Published in final edited form as:

Respir Physiol Neurobiol. 2012 January 15; 180(1): 69–78. doi:10.1016/j.resp.2011.10.013.

Hypothyroidism Stimulates D₂ Receptor-mediated Breathing in Response to Acute Hypoxia and alters D₂ Receptors Levels in Carotid Bodies & Brain

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Abstract

Hypothyroidism can depress breathing and alter dopamine D₂ receptor expression and function. We hypothesized that relative to euthyroid hamsters (EH), hypothyroid hamsters (HH) contain increased D₂ receptors in brain regions associated with breathing and carotid bodies (CB), and that stimulation of D₂ receptors would decrease ventilation more in the HH compared to the EH. Hamsters were treated with vehicle, carboxipropyl (peripherally acting D₂ receptor agonist), or bromocriptine (central and peripherally acting D₂ receptor agonist) and breathing was evaluated during exposure to air, hypoxia, and then air. HH exhibited increased D₂ receptor protein levels in the striatum and CB's, but decreased levels in the paraventricular hypothalamic nucleus. Relative to vehicle, carboxipropyl and bromocriptine stimulated ventilation in the HH during and following exposure to hypoxia. Only bromocriptine depressed ventilation in the EH during and after exposure to hypoxia. Thus, hypothyroidism impacts the expression of D₂ receptors in the carotid body, PVN and striatum, and D₂ stimulation affects ventilation remarkably differently than in EH.

Keywords

Hypothyroidism; Dopamine D₂ receptors; Hypoxia; Ventilation

1. Introduction

Hypothyroidism is a prevalent endocrine disorder affecting 2 to 15% of the population depending on gender and age (Aoki et al., 2007; Hollowell et al., 2002; Roos et al., 2010). Consequences of hypothyroidism include cognitive and mood dysfunctions as well as deleterious effects on cardiopulmonary function and sleep (Resta, 2004; Sakellaropoulou et al., 2011; Tews et al., 2005; Wu et al., 2006). In some cases thyroid hormone therapy reverses these dysfunctions, but not always (Jha et al., 2006; Misiolek et al., 2007). At the molecular level, thyroid hormones affect the expression and phosphorylation of various enzymes and the expression of pumps, channels and neurotransmitter systems that may

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affect ventilation including opioids, serotonin, gamma amino butyric acid, and dopamine (Burton and Kazemi, 2000; Diez et al., 2008; Kulikov and Zubkov, 2007; Sarkar et al., 2006; Schlenker, 1994; Slotkin and Slepatis, 1984; Varney and Schlenker, 2007).

Dopamine acts on specific receptors divided into two categories D₁-like (D₁ and D₅ subtypes) and D₂-like receptors (D₂, D₃ and D₄ subtypes (Beaulieu and Gainetdinov, 2011). Dopaminergic D₂ receptors are located in the carotid bodies in hamsters within the glomus cells (Schlenker, unpublished observations) and within several brain regions associated with control of breathing including nucleus tractus solitaires (NTS), the paraventricular nucleus of the hypothalamus (PVN) and in the striatum (Fagerlund et al., 2010; Kline et al., 2002; Weiner et al., 1991). Both the NTS and the PVN are integratory regions that help regulate ventilatory responses to hypoxia (Genest et al., 2007; Granjeiro and Machado, 2009; Reddy et al., 2005; Schlenker et al., 2001). Several studies suggest that the striatum is also involved in regulation of breathing (Ángyán and Ángyán, 1999; Evans et al., 1999; McKay et al., 2003).

In chemodennervated rabbits acute blockade of centrally located D₂ receptors prevented the depression of ventilation following the peak ventilatory responses to short-term hypoxia (Guner et al., 2002). Moreover, Nielsen and Bisgard administered bromocriptine, a D₂ receptor agonist, intravenously to decerebrate, vagotomized, paralyzed, and carotid body dennervated air breathing dogs (Nielsen and Bisgard, 1984). Following administration of the drug, phrenic nerve amplitudes decreased, but no effect was noted on the frequency of bursts. Treatment with the D₂ receptor antagonist haloperidol prevented the effects of bromocriptine confirming that D₂ receptors acting centrally influenced breathing. Several studies in conscious animals and human subjects have also verified the importance of D₂ receptors in the control of breathing, especially during hypoxic exposures (Bascom et al., 1991; Huey et al., 2003; Pedersen et al., 1997; Schlenker, 2007). Most studies suggest that stimulating D₂ receptors depresses breathing, although a few actually reported excitatory effects of D₂ receptor stimulation on ventilation (Bairam et al., 1993). Thus, D₂ receptors affect central pathways and carotid body function associated with the regulation of breathing (O'Halloran et al., 1998).

Hypothyroidism can affect the expression and/or function of D₂ receptors. For example, Cameron and Crocker induced hypothyroidism in rats by administration of PTU for four weeks (Cameron and Crocker, 1987). Although there was no change in the number of striatal D₂ receptors in the hypothyroid rats relative to euthyroid rats, the hypothyroid animals exhibited greater behavioral sensitivity to D₂ receptor agonists. If rats had been rendered hypothyroid for ten weeks, D₂ receptor numbers increased in the striatum and behavioral responses (stereotypic behavior in an open field, catalepsy, and operant responses) to apomorphine and haloperidol were enhanced relative to euthyroid rats (Crocker et al., 1986). Finally, Oh-Nishi and coworkers (2005) noted that in life-long methylmercaptoimidazole-treated rats or congenital hypothyroid rats, stimulation of hippocampal D₂ receptors with quinpirole caused marked increases in the field excitatory postsynaptic potentials, but in euthyroid rats quinpirole administration inhibited postsynaptic potentials (Oh-Nishi et al., 2005). The researchers concluded that these disparate findings could be, in part, attributed to reversion of D₂ receptors to a more immature form in hypothyroid rats and also that stimulation of these altered presynaptic receptors enhanced, rather than inhibited release of glutamate.

Although the role of D₂ receptors on ventilation in various species has been extensively studied, how hypothyroidism affects expression of this receptor subtype in areas associated with control of breathing and influences ventilation are unknown. The purposes of the present study are twofold. First, we evaluated protein levels of D₂ receptors in carotid

bodies, NTS, striatum, and PVN of euthyroid and propylthiourical (PTU)-induced hypothyroid hamsters. Secondly, we determined the role of peripheral and central D₂ receptors in control of ventilation during and following exposure of euthyroid and hypothyroid hamsters to hypoxia. A previous study from our laboratory showed that in hamsters D₂ receptors not only affect ventilation during hypoxia, but also following hypoxia (Schlenker, 2007). To stimulate peripherally located D₂ receptors we used carboxirole (Haase et al., 1991; Marchese et al., 2002). We utilized bromocriptine, a D₂ receptor agonist that permeates the central nervous system (Atsumi et al., 2003) to determine if central and peripheral D₂ receptor stimulation on affected control of breathing. We hypothesized that relative to euthyroid hamsters, hypothyroid hamsters would exhibit more D₂ receptors in the brain regions associated with control of breathing and within the carotid body as suggested by a study by Crocker and coworkers (Crocker et al., 1986). Furthermore, we hypothesized that stimulation of D₂ receptors should result in a greater decrease of ventilation in the hypothyroid compared to the euthyroid hamsters during and following exposure to hypoxia.

2. Experimental Procedures

2.1 Animals

Seven to eight week old male golden Syrian hamsters were obtained from Harlan Sprague Dawley, Inc. (Indianapolis, IN). For the study evaluating the effects of carboxirole on control of breathing, 17 animals were used. Nine animals received propylthiourical (PTU) to induce hypothyroidism as described below and 8 received tap water. For the bromocriptine study, 8 animals received PTU and 6 received tap water. Hypothyroidism was induced in the hamsters by administering 0.04% PTU in tap water. This is a technique previously used in our laboratory (Schlenker and Schultz, 2011) and resulted in decreased serum thyroid hormones levels (Olea et al., 2007). In both experiments animals were housed three to four per cage, exposed to 14 hours of light and 10 hours 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*. Three months following the start of PTU administration the experiments were started. Three days prior to the experiments, hamsters were acclimated to the barometric chamber for 30 minutes each day. The University of South Dakota Animal Care and Use Committee approved all procedures used in these studies.

2.2 Barometric Chamber and Ventilatory Measurements

For both studies respiratory measurements were made in hamsters placed into a 20.2 × 7.9 cm Plexiglas cylindrical chamber using the barometric method (Schlenker, 1984). Ports allowed air or 10% oxygen in nitrogen (hypoxia) to enter and exit the chamber. Barometric pressure was measured using a W.M. Welch (Chicago, IL) mercury barometer and airflow rate through the chamber was measured using a Gilmont rotameter. Chamber temperature was determined using a Fisher Scientific digital thermometer. Relative humidity within the chamber averaged 50%. Inspiratory and expiratory times were measured from 10–15 breaths and were averaged for each portion of the study. Frequency of breathing was calculated by adding inspiratory and expiratory times and dividing the sum into 60 (sec/min). The minute ventilation was calculated by multiplying frequency of breathing by tidal volume. Both tidal volume and minute ventilation were normalized by body weight (variable*1000/BW).

2.3 Ventilatory studies

For the carboxirole study the hamster was weighed and injected subcutaneously with either vehicle (5% DMSO in saline) or 0.2 mg/kg or 0.4 mg/kg carboxirole in vehicle (Tocris Bioscience, Ellisville, MO.). The doses of carboxirole were obtained from previous studies in the literature (Meyer et al., 1992). Each hamster received all three doses 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 minutes and exposed to room air. During the last minute of air exposure frequency, tidal volume, and minute ventilation were determined.

The hamster was then exposed to hypoxia for 5 minutes. During the last minute of exposure to hypoxia, tidal volume, frequency and minute ventilation were determined. Following the 5 minute influx of hypoxia, the chamber was washed out with room air for 5 minutes. 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 Sontek BAT-12 thermometer and a Physiotemp thermocouple.

For the bromocriptine study, hamsters received subcutaneous injections of either saline (vehicle) or 1.0 mg/kg bromocriptine in saline (Tocris Bioscience, Ellisville, MO). This dose was obtained from a study in the literature (Lane and Dunnett, 2010). The same procedures to evaluate control of ventilation and body temperature as described for carboxirle were used in this study.

2.4 Western blot studies

At the end of the study, hamsters were deeply anesthetized. The brains and carotid bodies were quickly removed, frozen on dry ice, and stored at -80°C . The protein in carotid bodies was extracted with a lysis buffer (10 mM Tris, 1 mM EDTA, 1 % SDS) containing a protease inhibitor cocktail (100 $\mu\text{l/ml}$). Following centrifugation at 12,000 g for 20 min at 4°C , the protein concentration in the supernatant was determined using a BCA protein assay kit (Pierce Chemical, Rockford, IL, USA). The sample was then mixed with loading buffer containing α -mercaptoethanol and heated at 100°C for 5 min. Equal amounts of protein for each brain region and carotid body were loaded on the 10% polyacrylamide gel along. Subsequently, the proteins and molecular weight standards were transferred to a PVDF membrane. For carotid bodies, the membrane was probed with a mouse D_2 dopamine receptor antibody 1–50 mapping at the N-terminal extracellular region of dopamine receptor type 2 of human origin (sc-9113, Santa Cruz, CA, USA; 1:500). A peroxidase-conjugated goat anti-mouse IgG (Pierce Chemical, Rockford, IL, USA) secondary antibody was used. The signal was detected using enhanced chemiluminescence substrate (Pierce Chemical, Rockford, IL, USA) and the bands were analyzed using UVP BioImaging Systems. Protein loading was evaluated by probing all Western blots with mouse anti- β -tubulin antibody (Santa Cruz, CA, USA) and normalizing D_2 dopamine receptor protein intensities to that of β -tubulin. There was no difference in the level of expression of β -tubulin between normal and PTU-treated groups.

To determine D_2 receptor protein levels in the three brain regions, brains were sliced at 300 μm using a cryostat and punches made using a 20-gauge stainless steel needle according to the hamster brain stereotaxic atlas (Morin and Wood, 2001) in the striatum, PVN, and NTS. Brain punches were frozen at -80°C . Western blot methods described above were used to determine D_2 receptor protein levels in the three brain regions. In these studies the D_2 receptor antibody used was a rabbit polyclonal antibody from Abcam (ab21218) made against a synthetic peptide: AARRAQELE, corresponding to amino acids 272–282 of Human Dopamine D_2 Receptor (1:4,000). Protein levels were normalized using glyceraldehyde 3-phosphate dehydrogenase (GAPDH).

2.5 Data Analysis and Statistics

For the ventialtory studies statistical analysis consisted of two-way analysis of variance (ANOVA) to test the effects of treatment (PTU versus tap water) and drug administration

(either carboxirole or bromocriptine). If the result was significant ($P < 0.05$), post hoc Holm-Sidak tests were conducted. Body weight, body temperature, and Western blot data were analyzed using unpaired Student's *t* tests or one way repeated ANOVA. If the data were skewed, we used nonparametric tests such as the Friedman test (analogous to repeated one way ANOVA), Mann-Whitney test (for unpaired comparisons) or the Wilcoxon sign rank test (for paired comparisons). Data were analyzed using Statmost programs (Dataxiom Software Inc. Los Angeles). All data are presented as means and standard error of the mean.

3. Results

3.1 D₂ receptor protein levels

D₂ receptor protein was present in all the areas investigated. In the carotid body and striatum (Fig. 1), D₂ protein levels were significantly higher in the hypothyroid compared to euthyroid hamsters ($P < 0.01$ for both regions), but in the PVN D₂ protein receptor levels were lower in the hypothyroid animals compared to the euthyroid hamsters ($P < 0.01$, Fig. 2). There were no statistically significant differences in the D₂ receptor protein levels in the NTS between the two groups.

3.2 Carmoxirole effects on Body Temperature and Control of Breathing

Body temperature and resting ventilation—Body temperature (in degrees Celsius) was lower in the hypothyroid relative to the euthyroid hamsters (32.6 ± 0.06 versus 33.1 ± 0.09 , $P < 0.01$). Treatment of hamsters with carboxirole had no effect on body temperature (data not shown). Ventilation (Fig. 3) was lower in hypothyroid relative to euthyroid hamsters ($P = 0.03$) due to a significantly lower frequency of breathing ($P = 0.01$). Carmoxirile did not affect baseline ventilation during air exposure in either group.

Hypoxic ventilation—With vehicle treatment during exposure to hypoxia (Fig. 3), ventilation was lower in the hypothyroid relative to the euthyroid group ($P = 0.02$). Neither dose of carboxirole affected ventilation in euthyroid hamsters relative to vehicle values. By contrast, carboxirole increased ventilation in the hypothyroid hamsters ($P < 0.01$) due to an increase of frequency (with both doses, $P < 0.05$) and an increased tidal volume with the 0.2 mg/kg dose ($P < 0.01$). Hypoxic responsiveness (ventilation during exposure to hypoxia divided by ventilation during exposure to baseline air) was not different between the groups and was not affected by carboxirile in the euthyroid group following treatment of either dose of carboxirile (Fig. 4A). In contrast, the 0.2 mg/kg dose of carboxirile, but not the higher dose, increased hypoxic responsiveness in the hypothyroid hamsters from 1.61 ± 0.06 to 1.97 ± 0.01 ($P < 0.01$).

Post-hypoxic ventilation—Following exposure to hypoxia, while hamsters were breathing air, frequency of breathing and minute ventilation were lower in the hypothyroid relative to the euthyroid hamsters ($P < 0.01$ for both). In the euthyroid hamsters carboxirile *decreased* minute ventilation ($P < 0.01$ for the low dose and $P = 0.05$ for the higher dose), due to a decrease in frequency of breathing ($P < 0.01$ for the low dose and $P = 0.05$ for the higher dose). By contrast, in the hypothyroid group both doses of carboxirole *increased* minute ventilation ($P < 0.01$ for the low dose and $P < 0.05$ for the higher dose) due to increase in frequency of breathing ($P < 0.02$ for both doses) and with the lower dose due to an increased tidal volume ($P < 0.03$).

Post-hypoxic ventilatory responsiveness (ventilation of animals during exposure to air following hypoxia divided by ventilation during exposure to baseline air) was not significantly different between the two groups when they were treated with vehicle (Fig. 4). However, relative to saline, the 0.2 mg/kg dose *decreased* post-hypoxic responsiveness

significantly in the euthyroid group from 1.11 ± 0.12 to 0.82 ± 0.034 ($P < 0.05$), but *increased* in the hypothyroid group from 0.90 ± 0.06 to 1.14 ± 0.05 ($P = 0.02$). There were no significant effects on this parameter at the 0.4 mg/kg dose relative to saline in either group.

3.3 Bromocriptine effects on body temperature and ventilation

Body temperature and resting ventilation—Relative to saline values bromocriptine decreased body temperature (in degrees Celsius) in the euthyroid group from 32.6 ± 0.1 to 32.1 ± 0.1 ($P = 0.03$) and in the hypothyroid group from 33.3 ± 0.3 to 31.8 ± 0.2 ($P = 0.015$). With saline treatment ventilation was lower in the hypothyroid relative to that the euthyroid hamsters ($P < 0.05$) due to a lower frequency of breathing ($P < 0.01$, Fig 5). Bromocriptine had no effect on ventilation during baseline air exposure in either group.

Hypoxic ventilation—With Saline treatment during exposure to hypoxia, ventilation ($P < 0.03$), frequency of breathing ($P < 0.01$), and tidal volume ($P < 0.03$) were lower in the hypothyroid relative to the euthyroid hamsters. Relative to saline, bromocriptine *decreased* ventilation in the euthyroid hamsters ($P = 0.002$), but *increased* it in the hypothyroid animals ($P < 0.001$) by affecting both frequency and tidal volume in opposite manners in the two groups. Hypoxic responsiveness was significantly lower in the hypothyroid relative to the euthyroid group (1.88 ± 0.09 compared to 2.22 ± 0.13 , $P < 0.05$), and was not affected significantly by bromocriptine (Fig. 4B).

Post-hypoxic ventilation—With saline treatment, following the hypoxic exposure, ventilation was lower in the hypothyroid relative to the euthyroid hamsters ($P = 0.003$). Bromocriptine *decreased* ventilation in the euthyroid group ($P < 0.01$), but *increased* it in the hypothyroid group ($P = 0.01$) by affecting frequency of breathing in opposite ways in the two groups. Post-hypoxic ventilatory responsiveness was not affected by bromocriptine in either group (data not shown). The ventilatory effects of bromocriptine and carboxirole treatments in both groups are summarized in Table 1.

4. Discussion

The major findings of this study were that hypothyroidism in hamsters treated with PTU resulted in increased levels of D₂ receptor protein in the striatum and the carotid body, decreased levels in the PVN, but had no effect on D₂ protein levels in the NTS. Body temperature tended to be lower in the PTU-treated groups and was decreased in both groups following bromocriptine, but not following carboxirole treatment. In the two groups of hamsters bromocriptine and carboxirole had no effect on baseline ventilation during air exposure. During exposure to hypoxia, relative to their respective vehicles, both carboxirole and bromocriptine *stimulated* ventilation in the hypothyroid hamsters. Only bromocriptine treatment depressed ventilation in the euthyroid hamsters. Following exposure to hypoxia, both agents *decreased* ventilation in the euthyroid hamsters, but *increased* ventilation in the hypothyroid hamsters. The potential mechanisms of these unique findings and their relevance to hypothyroid patients will be discussed below.

4.1 Effects of hypothyroidism on D₂ receptor protein levels

For the first time we showed that hypothyroidism in hamsters affected the levels of D₂ receptor protein in the carotid bodies, striatum and PVN. Crocker and colleagues found similar results in the striatal homogenates from rats treated with iodine⁻¹³¹ to induce hypothyroidism (Crocker et al., 1986). Levels of D₂ receptor protein may also be the result of the length of the hypothyroidism. In one study short term hypothyroidism (4 weeks) resulted in not changes in D₂ receptor concentrations in the striatum (Cameron and Crocker, 1990). Longer term treatment (12 weeks) of rats with PTU resulted in increased striatal D₂

receptor levels and increased sensitivity to a D₂ receptor agonist (Crocker et al., 1986). Why hypothyroidism in the present study decreased D₂ concentration levels in the PVN while increasing it in the carotid body and striatum and what these findings may have on control of breathing is not clear. Moreover, the lack of effect of hypothyroidism on D₂ receptors in the NTS mirrors findings from a recent study of D₁ receptors in hypothyroid hamsters (Schlenker and Schultz, 2011).

4.2 Effects of bromocriptine on body temperature

Centrally acting D₂ receptor agonism is associated with a decrease in body temperature (Mustafa, 1991). In humans Schwartz and Erk (Schwartz and Erk, 2004) showed that oral administration of bromocriptine relative to placebo pills resulted in a long lasting hypothermia. In the present study we found comparable decreases in body temperatures in the euthyroid and hypothyroid hamsters. Finally, the lack of effect of carboxirile on body temperature in the present study confirms the more peripherally acting nature of this drug relative to the central and peripherally acting bromocriptine (Marchese et al., 2002).

4.3 Effects of hypothyroidism on ventilation

Ventilation of hypothyroid hamsters exposed to air or hypoxia was lower than in the euthyroid animals. These results concur with previous findings in hypothyroid hamsters (Schlenker and Schultz, 2011) and rats (Peterson et al., 2006). This decreased ventilation may be the result of lower body temperatures and metabolic rates observed in the hypothyroid animals (Schlenker, 1994). Moreover, we observed similar results of Olea and coworkers (Olea et al., 2007) who showed that hypothyroid rats exhibited diminished carotid body hypoxic sensitivity relative to that noted in euthyroid animals.

4.4 D₂ modulation of breathing necessitates exposure to hypoxia

Bromocriptine did not affect ventilation in either group of hamsters without prior exposure to hypoxia. In a separate study we verified that hypoxic exposure was necessary to elicit the responses described in the present study. Bromocriptine-treated euthyroid hamsters exposed to three bouts of air corresponding to the same protocol used in the present study showed decreases in body temperature, but no differences in ventilation compared to the same hamsters were treated with saline (Schlenker and Sykora, unpublished observations). A study by Cocker and Cameron also found that baseline levels of cAMP in the striatum of rats were not different, but exposure to dopamine increased cAMP levels to a greater extent in hypothyroid relative to control rats (Crocker and Cameron, 1988). Thus, hypoxia may act to increase release of dopamine (Orset et al., 2005) or “prime the pump” to elicit the ventilatory responses to occur as noted in the present studies.

4.5 Stimulatory effects of hypothyroidism on D₂ modulation of breathing

During and after hypoxic exposures both carboxirile and bromocriptine increased ventilation in the hypothyroid hamsters, while only bromocriptine depressed ventilation in the euthyroid hamsters. Thus there appear to be distinct peripheral and central effects of D₂ receptor stimulation on ventilation in the euthyroid hamsters, but not in the hypothyroid hamsters. The unique findings of excitatory effects of D₂ receptor agonist stimulation on ventilation in the hypothyroid hamsters may be the result of several possible factors. The first may be that hypothyroidism actually changes the characteristics of D₂ receptors back to an earlier developmental status that is excitatory (Oh-Nishi et al., 2005). Using rats rendered hypothyroid using methylnitroimidazole (MMI) that blocks the synthesis of thyroid hormones, Oh-Nishi and coworkers found persistent hippocampal excitation with application of dopaminergic D₂ receptor agonists, quinpirole and 2-bromo- α -ergocryptine. This response mirrored what the investigators found in very young rats and subsequently

became inhibitory when animals reached adulthood. Moreover, treating rats concomitantly with MMI and T4 prevented the excitatory phenotype of the D₂ receptors.

The second manner that increased D₂ receptors in hypothyroid hamsters may become excitatory is by presynaptically blocking the release of gamma amino butyric acid (GABA). This mechanism was recently described by Xu and Yao (Xu and Yao, 2010). Of importance is that this effect is dependent on activity within the circuit, similar to our findings that hypoxic stimulation is necessary to elicit the ventilatory responses observed in the present study.

Another mechanism that could cause the differential effect of D₂ receptor agonism on ventilation in the two groups may be related to greater internalization of D₂ receptors in the hypothyroid hamsters when exposed to hypoxia. Regulation of D₂ function can occur by internalization, recycling and degradation of receptors. Using primary mouse hippocampal neurons, HEK293 cells transfected with neural cell adhesion molecule (NCAM) and D₂ receptors, and NCAM knockout mice, Xiao and coworkers demonstrated that NCAM (especially the 180 isoform) by direct interaction with D₂ receptors caused internalization of D₂ receptors (Xiao et al., 2009). Moreover, apomorphine stimulated dopamine-modulated locomotor behavior in NCAM knockout mice compared to the minimal effect of apomorphine on locomotion in wild type controls. Interestingly, NCAM is increased in various brain regions of hypothyroid animals compared to levels in similar regions of euthyroid animals (Gong et al., 2010).

Thus, a potential mechanism by which bromocriptine and carboxirile may stimulate ventilation in hypothyroid hamsters is that the release of dopamine during hypoxia can stimulate NCAM internalization of D₂ receptors. If hypothyroid hamsters have increased levels of NCAM, D₂ stimulation may cause greater internalization of D₂ receptors relative to euthyroid hamsters. This could cause dopamine-induced stimulation of D₁ receptors that would increase ventilation. At this juncture this hypothesis needs to be verified.

Another potential mechanism to explain the differential effects of the two groups to bromocriptine during and following hypoxia may be that D₂ receptors were decreased in the PVN, but increased in the striatum in the hypothyroid animals relative to the euthyroid hamsters. To determine if the decreased expression of D₂ receptors in the PVN affects stimulation of ventilation in hypothyroid hamsters, bromocriptine could be microinjected into the PVN of both groups and the effects on body temperature, ventilation and metabolism determined.

The clinical relevance of the findings in this study suggests that hypothyroid patients given agents that may act on D₂ receptors may respond very differently than predicted. Hypothyroidism is a common endocrine disorder (Roos et al., 2010). Moreover, thyroid hormones dysfunction also occurs in subclinical hypothyroidism and euthyroid sick syndrome (Duntas and Biondi, 2011; Economidou et al., 2011; Meyer et al., 2011). Recently bromocriptine was approved for use in patients with type 2 diabetes (DeFronzo, 2011) who may also be hypothyroid. What effects this treatment has on control of breathing in this population of patients is not known.

In summary, hypothyroidism in hamsters affects expression of D₂ receptors in the PVN, stratum and carotid bodies in a different way than noted in euthyroid hamsters. A dichotomy of effects of D₂ receptor stimulation is also noted in control of breathing during exposure of hypothyroid and euthyroid hamsters to hypoxia and air following hypoxia. Specifically, we showed that by using carboxirile and bromocriptine to stimulate D₂ receptors in the carotid body and also centrally that both were needed to elicit excitation of breathing in hypothyroid hamsters, but that D₂ receptors acting centrally depressed breathing in euthyroid hamsters.

The potential underlying mechanisms for these findings are proposed and need to be evaluated.

Acknowledgments

This project was funded as a subproject to NIH NCRR P20 RR-015567. We would like to acknowledge Xinying Niu for her technical help with the dopamine D₂ Western blots.

References

- Ángyán L, Ángyán Z. Characterization of cardiorespiratory responses to electrical stimulation of the globus pallidus in cat. *Physiol Behav.* 1999; 66:53–58. [PubMed: 10222473]
- Aoki Y, Berlin RM, Clickner R, Jeffries R, Phillips L, Mahaffey K. Serum TSH and total T4 in the United States population and their association with participant characteristics: National health and nutrition examination survey (NHANES 1999–2002). *Thyroid.* 2007; 12:1211–1223. [PubMed: 18177256]
- Atsumi M, Kawakami J, Sugiyama E, Kotaki H, Sawada Y, Sato H, Yamada Y, Iga T. Pharmacokinetic and pharmacodynamic analyses, based on dopamine D₂ receptor occupancy of bromocriptine, of bromocriptine-induced contralateral rotations in unilaterally 6-OHDA-lesioned rats. *Synapse.* 2003; 50:110–116. [PubMed: 12923813]
- Bairam A, Hannhart B, Chon C, Marchal F. Effects of dopamine on the carotid chemosensory response to hypoxia in newborn kittens. *Respir Physiol.* 1993; 94:297–307. [PubMed: 8108608]
- Bascom DA, Clement ID, Dorrington KL, Robbins PA. Effects of dopamine and domperidone on ventilation during isocapnic hypoxia in humans. *Respir Physiol.* 1991; 85:319–328. [PubMed: 1683713]
- Beaulieu JM, Gainetdinov R. The physiology, signaling, and pharmacology of dopamine receptors. *Pharmacol Rev.* 2011; 63:182–217. [PubMed: 21303898]
- Burton MD, Kazemi H. Neurotransmitters in central respiratory control. *Respir Physiol.* 2000; 122:111–121. [PubMed: 10967338]
- Cameron DL, Crocker AD. Post-synaptic changes and increased dopamine receptor sensitivity in hypothyroid rats. *Pharmacol Biochem Behav.* 1987; 28:193–193. [PubMed: 2825219]
- Cameron DL, Crocker AD. The hypothyroid rat as a model of increased sensitivity to dopamine receptor agonists. *Pharmacol Biochem Behav.* 1990; 37:627–632. [PubMed: 1982691]
- Crocker AD, Cameron DL. Evidence of post-synaptic changes mediating increased behavioral sensitivity to dopamine receptor agonists in hypothyroid rats. *Prog Neuropsychopharmacol Biol Psychiatry.* 1988; 12:607–615.
- Crocker AD, Overstreet D, Crocker JM. Hypothyroidism leads to increased dopamine receptor sensitivity and concentration. *Pharmacol Biochem Behav.* 1986; 24:1593–1597. [PubMed: 3090565]
- DeFronzo RA. Bromocriptine: a sympatholytic, D₂-dopamine agonist for the treatment of Type 2 Diabetes. *Diabetes Care.* 2011; 34:789–794. [PubMed: 21447659]
- Diez D, Grijota-Martinez C, Agretti P, De Marco G, Tonacchera M, Pinchera A, de Escobar GM, Bernal J, Morte B. Thyroid hormone action in the adult brain: Gene expression profiling of the effects of single and multiple doses of Triiodo-L-thyronine in the rat striatum. *Endocrinology.* 2008 en.2008–0350.
- Duntas L, Biondi B. New insights into subclinical hypothyroidism and cardiovascular risk. *Sem Thromb Hemostasis.* 2011; 37:27–34.
- Economidou F, Douka E, Tzanela M, Nanas S, Kotanidou A. Thyroid function during critical illness. *Hormones.* 2011; 10:117–124. [PubMed: 21724536]
- Evans KC, Shea SA, Saykin AJ. Functional MRI localisation of central nervous system regions associated with volitional inspiration in humans. *J Physiol.* 1999; 520:383–392. [PubMed: 10523407]

- Fagerlund M, Khlin J, Ebberyd A, Schulte G, Mkrtchian S, Eriksson L. The human carotid body: expression of oxygen sensing and signaling genes of relevance for anesthesia. *Anesthesiology*. 2010; 113:1270–1279. [PubMed: 20980909]
- Genest SE, Balon N, Laforest S, Drolet G, Kinkead R. Neonatal maternal separation and enhancement of the hypoxic ventilatory response in rat: the role of GABAergic modulation within the paraventricular nucleus of the hypothalamus. *J Physiol*. 2007; 583:299–314. [PubMed: 17569732]
- Gong J, Liu W, Dong J, Wang Y, Xu H, Wei W, Zhong J, Xi Q, Chen J. Developmental iodine deficiency and hypothyroidism impair neural development in rat hippocampus: involvement of doublecortin and NCAM-180. *BMC Neuroscience*. 2010; 11:50. [PubMed: 20412599]
- Granjeiro ÉM, Machado BH. NO in the caudal NTS modulates the increase in respiratory frequency in response to chemoreflex activation in awake rats. *Respir Physiol Neurobiol*. 2009; 166:32–40.
- Guner I, Yelmen N, Sahin G, Oruc T. The effect of intracerebroventricular dopamine administration on the respiratory response to hypoxia. *Tohoku Journal of Experimental Medicine*. 2002; 196:219–230. [PubMed: 12086150]
- Haase AF, Greiner HE, Seyfried CA. Neurochemical profile of EMD 45609 (carmoxirole), a dopamine DA2-receptor agonist. *Naunyn-Schmiedeberg's Arch Pharmacol*. 1991; 343:588–594.
- Hollowell JG, Staehling NW, Flanders WD, Hannon WH, Gunter EW, Spencer CA, Braverman LE. Serum TSH, T4, and thyroid antibodies in the United States population (1988 to 1994): National Health and Nutrition Examination Survey (NHANES III). *J Clin Endocrinol Metab*. 2002; 87:489–499. [PubMed: 11836274]
- Huey KA, Szewczak JM, Powell FL. Dopaminergic mechanisms of neural plasticity in respiratory control: transgenic approaches. *Respir Physiol Neurobiol*. 2003; 135:133–144. [PubMed: 12809614]
- Jha A, Sharma SK, Tandon N, Lakshmy R, Kadiravan T, Handa KK, Gupta R, Pandey RM, Chaturvedi PK. Thyroxine replacement therapy reverses sleep-disordered breathing in patients with primary hypothyroidism. *Sleep Medicine*. 2006; 7:55–66. [PubMed: 16198143]
- Kline DD, Takacs KN, Ficker E, Kunze DL. Dopamine modulates synaptic transmission in the nucleus of the solitary tract. *J Neurophysiol*. 2002; 88:2736–2744. [PubMed: 12424308]
- Kulikov AV, Zubkov EA. Chronic thyroxine treatment activates the 5-HT2A serotonin receptor in the mouse brain. *Neurosci Lett*. 2007; 416:307–309. [PubMed: 17316999]
- Marchese G, Ruii S, Casti P, Bartholini F, Saba P, Gessa GL, Pani L. Carmoxirole is able to reduce amisulpride-induced hyperprolactinemia without affecting its central effect. *Eur J Pharmacol*. 2002; 447:109–114. [PubMed: 12106810]
- McKay LC, Evans KC, Frackowiak RSJ, Corfield DR. Neural correlates of voluntary breathing in humans. *J App Physiol*. 2003; 95:1170–1178.
- Meyer S, Schuetz P, Wieland M, Nusbaumer C, Mueller B, Christ Crain M. Low triiodothyronine syndrome: a prognostic marker for outcome in sepsis? *Endocrine*. 2011; 39:167–174. [PubMed: 21210252]
- Misiolek M, Marek B, Namyslowski G, Scierski W, Zwirska-Korczala K, Kazmierczak-Zagorska Z, Kajdaniuk D, Misiolek H. Sleep apnea syndrome and snoring in patients with hypothyroidism with relation to overweight. *J Physiol Pharmacol*. 2007; 58(Suppl 1):77–85. [PubMed: 17443029]
- Mustafa A. Bromocriptine-induced hypothermia in Balb/C mice: its possible mechanism of action. *Gen Pharmacol*. 1991; 22:545–548. [PubMed: 1651267]
- Nielsen AM, Bisgard GE. Differential effects on phrenic output of two dopamine agonists, apomorphine and bromocriptine. *Eur J Pharmacol*. 1984; 106:69–78. [PubMed: 6529974]
- O'Halloran KD, Janssen PL, Bisgard GE. Dopaminergic modulation of respiratory motor output in peripherally chemodenervated goats. *J Appl Physiol*. 1998; 85:946–954. [PubMed: 9729568]
- Oh-Nishi A, Saji M, Furudate SI, Suzuki N. Dopamine D2-like receptor function is converted from excitatory to inhibitory by thyroxine in the developmental hippocampus. *J Neuroendocrinology*. 2005; 17:836–845.
- Olea E, Gonzalez C, Gallego R, Gejjo-Barrientos ELA. PTU-induced hypothyroidism reduces hypoxic ventilatory drive by impairing carotid body chemosensitivity in adult rats. *Acta Physiologica*. 2007; 190(Supplement 655):P51.

- Orset C, Parrot S, Sauvinet V, Cottet-Emard JM, Berod A, Pequignot JM, Denoroy L. Dopamine transporters are involved in the onset of hypoxia-induced dopamine efflux in striatum as revealed by in vivo microdialysis. *Neurochem Intern.* 2005; 46:623–633.
- Pedersen MEF, Dorrington KL, Robbins PA. Effects of haloperidol on ventilation during isocapnic hypoxia in humans. *J Appl Physiol.* 1997; 83:1110–1115. [PubMed: 9338418]
- Peterson A, Gilman TL, Banks ML, Sprague JE. Hypothyroidism alters striatal dopamine release mediated by 3,4-methylenedioxymethamphetamine (MDMA, ecstasy). *Synapse.* 2006; 59:317–319. [PubMed: 16421905]
- Reddy MK, Patel KP, Schultz HD. Differential role of the paraventricular nucleus of the hypothalamus in modulating the sympathoexcitatory component of peripheral and central chemoreflexes. *Am J Physiol Regul Integr Comp Physiol.* 2005; 289:R789–797. [PubMed: 15919733]
- Resta O, Pannacciulli N, Di Guoia G, Stefano A, Barbaro MP, De Pergola G. High prevalence of previously unknown subclinical hypothyroidism in obese patients referred to a sleep clinic for sleep disordered breathing. *Nutr Metab Cardiovas Dis.* 2004; 14:248–253.
- Roos A, Links T, de Jong-van den Berg LTW, Gans ROB, Wolffenbuttel BHR, Bakker SJL. Thyroid peroxidase antibodies, levels of thyroid stimulating hormone and development of hypothyroidism in euthyroid subjects. *Eur J Intern Med.* 2010; 21:555–559. [PubMed: 21111944]
- Sakellaropoulou AV, Hatzistilianou MN, Emporiadou MN, Aivazis VT, Rousso I, Athanasiadou Piperopoulou F. Evaluation of thyroid gland function in children with obstructive apnea hypopnea syndrome. *Inter J Immunopathol Pharmacol.* 2011; 24:377–386.
- Sarkar PK, Durga ND, Morris JJ, Martin JV. In vitro thyroid hormone rapidly modulates protein phosphorylation in cerebrocortical synaptosomes from adult rat brain. *Neuroscience.* 2006; 137:125–132. [PubMed: 16289831]
- Schlenker E, Barnes L, Hansen S, Martin D. Cardiorespiratory and metabolic responses to injection of bicuculline into the hypothalamic paraventricular nucleus (PVN) of conscious rats. *Brain Research.* 2001; 895:33–40. [PubMed: 11259757]
- Schlenker EH. In hamsters dopamine D2 receptors affect ventilation during and following intermittent hypoxia. *Comp Biochem Physiol- Part A: Mol Int Physiol.* 2007; 148:674–680.
- Schlenker EH, Means R, Burbach JA. Naloxone stimulates oxygen consumption but not ventilation in hypothyroid hamsters. *Physiol Behav.* 1994; 56:635–638. [PubMed: 7800724]
- Schlenker EH, Schultz HD. Hypothyroidism attenuates SCH 23390-mediated depression of breathing and decreases D1 receptor expression in carotid bodies, PVN and striatum of hamsters. *Brain Res.* 2011; 1405:40–51. [PubMed: 21669406]
- Schwartz PJ, Erk SD. Regulation of central dopamine-2 receptor sensitivity by a proportional control thermostat in humans. *Psychiatry Res.* 2004; 127:19–26. [PubMed: 15261701]
- Slotkin TA, Slepatis RJ. Obligatory role of thyroid hormones in development of peripheral sympathetic and central nervous system catecholaminergic neurons: effects of propylthiouracil-induced hypothyroidism on transmitter levels, turnover and release. *J Pharm Exper Ther.* 1984; 230:53–61.
- Tews M, Shah S, Gossain V. Hypothyroidism: mimicker of common complaints. *Emerg Med Clin N Am.* 2005; 23:649–667. vii.
- Varney AA, Schlenker EH. Thyroid status affects 5-HT_{2A} receptor modulation of breathing before, during, and following exposure of hamsters to acute intermittent hypoxia. *Am J Physiol Regul Integr Comp Physiol.* 2007; 293:R2070–2080. [PubMed: 17855493]
- Weiner DM, Levey AI, Sunahara RK, Niznik HB, O'Dowd BF, Seeman P, Brann MP. D1 and D2 dopamine receptor mRNA in rat brain. *PNAS.* 1991; 88:1859–1863. [PubMed: 1825729]
- Wu T, Flowers J, Tudiver F, Wilson J, Punyasavatsut N. Subclinical thyroid disorders and cognitive performance among adolescents in the United States. *BMC Pediatrics.* 2006; 6:12–18. [PubMed: 16623938]
- Xiao MF, Xu JC, Tereshchenko Y, Novak D, Schachner M, Kleene R. Neural cell adhesion molecule modulates dopaminergic signaling and behavior by regulating dopamine D2 receptor internalization. *J Neurosci.* 2009; 29:14752–14763. [PubMed: 19940170]

Xu TX, Yao WD. D1 and D2 dopamine receptors in separate circuits cooperate to drive associative long-term potentiation in the prefrontal cortex. PNAS. 2010; 107:16366–16371. [PubMed: 20805489]

Highlights

- Hypothyroidism affects expression of D2 receptors in the PVN, stratum and CB.
- D2 receptor stimulation and hypoxia depressed breathing in normal hamsters.
- D2 receptor stimulation and hypoxia stimulated breathing in hypothyroid hamsters.
- Carotid body and CNS D2 modulation of breathing are altered in hypothyroidism.

Figure 1.

Representative Western blots and analysis of D₂ protein levels in the carotid bodies (CB, n=8 per group) and striatum (n=3 per group). Lanes 1–3 are from euthyroid hamsters and lanes 4–6 are from hypothyroid hamsters. The graphs below summarize the results of data from carotid bodies and striatum of euthyroid (control) and hypothyroid (PTU) hamsters. Values are means $\pm$ SEM. The asterisk denotes significant differences in D₂ receptor protein levels between the two groups.

Figure 2.

Representative Western blots and analysis of D₂ protein levels in the paraventricular nucleus of the hypothalamus (PVN, n=3 per group) and nucleus tractus solitarius (NTS, n=3 per group). Lanes 1–3 are from euthyroid hamsters (control) and lanes 4–6 are from hypothyroid (PTU) hamsters. The graphs below summarize the data from the PVN and NTS of euthyroid and hypothyroid hamsters. Values are means $\pm$ SEM. The asterisk denotes significant differences in D₂ receptor protein levels between the two groups.

Figure 3.

Effects of vehicle (first dark bar), 0.2 mg/kg carboxirole (light, 2nd bar), and 0.4 mg/kg carboxirole (medium bar (3rd) on ventilation (A), frequency of breathing (B) and tidal volume (C) of euthyroid and hypothyroid hamsters exposed to air, hypoxia and air post-hypoxia. The asterisks denote significant effects ($P < 0.05$) of carboxirole relative to vehicle in each group and the pound sign denotes significant differences ($P < 0.05$) in the responses of the two groups of hamsters to each of the treatments.

Figure 4.

Hypoxic responsiveness of euthyroid (dark bar) and hypothyroid hamsters (light bar) treated with carmoxirole (A) or bromocriptine (B) relative to vehicle. The asterisk denotes significant effects ($P < 0.05$) of treatments (carmoxirole or bromocriptine) relative to vehicle in each group and the pound sign denotes significant differences ($P < 0.05$) in the responses of the two groups of hamsters to the treatments. The § sign indicates a P value of 0.05.

Figure 5. Effects of vehicle (dark bar) and 1.0 mg/kg bromocriptine (light bar) on ventilation (A), frequency of breathing (B) and tidal volume (C) of euthyroid and hypothyroid hamsters exposed to air, hypoxia and air post-hypoxia. The asterisks denote significant effects ($P < 0.05$) of carboxirole to vehicle in each group and the pound sign denotes significant differences ($P < 0.05$) in the responses of the two groups of hamsters to the treatments.

Table 1

Summary of Ventilatory Results following Treatment of Euthyroid (E) and Hypothyroid (H) Hamsters with Vehicle, Carmoxirole and Bromocriptine

	H versus E	Carmoxirole	Bromocriptine
<u>Baseline Air</u>			
Frequency	↓	H↔, E↔	H↔, E↔
Tidal Volume	↔	H↔, E↔	H↔, E↔
Minute Ventilation	↓	H↔, E↔	H↔, E↔
<u>Hypoxia</u>			
Frequency	↓	H↑, E↔	H↑, E↓
Tidal Volume	↓	H↑, E↔	H↑, E↓
Minute Ventilation	↓	H↑, E↔	H↑, E↓
*Hypoxic Responsiveness	↓	H↑, E↔	H↔, E↔
<u>Post-Hypoxia</u>			
Frequency	↓	H↑, E↓	H↑ E↓
Tidal Volume	↔	H↑, E↔	H↔, E↔
Minute Ventilation	↓	H↑ E↓	H↑ E↓
** Air/Post-Hypoxic	↔	H↑ E↓	H↔, E↔

A summary of ventilatory parameters of euthyroid and hypothyroid hamsters exposed to air, hypoxia and air following hypoxia.

The *denotes ventilation during hypoxia/ventilation during air,

** denotes ventilation following hypoxia/ventilation during baseline air exposure.

The ↓ denotes a decrease, ↑ increase, or ↔ no change of the parameter either comparing hypothyroid to euthyroid hamsters in the first column, effects of carmoxorile (0.2 mg/kg) relative to vehicle, or bromocriptine to vehicle.