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In Hamsters the D1 Receptor Antagonist SCH 23390 Depresses Ventilation during Hypoxia

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Abstract

During exposure of animals to hypoxia, brain and blood dopamine levels increase stimulating dopaminergic receptors which influence the integrated ventilatory response to low oxygen. The purpose of the present study is to test the hypothesis, that in conscious hamsters, systemic antagonism of D₁ receptors would depress their breathing in air and in response to hypoxic and hypercapnic challenges. Nine male hamsters were treated with saline or 0.25 mg/kg SCH-23390 (SCH), a D₁ receptor antagonist that crosses the blood-brain barrier. Ventilation was determined using the barometric method and oxygen consumption and CO₂ production were evaluated utilizing the flow-through method. During exposure to air, SCH decreased frequency of breathing. During exposure to hypoxia (10% oxygen in nitrogen), relative to saline, SCH-treated hamsters decreased minute ventilation by decreasing tidal volume and oxygen consumption but not CO₂ production. During exposure to hypercapnia (5% CO₂ in 95% O₂) frequency of breathing was decreased with SCH, but there was no significant effect on minute ventilation. Relative to saline treatment body temperature was lower in SCH treated hamsters by 0.6 degrees Celsius. These results demonstrate that in hamsters D₁ receptors can modulate control of ventilation in air and during hypoxia and hypercapnic exposures. Whether D₁ receptors located centrally or on carotid bodies modulate these effects is not clear from this study.

Keywords

hamster; dopamine D1 receptor; hypoxia; hypercapnia; body temperature

1. Introduction

Exposure of animals and human beings to hypoxia increases both plasma and brain levels of dopamine [34,43]. Ventilatory responses to hypoxia may be modulated by dopamine acting on dopaminergic receptors divided into D₁-like (D₁ and D₅) and D₂-like (D₂, D₃ and D₄) subtypes [28]. Although numerous studies investigated the role of D₂ receptors on regulation of breathing in various species including the golden Syrian hamster [17,29,42,45] considerably less research has examined the role of D₁ receptors in regulation of breathing. Relative to D₁, there is more mRNA or protein for D₂ receptors in carotid body and brain stem regions associated with control of breathing especially in the dorsal motor nucleus [1,3,18]. Although D₁ receptors are located within the dorsal vagal complex including the nucleus tractus solitarius (NTS) and carotid body, D₁ receptors are also found in the ventral tegmental area, striatum,

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locus cereolus, paraventricular nucleus of the hypothalamus (PVN) and parabrachial nuclei [11,18,47]. These regions also modulate ventilation, cardiovascular and metabolic functions [12,39].

Functional studies support the role of D₁ receptors in control of breathing. For example, transgenic mice that lack D₁ receptors generally died within 1 week after birth and exhibited periodic breathing [16]. Those that survived exhibited Parkinson characteristics including myoclonic jerks, tongue protrusion, and dystonia. Moreover, Srivivasan and coworkers [44] noted that intravenous administration of the brain permeable D₁ receptor antagonist SCH-23390 (SCH) [6] to anesthetized, paralyzed and artificially ventilated rabbits increased their phrenic nerve activity by releasing substance P within the NTS. In contrast, direct perfusion of the NTS with SCH had no effect on phrenic nerve activity suggesting that SCH acted on D₁ receptors located in more rostral brain regions. One such region may be the ventral tegmental area (VTA) where Deschamps and Couture [12] demonstrated in freely behaving rats that tachykinin receptor regulation of cardiovascular function was modulated by D₁ receptors. Stimulation of the VTA also modulated control of breathing in fetal unanesthetized sheep [19]. Moreover, functional magnetic resonance imaging in children exposed to hypoxia revealed that the VTA and other dopaminergic areas such as the caudate were involved in ventilatory responses to hypoxia [24].

Using anesthetized male cats, Lalley noted that intravenous administration of SCH prolonged inspiratory discharge, lengthened the expiratory silent period and reduced frequency [23]. In a separate study using similar methods in cats Lalley [22] demonstrated that intravenous administration of a D₁ receptor agonist increased respiratory network responses to CO₂ and decreased the apnea threshold. Thus, D₁ receptors can modulate control of breathing in the central nervous system and possibly carotid body in anesthetized or decerebrate preparations.

The role of D₁ receptors in regulating ventilation has not been evaluated in a conscious animal. The purpose of the present study is to test the hypothesis that systemic antagonism of D₁ receptors in conscious hamsters would depress their breathing in air, during and following exposure to intermittent hypoxia and during a hypercapnic challenge. This species was chosen for the studies, because our laboratory has a long history of investigating factors affecting control of breathing in normal, golden Syrian hamsters and also BIO 14.6 dystrophic hamsters [40–42].

2. Results

Baseline Ventilatory and Metabolic Parameters

While hamsters were exposed to air, frequency of breathing was decreased after a dose of 0.25 mg/kg SCH relative to saline treatment ($P=0.016$). The decrease in frequency was due to an increase of both inspiratory (TI) and expiratory time (TE). Thus, TI (in seconds) was 0.22 ± 0.03 with saline and 0.28 ± 0.03 with SCH ($P=0.01$), and TE (in seconds) was 0.26 ± 0.02 with saline and 0.29 ± 0.02 with SCH ($P=0.0068$). No significant effects of SCH were noted on tidal volume, minute ventilation, CO₂ production or O₂ consumption (Table 1).

Intermittent Hypoxia

In saline and SCH-treated hamsters ventilation increased relative to baseline due to an increase of tidal volume and not frequency of breathing (Table 2). However, at each of the three hypoxic episodes tidal volume ($P<0.01$) and minute ventilation ($P<0.0001$) were lower in the hamsters treated with SCH relative to saline treatment. Another way to analyze these data is to calculate the ratio of the minute ventilation value during the last minute of each hypoxic exposure to the corresponding baseline value (Figure 1). For minute ventilation, there was an interaction

between treatment (saline relative to SCH) and time (the three exposures) ($F_{2,32}=45.946$, $P<0.0001$) and a treatment effect ($F_{2,32}=41.02$, $P<0.0001$). Relative to saline, SCH administration resulted in depression of ventilatory responses to hypoxia.

After the third hypoxic exposure, oxygen consumption (in ml/min*1000/g) was 18.9 ± 8.1 after saline and 11.2 ± 4.7 after SCH treatment ($P=0.016$). In contrast, there was no significant effect of either treatment on CO_2 production (in ml/min*1000/g) saline (16.2 ± 9) and SCH (17.2 ± 6.7).

Post Hypoxic Exposure

A two way ANOVA with repeated measures revealed an overall effect of time (15, 30 and 45 minutes, $F_{2,32}=15.79$, $P=0.000017$) on frequency (Table 3), which was higher with saline compared to SCH treatment ($P=0.0383$) by 45 minutes. No significant effects of time, treatment, or interactions were noted with tidal volume following intermittent hypoxia (Table 3). However, the ratio of tidal volume at 15, 30, and 45 minutes following intermittent hypoxia to the baseline values demonstrated that both treatments were greater than 1.0, and that there was a significant overall time effect ($F_{2,32}=4.165$, $P=0.0247$, Fig. 2). With saline treatment the ratios decreased over time ($P=0.006$), which was most evident from 30 to 45 minutes ($P=0.0003$). There was no significant effect of time with SCH. Thus, SCH treatment prevented a drop of tidal volume over time.

Minute ventilation showed an overall time effect ($F_{2,32}=46.87$, $P<0.0001$, Table 3). That is minute ventilation decreased with time and was significant with SCH ($P=0.0189$), but not with saline treatment. Specifically, significant decreases of minute ventilation occurred from 15 to 30 minutes ($P=0.0067$).

Another way of examining the data is by calculating the ratio of minute ventilation at 15, 30 and 45 minutes following intermittent exposure to hypoxia to the baseline value (Figure 3). Results of these data showed an overall time effect ($F_{2,32}=61.524$, $P<0.0001$). At 15 and 45 minutes following intermittent hypoxia, there were no treatment differences. However, at 30 minutes the ratio was 1.00 ± 0.19 with SCH treatment and 1.25 ± 0.18 with saline treatment ($P=0.0386$). Thus, saline treatment increased minute ventilation following intermittent hypoxia relative to baseline at all time points, whereas SCH administration caused a decline in the ratio after 30 and 45 minutes following intermittent hypoxia. These results suggest that SCH may have inhibited the production of ventilatory long term facilitation.

Ventilatory Responses to Hypercapnia

Following the hypercapnic challenge, SCH decreased frequency ($102 \text{ bpm} \pm 13$) relative to saline ($126 \text{ bpm} \pm 19$, $P=0.0115$). The decreased frequency with SCH relative to saline values was due to an increase of both TI and TE. Thus, TI (in seconds) following saline was 0.23 ± 0.05 and with SCH 0.32 ± 0.07 ($P=0.015$) and TE (in seconds) following saline treatment was 0.26 ± 0.03 and with administration of SCH 0.28 ± 0.02 ($P=0.0142$). Ratios of frequency of breathing during CO_2 exposure relative to baseline were 1.04 ± 0.15 for saline treated animals and 0.93 ± 0.14 for SCH treated animals. These ratios were not significantly different.

In both groups tidal volume was increased above baseline values from 1.54 ± 0.35 to 2.60 ± 0.55 in the saline-treated group and from 1.61 ± 0.175 to 2.69 ± 0.20 with SCH treatment ($P<0.01$ for both groups). There was no significant effect of treatment on tidal volume or minute ventilation or of the ratios of tidal volume or minute ventilation during CO_2 relative to baseline (data not shown). Thus, saline and SCH treatment increased minute ventilation following exposure to hypercapnia due to an increase of tidal volume.

Effects of SCH on Body Temperature

With a dose of 0.25 mg/kg of SCH, body temperature in degrees Celsius was significantly lower (33.7 ± 0.3) than with saline (34.3 ± 0.33 , $P < 0.05$). Thus, in hamsters this dose of SCH decreased body temperature relative to the effect of saline treatment.

3. Discussion

The major findings in this study in conscious hamsters were that 0.25 mg/kg SCH relative to saline decreased frequency during air and hypercapnic exposures and decreased tidal volume and minute ventilation during each of the three hypoxic episodes. Following the last bout of intermittent hypoxia, oxygen consumption, but not CO₂ production was lower with SCH treatment. Moreover, following intermittent hypoxic exposures, minute ventilation was lower than baseline with SCH treatment, suggesting that blockade of D₁ receptors may attenuate the development of ventilatory long term facilitation. Moreover, body temperature decreased following SCH treatment.

Effects of SCH on Body Temperature

A number of studies used SCH to investigate the role of D₁ receptors in modulating body temperature. In rats or mice SCH had no effect on body temperature when administered subcutaneously or intracerebroventricularly at various doses up to 0.5 mg/kg ([7,9,33,37,38]. Faunt and Crocker [13] evaluated the effects of various doses of SCH on body temperature in male Sprague Dawley rats over a period of three hours. There was no effect of the lowest dose (0.04 μ mol/kg) at any time. Higher doses ranging from 0.17 to 1.74 μ mol/kg produced an increase in body temperature after 30 minutes, but then a decrease in body temperature by 150 minutes. Since we did not measure body temperature continuously in the hamsters, if SCH caused an initial increase in temperature, we were not aware of this. We did, however, find a decrease of body temperature by two hours after administration of SCH.

Mechanisms whereby SCH may decrease body temperature include peripheral vasodilatation, and/or by acting in other brain regions associated with regulation of temperature and metabolism such as in the PVN and anteroventral preoptic region [5,36]. In the present study, SCH did not affect oxygen consumption and CO₂ production of hamsters exposed to air. Moreover in hamsters a dose of SCH of 0.10 mg/kg also had no effect on body temperature (unpublished observations, Schlenker). However, the present experiment noted that approximately two hours following administration of 0.25 mg/kg SCH decreased body temperature in hamsters. Thus, in hamsters the effects of SCH on body temperature appear to be dose dependent and not related to changes in metabolic rate, suggesting that other factors such as changes in vasomotor tone may contribute to the decrease in body temperature at the higher dose.

Effects of SCH on ventilation

Few studies have investigated the role of D₁ receptors on regulation of breathing. Fujii and coworkers [15] noted that bath application of dopamine to an isolated pons-medulla-spinal chord (PMS) neonatal rat preparation enhanced firing rate in the C4 (phrenic nerve) rootlet, but application of dopamine or a D_{1/5} receptor agonist to a preparation lacking the pons actually depressed firing rate. Effects of specific dopamine receptor agonists were not tested in the PMS preparation. These results suggest that the excitatory effects of dopamine on C4 firing maybe influenced by dopamine acting on D₁ receptors in supra-medullary regions.

In pentobarbital-anesthetized, vagotomized cats Lalley determined that intravenous administration of different doses of the D₁ receptor blocker SCH slowed phrenic nerve burst discharge frequency, minute phrenic nerve activity and spike augmentation[23]. The decreased

frequency was due to a prolongation of both the inspiratory discharge and the expiratory silent period. The results on frequency are similar to those found in the present study. In addition, there were no effects on blood pressure or heart rate, but the depressive effects of SCH on ventilation lasted over 2 hours. In contrast, Srinivasan [44] noted that in anesthetized rabbits, SCH enhanced phrenic nerve activity. . These results suggest that there may be species differences in how SCH affects control of breathing, and possibly an effect of anesthesia.

In the present study SCH relative to saline administration in hamsters exposed to intermittent hypoxia exhibited a decrease of ventilation due to decrease in tidal volume. During hypoxia dopamine is released by activating neuronal sodium channels and dopamine transporters [34]. The inhibitory effect of SCH on ventilation during hypoxia may have occurred by blocking D₁ receptors at the carotid body and/or brain regions affected by hypoxia. Almaraz and coworkers [1] reported that rabbit carotid bodies exposed to exogenous dopamine, or increased dopamine release induced during exposure to hypoxia, increased cyclic AMP levels that could be blocked by SCH. Moreover, exposure of adult New Zealand rabbits to hypoxia for 6 hours decreased D₁-R mRNA expression in the carotid body, but increased expression in the petrosal ganglion and the superior cervical ganglion[3]. These studies suggest dopamine released during hypoxia may act through chemoreflex pathways containing D₁ receptors to affect ventilation.

In the central nervous system activation of D₁ receptors can have stimulatory or depressive effects on neurons by affecting both channels and other receptor function [31,46]. For example D₁ receptors cause excitation by decreasing gamma aminobutyric acid (GABA_A) currents in striatal neurons and cortex and glutamatergic signaling in striatal neurons [14,48]. In the ventral respiratory group neurons application of D₁ agonists stimulated neurons by elevating intracellular levels of cyclic AMP and countered effects of fentanyl[4]. In contrast, in the rat parabrachial nucleus dopamine attenuated the non-NMDA receptor evoked excitatory postsynaptic potentials [10]. Application of SCH completely prevented the attenuated response. Thus, SCH may modulate effects of central release of dopamine to modulate ventilation.

Although D₁ receptors have not been investigated in the production of acute long term ventilation, D₁ receptors are implicated in the production of long term potentiation. Kusuli and colleagues [20] found that oral administration of L-DOPA or a D1/D5 agonist to anesthetized rats induced LTP in the dentate gyrus. These responses were inhibited by prior treatment with SCH. More recently Navakkode and coworkers [30] induced LTP in rat hippocampal slices. The LTP was also induced by application of D1/D5 agonists and was inhibited by bath application of SCH. The mechanism of the D1/D5 induced LTP was associated with activation of NMDA receptor function. Since activation of D1/D5 receptors increase levels of c-AMP and activation of protein kinase A that phosphorylates NMDA receptors[35], this may be a mechanism by which D₁ receptors may also cause ventilatory LTF. Future studies will investigate these possibilities.

Potential Limitations

There are a number of limitations to this study. For one, since SCH was given systemically, it is not clear where it may act to affect ventilation, body temperature, and oxygen consumption. Using radiolabeled SCH Nobrega and colleagues [32] noted that D₁ receptors were present at high levels in the caudate-putamen, and substantia nigra pars reticulata and to a lesser extent in other forebrain regions of hamsters. A careful immunohistochemical study to determine location and densities of D₁ receptor proteins in hypothalamic and brain stem nuclei of hamsters is needed.

Another potential limitation is that although SCH clearly antagonizes D1 receptor mediated functions in many studies[1] including those that employ D1 null mice [8], SCH may also affect serotonin receptors, release of corticotrophin releasing factor, and potassium channel function. For example, SCH may act as an agonist at serotonergic (5-HT) 1C receptors and possibly 5-HT₂ receptors. Studies in *Xenopus* oocytes expressing cloned 5-HT receptors exposed to SCH elicited only 10 to 25% of the maximum response elicited by 5-HT [49]. Using the techniques in the present study, the potential effects of SCH on 5-HT receptor function is not possible

In addition, SCH may decrease release of corticotrophin releasing factor (CRF) in rats exposed to stressors that increase anxiety([27]. Arakawa and coworkers[2] noted that chronic administration of doses of SCH than used in our study given to BIO 14.6 dystrophic hamsters was able to prevent stress-induced sudden death in these animals. How interactions of SCH and CRF may affect control of breathing in hamsters is not known.

SCH also inhibits G-protein-coupled inwardly rectifying potassium channels (GIRK) in AtT-20 and in Chinese hamster ovary cells expressing human GIRK channels[21]. According to Kuzhikandathil and Oxford [21], these results suggest that D1-like receptors couple to GIRK. Thus, SCH may indirectly influence D₁-like receptor responses; physiological proof of this hypothesis was not presented in their study.

In summary, this is the first report demonstrating the significant role of D₁ receptors in regulation of breathing in conscious hamsters. SCH decreased frequency of breathing in normoxia and hypercapnia, decreased ventilation during exposure to intermittent hypoxia due to a decrease in tidal volume, and attenuated the increase of ventilation following intermittent hypoxia. Future studies are needed to investigate the mechanisms responsible for these observations.

4. Experimental Procedure

Nine two month old male golden Syrian hamsters obtained from Harlan Sprague Dawley, Inc., Indianapolis, IN were used in this study. Before and after experimental procedures animals were housed in Ancare cages (22.9 cm x 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, Inc., Indianapolis, IN) and water were available *ad libitum*. Two days before all experimental procedures commenced, animals were acclimated to the laboratory and the respiratory-metabolic chamber. The University of South Dakota Animal Care and Use Committee approved all procedures used in this experiment.

Respiratory metabolic chamber

Respiratory and metabolic measurements were made in a 20.2 cm long x 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, 5% CO₂ in oxygen (hypercapnia) or 10% oxygen in nitrogen (hypoxia). The other end of the cylinder was closed and contained two openings. One opening was used to measure flow rate or gases through the chamber (using a Gilmont rotameter). The other opening was connected to a "leak" (used to stabilize recordings) or to gas analyzers (Vacu-Med Model 17515^A gas analyzers) to measure the fractional content of O₂ and CO₂.

Respiratory parameters were determined using the barometric method [40]. Tidal volume and inspiratory and expiratory time were determined directly from ten breaths and averaged. Frequency of breathing was calculated by dividing sixty seconds by the sum of inspiratory (TI) plus expiratory times (TE). Minute ventilation was determined by multiplying tidal volume by frequency.

O₂ consumption was calculated by subtracting the fractional content of O₂ in the air exiting the chamber from the fractional content of O₂ the air entering and multiplying the difference by the flow rate. CO₂ production was calculated 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, CO₂ production, and O₂ consumption were normalized by body weight of the hamsters by multiplying each variable by 1000/body weight in grams. Flow rates through the chamber averaged 680 ml/s. Barometric pressure, determined using a W. M. Welch Scientific Company barometer averaged 720 Torr.

Protocol

Initially a hamster was weighed and then injected subcutaneously with approximately 0.2 ml of the saline (vehicle), or 0.25mg/kg SCH-3390, a D₁ receptor antagonist (Tocris Bioscience). This dose was determined from the literature and preliminary dose response studies where we found that administration of 0.1 mg/kg had comparable effects to administration of saline on all parameters measured.

After receiving an injection the hamster was placed into the chamber for 30 minutes of acclimatization while the chamber was flushed with air. SCH permeated the brain within this time interval [25]. At the end of thirty minutes metabolic and ventilatory parameters were evaluated and constituted baseline values. Studies for each agent were conducted at least 4 days apart.

To evaluate ventilatory responses of hamsters to intermittent hypoxia, animals were exposed to three five minute alternating bouts of air and hypoxia (10% O₂ in nitrogen). This protocol is a modification of to that used by McGuire and coworkers in conscious rats [26]. After the last minute of each five minute exposure to hypoxia (H₁-H₃), ventilatory parameters were measured. In addition, O₂ consumption and CO₂ production were determined after H₃.

Following the intermittent hypoxic bouts, animals were exposed to air and ventilatory measurements were made after three fifteen-minute increments (15, 30, 45 minutes). O₂ consumption and CO₂ production were evaluated at fifteen and forty-five minutes. Subsequently the hamster was exposed to five minutes of 5% CO₂ in oxygen, ventilatory measurements made, and then removed from the chamber. Subsequently its rectal temperature was measured using a Physiotemp thermocouple and a Sentsortek BAT-12 thermometer.

Data analysis

Data analysis consisted of repeated two-way analysis to determine effects treatments (saline and SCH) and time for the intermittent hypoxia and post hypoxia measurements on each variable. One way ANOVA was employed to compare the effects of treatments relative to respective baseline values for ventilation, tidal volume, O₂ consumption, CO₂ production, frequency, body temperature and ventilatory responses to CO₂. Post hoc tests consisted of Holms t-tests were corrected for multiple comparisons. Significance was accepted at P<0.05. Data were analyzed using the Statmost program by Dataxiom Software, Inc (Los Angeles). Values are presented as means $\pm$ SD.

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Figure 1.

The ratio of minute ventilation after each of three bouts of hypoxia to baseline ventilation (H1/B, H2/B and H3/B) in 9 hamsters treated with saline (gray bars) or SCH23390 (white bars). Values are means $\pm$ SD. The asterisks denote a significant difference between the two treatments ($P < 0.05$).

Tidal Volume following IH normalized by Baseline Values

Figure 2.

The ratio of tidal volume after 15, 30, and 45 minutes following the intermittent hypoxic exposures to baseline ventilation (15/B, 30/B and 45/B) in 9 hamsters treated with saline (gray bars) or SCH23390 (white bars). Values are means $\pm$ SD. The # denotes a significant difference between the values in the saline-treated group at the 30 relative to the 45 minute time point ($P < 0.05$).

Figure 3.

The ratio of minute ventilation after 15, 30, and 45 minutes following the intermittent hypoxic exposures to baseline ventilation (15/B, 30/B and 45/B) in 9 hamsters treated with saline (gray bars) or SCH23390 (white bars). Values are means $\pm$ SD. The asterisk denotes a significant difference between the two treatments at 30 minutes ($P < 0.05$).

Table 1
Ventilatory and Metabolic Values Obtained during Initial Exposure to Air

Variable	Saline	SCH
Frequency	120 ± 8	111 ± 8 *
Tidal Volume	1.54 ± 0.35	1.61 ± 0.18
Ventilation	186.3 ± 41.8	177.9 ± 21.6
O ₂ consumption	20.2 ± 7.1	17.2 ± 3.5
CO ₂ production	14.5 ± 7.8	13.2 ± 4.5

Values are means ± SD of nine animals. Frequency is in breaths per minute, tidal volume in ml/breath*1000/BW, ventilation, CO₂ production and O₂ consumption are in ml/min*1000/BW. The asterisk denotes that frequency was lower with SCH treatment relative to saline treatment (P<0.05).

Table 2
Breathing Variables during Intermittent Hypoxia

Variable	Saline	SCH
Frequency		
H1	127 ± 17	122 ± 12
H2	121 ± 9	118 ± 13
H3	122 ± 8	116 ± 13
Tidal Volume		
H1	2.56 ± 0.21	1.83 ± 0.20 **
H2	2.38 ± 0.28	1.85 ± 0.31 **
H3	2.32 ± 0.29	1.85 ± 0.27 **
Minute Ventilation		
H1	322.8 ± 50.3	229.9 ± 25.6 **
H2	286.7 ± 38.8	218.2 ± 37.5 **
H3	281.4 ± 35.4	213.1 ± 30.4 **

Values are means ± SD of nine animals. Frequency is in breaths per minute; tidal volume is in ml/breath*1000/BW; and ventilation is in ml/min*1000/BW. The asterisks denotes that SCH values are significantly lower ($P < 0.01$) relative to the saline values. H1-H3 denote the three periods of intermittent hypoxia.

Table 3
Tidal Volume, Frequency of Breathing, and Minute Ventilation following

Variable	Saline	Intermittent Hypoxia SCH
Frequency		
15	118 ± 8	109 ± 16
30	111 ± 10	102 ± 13
45	112 ± 8	102 ± 11*
Tidal Volume		
15	1.87 ± 0.22	1.99 ± 0.31
30	2.00 ± 0.29	1.79 ± 0.35
45	1.86 ± 0.29	1.91 ± 0.19
Minute Ventilation		
15	218.4 ± 25.3	217.2 ± 35.9
30	222.6 ± 42.7	182.2 ± 32.4
45	208.5 ± 5.7	194.9 ± 8.3*

Values are means ± SD of nine animals following 15, 30 and 45 minutes after intermittent hypoxia. Frequency is in breaths per minute; tidal volume is in ml/breath*1000/BW and ventilation is in ml/min*1000/BW. The asterisk denotes that frequency and minute ventilation were lower after SCH treatment relative to saline ($P < 0.05$). The times 15, 30 and 45 denote times after exposure of hamsters to intermittent hypoxia when measurements were conducted.