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Hypothyroidism affects D2 receptor-mediated breathing without altering D2 receptor expression

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ABSTRACT

Bromocriptine depressed ventilation in air and D2 receptor expression in the nucleus tractus solitaries (NTS) in male hypothyroid hamsters. Here we postulated that in age-matched hypothyroid female hamsters, the pattern of D2 receptor modulation of breathing and D2 receptor expression would differ from those reported in hypothyroid males. In females hypothyroidism did not affect D2 receptor protein levels in the NTS, carotid bodies or striatum. Bromocriptine, but not carmoxirole (a peripheral D2 receptor agonist), increased oxygen consumption and body temperature in awake air-exposed hypothyroid female hamsters and stimulated their ventilation before and following exposure to hypoxia. Carmoxirole depressed frequency of breathing in euthyroid hamsters prior to, during and following hypoxia exposures and stimulated it in the hypothyroid hamsters following hypoxia. Although hypothyroidism did not affect expression of D2 receptors, it influenced central D2 modulation of breathing in a disparate manner relative to euthyroid hamsters.

1. Introduction

Hypothyroidism is a common endocrine disorder. Moreover, hypothyroidism is more prevalent among women and increases with age. For example, Empson et al. (2007) noted that of 3504 subjects over the age of 49 years, hypothyroidism was present in 7.1% of women and 3.7% of men. Lucas et al. (2010) reported a prevalence of hypothyroidism of 8.9% with 71% of hypothyroid individuals being women. In a large population study in Colorado, Canaris et al. (2000) reported a higher prevalence of women with hypothyroidism than men that increased with age. Thus, females are more likely to develop hypothyroidism than males which becomes more common with age.

Clinically, hypothyroidism causes depression of breathing and contributes to the development of sleep apnea, insulin insensitivity and increases the risk for cardiovascular disease (Braverman and Utiger, 2005; Kansagra et al., 2010; Lakshmi et al., 2009; Mainenti et al., 2010). Moreover, the development of sleep apnea includes an interplay of many players including factors that globally or selectively (i.e. diaphragm versus upper airway muscles) diminish central nervous system drive, affect the function of reflexes involving inputs from mechanoreceptors as well as chemoreceptors (such as the carotid body) as well as changes in various neurotransmitter and receptor levels. These include gamma aminobutyric acid, acetylcholine, serotonin, norepinephrine and dopamine (see recent review by Ramirez et al., 2013 (Chenuel et al., 2005; Dempsey et al., 2012)).

Dopaminergic receptors are G protein coupled receptors can be divided into two groups D1-like that result in stimulatory responses and D2-like receptors (D2 and D3) whose stimulation cause depression (Beaulieu and Gainetdinov, 2011). Dopaminergic D2 receptors are located in the carotid bodies and also several brain regions associated with control of breathing, including nucleus tractus solitaires (NTS), the paraventricular nucleus of the hypothalamus (PVN) and in the striatum (Bairam and Carroll, 2005; Hyde et al., 1996; Nobrega et al., 1996). Both the NTS and the PVN are integratory regions involved with modulating hypoxic responses and autonomic function (Reddy et al., 2005). Although less investigated, several studies have shown that the striatum is involved in regulation of breathing (Evans et al., 1999; Nattie et al., 2001).

Stimulation of D2 receptors generally depresses ventilation. For example, Nielsen and Bisgard (1984) administered bromocriptine, a D2 receptor agonist, intravenously to decerebrate, vagotomized, paralyzed, carotid body dennervated air breathing dogs. Following administration of the drug, the investigators reported decreased phrenic nerve amplitudes without an effect on the frequency of bursts. Co-administration with the D2 receptor antagonist haloperidol with bromocriptine prevented these effects. More

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recently Lalley and Mifflin (2012) investigated the effects of piribedil, a D2/D3 receptor agonist on phrenic nerve response in anesthetized, paralyzed and ventilated cats whose vagi were cut. Results indicated that in dose dependent manner intravenously administered pirbedil depressed peak action potential frequency and rate of discharge. Most studies suggest that D2 receptors depress breathing, although a few actually had an excitatory effect of D2 receptors on ventilation (Burton and Kazemi, 2000). Moreover, the D2 receptors can affect ventilation by modulating the carotid body activity (Kumar and Prabhakar, 2011; O'Halloran et al., 1998).

Hypothyroidism also has profound effects on the expression and function of various neurotransmitters that can modulate breathing. For example, Schlenker et al. (1994) showed that naloxone (a mu opioid receptor antagonist) caused depression of breathing in male hypothyroid hamsters but stimulated breathing in euthyroid males. Varney and Schlenker (2007) reported that a specific serotonin 2A receptor antagonist resulted in long-term depression of ventilation following intermittent hypoxia relative to baseline values in male euthyroid, but not in hypothyroid hamsters. More recently, studies from our laboratory demonstrated that hypothyroidism induced for 5 months in male hamsters had profound effects on D2 receptor modulation of breathing especially following exposure to hypoxia (Sykora et al., 2013). Specifically administration of the D2 receptor agonist bromocriptine (relative to vehicle-treatment) depressed ventilation in both groups exposed to air or to hypoxia, but hypothyroid bromocriptine-treated hamsters increased ventilatory responsiveness to hypoxia, while euthyroid hamsters decreased ventilatory responsiveness to hypoxia and exhibited a post-hypoxic depression. Moreover, in that study, hypothyroidism increased D2 receptor levels in the nucleus tractus solitaries (NTS), but D2 receptor levels were comparable in the striatum and carotid bodies of both groups of hamsters. Interestingly, the ventilatory and D2 receptor results were dissimilar to those previously reported in younger male hypothyroid hamsters (Schlenker and Schultz, 2011). In that study bromocriptine had no effect on ventilation during air exposure in hypothyroid or euthyroid hamsters, but depressed ventilation following hypoxic exposures in the euthyroid animals while stimulation it in the hypothyroid hamsters. Moreover, hypothyroid males exhibited increased D2 receptor protein levels in the striatum and CB's, but decreased levels in the paraventricular hypothalamic nucleus relative to euthyroid hamsters. No effect of hypothyroidism was seen on D2 receptor levels in the NTS. Thus, age may also affect hypothyroidism dopaminergic mediated control of breathing and dopamine receptor expression.

What effects hypothyroidism has on D2 receptor expression and D2 receptor modulation of breathing in female hamsters is the purpose of the present study. We postulated that the pattern of ventilatory responses to a central D2 receptor agonist bromocriptine would be different to that previously found in the older hypothyroid males. In the present study we also used carmoxirole, a peripherally acting D2 receptor agonist to determine if carotid body D2 receptor regulation of breathing was altered by hypothyroidism in female hamsters. Moreover, we posited that in hypothyroid females the expression of D2 receptors in the striatum, NTS, and the carotid bodies should be increased relative D2 expression in the same regions of euthyroid hamsters.

2. Methods

2.1. Animals

Seven- to eight-week-old female golden Syrian hamsters were obtained from Harlan Sprague Dawley, Inc. (Indianapolis, IN, USA). Ten animals received propylthiouracil (PTU) in drinking water to induce hypothyroidism as described below and 9 received tap water. This is a technique previously used in our laboratory (Schlenker and Schultz, 2011) and resulted in decreased serum thyroid hormones levels In both experiments animals were housed three to four per cage, exposed to 14 h of light and 10 h of dark, and received rodent pellets (8604, Harlan Sprague Dawley, Inc.). Food and fluids (tap water or PTU in tap water) were available ad libitum. Five months following the start of PTU administration the experiments outlined below were started. Three days prior to commencement of the experiments, hamsters were acclimated to the barometric chamber for 30 min each day. The University of South Dakota Animal Care and Use Committee in accordance to the "Guide for the Care and Use of Laboratory Animals" approved all procedures used in these studies.

2.2. Ventilatory and metabolic measurements

Oxygen consumption and respiratory measurements were made in hamsters placed into a 20.2 cm × 7.9 cm Plexiglas cylindrical chamber. Ventilatory measurements used the barometric method previously reported in hamsters (Schlenker, 1984). Ports allowed air or 10% oxygen in nitrogen (hypoxia) to enter and exit the chamber. Chamber pressure was measured using a Statham pressure transducer and airflow rate through the chamber was measured using a Gilmont rotameter. The barometric pressure was measured using a W.M. Welch (Chicago, IL, USA) mercury barometer. Chamber temperature was measured using a Fisher Scientific digital thermometer and the relative humidity within the chamber averaged 50%. The average of 15–20 inspiratory and expiratory times was measured and was averaged for each portion of the study. Frequency of breathing was calculated by adding inspiratory and expiratory times and dividing the sum into 60 s/min. The minute ventilation was calculated by multiplying frequency of breathing by tidal volume. Tidal volume and minute ventilation were normalized by body weight (variable × 1000/BW). Oxygen consumption was determined by subtracting the fractional content of O_2 in the output air from the fractional content of O_2 in the input air and multiplying the difference by the flow rate. Values were corrected to STPD and by body weight as described for the ventilatory parameters.

2.3. Protocols for ventilatory studies

For the carmoxirole study the hamster was weighed and injected subcutaneously with either vehicle (5% DMSO in saline) or 0.2 mg/kg carmoxirole in vehicle (Tocris Bioscience, Ellisville, MO, USA). The dose of carmoxirole was obtained from previous studies in our laboratory (Schlenker, 2007). Each hamster received vehicle or carmoxirole in a random manner at the same time of day with 2 days between treatments. Following drug administration, the hamster was placed into the chamber for 35 min and exposed to room air. During the last minute of air exposure oxygen consumption, frequency, tidal volume, and minute ventilation were determined. The hamster was then exposed to hypoxia for 5 min. During the last minute of exposure to hypoxia, tidal volume, frequency and minute ventilation were determined. Following the hypoxic exposure, the chamber was washed out with room air for 5 min. During the last minute of the washout, tidal volume, frequency and minute ventilation were determined in the hamster. Afterwards the hamster was removed from the chamber and its body temperature was measured using a Sensortek BAT-12 thermometer and a Physiotemp thermocouple.

For the bromocriptine study, hamsters received subcutaneous injections of either saline or 1.0 mg/kg bromocriptine in saline (Tocris Bioscience, Ellisville, MO, USA). This dose was used in a previous study in our laboratory (Schlenker and Schultz, 2012a). The same procedures to evaluate oxygen consumption, control of