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In Hamsters Dopamine D₂ Receptors affect Ventilation during and following Intermittent Hypoxia

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Abstract

We tested the hypothesis that in golden Syrian hamsters (*Mesocricetus auratus*) carotid body dopaminergic D₂ receptors modulate ventilation in air, during exposure to intermittent hypoxia (IH) and reoxygenation. Ventilation was evaluated using the barometric method and CO₂ production was determined using the flow through method. Hamsters (n=8) received either subcutaneous injections of vehicle, haloperidol (0.5 mg/kg) or domperidone (0.5 mg/kg). Ventilatory and metabolic variables were determined 30 minutes following injections, after each of 5 bouts of 5 minutes of 10% oxygen interspersed by normoxia (IH), and 15, 30, 45 and 60 minutes following IH when hamsters were exposed to air. Haloperidol, but not domperidone decreased body temperature in hamsters. Neither treatment affected CO₂ production. Vehicle-treated hamsters exhibited ventilatory long term facilitation (VLTF) following IH. Haloperidol or domperidone decreased ventilation in air, during IH and eliminated VLTF due to changes in tidal volume and not frequency of breathing. Thus, in hamsters D₂ receptors are involved in control of body temperature and ventilation during and following IH

Keywords

hamster; D₂ receptor; haloperidol; domperidone; hypoxia; body temperature; ventilation; CO₂ production

1.0 Introduction

During exposure to hypoxia blood and central nervous system dopamine levels increase in rats (Orset et al. 2005). Moreover, in human subjects higher blood dopamine levels correlate with lower hypoxic ventilatory responses (Serebrovskaya et al. 2000). Dopamine binds to various dopamine receptor subtypes including the D₂ receptors that are located in the carotid body and brain regions associated with control of breathing including the nucleus tractus solitarius (Huey et al. 2000b; Kline et al. 2002b). Not only are D₂ receptors involved in regulating dopamine mediated ventilatory responsiveness to hypoxia, but dopamine transporters (DAT) may modulate the level of dopamine released during hypoxia. Recently Vincent and coworkers (Vincent et al. 2007) reported that DAT knock-out mice that had high brain levels of dopamine exhibited lower body temperatures and blunted ventilatory responses to hypoxia relative to those of wild type controls.

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Dopaminergic D₂ receptor regulation of breathing may vary according to species, strains and animal models of disease. For example, Nakano and coworkers (Nakano et al. 2002) evaluated the effects of blocking D₂ receptors on ventilation in air and in response to hypoxia in lean and obese Zucker rats. The investigators noted that while both strains of rats breathed air, haloperidol (a D₂ receptor antagonist that crosses the blood-brain barrier and acts predominantly on D₂ receptors (Seeman 2002), but not domperidone (a peripherally acting D₂ receptor antagonist (Barone 1999)) decreased frequency of breathing, but increased tidal volume. Thus, minute ventilation relative to vehicle treatment was not altered. During exposure of the obese Zucker rats to 10% oxygen domperidone, but not haloperidol, stimulated ventilation suggesting that the decreased hypoxic ventilatory responses in obese rats relative to lean controls were due to the inhibitory role of D₂ on the carotid body.

Subramanian and coworkers (Subramanian et al. 2007) compared the effects of domperidone on ventilation in Brown Norway (BN) and Sprague-Dawley (SD) rats. During exposure to 5 minutes of 13% O₂/ 3% CO₂/N₂ (non-poikilothermic hypoxia) and immediately following increasing doses of domperidone depressed frequency in SD, but not in BN rats. Interestingly domperidone decreased metabolism only in BN rats. These results suggest that genetic factors affecting dopaminergic D₂ receptors in the carotid body and central nervous system may contribute to ventilatory and metabolic responses during and following hypoxia.

Although the studies cited above utilized “constant” hypoxic exposures, the effects of intermittent bouts of hypoxia on ventilation may result in long term facilitation (VLTF) of ventilation or what Millhorn and coworkers (Millhorn et al. 1981) called “respiratory afterdischarge”. Thus, following exposure of animals to several bouts of intermittent hypoxia, ventilation (or phrenic nerve output) increases above baseline values for minutes to hours (Mitchell et al. 2001). Several studies indicate that serotonergic receptors can modulate this response, although in rats glutamate acting on NMDA receptors can elicit phrenic long-term facilitation (Mitchell et al. 2001; McGuire et al. 2004; McGuire et al. 2005). Moreover, Kline and colleagues (Kline et al. 2002a) noted that mice lacking nitric oxide synthase -1 (NOS-1) exhibited attenuated ventilation following intermittent hypoxic exposures relative to responses of wild type mice. These studies and more recent work by Peng (Peng et al. 2006) in mice heterozygotic for hypoxic inducible factor 1 α indicate that multiple factors in the carotid body and central nervous system may be responsible for producing VLTF.

Although evidence cited in preceding paragraphs indicates that dopaminergic receptors are involved in regulation of breathing in air and in response to chronic hypoxia, their role in modulating ventilatory LTF has not been investigated. Preliminary studies in our laboratory indicate that hamsters can elaborate VLTF following exposure to intermittent hypoxia that can be attenuated by systemic administration of a serotonergic receptor antagonist or haloperidol (Schlenker 2006). Our laboratory has a long history of investigating underlying mechanisms responsible for control of breathing in the golden Syrian hamster and the BIO 14.6 dystrophic hamster (Schlenker 1984; Singh 2004). The present study was designed to test the hypothesis that D₂ receptors acting on peripheral chemoreceptors in hamsters modulate VLTF. Thus we compared the effects of haloperidol and domperidone on ventilation prior to, during, and following exposure to intermittent hypoxia in conscious hamsters.

2.0 Methods

2.1 Animals

Eight male golden Syrian hamsters approximately 2 months old obtained from Harlan Sprague Dawley, Inc., Indianapolis, IN (USA) were used in this study. Before and after experimental procedures animals were housed in Ancare cages (22.9 cm $\times$ 43.2 cm) with a micro-isolator top. Bedding consisted of Aspen chips. Animals were housed in groups of two or three on a

14:10 light/dark cycle. Food (8604 rodent pellets obtained from Harlan Sprague Dawley) and water were available *ad libitum*. For two days before all experimental procedures commenced, animals were acclimated to the respiratory-metabolic chamber. All studies were conducted during the hours of 08:00 h to 14:00 h while the animals were resting. To ensure that the hamsters were not asleep, their cage was gently tapped. The University of South Dakota Animal Care and Use Committee approved all procedures used in this experiment.

2.2 Respiratory - metabolic chamber

Respiratory and metabolic measurements were made in a 20.2 cm long $\times$ 7.9 cm diameter Plexiglas cylinder. One end of the cylinder was closed and contained openings for calibration (using a 1 mL glass syringe), measurement of chamber temperature (using a Taylor series 9940 thermometer), determination of pressure changes within the chamber associated with breathing using a Statham low pressure transducer (coupled to an BioPac Acknowledge System data acquisition system), and input for air or 10% oxygen in nitrogen. The other end of the cylinder was closed and contained two openings. One opening was used to determine flow rate through the chamber (using a Gilmont rotameter). The other opening could be connected to a "leak" or to a gas analyzer (Vacu-Med Model 17515^A gas analyzer) to measure the fractional content of CO₂.

Respiratory parameters were determined using the barometric method (Schlenker 1984). Tidal volume and inspiratory and expiratory time were determined directly from twenty consistent breaths and averaged. Sniffs and sighs were eliminated from consideration. Frequency of breathing was calculated by dividing sixty seconds by the sum of inspiratory plus expiratory time. Minute ventilation was determined by multiplying tidal volume by frequency. CO₂ production was determined by subtracting the fractional content of CO₂ in the air entering the chamber from the fractional content of CO₂ the air exiting and multiplying the difference by the flow rate. Minute ventilation, tidal volume, and CO₂ production were normalized by multiplying each variable by 1000/body mass in grams. The convection ratio or ventilatory equivalent was calculated by dividing ventilation by CO₂ production. Flow rates through the chamber averaged 680 mL/s. Barometric pressure was determined using a W. M. Welch Scientific Company barometer and averaged 720 Torr. Chamber temperature averaged 24 degrees Celsius.

2.3 Protocol

Initially a hamster was weighed and injected subcutaneously with approximately 0.2 mL of the vehicle (5% DMSO in saline), haloperidol (0.5 mg/kg) or domperidone (0.5 mg/kg). The doses of haloperidol and domperidone were determined from the literature and preliminary dose response studies. Moreover, the dose of haloperidol we used in this study does not cause catalepsy in hamsters (Richter et al. 1997). The hamster was then placed into the chamber for 30 minutes of acclimatization while the chamber was flushed with air. At the end of the acclimatization period, flow rate and CO₂ content were measured and ventilatory parameters were evaluated. Studies for each agent were conducted at least 4 days apart.

To determine the ventilatory responses of hamsters to intermittent hypoxia, animals were exposed to five alternating bouts of five minutes each of air and hypoxia (10% O₂ in nitrogen). This protocol is similar to that used by McGuire and coworkers in conscious rats (McGuire et al. 2004). After each exposure to hypoxia (H₁-H₅) ventilatory variables were measured and, CO₂ production was determined after H₅.

Following the five intermittent hypoxic bouts, while the animals were breathing air ventilatory measurements were made after four fifteen-minute increments (15, 30, 45, 60 minutes). CO₂ production was evaluated, and the convection ratios were calculated fifteen, forty-five and

sixty minutes following exposure to intermittent hypoxia. Subsequently the hamster was removed from the chamber and its rectal temperature was measured using a Physiotemp thermocouple and a Sentsortek BAT-12 thermometer.

2. 4 Data analysis

Data analysis consisted of repeated two-way analysis to determine effects of drugs (treatments) and time for the intermittent hypoxia and post hypoxia measurements. One way ANOVA was employed when comparing the effects of drugs relative to baseline ventilation, tidal volume, CO₂ production, the convection ratio, frequency and body temperature. If the ANOVA was significant ($P < 0.05$), post hoc tests using Student-Newman Keuls or Holms t-tests, corrected for multiple comparisons, were used. For data that were not normally distributed a Friedman test was conducted followed by a Wilcoxin Signed Rank Test. The data were analyzed using the Statmost program by Dataxiom Software, Inc (Los Angeles, CA, USA). Values are presented as means $\pm$ SD.

3.0 Results

3.1 Body temperature Baseline Ventilatory Variables and CO₂ production

Results from the one way repeated ANOVA indicated that there was an effect of treatments ($F_{2, 14} = 7.80$, $P = 0.0053$). Body temperature was decreased in hamsters treated with haloperidol (H), $33.5^{\circ}\text{C} \pm 0.38$, $P = 0.0011$, but not domperidone (D), $33.9^{\circ}\text{C} \pm 0.43$ relative to vehicle (V) controls ($34.1^{\circ}\text{C} \pm 0.22$). In contrast, there were no significant differences in body mass corrected CO₂ production (mL/min*1000/bw in grams) with the three treatments (V 15.3 ± 3.4 , H 13.5 ± 4.4 , and D 13.0 ± 4.9). While hamsters were exposed to room air both D and H decreased ventilation ($F_{2, 14} = 5.098$, $P = 0.0217$) and tidal volume relative (Friedman test $P = 0.0208$) to V (Table 1). There were no effects of D or H on frequency of breathing (Table 1), or on inspiratory or expiratory times (data not shown)

3.2 Ventilatory Responses to Intermittent Hypoxia

Relative to baseline values, hamsters treated with V and exposed to intermittent hypoxia increased minute ventilation ($F_{5, 35} = 13.7$, $P < 0.0001$) as did treatment with H ($F_{5, 35} = 9.95$, $P < 0.0001$) and D ($F_{5, 35} = 4.9184$, $P = 0.0016$). Since baseline values varied with treatment, data were normalized by baseline by dividing each ventilation value during each of the five exposures to hypoxia by the corresponding baseline value or ventilation ratio (Fig. 1). A two way repeated ANOVA for effects of treatment and hypoxic period on the ventilation ratio indicated an interaction ($F_{8, 70} = 9.9245$, $P < 0.0001$) and effect of treatment ($P < 0.0001$). Both V and H, but not D exhibited a decrease in the ventilation ratio (C, $P = 0.0107$, H, $P = 0.0249$, D, $P > 0.05$). With all three treatments the ventilatory response to hypoxia was due to an increase in tidal volume (Table 2), but not frequency of breathing (Table 3). There was no effect of treatment or time on inspiratory time during the hypoxic bouts relative to the respective baseline values (data not shown). In contrast, there was a treatment effect on TE ($F_{3, 64} = 9.345$, $P = 0.002$) in that the overall expiratory times of the V group were somewhat lower than that of the other two groups. Moreover, only the V exhibited an increase in expiratory time from comparing H1 to H3-H5 (Table 4). At H₅ there were no significant differences in CO₂ production (mL/min*1000/g) with the three treatments (V, 14.6 ± 3.3); (H, 14.2 ± 5.5); and (D 11.5 ± 4.3)).

3.3 Ventilatory Responses following Intermittent Hypoxia

Following intermittent hypoxic exposures, hamsters were exposed to air and ventilation was assessed at 15, 30, 45 and 60 minutes (Fig. 2). When hamsters received vehicle, ventilation increased above baseline values ($F_{4, 28} = 4.43$, $P = 0.0064$) at all time points. In contrast, ventilation did not increase with either H or D. In fact at 15 minutes following intermittent

hypoxia, ventilation decreased below baseline values with D. The major factor increasing ventilation following intermittent hypoxia with vehicle treatment was an increase in tidal volume at 30 to 60 minutes ($F_{4,28}=7.285$, $P=0.0004$; Fig. 3). Frequency of breathing following intermittent hypoxia was not different among treatments at any time point (data not shown). CO_2 production was not different among treatments or with time (Table 5). Thus, the ventilatory effects were not related to changes in CO_2 production.

4.0 Discussion

The major findings of this study were that relative to vehicle treatment haloperidol, but not domperidone decreased body temperature in hamsters. Neither treatment affected CO_2 production. Following exposure to intermittent hypoxia hamsters treated with vehicle exhibited VLTF, but treatment with H or D decreased ventilation in air, during hypoxia and eliminated VLTF following hypoxia. Most effects of the D_2 receptor antagonists were due to changes in tidal volume with no significant effects on frequency of breathing. These results suggest that in hamsters D_2 receptors in the carotid body are involved in control of ventilation in hamsters during and following intermittent hypoxia. Each of these findings will be discussed below.

4.1 Effects of D_2 receptors on Body Temperature and Metabolism

Dopamine and its receptors have been implicated in regulation of body temperature. In mice D_2 agonists administered either intraperitoneally (IP) or intracerebroventricularly (ICV) decreased body temperature that could be reversed by D_2 antagonists acting centrally (Nunes 1991). In that study domperidone had no effect on body temperature when given systemically, but countered the hyperthermic effect of the D_2 agonist. Using biotelemetry Barros and coworkers (Barros et al. 2004) investigated the role of D_2 receptors in the anteroventral preoptic (AVP) region of adult male Wistar rats. Microinjection of 500 ng, but not 50 ng of haloperidol into the AVP region increased core body temperature. Haloperidol also attenuated the decrease of body temperature resulting from exposure of the rats to 30 minutes of 7% oxygen in nitrogen. Nakano and co-workers (Nakano et al. 2002) found no effect of IP administration of 1 mg/kg haloperidol or 0.5 mg/kg domperidone into lean or obese male Zucker rats. Likewise, Chipkin (Chipkin 1988) found no effect of various doses of haloperidol on body temperature of mice. In that study, haloperidol was able to antagonize the hyperthermic effect of apomorphine - induced hyperthermia. In contrast Fick and colleagues (Fick et al. 2005) found that intramuscular injections of 0.5 mg/kg haloperidol decreased body temperature in female rats. Doses higher or lower had no effect on body temperature. In the present study, haloperidol but not domperidone decreased body temperature in hamsters suggesting that this species regulation of body temperature is modulated by centrally located D_2 receptors. Moreover, unlike in the studies in male Zucker rats and mice, haloperidol decreased body temperature in hamsters suggesting a species difference D_2 receptor modulation associated with temperature regulation.

Although haloperidol decreased body temperature in hamsters, there was no effect of haloperidol or domperidone on CO_2 production. Subramanian and coworkers (Subramanian et al. 2007) evaluated the effects of domperidone on metabolism in Sprague-Dawley and Brown Norway rats. Administration of 0.5mg/kg domperidone to Sprague-Dawley rats decreased oxygen consumption and CO_2 production, whereas this dose was ineffective in Brown Norway rats. Body temperature was not reported.

In lean and obese Zucker rats the same dose of domperidone had no effect on oxygen consumption or CO_2 production (Nakano et al. 2002). Thus, effects of D_2 antagonists on metabolism may be related to strain differences. Moreover, the lack of effect of haloperidol on CO_2 production, but a decreased body temperature in hamsters, suggests that other mechanisms may be responsible for heat loss, such as vasodilatation. For example in rats dopamine receptor

antagonists acting centrally increase blood flow to peripheral vascular beds, thus contributing to heat loss and decreased body temperature (Blessing et al. 2007). If this is also the case in the hamster, needs to be determined.

4.2 Ventilation Following Intermittent Hypoxia in Hamsters

This study demonstrated that conscious hamsters develop VLTF. This phenomenon has been previously described in conscious rats and mice. In the hamster an increase in tidal volume, but not frequency was responsible for VLTF. In contrast, McGuire and coworkers (McGuire et al. 2002) found that after rats were subjected five bouts of 10% oxygen and air, ventilation increased above baseline values because frequency of breathing increased, but not tidal volume. A similar finding was reported in mice (Kline et al. 2002a). However, in that study when mice were anesthetized and vagotomized VLTF was elicited by an increase in tidal volume rather than an increase of frequency. In contrast, Olsen and coworkers (Olsen et al. 2001), reported that a in conscious Sprague Dawley rats exposed to an IH protocol similar to ours resulted in the development of VLTF due to increase of both frequency and tidal volume. Moreover, to determine the role of CO₂ in the development of VLTF, rats in separate groups were exposed to either isocapnic poikilocapnic intermittent hypoxia. Both exposure protocols elicited VLTF in the rats, suggesting that CO₂ levels themselves did not modify VLTF. However, in an earlier study, Bach and Mitchell (Bach et al. 1998) demonstrated that exposure of anesthetized, vagotomized and artificially ventilated rats to intermittent bouts of 10%, but not 3 to 5% CO₂ did elicit phrenic and hypoglossal long term depression (LTD). Since in the present experiment in hamsters following IH ventilation decreased with both D₂ antagonists, but CO₂ production remained constant, an increase of arterial PCO₂ may have contributed to the development of ventilatory LTD. Future studies measuring arterial PCO₂ in hamsters treated with D₂ receptor antagonists, and exposure of hamsters to intermittent hypercapnia at various levels will help address this issue.

4.3 D₂ Receptor Modulation of Ventilation

D₂ receptors are present in brain stem areas associated with regulation of ventilation as well as in the carotid body affect breathing in air and during exposure to either acute or chronic hypoxia (Bairam et al. 1998; Huey et al. 2000b; Carroll et al. 2005). Huey and coworkers ((Huey et al. 2000a) investigated the role of D₂ receptors in the carotid body and central nervous system in response to acute hypoxic ventilatory responses (HVR) prior to and during exposure of rats to chronic hypoxia using domperidone and haloperidol. Prior to testing the HVR rats were exposed to 30% oxygen before chronic hypoxic exposure (Day 0), systemically administered domperidone increased frequency of breathing and ventilation in 30% oxygen, but had no effect when rats were exposed to hypoxia, whereas frequency was decreased during hypoxia with haloperidol treatment. At the same time arterial PCO₂ was decreased in 30% oxygen by both D₂ receptor antagonists. By day 8 of exposure to 10% oxygen, domperidone and haloperidol had opposite effects on HVR. Domperidone increased ventilation and haloperidol decreased ventilation in response to acute hypoxia. Intracerebroventricular administration of domperidone decreased ventilatory responses to hypoxia, suggesting central excitatory effects of dopamine acting on D₂ receptors. The author concluded that in rats exposed to hypoxia dopamine acting on peripheral chemoreceptors was inhibitory, whereas centrally acting dopamine stimulated ventilation. Since haloperidol and domperidone depressed ventilation in air in hamsters, dopamine appears be excitatory in carotid bodies. These results suggest that D₂ receptor modulation of breathing in the carotid body is not similar to that previously described in rats or mice (Huey et al. 2003). Whether the species differences are associated with the fossorial characteristics of the hamsters needs to be considered (Frappell et al. 1994). For example, rats exposed to chronic hypoxia decreased their oxygen consumption, whereas hamsters did not. Animals of both species increased ventilation, but the ventilatory equivalent of the hamster was lower than that of the rat. Additional *in vitro* studies in hamster

carotid bodies similar to those of Carroll and colleagues (Carroll et al. 2005) may elucidate the function of dopamine in carotid body during exposure to air and during and following intermittent hypoxia. In addition, since carotid body afferents are integrated in the nucleus tractus solitarius that contain D₂ receptors (Kline et al. 2002b), microinjection of domperidone into this region in hamsters exposed to air and hypoxia may help determine the role of this brain region in D₂ receptor regulation of breathing in the hamsters. Parallel studies in rats may provide insight into mechanisms related to species differences.

The role of D₂ receptors in modulating ventilation following intermittent hypoxia has been investigated to a lesser extent than during constant hypoxia. Serebrovskaya and coworkers (Serebrovskaya et al. 1999) evaluated oscillations in blood levels of dopamine and its precursor dihydroxyphenylalanine (DOPA) in healthy young male subjects who underwent a 14 day intermittent hypoxia condition training protocol consisting of three 5 to 6 minute long rebreathing episodes of hypoxia per day interspersed with 2 five minute periods of air breathing. Ventilatory responses to hypoxic rebreathing tests indicated a greater ventilatory response to severe hypoxia following the intermittent training compared to pre-training values. Moreover, venous blood DOPA levels were twice as high following intermittent hypoxia training compared to pre-training values. The investigators hypothesized that increased blood dopamine levels may be due to hypoxia-mediated increases in tyrosine hydroxylase activity. Moreover, increased dopamine levels could provoke dopamine “autoreceptor-mediated inhibition” of endogenous carotid body dopamine release of dopamine producing decreased hypoxic ventilatory responses.

Several studies investigated the underlying mechanisms responsible for ventilatory long term facilitation following intermittent hypoxia and demonstrated that brain derived neurotrophic factor (BDNF) was a “necessary and sufficient” factor for the development of phrenic LTF (Baker-Herman 2004). In rats intrathecal administration of small interfering RNA against BDNF blocked the development of phrenic LTF following intermittent hypoxia. Thus, knocking down protein production of BDNF eliminated phrenic LTF.

Additional support that BDNF may be a factor in affecting D₂ receptor modulation of VLTF comes from work by Wang and coworkers (Wang et al. 2006) who investigated cellular mechanisms associated release of BDNF in PC12 cells, a model of catecholaminergic neurosecretion. In response to 30 second bouts of hypoxia (PO₂ 50 mmHg) repeated every 10 minutes for a total of 120 cycles BDNF secretion increased 3 fold increase of BDNF relative to that of cells in normoxic conditions. Preincubating cells with N-acetyl-L-cysteine, a potent reactive oxygen scavenger, a dopamine synthesis inhibitor or D₂ receptor blockers prevented the enhanced release of BDNF following intermittent hypoxia. Thus, a potential mechanism by which D₂ receptors may modulate ventilatory LTF, as noted in the present study, maybe through the release of BDNF or oxygen radicals during intermittent hypoxia.

In summary, this study demonstrated in hamsters that central dopaminergic D₂ receptors modulate body temperature independent of effects on CO₂ production and that peripherally acting D₂ receptors affect ventilation. Since domperidone can prevent VLTF, carotid body D₂ receptors influence the production of VLTF in hamsters. The underlying mechanisms for the unique species responses of hamsters to D₂ modulation of breathing, metabolism and body temperature need further investigation.

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Ratio of Ventilation during IH to Baseline

Figure 1.

The ratio of ventilation during intermittent hypoxia (IH) bouts to the corresponding baseline values of hamsters treated with vehicle (dark gray bars), haloperidol (Halo, white bars), or domperidone (Dom, vertical striped bars). The ratio was determined by dividing the ventilation during an IH bout by the corresponding baseline value for each treatment. Values are means $\pm$ SD. The broken line indicates a line of identity between ventilation at baseline and during hypoxia. Note that in all groups ventilation increased above baseline values with hypoxia. The asterisk denotes that with haloperidol treatment the ratio was significantly less relative to vehicle treatment.

Ventilation following Intermittent Hypoxia

Figure 2.

Minute ventilation at baseline and 15, 30, 45 and 60 minutes following intermittent hypoxic exposures in hamsters treated with vehicle (dark gray bars), haloperidol (Halo, white bars), or domperidone (Dom, vertical striped bars). Values are means $\pm$ SD. The asterisks denote significant differences between Halo and Dom and Vehicle treatments. The pound sign denotes a difference between Dom and Halo treatments. The † indicates that ventilation with vehicle treatment was significantly enhanced following intermittent hypoxia.

Tidal Volume following IH

Figure 3.

Tidal volume at baseline and 15, 30, 45 and 60 minutes following intermittent hypoxic exposures in hamsters treated with vehicle (dark gray bars), haloperidol (Halo, white bars), or domperidone (Dom. vertical striped bars). Values are means $\pm$ SD. The asterisks indicate that with vehicle treatment tidal volume was significantly enhanced following intermittent hypoxia.

Table 1
Baseline Ventilatory Variables for Hamsters Treated with Vehicle, Haloperidol or Domperidone

<u>Treatments</u>	Frequency	<u>Variables</u>	Ventilation
		Tidal Volume	
Vehicle	113 ± 7	2.27 ± 0.45	255 ± 38
Haloperidol	118 ± 13	1.75 ± 0.27*	205 ± 36*
Domperidone	113 ± 14	1.73 ± 0.24*	194 ± 48*

Values are means ± SD of 8 animals per treatment. The asterisks denote significant differences relative to vehicle for each variable. Units for frequency are breaths per minute, tidal volume are mL per breath*1000/gram and units for ventilation are mL/min*1000/gram.

Table 2

Tidal Volume during Intermittent Hypoxia of Hamsters Treated with Vehicle, Haloperidol or Domperidone

	Vehicle	Treatments	
		Haloperidol	Domperidone
<u>Condition</u>			
Baseline	2.27 ± 0.45	1.74 ± 0.27	1.74 ± 0.43
H1	3.84 ± 0.44*	2.43 ± 0.31*	2.50 ± 0.35*
H2	3.44 ± 0.71*	2.29 ± 0.23*	2.30 ± 0.26*
H3	3.49 ± 0.42*	2.24 ± 0.32*	2.29 ± 0.31*
H4	3.68 ± 0.59*	2.16 ± 0.33*	2.28 ± 0.35*
H5	3.53 ± 0.36*	2.32 ± 0.33*	2.26 ± 0.41*

Values are means ±SD of 8 animals per treatments. Units for tidal volumes are in mL/ breath*1000/body mass in grams. Asterisks designate significant differences between baseline and values at intermittent hypoxic exposures (H1-H5) at P<0.01 for each treatment relative to respective baseline values.

Table 3

Frequency of Breathing during Intermittent Hypoxia of Hamsters Treated with Vehicle, Haloperidol or Domperidone

	Vehicle	<u>Treatments</u> Haloperidol	Domperidone
<u>Hypoxic Bout</u>			
H1	123 ±12	119 ± 12	120 ±13
H2	126 ±16	115 ±11	117 ±16
H3	118 ±16	116 ±10	121 ±19
H4	121 ±16	117 ±14	114 ±16
H5	116 ±12	115 ±10	114 ±12

Frequency is in breaths per minute. Values are means ±SD of 8 animals per treatments. There were no significant differences during hypoxic bouts (H1-H5) or between treatments.

Table 4

Expiratory Time relative to Baseline Values during Intermittent Hypoxia of Hamsters Treated with Vehicle, Haloperidol or Domperidone

	Vehicle	<u>Treatments</u>	Haloperidol	Domperidone
<u>Hypoxic Bout</u>				
H1	0.94 ± 0.08		1.08 ± 0.22	1.02 ± 0.10
H2	0.93 ± 0.07		1.14 ± 0.21	1.09 ± 0.16
H3	1.07 ± 0.14*		1.11 ± 0.20	1.07 ± 0.17
H4	1.05 ± 0.18*		1.11 ± 0.22	1.14 ± 0.13
H5	1.05 ± 0.11*		1.09 ± 0.19	1.14 ± 0.16

Expiratory times during each bout of hypoxia (H1-H5) were divided by the baseline value for each treatment group. Values are means ±SD of 8 animals per treatments. The asterisks denote a significant difference between H1 and H3-H5 in the vehicle-treated hamsters.

Table 5CO₂ Production Following Intermittent Hypoxia in Hamsters Treated with Vehicle, Haloperidol or Domperidone.

	Vehicle	<u>Treatments</u> Haloperidol	Domperidone
15 minutes	11.1 ± 2.0	10.4 ± 5.2	11.2 ± 4.4
45 minutes	11.7 ± 3.4	11.5 ± 5.6	11.3 ± 2.9
60 minutes	14.6 ± 4.8	12.0 ± 4.0	10.9 ± 2.8

Values are means ± SD of 8 animals per treatment group. CO₂ production units are mL/min*1000/g. There was no significant effect of treatment or time.