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In Adult Female Hamsters Hypothyroidism Stimulates D1 Receptor-mediated Breathing without altering D1 Receptor Expression

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Abstract

Hypothyroidism affects cardiopulmonary regulation and function of dopaminergic receptors. Here we evaluated effects of 5 months of hypothyroidism on dopamine D1 receptor modulation of breathing in female hamsters using a D1 receptor antagonist SCH23390. Euthyroid hamsters (EH) served as controls. Results indicated that hypothyroid female hamsters (HH) exhibited decreased body weights and minute ventilation (V_E) following hypoxia due to decreased frequency of breathing (F). Moreover, SCH 23390 administration in HH increased V_E by increasing tidal volume during exposure to air, hypoxia and following hypoxia. Relative to vehicle, SCH 23390 treatment decreased body temperature and hypoxic V_E responsiveness in both groups. In EH, SCH 23390 decreased F in air, hypoxia and post hypoxia, and V_E during hypoxia trended to decrease ($P=0.053$). Finally, expression of D1 receptor protein was not different between the two groups in any region evaluated. Thus, hypothyroidism in older female hamsters affected D1 receptor modulation of ventilation differently relative to euthyroid animals, but not expression of D1 receptors.

Keywords

hypothyroidism; female; hypoxia; ventilation; D1 receptor expression

1.0 Introduction

Hypothyroidism is a prevalent endocrine disorder that affects metabolism, cardiopulmonary function, cognition, and behavior (Carle et al., 2014; Demartini et al., 2014; Hennessey and Espaillat, 2015; Kim, 2000; Vargas-Uricoechea et al., 2014; Vargas et al., 2006). With age, the prevalence of hypothyroidism is higher in females than in males (Shinkov et al., 2014). The underlying mechanism for these gender differences is currently unknown. Hypothyroidism also affects the respiratory system and control of breathing (Duranti et al., 1993) and (see references (Schlenker, 2012; Schlenker et al., 1994)). Several studies from

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our laboratory indicate that hypothyroidism specifically affects modulation of breathing by altering the function of various neurotransmitter systems and receptors including opioids, serotonin and dopamine (Schlenker et al., 1994; Schlenker and Schultz, 2011a; Varney and Schlenker, 2007).

Dopamine acts on receptors that are divided into various subtypes: the D1-like receptors include D1 and D5 subtypes and the D2-like receptors include D2, D3 and D4 subtypes (Beaulieu and Gainetdinov, 2011). Generally stimulation of D2 receptors results in depression of breathing (Huey et al., 2000), although this may not be the case in hypothyroid hamsters. For example, we showed in 8 month old female hamsters rendered hypothyroid for 5 months that stimulation of the dopamine D2 receptor using central and peripherally acting bromocriptine stimulated breathing in the hypothyroid, but not in the control age-matched euthyroid hamsters (Schlenker and Schultz, 2012a). By contrast, in younger two month old hypothyroid females bromocriptine had minor effects on ventilation and necessitated a higher dose to affect breathing, suggesting effects of age and length of hypothyroidism influence D2 receptor control of breathing in female hamsters (Padilla and Schlenker, 2014).

Interestingly age also affected D2 modulation of breathing in male hypothyroid hamsters. Thus, in two month old male hypothyroid hamsters, relative to vehicle, bromocriptine stimulated ventilation during and following exposure to hypoxia, while bromocriptine treatment depressed ventilation during the same exposures in age-matched euthyroid male hamsters (Schlenker and Schultz, 2012a). By contrast, eight month old male hamsters rendered hypothyroid for 5 months bromocriptine had little effect on ventilation in hypothyroid hamsters, but decreased it in the euthyroid hamsters (Sykora et al., 2013). Thus in males there also appears to be an age and hypothyroid related effect of D2 receptors modulation of breathing.

Another dopamine receptor subtype that affects breathing is the D1 receptor. Previous studies indicate that upregulation of D1 receptors and either stimulation or blockade of D1 receptors can modulate control of breathing in hamsters, rats, cats, and mice (Iwase et al., 2013; Lalley, 2005; Lalley, 2008; Schlenker and Schultz, 2011b). Iwase and his colleagues (Iwase et al., 2013) showed that treatment of with the D1 receptor antagonist, SCH 23390 depressed ventilation, oxygen uptake, CO₂ production and body temperature at rest and attenuated the increased in exercise-induced hyperpnea. They went on to discuss that the paraventricular nucleus of the hypothalamus (PVN) was a potential substrate for the D1 mediated physiological changes observed in their study.

Moreover, hypothyroidism can also affect express of D1 receptors and modulate ventilatory responses of male hamsters. For example, relative to euthyroid controls, rendering adult male hamsters hypothyroid for 3 months exhibited decreased D1 protein levels in the carotid body, paraventricular nucleus of the hypothalamus (PVN), and striatum, but not in the nucleus tractus solitarius (Schlenker and Schultz, 2011b). Administration of SCH 23390, a D1 receptor antagonist, depressed ventilation during exposure of euthyroid male hamsters to air, but stimulated ventilation in the hypothyroid male hamsters. Moreover, exposure of SCH 23390-treated euthyroid hamsters to hypoxia and to hypercapnia caused depression of

breathing, but had no effect on ventilation in hypothyroid hamsters. Thus, hypothyroidism, age and sex may modify the effects of dopamine receptor modulation of breathing.

The purpose of the present study was to determine how hypothyroidism affected D1 receptor expression and modulation of metabolism and ventilation in adult female hamsters rendered hypothyroid for 5 months. We hypothesized that 1. there would be a sexually dimorphic pattern difference in both D1 receptor protein expression in the nucleus tractus solitarius (NTS), striatum, PVN and carotid bodies, all regions associated with regulation of breathing (King et al., 2015; Kline et al., 2002; Nattie, 2001; Semenza and Prabhakar, 2012) and 2. also in ventilation in the females compared to the previous study reported in males.

2.0 Methods

2.1 Animals

Seven to eight week old female golden Syrian hamsters were obtained from Harlan Sprague Dawley, Inc. (Indianapolis, IN). Ten animals received 0.04% propylthiouracil (PTU) to induce hypothyroidism and 9 hamsters received tap water. This is a technique previously used in our laboratory in the same animals (Schlenker and Schultz, 2012b) and resulted in decreased serum thyroxine hormones levels. Hamsters 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.). Tap water or PTU in tap (Schlenker and Schultz, 2012b) water were available *ad libitum*. When the hamsters had been on PTU for 5 months, the ventilatory experiments started. For 3 days prior to the ventilatory studies, all hamsters were acclimated to the cylindrical Plexiglas chamber (see below) for 30 minutes each day. The University of South Dakota Animal Care and Use Committee approved all procedures used in these studies. These hamsters had been used in a previous study to investigate effects of hypothyroidism on D2 modulation of breathing and expression of D2 receptors in several brain regions and the carotid body as described in the introduction. The current experiments investigating D1 modulation of breathing in these same animals were conducted 2 weeks following the conclusion of the D2 experiments while hamsters were still receiving PTU.

2.2 Ventilatory and Metabolic Measurements

Oxygen consumption, CO₂ production and respiratory measurements were made in hamsters placed into a 20.2 × 7.9 cm Plexiglas cylindrical chamber. The barometric method was used to evaluate ventilatory parameters as previously described in hamsters (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 pressure was measured with a Statham pressure transducer and chamber temperature was determined using a Fisher Scientific digital thermometer. Relative humidity within the chamber averaged 50%.

Inspiratory and expiratory times were measured from approximately 15 breaths, for each portion of the study. Frequency of breathing (F) was calculated by adding inspiratory and expiratory times and dividing the sum into 60 (sec/min). The minute ventilation (V_E) was

calculated by multiplying F by tidal volume (V_T). Both V_T and V_E were normalized by body weight (variable*1000/g). Oxygen consumption (Vo_2) was calculated 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 of air through the chamber. CO_2 production (Vco_2) was determined by subtracting the fractional content of CO_2 incoming gas to that leaving the chamber. VacuMed CO_2 and O_2 gas analyzers were used to measure levels of CO_2 or O_2 in the incoming and outgoing air. Vo_2 and Vco_2 were corrected to STPD and BW (g).

2.3 Protocols for Ventilatory Studies

To evaluate the effects of D1 receptor modulation of ventilation a subcutaneous injection of 0.25 mg/kg SCH 23390 (SCH 23390, Tocris Bioscience), a D1 receptor antagonist (Bourne, 2001) in saline was administered. This dose of SCH 23390 was used in a previous study (Schlenker and Schultz, 2011a). All animals also received saline at a separate week. Following drug or saline administration, the hamster was placed into the chamber for 35 minutes, and exposed to room air. First, Vo_2 and Vco_2 were determined and then F , V_T , and V_E were evaluated. The hamster was then exposed to hypoxia for 5 minutes. During the last minute of exposure to hypoxia, F , V_T , and V_E were determined. Subsequently, the chamber was washed out with room air for 5 minutes. During the last minute of the washout, F , V_T , and V_E were evaluated in the hamster. Within one minute after the last air reading the hamster was removed from the chamber and its body temperature was measured using a Sentsortek BAT-12 thermometer and a Physiotemp thermocouple.

2.4 Evaluation of Body Weights and Western Blot Analysis for D1 Receptors

At the end of the physiological studies, hamsters were deeply anesthetized and weighed. The brain and carotid bodies were quickly removed, frozen on dry ice, and stored at -80 degrees Celsius. Punches from striatum, PVN, NTS and carotid bodies from euthyroid and hypothyroid female hamsters were obtained as previously described (Schlenker and Schultz, 2011b).

The protein from brain punches (Striatum, PVN, NTS) and carotid bodies was extracted with the Radio-Immunoprecipitation Assay lysing buffer (RIPA containing 150 mM NaCl, 1.0% IGEPAL[®] CA-630, 0.5% sodium deoxycholate, 0.1% SDS, and 50 mM Tris; pH 8.0, Sigma, USA) plus 1% complete protease inhibitor cocktail (Sigma, USA). Following ultrasonic disruption samples were centrifuged at 12,000g for 30min at 4 °C. Protein concentration in the supernatant was determined using a BCA protein assay kit (ThermoScientific, USA). The protein sample was mixed with loading buffer containing β -mercaptoethanol and heated at 100 °C for 5 min. Equal amount of protein (35 μ g) was loaded into 10% polyacrylamide gel along with molecular weight standards (PrecisionPlus Protein Standards, Bio-Rad, USA) for electrophoretic separation. Gels containing the proteins were then transferred to PVDF membrane (Immobilon-P, Millipore, USA) at 4°C. To determine D1 receptor protein levels in the three brain regions, the membranes were first incubated with blocking buffer (Odyssey Blocking Buffer, Li-Cor, USA) and then incubated overnight at 4°C with prepared in the same blocking solution: rabbit polyclonal antibody (1:4000, against a sequence corresponding to amino acids 9–21 of human D1DR, Abcam,

USA) Then, membranes were washed with cold PBS (Sigma, USA) containing 0.2% Tween-20 (Sigma, USA) and incubated for 1h at room temperature with a secondary goat anti-rabbit antibody (1:10000, IRDye 800CW, Li-Cor, USA). Membranes were then developed using the Odyssey infrared fluorescence imaging systems (Li-Cor, USA).

For carotid bodies, the membrane was probed with mouse anti D1 dopamine receptor antibody (1:500 against the last 123 C-terminal amino acids of D1DR of rat origin, Santa Cruz, USA). Membranes were then washed with cold PBS (Sigma, USA) containing 0.2% Tween-20 (Sigma, USA), incubated for 1h at room temperature with a secondary donkey anti-mouse antibody (1:10000, IRDye 800CW, Li-Cor, USA) and developed using infrared fluorescence systems (Li-Cor, USA).

Following stripping of the membranes, protein loading was evaluated by probing all membranes with mouse anti- β -actin antibody (1:2500, Sigma, USA). Stripping protocol consist in 30 min incubation with the proper stripping buffer solution (NewBlot™, Li-Cor, USA). Stripping efficacy was determined by the absence of fluorescence signal after the incubation with the stripping buffer. Then, membranes were incubated with the anti- β -actin antibody and develop as previously described.

Analysis of the specific bands optical densities were performed using the Odyssey Infrared Fluorescence Imaging Software (Li-Cor, USA). All data were normalized using dopamine receptor protein intensities to that of β -actin. No difference in the level of expression of β -actin was found between euthyroid and hypothyroid samples.

2.5 Statistical Analysis

Statistical analysis of the body temperature, ventilatory, and metabolic data consisted of two-way analysis of variance (ANOVA) to test the effects of treatment (PTU versus tap water) and drug (SCH 23390 relative to saline). If the result was significant ($P < 0.05$), post hoc Holm-Sidak tests comparisons were conducted. Body weights and Western blot data were analyzed using unpaired Student's t tests. If the data were skewed, we used nonparametric tests such as the Mann-Whitney test (for unpaired comparisons) or the Wilcoxon sign rank test (for paired comparisons). Data were analyzed using IBM SPSS Software Version 19.0. All data are presented as means and standard error of the mean.

3.0 Results

3.1 Body Weights, Body Temperatures, Metabolic Results

Body weights were significantly lower in the hypothyroid relative to the euthyroid hamsters (149.2 ± 2.2 versus 160.4 ± 4.1 g, $P = 0.025$) and as also reported in a previous paper on the same animals, T4 levels were significantly lower in the hypothyroid hamsters relative to the euthyroid animals (Schlenker, et. al, 2014). There was no effect of thyroid status on body temperature, but SCH treatment decreased body temperatures in in both groups (Table 1). There were no effects of thyroid status or SCH treatment on $\dot{V}O_2$ or $\dot{V}CO_2$ (Table 1).

3.2 Effects of Thyroid Status on D1 Receptor Expression

Dopamine D1 receptor protein levels were determined in the carotid bodies, paraventricular nucleus of the hypothalamus, nucleus tractus solitaries, and striatum in both groups as indicated in Figure 1. No differences in D1 receptor protein levels were noted as a function of thyroid status in any region.

3.3 Ventilatory Responses to Air Exposure

During exposure to air, there were no differences due to thyroid status on V_E , V_T or F (Figure 2). In contrast, administration of SCH significantly stimulated V_E in the hypothyroid hamsters relative to saline treatment ($P=0.018$), but had no effect in euthyroid hamsters. In both groups relative to saline, SCH increased V_T ($P=0.045$), but to a greater extent in the hypothyroid than in the euthyroid hamsters ($P<0.05$). SCH decreased F in the euthyroid hamsters ($P<0.01$), but had no effect on this parameter in the hypothyroid animals. Relative to saline, SCH increased the O_2 ventilatory equivalent (V_E/V_{O_2}) as shown in Figure 3B in the hypothyroid hamsters ($P=0.02$) without having an effect in the euthyroid animals. The same stimulatory effect of SCH was noted for the V_E/V_{CO_2} ($P=0.015$, data not shown).

3.4 Ventilatory Responses to Hypoxia Exposure

During exposure to hypoxia (Figure 2) V_E increased relative to air values in hypothyroid and euthyroid groups ($P<0.01$ for both groups). SCH treatment further increased V_E in the hypothyroid hamsters relative to saline treatment ($P<0.05$) and to a greater extent than seen in the SCH-treated euthyroid hamsters ($P<0.05$) due predominantly to an increase in V_T ($P=0.01$). In both groups hypoxic V_E responsiveness (determined as the ratio of V_E during exposure to hypoxia divided by V_E during exposure to air) was similar following saline administration and decreased to the same extent with SCH administration ($P<0.01$, Figure 3A).

3.5 Ventilation Following Exposure to Hypoxia

Administration of saline resulted in a lower V_E in the hypothyroid hamsters relative to the euthyroid hamsters ($P<0.05$) due to a lower F ($P<0.002$) during exposure to air following hypoxia. Following SCH treatment V_E increased in the hypothyroid group, but not in the euthyroid group. In both groups SCH significantly increased V_T ($P<0.01$), but decreased F in the euthyroid group ($P=0.02$) and marginally the hypothyroid group ($P=0.058$) relative to saline.

4.0 Discussion

The major findings of the present study were that hypothyroidism in adult female hamsters induced a decrease in body weight and decreased their ventilation following exposure hypoxia due to a decrease in frequency of breathing. For the first time we demonstrate that SCH 23390 administration to female hamsters rendered hypothyroid for 5 months increased their ventilation in air, during hypoxic exposure and following hypoxia due to an increase of V_T . Moreover, during baseline air exposure SCH 23390 increased the ventilatory equivalents for O_2 and CO_2 in hypothyroid hamsters. In both groups relative to saline treatment, SCH 23390 decreased body temperatures and decreased hypoxic responsiveness.

In euthyroid hamsters SCH 23390 decreased frequency of breathing during exposure to in air, hypoxia and post hypoxia, while ventilation during hypoxia showed a decreasing trend ($P=0.053$). Finally, expression of D1 receptor protein levels evaluated by Western blot were not different between the two groups in any region evaluated.

4.1 Effects of Hypothyroidism on Ventilation and D1 Receptor Expression

The few physiological manifestations of hypothyroidism in these older females were a decreased body weight, lower body temperature, and post hypoxic frequency decline compared to the euthyroid hamsters. Moreover, relative to euthyroid hamsters, there were no effects of hypothyroidism on the D1 protein expression in any region evaluated. This contrasts with the decreased D1 receptor protein expression in carotid bodies, PVN and striatum previously reported 3 month old adult hypothyroid male hamsters (Schlenker and Schultz, 2011a).

There were significant differences between the hypothyroid and euthyroid hamsters in the ventilatory responses to blocking the D1 receptor described in detail within sections 3.3–3.5 above, but no differences in total receptor expression. The underlying mechanisms for these functional differences are unknown, but may involve the trafficking of D1 and D2 receptors to and from the membranes of neurons in areas associated with control of breathing. Whether the receptors are located on the plasma membrane or internalized into the cytoplasm can influence D1 receptors' availability to interact with extracellular dopamine. The Western blot method for D1 receptor evaluation utilized in the present study was unable to distinguish the cellular location of the receptor. In a study done in PC12 cells (a catecholaminergic cell used as a model of carotid bodies (Patel et al., 2009; Peers, 2004) and contain both D1 and D2 receptors (Raghuraman et al., 2010; Zhu et al., 1997)) we observed that D1 receptors were located in the cytoplasm and on the plasma membrane (unpublished results, Schlenker, Padilla and Rezvani). When PC12 cells (102 cells used) were stimulated with a D1 receptor agonist, SKF38393 (Tocris), an increased number of receptors were localized to the plasma membrane compared to the cytoplasm, increasing the total number of D1 receptors in PC12 cells.

Possibly hypothyroidism affected the localization and therefore ability of D1 receptors to interact with dopamine. To investigate if this shift of D1 receptor localization is also applicable to hypothyroid and euthyroid hamsters and possibly the mechanisms involved, future studies on brain punches from specific regions and carotid bodies will be utilized. Methods may include using differential centrifugation methods followed by Western blotting as well as altering membrane permeability and using immunohistochemical methods (Ha et al., 2012; Porras et al., 2012).

4.2 Role of D1 Receptors on Control of Breathing

In a recent study resting and exercising male mice treated with SCH23390 decreased their ventilation at rest due to decreased frequency of breathing (Iwase, et al., 2013). Both inspiratory and expiratory times were increased in that study. Moreover, as a result of SCH 23390 treatment, there were concomitant decreases of V_{CO_2} and V_{O_2} that matched the changes in ventilation. Finally, when mice were exercised, SCH23390 did not affect the

initial increase in ventilation during onset of exercise, but gradually decreased ventilation over the 30 minute exercise bout and decreased body temperature. No effect of SCH 23390 was noted on ventilation during exposure of mice to hypercapnia.

By contrast, in the present study female euthyroid hamsters treated with SCH 23390, relative to saline, exhibited decreased frequency of breathing during exposure to air and air following hypoxia, but at the same time tidal volumes increased resulting in no net effect on minute ventilation. In addition, body temperatures decreased, but metabolism did not following SCH 23390 treatment.

4.3 Effects of D1 Receptors on Ventilation in Female Hypothyroid Hamsters

In the present study we are the first to show that blocking D1 receptors had stimulatory effects on ventilation in the hypothyroid females as noted in the increased ventilatory equivalent, and ventilation during exposure to room air, hypoxia and air following hypoxia. The increase in ventilation during gas exposures was due to increases in V_T . By contrast, in a recent study of younger 4 month old females rendered hypothyroid for 2 month, SCH 23390 stimulated V_{CO_2} and hypoxic responsiveness, but depressed V_E due to decreases in F and not V_T (Schlenker and Wilson, 2015). In that study euthyroid hamsters treated with SCH 23390 decreased body temperature and ventilation during exposure to baseline air and to hypercapnia due to a decrease in F . Thus, the length of time that female hamsters are rendered hypothyroid may affect D1 receptor modulation of breathing. A summary of the effects of hypothyroidism on D1 and D2 modulation of breathing in male and female hamsters at various ages from a number of published studies including results from the current study is presented (Tables 2 and 3) to support this statement.

4.4 Conclusion

In conclusion, D1 receptors modulate body temperature, control of breathing in conscious eight month old female hamsters, and affected ventilation differently in hypothyroid compared to euthyroid hamsters. Furthermore, expression of D1 receptors in carotid bodies, PVN, striatum and NTS were not different between the two groups suggesting the cellular localization and possibly signaling of D1 receptors may affect differences in D1 modulation of breathing in euthyroid and hypothyroid female hamsters.

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Figure 1.

Dopamine D1 receptor (D1DR) protein levels normalized to beta-actin. The top graph (A) denotes protein levels in the striatum, paraventricular nucleus of the hypothalamus (PVN), nucleus tractus solitarius (NTS) and carotid bodies (CB) of hypothyroid (white bar) and euthyroid (black bar) hamsters. Values are means and SEM. In B, representative Western blots of D1 receptor and beta-actin are shown for the striatum, NTS and CB from a hypothyroid and a euthyroid hamster.

Figure 2.

Ventilatory responses of hypothyroid (gray bars) and euthyroid (black bars) hamsters to air, hypoxia and air following hypoxia (post-hypoxia) following treatment with vehicle or SCH 23390 (SCH). Ventilatory parameters include minute ventilation (A), tidal volume (B) and frequency of breathing (C). The asterisks denote significant effects of SCH and the # denotes significant effects of thyroid status. Values are means and SEM of 8 euthyroid and 9 hypothyroid hamsters.

A.

B.

Figure 3.

Hypoxic responsiveness (A) and ventilatory equivalent for O₂ (B) hypothyroid (gray bars) and euthyroid (black bars) hamsters following treatment with vehicle or SCH 23390 (SCH). (V_E or Ventilation: ml/min*1000/BW) The asterisks denote significant effects of SCH treatment relative to vehicle for each group.

Table 1

Body Temperature, CO₂ production and Oxygen Consumption Data for Female Hypothyroid and Euthyroid Hamsters Receiving Saline or SCH29930.

Body Temperature (°C)		
<u>Treatment</u>	<u>Euthyroid</u>	<u>Hypothyroid</u>
Saline	36.8 (0.2)	36.1 (0.3)
SCH29930	35.3 (0.3) *	34.3 (0.4) *
Oxygen Consumption (ml/min *1000/BW)		
<u>Treatment</u>	<u>Euthyroid</u>	<u>Hypothyroid</u>
Saline	18.1 (1.6)	16.9 (1.3)
SCH29930	21.3 (1.5)	18.5 (1.2)
CO ₂ Production (ml/min *1000/BW)		
<u>Treatment</u>	<u>Euthyroid</u>	<u>Hypothyroid</u>
Saline	20.0 (1.4)	18.7 (1.6)
SCH29930	19.4 (1.2)	17.1 (1.1)

Values are means and SEM (in parentheses) of 9 euthyroid and 10 hypothyroid hamsters. The asterisks denote significant effects of treatments relative to saline ($P < 0.01$). Relative to saline SCH23390 decreased body temperatures in both groups, but had no effect on oxygen consumption or CO₂ production.

Table 2

Effects of SCH23390, a D1 receptor antagonist relative to saline on ventilatory parameters (tidal volume or V_T , frequency or F and minute ventilation or V_E) of hamsters: young male (YM), young males that were hypothyroid (YMH), young female (YF), young females that were hypothyroid (YFH), old female (OF), and old females that were hypothyroid (OFH) in response to exposure to air, hypoxia and air following hypoxia or post hypoxia.

SCH 23390	YM	YMH	YF	YFH	OF	OFH
<u>Air</u>						
V_T	NC	I	NC	NC	I	I
F	D	NC	D	D	D	NC
V_E	D	I	D	D	NC	I
<u>Hypoxia</u>						
V_T	NC	NC	NC	NC	NC	I
F	DD	D	NC	D	NC	NC
V_E	D	NC	NC	NC	NC	I
<u>Post Hypoxia</u>						
V_T	NC	NC	NC	NC	I	I
F	DD	D	NC	DD	D	NC
V_E	D	NC	NC	D	NC	I

Values relative to vehicle

D = decrease

DD = large decrease

NC = no change

I = increase

Table 3

Effects of bromocriptine (a D2 receptor agonist) on ventilatory parameters (tidal volume or V_T , frequency of breathing or F and minute ventilation or V_E) of hamsters: young male (YM), young males that were hypothyroid (YMH), young female (YF), young females that were hypothyroid (YFH), old female (OF), old females that were hypothyroid (OFH) old male (OM), and old males that were hypothyroid (OMH), in response to exposure to air, hypoxia and air following hypoxia or post hypoxia

Bromocriptine	YM	YMH	YF*	YFH*	OF	OFH	OM	OMH
<u>Air</u>								
V_T	NC	NC	NC	NC	NC	NC	D	D
F	NC	NC	D	NC	D	I	D	NC
V_E	NC	NC	NC	NC	NC	I	D	D
<u>Hypoxia</u>								
V_T	D	I	NC	NC	NC	NC	D	D
F	D	I	NC	NC	NC	NC	D	NC
V_E	D	I	NC	NC	NC	NC	D	D
<u>Post Hypoxia</u>								
V_T	NC	NC	NC	NC	I	NC	NC	NC
F	D	I	D	D	D	I	D	NC
V_E	D	I	NC	NC	NC	I	D	NC

The asterisk denotes effect of higher dose of bromocriptine (3 mg/kg) used in young females vs. 1 mg/kg in all other studies.

Values relative to vehicle

D = decrease

NC = no change

I = increase