/2 expression. Finally, ERK1/2 and its phosphorylation were reduced in response to IH. These data suggest that IH induces significant alterations to body energy balance through changes in the secretion of leptin which exert effects on satiety-inducing pathways within the hypothalamus.

Key words: intermittent hypoxia, food intake, pro-opiomelanocortin, arcuate nucleus, leptin, adiponectin.

INTRODUCTION

While white adipose tissue is the major storage site for triglycerides in the body, this organ also appears to play a role in endocrine function, partly through the production and release of a class of hormones known as adipokines. Adipokines are used by the body to provide information regarding energy balance, which in turn may influence thermogenic activity, food intake and glucose homeostasis (Ahima and Osel, 2008). Leptin, a 16-kDa hormone produced by the *ob* gene (Halaas et al., 1995), is an adipokine that functions as a satiety-inducing hormone. The primary site of secretion of leptin into the bloodstream is the white adipose tissue, and this secretion generally occurs directly in relationship to the white adipose tissue mass (Maffei et al., 1995). However, the release of leptin into the circulation can be altered by a number of different stimuli, including hypoxia (Sherry et al., 2009; Reinke et al., 2011) and acute intermittent hypoxia (IH) (Messenger et al., 2012). Hypoxia elevates leptin production and release from white adipose tissue both *in vitro* (Grosfeld et al., 2002) and *in vivo* (Sherry et al., 2009). Similarly, acute IH has been shown to increase the circulating levels of leptin (Messenger et al., 2012). Although the functional role of this upregulation of the leptinergic system is unknown, it may be involved in the regulation of the sympathetic nervous system activity (Mark et al., 2009; Ciriello and Moreau, 2012), and this may be mediated partly through its effects on hypothalamic neurons (Harlan et al., 2011). Leptin not only reduces food intake largely by acting on pro-opiomelanocortin (POMC) neurons of the arcuate nucleus (ARC; Hill et al., 2008), but these POMC neurons may also affect blood glucose levels (Shi et al., 2008; Berglund et al., 2012). Leptin binds to POMC neurons through the long-form leptin receptor (Ob-Rb), resulting in the homodimerization and phosphorylation of signal transducer and the activator of transcription-3 (STAT3; Calvino et al., 2012). Phosphorylated STAT3 (pSTAT3) translocates to the nucleus of these neurons and alters gene transcription of various targets including *Pomc* and immediate early genes (Bousquet et al., 2000).

As adipose tissue is responsive to hypoxia, and that factors produced by the adipose tissue are related to energy balance, this study was done to determine whether acute 8-h IH altered the signaling of the adipose. These data suggest that IH induces significant alterations to body energy balance through changes in the secretion of leptin which in turn exerts effects on satiety-inducing pathways within the hypothalamus.

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Abbreviations: ARC, arcuate nucleus; IH, intermittent hypoxia; Norm, normoxic; Ob-Rb, long-form leptin receptor; PBS, phosphate-buffered saline; POMC, pro-opiomelanocortin; pSTAT3, phosphorylated STAT3; SDS, sodium dodecyl sulfate; STAT3, signal transducer and activator of transcription-3; TBST, Tris-buffered saline + Tween-20.

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EXPERIMENTAL PROCEDURES

Animals

Adult, male Sprague–Dawley rats (320–400 g: $n = 48$) were purchased from Charles River Canada and housed singly at a temperature of 22 °C and 60% relative humidity with access to food and water available *ad libitum*, except during the acute 8-h IH or normoxic (Norm) exposure, in 12-h light/dark cycle conditions. Animals were handled in accordance with the guidelines set forth by the Canadian Council on Animal Care and the Animal Use Committee at the the University of Western Ontario.

Groups

Animals were divided into different subsets: the first subset ($n = 7$ per condition) was used to determine physiological measures including food and water intake and body weight changes; the second subset ($n = 7$ per condition) was used for active cycle locomotion studies; the third subset ($n = 7$ per condition) was used for immediate sacrifice following exposure, from which plasma samples (used for hormone analysis), blood glucose, adipose tissue and brains were removed; the final subset of animals ($n = 3$ per condition) were perfused immediately following exposure, and were used for immunohistochemistry and immunofluorescence.

IH or Norm exposure

Animals were exposed to 8 h (0900–1700) of acute IH or Norm during their sleep cycle, as previously described (Messenger et al., 2012). In brief, animals were placed in a chamber (total volume, 35 L) that consisted of four separate animal tubes (10 cm diameter by 35 cm length) within the chamber, and a zero-pressure escape valve. For IH-exposed animals, a computer that regulated solenoid valves altered the input of N_2 or room air to generate IH and Norm conditions. The gases were pushed through the system using fans and passed through a mixing chamber prior to entering the animal tubes. Flows of N_2 and room air were set as that required for the same amount of time to drop from 21% to 6.5% O_2, and returning to the 21% O_2. Animals were exposed to 80-s hypoxia (6.5% O_2) followed by 120 s normoxia. The levels of O_2 and CO_2 were monitored by sensors on the chamber, which relayed information back to the computer to ensure proper cycling. Conditions within the chamber were isobaric (770 ± 11 mmHg) and eucapnic (0.04–0.1% CO_2). Norm animals were exposed to identical chambers with only room air input during cycling. During either IH or Norm exposure, no alterations in sleep duration or locomotion were observed between IH or Norm animals during exposure. Animals were observed to lay-down within the tubes and remained in that relative position throughout the 8-h experimental period.

Measures of body weight, food intake and water intake

Animals were weighed immediately before and after IH or Norm exposure. These values were used to calculate body weight changes during the IH or Norm exposure period and 24-h body weight change. Additionally, food and water intake measurements were recorded for the 16-h period immediately following exposure.

Locomotion assay

In a subset ($n = 7$ per group), immediately following acute IH or Norm exposure, animals were placed in home cages with *ad libitum* access to food and water. Thirty minutes into the dark (active) cycle (1930 h), animals were placed into large cages (60 cm × 40 cm) with a floor 4 × 5 grid system in the dark (red light on). Animals were acclimatized for 10 min to the testing cage. Over a 5-min period, the number of crosses of a line by an animal was determined by two independent, blinded observers to determine horizontal locomotion. At the same time, vertical locomotion was determined by the number of rearing events. An average value was then calculated from these two observers for both horizontal and vertical locomotion. These animals were not used for the determination of food or water intake.

Blood glucose measurement

Immediately following exposure conscious animals had blood glucose measured three times using an Accu-Check Aviva glucometer (Roche Diagnostics Canada; Laval, QC, Canada) from a tail vein puncture.

Plasma collection and enzyme immunoassays

Subgroups of rats exposed to IH ($n = 7$) or Norm ($n = 7$) were sacrificed under equithesin anesthesia (0.3 ml/100 g b.w.; i.p.; [2.88 mg/100 g b.w. sodium pentobarbital; 12.79 mg/100 g b.w. chloral hydrate; 6.37 mg/100 g b.w. MgSO4; 0.12 ml/100 g b.w. propylene glycol; 0.03 ml/100 g b.w. ethanol]; Gandal, 1969) immediately following exposure. Blood samples were collected by cardiac puncture in the presence of 7% ethylenediaminetetraacetic acid at a volume of 10 µl/ml blood. This blood was immediately centrifuged at 10,000 RPM for 10 min at 4 °C to isolate the aqueous plasma. This aqueous plasma phase was removed and stored frozen at −80 °C until analyzed for hormone content. Plasma samples were analyzed using enzyme immunoassays for rat leptin (sensitivity: 67.2 pg/ml; Enzo Life Sciences; Farmingdale, NY, USA), adiponectin (sensitivity: 0.12 ng/ml; Phoenix Pharmaceuticals; Burlingame, CA, USA), corticosterone (sensitivity: 26.99 pg/ml; Enzo Life Sciences; Farmingdale, NY) and insulin (sensitivity: 0.12 ng/ml; Alpco Diagnostics; Salem, NH, USA), according to the manufacturer instructions. Enzyme immunoassay plates were read on a SpectraMax M5 plate reader using SoftMax Pro v.5 microplate analysis software (Molecular Devices; Sunnyvale, CA, USA).