GABA-mediated neurotransmission in the nucleus of the solitary tract alters resting ventilation following exposure to chronic hypoxia in conscious rats

Sean Chung, Gwen O. Ivy, and Stephen G. Reid

Centre for the Neurobiology of Stress, Department of Life Sciences, University of Toronto at Scarborough, Toronto, Ontario, Canada

Submitted 2 September 2005; accepted in final form 8 June 2006

EXPOSURE TO CHRONIC HYPOXIA (CH) can result in ventilatory acclimatization to hypoxia (VAH) that is defined as a time-dependent increase in ventilation beginning after a few hours of continuous hypoxia and continuing for days until full acclimatization occurs (26). This phenomenon has been observed in many species, including humans (1, 5, 26, 28). VAH is characterized by an increased level of resting breathing, an increase in CO$_2$ sensitivity (i.e., a decrease in the CO$_2$ set point for triggering ventilation) and usually an increase in the acute hypoxic ventilatory response (HVR) (26, 36).

Two mechanisms have been proposed to explain the changes in ventilation associated with VAH (25). The first of these mechanisms is an increase in the sensitivity of peripheral O$_2$ chemoreceptors, located within the carotid body, to reduced O$_2$ levels in the arterial blood (2, 5). Afferent input from the carotid body is conveyed to the central nervous system (CNS) via the carotid sinus nerve that joins the glossopharyngeal nerve before reporting to the brain stem. Whereas a small number of these afferent fibers report to the ventral lateral medulla, the primary site of afferent projection to the CNS is the nucleus of the solitary tract (NTS) (9, 13, 14). In turn, the NTS contains bulbospinal neurons that project to the phrenic motor nucleus and propriobulbar (premotor) neurons that project to the ventral respiratory group.

The second mechanism that has been proposed to explain VAH is an increased responsiveness of the CNS to afferent input from the carotid body (25). Although first proposed by Forster and Dempsey (10), this phenomenon was only recently observed by Dwinnell and Powell (7) who demonstrated that CH significantly augmented phrenic nerve burst activity when the carotid sinus nerve of anesthetized rats was electrically stimulated. Given that the peripheral O$_2$ chemoreceptors were not present, these authors concluded that CH produced changes within the CNS that caused the increase in CNS sensitivity to carotid body input.

Glutamate release in the NTS is important for the carotid chemoreceptor-induced HVR. For example, Mizusawa et al. (22) reported that microinjection of MK-801 into the caudal NTS attenuated the increase in tidal volume (VT), but not breathing frequency (fR), to 10% O$_2$. Microinjection of kynurenic acid further attenuated the VT response during hypoxia. Vardhan et al. (34) demonstrated that simultaneous antagonism of both N-methyl-D-aspartate (NMDA) and non-NMDA receptors, within the NTS, abolished the ventilatory response to carotid body stimulation. GABA is the major inhibitory neurotransmitter in the CNS and plays an important role, at different sites, in respiratory control (4, 11, 17, 29, 31, 37) including regulation of hypoxic ventilatory decline (3, 12).

Currently it is not known whether or not amino acid neurotransmitters are involved in VAH (3). This study addressed the hypothesis that CH induces changes in GABA-mediated neurotransmission within the NTS, which, in turn, lead to the changes in breathing associated with VAH. To address this hypothesis, we exposed rats to 9 days of CH followed by measurements of resting ventilation and acute hypoxic breathing trials. These measurements and trials were performed before and after administration of GABA, as well as GABA$_A$ and GABA$_B$-receptor antagonists (bicuculline and CGP-35348, respectively) into the NTS through stereotaxically implanted microinjection cannula.

MATERIALS AND METHODS

Experimental animals. Male Sprague-Dawley rats weighing 250–300 g (n = 52) were obtained from Charles River Canada and housed in the University of Toronto, Scarborough vivarium. The ambient...
temperature was maintained at 20°C, and the photoperiod was 12:12 h light-dark. Rats were fed LabDiet 5001 Rodent Diet ad libitum and housed in individual cages on paper chip bedding. All experiments were approved by the University of Toronto Animal Care Committee and conformed to the Canadian Council for Animal Care standards for the care and use of experimental animals.

Animal preparation. Rats were anaesthetized with 3.25% isoflurane in O2 and kept under anesthesia with 2.0–2.5% isoflurane in O2. Nerve fibers were implanted into the abdominal cavity. These telemeters emit a radio measurement of body temperature. To measure body temperature, a radio plethysmography (see Breathing measurements using plethysmography). To calculate VT (6), it is necessary to obtain a measure of body temperature. To measure body temperature, a radio telemeter (model TA10OTA-F20; Data Science International) was implanted into the abdominal cavity. These telemeters emit a radio signal proportional to body temperature and were factory calibrated to a temperature range of 35 to 39°C. Fur was removed from an ∼3 × 2 cm area of the abdomen using a hair clipper. The exposed skin was cleansed with Prepodyne surgical scrub and alcohol swabs before disinfection with a 10% Betadine solution. A 2-cm incision was made along the rostral-caudal axis of the abdomen, and the telemeter was inserted into the abdominal cavity. The muscle and skin were sutured separately, and the wound was sterilized with 10% Betadine.

Microinjection cannulas (model 81C23SG12SPC; Plastics One) were implanted into the NTS using standard stereotaxic procedures. Fur was removed from the head, and the skin was cleansed and disinfected as described above. A 2-cm incision was made along the rostral-caudal axis; any bleeding was controlled by cautery. Two holes were drilled in the skull 4 mm lateral to the sagittal fissure, between bregma and lambda, and anchor screws were inserted. Entrance holes for the NTS microinjection cannulas were drilled bilaterally, 10.2 mm caudal to bregma and 0.6 cm lateral to midline. A guide cannula (containing a dummy cannula) was inserted into the brain at a 24° angle backwards from the vertical axis perpendicular to the skull such that the tips of the microinjection cannula were 9.0 mm below the level of bregma (22). A mixture of cross-linked flash acrylic liquid and fiber was used to secure the guide cannula to the skull and the previously installed anchor screws. The skin was sutured, and the animals were allowed to recover for 5–7 days before experimentation. CH conditions were maintained for 9 days in a custom-built laboratory hypobaric chamber (Submersible Systems Technology) at a pressure of ~0.5 atm. These conditions simulate high altitude and correspond to an inspired O2 level of ~10% at sea level. Within the hypobaric chamber, rats were housed in individual cages and provided with food and water ad libitum. The hypobaric chamber was housed in a room with a 12:12 h light-dark cycle identical to the main vivarium. The chamber was opened once a day for cage maintenance or whenever an animal was taken for an experiment.

Breathing measurements using plethysmography. Breathing was measured using the flow-through barometric method of plethysmography (1, 16, 23). Animals were placed into a sealed custom-made Plexiglas plethysmograph. Inflowing gas mixtures (2 liters/min) were humidified in a mixing chamber filled with 250 ml of H2O before entering the plethysmograph. N2 and O2 were mixed to desired concentrations using command modules (Commander 350; ABB) and digital mass flow controllers (TopTrak 110, TopTrak 210, and SmartTrak 100; Siemens Instruments) that maintained a set ratio of O2 flow-to-a preset level of N2 flow. O2 and CO2 concentrations within the plethysmograph were continuously monitored using gas analyzers for O2 (model S-3A/F; AEI Technologies) and CO2 (model CD-3A; AEI Technologies). The pressure within the plethysmograph was maintained at an atmospheric equivalent of ±0.5 cmH2O using a manometer and by adjusting outflow through a three-way valve (1). Temperature and relative humidity were measured using a hygrometer and temperature probe sealed within the plethysmograph.

Breathing was measured as the change in pressure associated with the change in volume caused by the warming and humidifying of air during inspiration. Pressure changes within the plethysmograph were measured using a differential volumetric pressure transducer (Valdyne DP45) in conjunction with a carrier demodulator amplifier (Valdyne CD12) that filtered and amplified the signal and then converted it from an analog to digital signal. The output from the carrier demodulator was sent to a computer data acquisition system (model MP100; BIOPAC) that recorded the signal at a sampling rate of 200 Hz. Figure 1 illustrates a typical pressure (breathing) trace from an animal exposed to both 30% and 10% inspired O2.

The plethysmograph/pressure transducer was calibrated following each experiment by using a series of 1.5-ml pulses of air originating from a gas-tight syringe. Pulses were administered at different rates [to approximate a range of inspiratory times (ITs)] and a best-fit regression line of simulated inspiratory time vs. pressure was plotted to obtain a line equation that was used to calibrate the plethysmograph/pressure transducer and calculate VT (see below).

Experimental protocol. All animals were subjected to acute breathing trials before and following an injection of saline and following an injection of either 1) bicuculline (200 μmol/l; a GABAA receptor antagonist), 2) bicuculline plus CGP-35348 (100 μmol/l; CGP-35348 is a GABAβ receptor antagonist), or 3) GABA (1 mM) into the NTS. In all cases, a volume of 200 nl was administered bilaterally over a 30-s period by using two 2 μl gas-tight Hamilton syringes that were connected via polyethylene (PE50) tubing to the microinjection cannula. The doses were chosen on the basis of previously published studies (29, 34) and our own pilot experiments. Six groups of rats were studied: three CH groups (bicuculline-injection, bicuculline/CGP-injection or a GABA-injection) and three control groups (bicuculline-injection, bicuculline/CGP-injection or a GABA-injection). A control group was always studied concurrently with a CH group. Each animal also received a saline injection (see below).

Rats were placed into the plethysmograph within 10 min of removal from the hypobaric chamber or vivarium. They were left in the plethysmograph for a 1-h acclimation period during which they were exposed to either 21% O2 (control rats) or 10% O2 (CH rats). This was followed by a series of 15-min exposures to 30% O2, 10% O2, and a final 30% O2. The animals were then removed from the plethysmograph and given a saline injection into the NTS. They were immediately returned to the plethysmograph and exposed to the same series of 15-min exposures to 30% and 10% O2. The animals were then removed a second time and given an injection of either bicuculline (controls, n = 7; CH, n = 12), bicuculline/CGP-35348 (controls, n = 7; CH, n = 12), or GABA (controls, n = 7; CH, n = 7), into the NTS, before repeating the 15-min exposures to 30% and 10% O2.

Histological localization of the microinjection site. Following the experiment, a 200-nl mixture of 4% Pontamine Sky Blue 6B (Sigma)
and 10% peroxidase-labeled lectin from *Triticum vulgaris* [horseradish peroxidase (HRP); Sigma] was administered into the NTS in an identical manner to the administration of the pharmacological agents described above. Three hours later, the rats were euthanized with sodium pentobarbital intraperitoneally (2.5–3.0 ml; 65 mg/ml). The rats were perfused transcardially with 150 ml of 0.1 M PBS (pH 7.4) followed by 400 ml of 4% paraformaldehyde. The brains were removed, submerged in 4% paraformaldehyde, and stored at 4°C for 18–24 h. Following this, they were placed into 30% sucrose in PBS at 4°C for 24 h. Brains were then frozen by immersion in 2-methylbutane at −45°C and stored at −70°C until sectioning.

Sections (40 μm) were obtained using a sliding freezing microtome and stored in 0.1 M PBS (pH 7.4). Every second section was removed and underwent a dianaminobenzidine reaction and a neutral red (1%) counterstain. All sections were mounted on slides and covered with a coverslip. Slides were allowed to dry for 24 h at room temperature before viewing. Images were recorded using an Axioplan 2 imaging system. Staining with both Pontamine Sky Blue and HRP was used to assess the location of each cannula tip. Figure 2 illustrates the site of the injection cannula tips in the control and CH rats. Figure 2, top, illustrates a relatively large staining area. Viewing of serial sections and the relative intensity of staining led to the determination of the cannula tip locations illustrated in the serial sections within this figure.

**Data and statistical analysis.** Data were analyzed for a 2-min period at the end of each 15-min period of acute exposure to 30% and 10% O₂ to determine a steady-state two-point HVR and facilitate comparisons with other studies (1, 27) that measured breathing at this time point. In all cases, the values recorded during the first 30% O₂ exposure were used to determine the HVR. fR, TI, total time of the respiratory cycle (TTOT), and breath amplitude were determined. Expiratory time (TE) was calculated as the difference between TTOT and TI. VT was calculated according to the equations reported by Drorbaugh and Fenn (6). Minute ventilation (VI) was calculated as the product of fR and VT. Data from any given animal were collected in a single trial.

The signal from the abdominally implanted radio telemeters was relayed to a DSI data exchange matrix via a DSI RPC-1 receiver placed directly underneath the plethysmograph. The signal was converted to a temperature value by the Dataquest acquisition and analysis software. Body temperature, as well as plethysmograph temperature and relative humidity, was recorded during the 2-min period in which breathing was analyzed.

The data are plotted as means ± SE. All statistical testing, including the determination of normality and equal variance, was performed using commercial software (SigmaStat 3.0; SPSS). The software determined the appropriate parametric or nonparametric tests, as well as the post hoc test that followed all ANOVA. A two-way repeated-measures ANOVA was used to compare the effects on breathing (the repeated measure), of acute hypoxia (i.e., 30% O₂ vs. 10% O₂), and of bicuculline (or bicuculline/CGP or GABA, the categorical variable) vs. saline in both the control and CH groups. To examine the effects of CH, a two-way nonrepeated-measures ANOVA was used in which the two factors were control/CH and saline/pharmacological treatment. In all cases, the limit of significance was taken to be 5% (P < 0.05).

**RESULTS**

In all cases, the values recorded before and following the saline injection were not different. Given this, the presaline treatment values are not reported in **RESULTS**.

**Effects of acute hypoxia in control rats before GABA receptor antagonism.** In the control animals, acute hypoxia (breathing 10% O₂) following saline treatment (i.e., before bicuculline or bicuculline/CGP treatment) caused an increase in fR (two-way repeated-measures ANOVA; Figs. 3A and 4A) and VI- (Figs. 3E and 4E), as well as a decrease in VT (Figs. 3C and 4C). The increases in fR were caused by decreases in both TI and TE (Tables 1 and 2).
Effects of CH before GABA receptor antagonism. When the rats were breathing 30% O₂ following the saline injection before bicuculline (Fig. 3) and bicuculline/CGP (Fig. 4) treatment, CH caused an increase in fR (two-way ANOVA; compare Fig. 3A with 3B and Fig. 4A with 4B) and VI (compare Fig. 3E with 3F and Fig. 4E with 4F). The increase in fR was due to a decrease in both TI and TE (Tables 1 and 2). There was no effect of CH on VT while breathing 30% O₂.

CH had no effect on fR, VT, or VI when the rats were breathing 10% O₂ (i.e., during acute hypoxia) (in Figs. 3A and 4A, respectively). In the CH group, bicuculline and bicuculline/CGP had no effect when the animals were breathing 10% O₂. When breathing 30% O₂, bicuculline and bicuculline/CGP caused a reduction in VI (Figs. 3F and 4F, respectively). In the bicuculline-treated group, the decrease in VI was caused by a decrease in VT (Fig. 3D) and a nonsignificant decrease in fR (Fig. 3B).

Effects of bicuculline and bicuculline/CGP. With the exception of a small decrease in fR at 30% O₂ following bicuculline/CGP (two-way repeated-measures ANOVA; Fig. 4A), bicuculline and bicuculline/CGP had no effect on any variable in the normoxic control groups (Figs. 3A, C, and E and 4A, C, and E). In the CH group, bicuculline and bicuculline/CGP had no effect when the animals were breathing 10% O₂. When breathing 30% O₂, bicuculline and bicuculline/CGP caused a reduction in VI (Figs. 3F and 4F, respectively). In the bicuculline-treated group, the decrease in VI was caused by a decrease in VT (Fig. 3D) and a nonsignificant decrease in fR (Fig. 3B).
the bicuculline/CGP-treated group, the decrease in VI was a result of a decrease in fR (Fig. 4B), which, in turn, was caused by increases in both TI and TE (Table 2).

**Effects of GABA.** Administration of GABA (data not shown) into the NTS had no effect on any variable associated with breathing in either the control or CH groups at either level of O2 (10 or 30%).

**DISCUSSION**

**GABA-mediated neurotransmission in the NTS modulates resting breathing following CH.** The results of this study indicate that exposure to 9 days of CH altered the effects of GABA-mediated neurotransmission in the NTS when the rats were acutely exposed to 30% but not 10% O2. Specifically, treatment with bicuculline and bicuculline/CGP-35348 caused a reduction in VI at 30% O2. The implication of this data is that changes in GABA-mediated neurotransmission, in the caudal NTS, may account, at least in part, for the increase in resting ventilation that occurs following exposure to CH. Although the effects of bicuculline and bicuculline/CGP-35348 on VI at 30% O2 were the same, the reduction in response to bicuculline was mediated by a decrease in VT (and a nonsignificant decrease in fR), whereas the decrease in response to bicuculline/CGP was mediated by a decrease in fR. Both GABA<sub>A</sub> and

![Fig. 4. Breathing frequency (A and B), tidal volume (C and D), and minute ventilation (E and F) in control (A, C, and E; n = 7) and chronically hypoxic (B, D, and F; n = 12) rats following either saline (circles) or bicuculline/CGP-35348 (squares) administration into the NTS. Symbols for P values are the same as for Fig. 3.](image-url)
GABA receptors are present in the caudal NTS (GABA_A on processes; GABA_B on cell bodies) (35), and the contribution of both on the regulation of resting ventilation appears to have been altered by CH.

In the present study, GABA and the GABA antagonists were administered into the NTS as a single bolus dose. It is possible that a continuous infusion of these drugs may have produced a different effect or may have accentuated the effects observed in response to the bolus dose. However, given that the bolus drug injections did have an effect in the CH group, it appears that this is an effective method for drug delivery, even if it only produced small changes in breathing.

In this study, we were not equipped to measure blood gas levels. It was possible that the level of respiratory drive (blood gas levels) was different in the control and CH animals. However, Aaron and Powell (1) observed, using an almost identical plethysmography (breathing trial) experimental protocol to the one used in the present study, that arterial P\textsubscript{CO}_2 and P\textsubscript{O}_2 levels were not different in control and CH rats during acute exposure to both 10% and 30% O\textsubscript{2} under poikilocapnic conditions. In our present study, it was not possible to conclude whether the differential effects of bicuculline/CGP-35348 were due to differences in respiratory drive arising from different levels of arterial P\textsubscript{CO}_2 and P\textsubscript{O}_2. However, there was no effect of bicuculline or bicuculline/CGP in the control group as the level of inspired O\textsubscript{2} was lowered from 30% to 10%. This observation would indicate that the effects of these GABA receptor antagonists are not dependent on the level of respiratory drive (i.e., blood gas levels). Tolstykh et al. (32) demonstrated that normobaric CH caused a reduction in the sensitivity of isolated NTS neurons to GABA\_A receptor stimulation. These authors suggested that this may lead to increased ventilatory responses to hypoxia following exposure to CH. This desensitization was observed in cultured neurons under normoxic conditions. The fact that NTS neurons can change their sensitivity (evoked current) in response to GABA receptor stimulation is consistent with the notion that GABA-mediated regulation of resting ventilation can be altered following exposure to CH. On the other hand, GABA in the NTS has been shown to cause a decrease in breathing (albeit under acute hypoxic conditions or in response to carotid chemoreceptor stimulation) in rats that were not exposed to CH. Given this, one may have initially hypothesized that bicuculline/CGP would have resulted in the opposite effect as that seen in this study (see below).

Wasserman et al. (35), working with anaesthetized and vagotomized rats, demonstrated that injections of nipecotic acid (a GABA\_A receptor inhibitor), muscimol (a GABA\_A agonist), and baclofen (a GABA\_B agonist) into the ventrolateral NTS caused a reduction in fR with apneustic breathing occurring in some animals following this treatment. However, these authors also noted that bicuculline either alone or in conjunction with CGP-35348, into this region caused a very rapid onset of apnea. This later observation (35) is consistent with the results of the present study (i.e., a reduction in breathing), albeit not to the extent of producing apnea. They (35) suggested that the bicuculline-induced apnea arose due to the unopposed action of glutamate-mediated neuronal excitation. In the present study, it is possible that the reduction in fR, observed when breathing 30% O\textsubscript{2} following bicuculline and bicuculline/CGP in the CH group, may have been caused by enhanced glutamatergic neurotransmission. If this were the case, then changes in glutamate-mediated neurotransmission, within the NTS, may account for the increase in resting ventilation that occurs during CH. Reid and Powell (27) demonstrated that systemic (ip) injection of the NMDA-receptor channel blocker, MK-801, caused changes in fR at 30% O\textsubscript{2} in both control and CH rats. Therefore, changes in amino acid-mediated neurotransmission within the NTS do appear to be involved in the increase in resting ventilation that accompanies VAH.

**CH did not cause an augmentation of the acute HVR.** The absence of an increase in the magnitude of the acute HVR following CH was not unexpected given that the acute hypoxic breathing trials were performed under poikilocapnic conditions (1). In this study, VT was reduced during acute hypoxia under all conditions. The VT response to acute hypoxia can be variable with either an increase or no change as the usual response. It is likely that in these poikilocapnic breathing trials, hypoxia-induced hyperventilation caused a reduction in arterial P\textsubscript{CO}_2 (P\textsubscript{ACO}), which, in turn, lowered VT values. Weil (36) reported that some studies show an enhanced HVR following CH while others do not. Hsieh et al. (15) report that chronic sustained hypoxia (14 days, 0.5 atm) caused a reduction in the HVR (measured as phrenic nerve activity in anaesthetized rats). Furthermore, Aaron and Powell (1) indicate that the increase in resting ventilation is the most important defining

---

**Table 1. The effects of chronic hypoxia and bicuculline-treatment on inspiratory and expiratory time during acute exposure to 30% and 10% O\textsubscript{2}**

<table>
<thead>
<tr>
<th></th>
<th>TI</th>
<th>TE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control, saline, 30% O\textsubscript{2}</td>
<td>0.270±0.014</td>
<td>0.390±0.011</td>
</tr>
<tr>
<td>Control, saline, 10% O\textsubscript{2}</td>
<td>0.174±0.009*</td>
<td>0.193±0.008*</td>
</tr>
<tr>
<td>Control, bicuculline, 30% O\textsubscript{2}</td>
<td>0.265±0.010</td>
<td>0.381±0.015</td>
</tr>
<tr>
<td>Control, bicuculline, 10% O\textsubscript{2}</td>
<td>0.174±0.006*</td>
<td>0.197±0.010*</td>
</tr>
<tr>
<td>CH, saline, 30% O\textsubscript{2}</td>
<td>0.184±0.004†</td>
<td>0.253±0.008†</td>
</tr>
<tr>
<td>CH, saline, 10% O\textsubscript{2}</td>
<td>0.172±0.005</td>
<td>0.205±0.013*</td>
</tr>
<tr>
<td>CH, bicuculline, 30% O\textsubscript{2}</td>
<td>0.203±0.011†</td>
<td>0.277±0.018†</td>
</tr>
<tr>
<td>CH, bicuculline, 10% O\textsubscript{2}</td>
<td>0.170±0.004*</td>
<td>0.188±0.012*</td>
</tr>
</tbody>
</table>

Values are means ± SE in seconds. CH, chronic hypoxia; TI, inspiratory time; TE, expiratory time. Symbols indicate there is a *difference between the value at 30% O\textsubscript{2} and 10% O\textsubscript{2} (within any group) or a †difference between the control and CH groups.

---

**Table 2. The effects of chronic hypoxia and bicuculline/CGP-35348 treatment on TI and TE during acute exposure to 30% and 10% O\textsubscript{2}**

<table>
<thead>
<tr>
<th></th>
<th>TI</th>
<th>TE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control, saline, 30% O\textsubscript{2}</td>
<td>0.286±0.024</td>
<td>0.350±0.014</td>
</tr>
<tr>
<td>Control, saline, 10% O\textsubscript{2}</td>
<td>0.199±0.016*</td>
<td>0.192±0.011*</td>
</tr>
<tr>
<td>Control, bic/CGP, 30% O\textsubscript{2}</td>
<td>0.303±0.025</td>
<td>0.377±0.007</td>
</tr>
<tr>
<td>Control, bic/CGP, 10% O\textsubscript{2}</td>
<td>0.191±0.010*</td>
<td>0.213±0.017*</td>
</tr>
<tr>
<td>CH, saline, 30% O\textsubscript{2}</td>
<td>0.179±0.004‡</td>
<td>0.217±0.005‡</td>
</tr>
<tr>
<td>CH, saline, 10% O\textsubscript{2}</td>
<td>0.163±0.005‡</td>
<td>0.189±0.007*</td>
</tr>
<tr>
<td>CH, bic/CGP, 30% O\textsubscript{2}</td>
<td>0.193±0.006‡</td>
<td>0.257±0.009‡</td>
</tr>
<tr>
<td>CH, bic/CGP, 10% O\textsubscript{2}</td>
<td>0.163±0.002‡</td>
<td>0.183±0.005*</td>
</tr>
</tbody>
</table>

Values are means ± SE in seconds. bic/CGP, bicuculline/CGP-35348. Symbols are the same as for Table 1, except † indicates the difference between saline and bicuculline/CGP.
characteristic of VAH Regardless, the results of this study clearly indicate that, following CH, GABA receptor antagonism altered resting ventilation and not the level of breathing in response to acute hypoxia (10% O2).

Bicuculline and bicuculline/CGP-35348 treatment did not alter breathing in control animals. With the exception of a small decrease in fR at 30% O2 (Fig. 4A), bilateral administration of bicuculline alone or in combination with CGP-35348 into the NTS did not alter any variable associated with breathing at either level of inspired O2 (10% or 30%) in the control group. Other investigators have reported that GABA-mediated neurotransmission has a depressive effect on respiration and on neurons that influence respiratory timing. Specifically, Mc-Crimmon et al. (21) demonstrated that single inspiratory and expiratory neurons from the NTS of anaesthetized dogs exhibited enhanced and reduced spontaneous neural discharge frequencies, respectively, when picomoles of bicuculline were injected into NTS neurons. Chitravanshi et al. (4) reported that hypoxia-induced increases in phrenic nerve activity were inhibited enhanced and reduced spontaneous neural discharge frequencies, respectively, when picomoles of bicuculline were injected into NTS of anaesthetized, vagotomized, paralyzed and artificially ventilated rats. Tabata et al. (31) reported that in conscious normoxic rats, both GABA agonists and antagonists administered into the NTS via microinjection were able to modulate VI during acute hypoxia (10% O2). They injected the GABA receptor agonists muscimol and baclofen into the NTS 10 min before initiating acute hypoxia and found that on immediate exposure to hypoxia, there was a significant decrease in VI and VT compared with values obtained at room air. Based on the Tabata et al. (31) study, we would predict that bicuculline and CGP-35348 would have caused an increase in VI during acute hypoxia mediated by an increase in VT. However, in our study, VT decreased during acute hypoxia and as such, any stimulatory effect of these drugs on VT may have been masked. Furthermore, our measurements were made 15 min after the onset of acute hypoxia rather than the immediate measurements made by Tabata et al. (31).

Trippenbach (33) demonstrated that, in chronically normoxic anaesthetized Wistar rats under normoxic conditions, microinjection of CGP-35348 into the caudal NTS shortened expiratory time but only after the administration of the GABA_A receptor agonist muscimol into the NTS of anaesthetized, vagotomized, paralyzed and artificially ventilated rats. Tabata et al. (31) reported that in conscious normoxic rats, both GABA agonists and antagonists administered into the NTS via microinjection were able to modulate VI during acute hypoxia (10% O2). They injected the GABA receptor agonists muscimol and baclofen into the NTS 10 min before initiating acute hypoxia and found that on immediate exposure to hypoxia, there was a significant decrease in VI and VT compared with values obtained at room air. Based on the Tabata et al. (31) study, we would predict that bicuculline and CGP-35348 would have caused an increase in VI during acute hypoxia mediated by an increase in VT. However, in our study, VT decreased during acute hypoxia and as such, any stimulatory effect of these drugs on VT may have been masked. Furthermore, our measurements were made 15 min after the onset of acute hypoxia rather than the immediate measurements made by Tabata et al. (31).

Trippenbach (33) demonstrated that, in chronically normoxic anaesthetized Wistar rats under normoxic conditions, microinjection of CGP-35348 into the caudal NTS shortened expiratory time but only after the administration of the GABA_B receptor agonist baclofen had previously increased expiratory time. Given this, it is not surprising that, in the present study, the administration of GABA antagonists into the NTS did not alter breathing in the chronically normoxic control rats. On the other hand, Chitravanshi et al. (4) reported that microinjections of muscimol into NTS abolished the increase in phrenic nerve discharge in response to artificial ventilation with 100% N2 for 7–10 s. This effect was reversed by the administration of bicuculline. This is not consistent with our study, although the brief anoxic stimulus of Chitravanshi et al. (4) was different from our current acute hypoxia regimen. Wasserman et al. (35) demonstrated, using anaesthetized and vagotomized Sprague-Dawley rats, that bilateral injection of a GABA uptake inhibitor, nipecotic acid, into the ventrolateral NTS caused an increase in breath duration and decrease in fR that could be reversed by the combined action of bicuculline and CGP-35348. This effect was not observed when the drugs were injected into the medial nucleus of the NTS. Our present study did not target any specific region of the NTS. Indeed, we used a relatively large injection volume in an attempt to target a large area of the NTS. Suzuki et al. (30) demonstrated that injection of nipecotic acid into the commissural subunit of the NTS caused a very brief attenuation of the carotid respiratory chemoreflex triggered by NaCN injection into the carotid artery in urethane anaesthetized rats. This attenuation was inhibited by bicuculline but not by the GABAB receptor antagonist, saclofen. The effects observed in that study were subtle with changes in fR of 12 and 5 breaths/min in response to NaCN in the presence of nipecotic acid and nipecotic acid/bicuculline, respectively. Our present study indicated that bicuculline/CGP-35348 caused a small (7 breaths/min), but not statistically significant, decrease in fR during exposure to 10% O2 in the control rats. Furthermore, our microinjections were targeted to positions slightly lateral of the majority of the commissural subnucleus, although the relatively large volume of the drug likely resulted in effects throughout the NTS (see Fig. 2). On the other hand, Lin et al. (18) demonstrated that bicuculline treatment (sc) had no effect on resting ventilation (while breathing room air) or the HVR (30 min of hypoxia) in conscious lean Zucker rats. Furthermore, in obese Zucker rats, bicuculline treatment caused a reduction in fR and an elevation in VT during exposure to acute hypoxia.

It is possible that the differences between our study and others reflect the experimental approach. We used freely moving conscious rats, whereas many other studies worked with anaesthetized animals (4, 15, 30, 33, 35), which in some cases were also vagotomized (4, 35) and artificially ventilated (4). In addition to receiving afferent input from peripheral O2 chemoreceptors, the (rostral) NTS is also the site of synapse of afferent fibers arising from arterial baroreceptors. Machado and Bonagamba (19) observed that glutamate injections into the NTS caused an increase in blood pressure in conscious rats but a decrease in anaesthetized rats, indicating that anesthesia can indeed alter NTS-regulated responses. In the present study, we cannot discount the possibility that the NTS microinjections altered blood pressure. Furthermore, the large target area may have resulted in altered GABA-mediated neurotransmission in different subregions of the NTS. Regardless, the primary objective of the present study was to compare the effects of GABA receptor antagonism in control vs. CH rats. It is clear from this study that CH altered the effects of GABA antagonism on resting ventilation.

Given that bicuculline and bicuculline/CGP-35348 caused a reduction in resting ventilation following CH, we predicted that GABA treatment would have caused an enhancement of resting ventilation. However, GABA injection into the NTS had no effect on breathing at either 30% O2 or 10% O2 in either the control or CH group. Although this result appears paradoxical, one possible explanation for this result is that GABA is already present in NTS synapses during exposure to 30% O2, such that application of exogenous GABA exerts no additional effect. However, the antagonists (bicuculline and CGP-35348) would have been able to cause a reduction in breathing by inhibiting the endogenous GABA that was already present. This explanation is consistent with the results reported by Trippenbach (33). The efficacy of the GABA was confirmed in experiments not related to this study.

GABA-mediated signals from the NTS may alter the function of other central respiratory control centers. Neurons from the NTS have been shown to synapse with the rostral part of...
the ventrolateral medulla (38) in the general vicinity of the pre-Bötzinger Complex (8, 24). It is not clear from our study whether the ultimate effect on breathing, of GABA receptor antagonism in the NTS, is due specifically to its action within the NTS or from downstream modulation of other respiratory-related centers.

NOTE ADDED IN PROOF

The upper panel of Figure 2 in this article contains several pieces of loose tissue as well as pink imaging artifacts in the corners of the rectangular white area that surrounds the photograph of the brain slice. In the Articles in Press version of this paper, these pieces of tissue and imaging artifacts were covered with white boxes for aesthetic purposes. I apologize if this was in any way misleading; the intent was only to tidy up the white background area surrounding the picture.—S. G. Reid

GRANTS

This study was supported by Natural Sciences and Engineering Research Council (NSERC) of Canada Discovery Grants (to S. G. Reid and G. O. Ivy) and by an NSERC Research Tools Grant (to S. G. Reid). Infrastructure support to S. G. Reid was provided by Canadian Foundation for Innovation and the Ontario Innovation Trust. During the course of this study, S. G. Reid held a Parker B. Francis Fellowship in Pulmonary Research from the Francis Families Foundation.

REFERENCES