Chem. Senses 32: 263–271, 2007

doi:10.1093/chemse/bjl054
Advance Access publication January 10, 2007

Topical Inhibition of Nasal Carbonic Anhydrase Affects the CO_2 Detection Threshold in Rats

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Abstract

Previous studies indicate that Long-Evans rats can be operantly trained to discriminate inspired CO_2 concentrations as low as 0.5%. This ability has been proposed to be due to the presence of CO_2-sensitive olfactory receptors that contain the enzyme carbonic anhydrase (CA). The objectives of the present study were as follows: 1) to determine whether Zucker rats could be operantly conditioned to discriminate low concentrations of CO_2 from control air and 2) to determine the rats' CO_2 detection thresholds before and after nasal perfusion of mammalian Ringers or methazolamide, a CA inhibitor. Rats were operantly trained to discriminate between 25% CO_2 and control air (0% CO_2) and were then subjected to various CO_2 concentrations (0.5–12.5%) to determine their CO_2 detection thresholds. The average (±standard error of mean) baseline CO_2 detection threshold of 7 Zucker rats was 0.48 ± 0.07% CO_2, whereas the average CO_2 detection thresholds after nasal perfusion of either mammalian Ringers or 10^{-2} M methazolamide were 1.41 ± 0.30% and 5.92 ± 0.70% CO_2, respectively. The average CO_2 detection threshold after methazolamide was significantly greater ($P < 0.0001$) than the baseline detection threshold. These findings demonstrate that like Long–Evans rats, Zucker rats can be trained to discriminate low concentrations of CO_2 and that inhibition of nasal CA reduces the ability of the rats to detect low concentrations (3.5% and below) but not higher concentrations of CO_2 (12.5%). These results add to the growing evidence that olfactory neurons exhibiting CA activity are CO_2 chemoreceptors sensitive to physiological concentrations of CO_2.

Key words: chemoreceptors, olfaction, trigeminal, upper airways

Introduction

Carbon dioxide is often used as a stimulant in studies on nasal chemesthesis because it is thought to selectively activate trigeminal nerves with no parallel affect on the olfactory system (Cain and Murphy 1980; Bryant and Silver 2000; Hummel and Livermore 2002; Shusterman 2002; Thürauf et al. 2002). Carbon dioxide concentrations of 25% or above have been shown to elicit a response from the ethmoid branch of the trigeminal nerve in rats (Alimohammadi and Silver 2001), from the nasal mucosa in rats (Thürauf et al. 1991) and humans (Thürauf et al. 1993; Kobal and Hummel 1994), from the trigeminal brain stem neurons in rats (Anton et al. 1991), and in studies where chemosensory event-related potentials or psychophysical responses in humans were measured (see review by Shusterman 2002).

Although noxious concentrations of nasal CO_2 stimulate trigeminal nerves, it appears that physiological concentrations of CO_2 can selectively stimulate olfactory receptors in a variety of animals (Getchell and Shephard 1978; Coates and Ballam 1990; Youngentob et al. 1991; Coates 2001). A behavioral study by Youngentob et al. (1991) demonstrated that Long–Evans rats can be operantly conditioned to discriminate between control air (0% CO_2) and CO_2 concentrations as low as 0.52%, which is well below the end-tidal CO_2 concentration of a typical rat (4–5%). These authors speculated that olfactory receptors in the nasal epithelium were responsible for the ability of the rat to detect and respond to the presence of CO_2.

Electrophysiological studies of olfactory receptors in salamanders (Getchell and Shephard 1978), frogs (Coates and Ballam 1990), and rats (Coates 2001) show that single olfactory receptor activity or electroolfactograms (EOGs), which measure summated receptor responses, can be recorded in response to CO_2 concentrations ranging from 0.5% to 15%. In addition, studies measuring ventilation in bullfrogs (Sakakibara 1978; Kinkead and Milsom 1996), lizards (Coates and Ballam 1987), and snakes (Coates and Ballam

1989) show that CO_2 delivered to the isolated upper airways causes a depression in ventilation. Transection of the olfactory nerves of bullfrogs (Sakakibara 1978) and garter snakes (Coates and Ballam 1989) eliminates the ventilatory response to upper airway CO_2, whereas transection of the trigeminal nerves of bullfrogs (Sakakibara 1978) or the vomeronasal nerves of garter snakes (Coates and Ballam 1989) does not affect the response. The results from the studies cited above provide further support for the presence of CO_2-sensitive olfactory receptors in the amphibians, reptiles, and mammals.

CO_2-sensitive olfactory neurons are thought to contain carbonic anhydrase (CA), an enzyme that catalyzes the reversible hydration of CO_2 to bicarbonate and protons, indicating a possible role for CA in the transduction mechanism of CO_2 chemoreceptors. CA has been found in a small subset of olfactory receptor neurons of frogs (Coates et al. 1998), mice (Kimoto et al. 2004), rats (Brown et al. 1984; Coates 2001), and guinea pigs (Okamura et al. 1996, 1999). In addition, topical or systemic inhibition of CA has been shown to attenuate the ventilatory or receptor response to CO_2 in respiratory chemoreceptors located in the brain stem (Coates et al. 1991; Nattie 1999), larynx (Coates et al. 1996), carotid bodies (Iturriaga et al. 1993), and lungs (Hempleman et al. 2000).

The first objective of the present study was to determine whether Zucker rats, like Long–Evans rats (Youngentob et al. 1991), could be operantly conditioned to discriminate between control air (0% CO_2) and low concentrations of CO_2. For each rat tested, a CO_2 discrimination (detection) threshold was determined, which was the lowest concentration of CO_2 that the rats could reliably discriminate from control air. The second objective of this study was to determine whether topical application of the CA inhibitor methazolamide or mammalian Ringers, a control solution, would affect the rats' CO_2 detection thresholds. The hypothesis for this portion of the study was that nasal CA inhibition would increase CO_2 detection thresholds, whereas perfusion of the nasal cavities with mammalian Ringers would not affect CO_2 detection thresholds.

Materials and methods

Animals

Initially, 10 adult female and 5 adult male lean Zucker rats were tested to determine whether they could be trained to lever press for reinforcement. From this group, the 7 rats that performed the best (6 females and 1 male) were selected to be used in subsequent experiments. The 6 female rats were designated Z1–Z6, and the male rat was designated Z7.

The lean Zucker rat strain was chosen because these rats are bred on-site, are routinely used in behavioral experiments at Allegheny College, and are different from the Long–Evans rat strain used by Youngentob et al. (1991). Rats were housed in a temperature-controlled room (21–23 °C) and kept on a 12:12 light:dark schedule. The rats were provided with food and water ad libitum, except on the day prior to testing when they were deprived of water for 23 h. All procedures used in this study were approved by the Allegheny College Animal Research Committee.

Experimental setup

Training and testing of the rats were performed in an operant conditioning chamber (modified size: 19.1 L × 13.3 W × 19.7 H; Lafayette Instruments Co., Lafayette, IN, Model 80201) (Figure 1). The chamber was connected to an operant conditioning console (Lafayette Instrument Co., Model 81355) that was used to record the number of lever presses (responses) and reinforcement. A 3-way solenoid valve was used to switch between air (0% CO_2, 21% O_2, balance N_2) and the various CO_2 concentrations used. The air and CO_2 were purified using silica and charcoal filters and were humidified before entering the chamber via the gas delivery nozzle. Flow rates of the air and CO_2 streams were carefully regulated at 2 l/min using flow meters (Cole-Parmer, Vernon Hills, IL). The various CO_2 concentrations were created by mixing 100% CO_2 with an air source using a gas proportioner (Cole-Parmer). The CO_2 concentrations were measured, prior to leaving the gas delivery nozzle, using a CO_2 analyzer (BCI, 9000). A fan was placed below the operant conditioning chamber for continuous air circulation and to prevent a buildup of CO_2.

Behavioral training

Standard operant conditioning techniques were used to develop an association between the click of the water delivery system and water reinforcement. After this was accomplished,

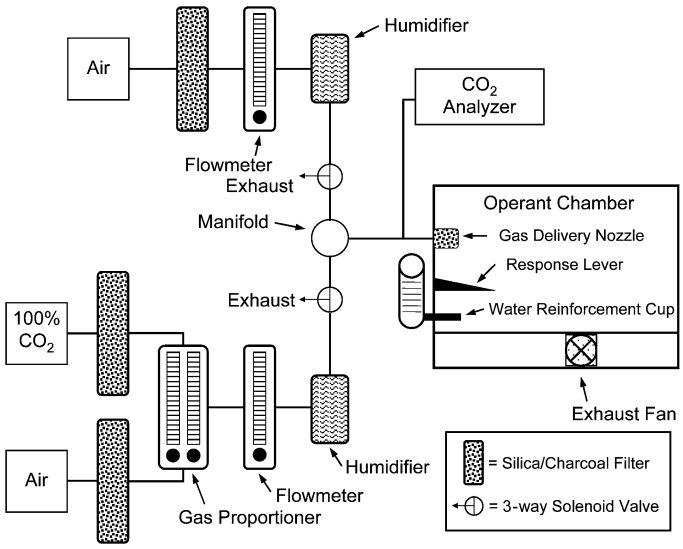

Figure 1 Experimental setup showing operant conditioning chamber and CO_2 and air delivery system.

an association between lever pressing and water reinforcement was developed. Initially, a fixed ratio (FR) schedule of one was used where one drop of water was presented for every lever press. As the rate of behavior increased, the FR schedule was incrementally increased up to FR 10, with the rat pressing the lever 10 times for one drop of water.

Rats were then conditioned to discriminate between 25% CO_2 (25% CO_2, 21% O_2, and balance N_2) and control air (0% CO_2, 21% O_2, and balance N_2). Training sessions consisted of five, 5-min trials of either 25% CO_2 or air. The first 5-min trial was used as an acclimation period and was either CO_2 or air. The remaining four 5-min trials contained 2 trials of 25% CO_2 and 2 trials of air, presented in random order. Reinforcement was set at FR 5 for CO_2 trials, whereas no reinforcement was given during the air trials. For each 5-min trial, the number of responses (lever presses) was recorded.

Training continued until rats discriminated between the 25% CO_2 and air correctly at least 90% of the time for 5 consecutive sessions. For most rats, this took between 20 and 30 testing sessions. The percent correct response was determined using only the last four 5-min trials and was calculated by adding the number of responses that occurred during the 2 CO_2 trials and dividing by the total number of responses that occurred during the last 4 trials. The first 5-min trial was used as an acclimation period and therefore was not used in any calculations.

CO_2 detection threshold

Once the rats were able to reliably discriminate 25% CO_2 from control air, testing was initiated to determine their ability to discriminate the following CO_2 concentrations: 0%, 0.5%, 1.0%, 2.0%, 3.5%, 5.0%, and 12.5% CO_2. The CO_2 concentrations were presented in random order, and only one CO_2 concentration was tested each testing session. Each CO_2 concentration was tested in a 9-min session, consisting of three 3-min trials. During the first 3-min trial, the rat was exposed to air. The next two 3-min trials were either the air or the specific CO_2 concentration used during that trial. No water reinforcement was given during any of these sessions.

The percent correct response was determined using only the last two 3-min trials and was calculated by dividing the number of responses during the CO_2 trial by the total number of responses that occurred during both the CO_2 and air trial. The detection threshold was defined as the CO_2 concentration corresponding to a 65% correct response on the curve relating CO_2 concentration to the percent correct response. The precise CO_2 detection threshold was determined by generating an equation for the line that ran through a point representing 65% correct response. This definition of the detection threshold is identical to that used by Youngentob et al. (1991) and was based on a study by Walker and O'Connell (1986). In the present study if a rat did not press the lever at least 10 times, the trial was considered to contain insufficient behavior and the trial was given a value of 50%. Each CO_2 concentration was tested at least twice for each rat.

Nasal CA inhibition

After baseline CO_2-response curves were generated for each rat and baseline CO_2 detection thresholds were calculated, rats were tested to determine if topical application of methazolamide to the nasal cavities would affect CO_2 detection thresholds. Prior to testing, rats were placed in a chamber and anesthetized with 5% isoflurane mixed with 100% O_2 delivered at 2 l/min. Once the rats were lightly anesthetized, they were removed from the chamber and placed, facing downward, on a platform with a 30° head-down tilt. This was done to prevent possible aspiration of the fluid perfused into the nasal cavities. A nose cone delivering the isoflurane and O_2 mixture was placed near the rat to keep them anesthetized once they were removed from the chamber. A small tube connected to a 1-cc syringe was inserted into the external nares and 0.3 cc of either 10 mM methazolamide mixed in phosphate-buffered mammalian Ringers (pH 7.6) or buffered mammalian Ringers alone (pH 7.6) was slowly perfused into each nasal cavity. Excess perfusion solution dripped out of the mouth. A relatively high concentration (10 mM) of methazolamide was used to improve the chances that the drug would diffuse through the nasal mucosa and inhibit intracellular CA.

After perfusion, the isoflurane was turned off, and 100% O_2 was delivered for 1 min or until the rats began to recover from the anesthesia. They were observed and allowed to fully recover for 90 min before beginning the CO_2 detection threshold experiments. Preliminary trials showed that at least 60–90 min was needed for the rats to fully recover from the isoflurane and to exhibit typical preanesthetic behavior in the operant conditioning chamber.

To minimize the number of times that rats were anesthetized, testing sessions after the nasal perfusions of methazolamide or mammalian Ringers were extended to 14 min, consisting of seven 2-min trials. With this protocol, 3 of the CO_2 concentrations could be tested each session instead of just one. Trials 1, 3, 5, and 7 were assigned control air and trials 2, 4, and 6 were randomly assigned 3 of the CO_2 concentrations. Once a pattern of CO_2 concentrations was established for an individual rat, the same pattern was used for both the methazolamide and mammalian Ringers experiments. Because rats never responded to 0% CO_2 above chance during baseline experiments, this CO_2 concentration was not used in the nasal perfusion experiments. Each CO_2 concentration was tested once to limit the number of times the animals had to be subjected to the anesthesia and nasal perfusion. No reinforcement was given during these testing sessions.

The percent correct response was calculated for each period by using the number of responses from a CO_2 trial

and the air trial immediately following. For example, CO_2 trial 2 was compared with air trial 3, CO_2 trial 4 was compared with air trial 5, and CO_2 trial 6 was compared with air trial 7. The first air trial was used as an acclimation period and was not included in the calculations. Discrimination training with 25% CO_2 and control air was carried out every third day of testing to reinforce the task.

Data analysis

Testing sessions were recorded using Biopac software (BSL Pro 3.7) and hardware (MP30). A computer recorded the operant conditioning console reinforcement and response outputs and the output of the CO_2 analyzer (BCI 9000). Results were analyzed using a 1-way repeated measures analysis of variance (ANOVA) and Fisher's post hoc test for comparing thresholds and a 2-way repeated measures ANOVA and Fisher's post hoc test for comparing differences at each CO_2 concentration. Significant differences were defined as $P < 0.05$. Values in Table 1 and throughout the text are reported as averages ± standard error of mean.

Results

Behavioral training

Initially, 5 male and 10 female Zucker rats were tested at FR1 to determine whether they could lever press for reinforcement. Seven of the rats, 6 females and 1 male, were determined to perform well enough to be used in subsequent experiments. On average, it took these 7 rats 25 trials to reach the 90% correct response rate for 5 consecutive trials at FR10, although the number of trials needed to reach 90% ranged from 11 to 31.

Table 1 CO_2 detection thresholds

Rat	Detection threshold (% CO_2)		
	Baseline	Mammalian Ringers	Methazolamide
Z1	0.44	0.76	3.95
Z2	0.26	2.45	7.25
Z3	0.30	1.30	3.95
Z4	0.34	0.65	7.25
Z5	0.67	2.45	7.25
Z6	0.69	1.60	3.95
Z7	0.65	0.65	7.82
Average	0.48 ± 0.07	1.41 ± 0.30 ($P = 0.15$)	5.92 ± 0.70 ($P < 0.0001$)

Individual and average (±standard error of mean) CO_2 thresholds before (baseline) and after nasal perfusion of mammalian Ringers or methazolamide for each Zucker rat. *P* values are given for mammalian Ringers and methazolamide compared with baseline CO_2 detection thresholds (repeated measures ANOVA and Fisher's post hoc test).

CO_2 detection threshold

Figure 2 shows the baseline CO_2-response curve generated for each rat prior to nasal perfusion of mammalian Ringers or methazolamide. Generally, rats were able to reliably discriminate CO_2 concentrations above 3.5%, whereas the percent correct response for CO_2 concentrations below 3.5% was quite variable. The average baseline CO_2-response curve shows (Figure 3), however, that as a group, the rats were able to discriminate, above a 65% correct response rate, all the CO_2 concentrations used in this experiment, including 0.5% CO_2. All rats failed to discriminate 0% CO_2 from control air.

From the baseline CO_2-response curves, detection thresholds were calculated for each rat (Table 1). The baseline detection thresholds ranged from 0.26% to 0.69%, with an average detection threshold of 0.48 ± 0.07.

Nasal CA inhibition

A CO_2-response curve was generated for each rat after nasal perfusion of mammalian Ringers or methazolamide (Figure 2). In general, perfusion of mammalian Ringers did not affect a rats' ability to discriminate the various CO_2 concentrations from control air. Figure 3 shows that the average CO_2-response curve after nasal perfusion of mammalian Ringers is only significantly different from baseline for 0.5% and 12.5% CO_2. The CO_2 detection thresholds after nasal perfusion of mammalian Ringers were higher in most rats, ranging from 0.65% to 2.45% CO_2 (Table 1); however, the average detection threshold (1.41 ± 0.30) was not significantly different ($P = 0.15$) from the average baseline CO_2 detection threshold (0.48 ± 0.07).

Nasal perfusion of methazolamide affected the rats' ability to discriminate low concentrations of CO_2 from control air (Figure 2). After nasal perfusion of methazolamide, none of the rats were able to discriminate CO_2 concentrations of 3.5% or below, whereas 3 of the rats (Z1, Z3, and Z6) were able to discriminate 5% CO_2. All rats were able to discriminate 12.5% CO_2 from control air after nasal perfusion of methazolamide. The average CO_2-response curve (Figure 3) shows that nasal CA inhibition significantly reduced the rats' ability to discriminate CO_2 from control air for all the CO_2 concentrations used except 12.5% CO_2.

Nasal perfusion with methazolamide increased the CO_2 detection thresholds of each rat with the detection thresholds ranging from 3.95% to 7.82% CO_2. The average CO_2 detection threshold of 5.92 ± 0.70 after methazolamide was significantly ($P < 0.0001$) greater than the baseline CO_2 detection threshold of 0.48 ± 0.07.

Discussion

The 2 main objectives of this study were as follows: 1) to determine whether Zucker rats could be operantly conditioned to discriminate low concentrations of CO_2 from control air and 2) to determine the rats' CO_2 detection thresholds before

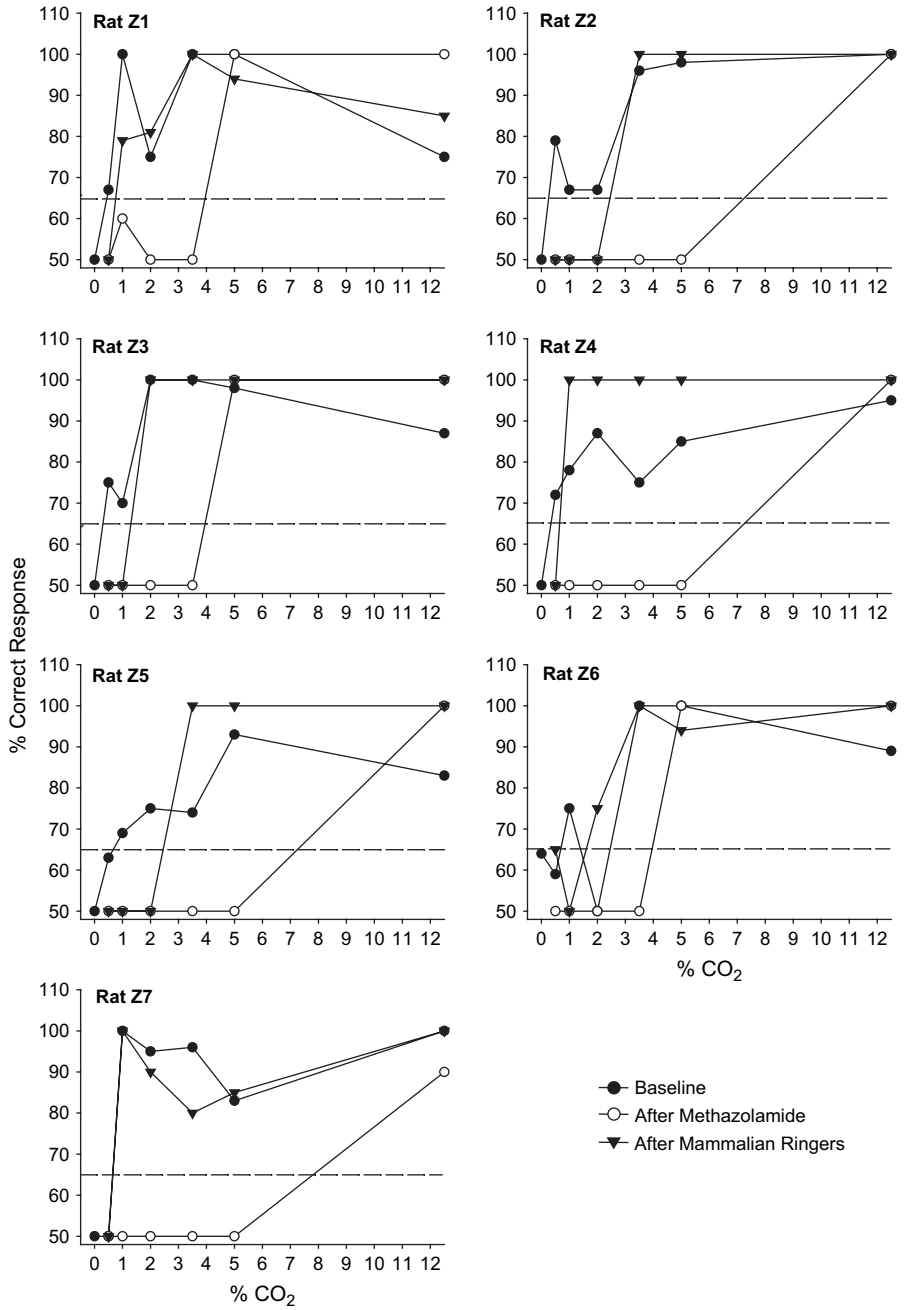

Figure 2 Correct responses (%) at each CO_2 concentration tested for the Zucker rats (Z1–Z7). Baseline responses are shown as filled circles. The percent correct responses after topical mammalian Ringers application are shown as filled triangles, whereas the percent correct responses after topical methazolamide application are shown as open circles. The intersections of the lines with the horizontal dashed line at 65% represent the CO_2 detection thresholds.

and after nasal perfusion of mammalian Ringers or the CA inhibitor, methazolamide. Rats were able to discriminate CO_2 concentrations ranging from 25%, the concentration used for the initial discrimination trials, down to 0.5%. None of the rats were able to discriminate 0% CO_2 from the control air (also 0% CO_2) indicating that they were not detecting subtle differences in air flow, temperature, or humidity between the 2 air streams.

CO_2 detection thresholds

The results show that like Long–Evans rats (Youngentob et al. 1991), Zucker rats can be trained to discriminate low concentrations of CO_2 from air. The CO_2-response curves generated in the present study are remarkably similar to those reported by Youngentob et al. (1991) in that both Long–Evans and Zucker rats responded correctly 90–100% of the time for CO_2 concentrations above 3%, correctly 80%

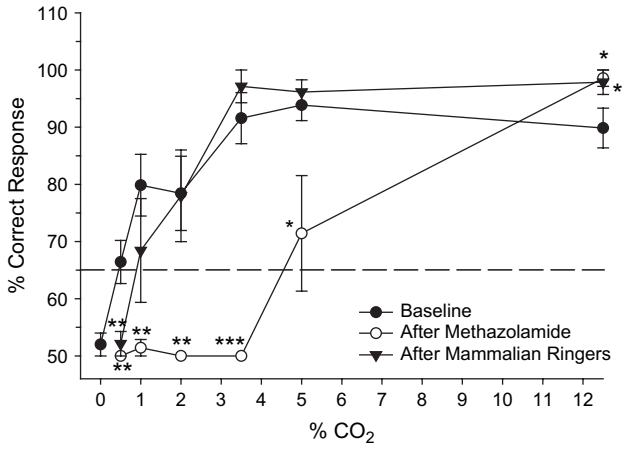

Figure 3 Average (±standard error of mean) percent correct responses at each CO_2 concentration for the Zucker rats (Z1–Z7). Baseline responses are shown as filled circles. The percent correct responses after topical mammalian Ringers application are shown as filled triangles, whereas the percent correct responses after topical methazolamide application are shown as open circles. The intersections of the lines with the horizontal dashed line at 65% represent the CO_2 detection thresholds. Significant differences from baseline responses are indicated by an asterisk. * = $P < 0.05$, ** = $P < 0.01$, *** = $P < 0.001$.

of the time for CO_2 concentrations between 1% and 2%, and correctly 50–80% of the time for CO_2 concentrations below 1% (Figure 3). The average baseline CO_2 detection threshold in the present study was 0.48 ± 0.07 and ranged from 0.26% to 0.69%. This value is similar to the average CO_2 detection threshold of 0.52 ± 0.27 (ranging from 0.04% to 1.7%) reported for Long–Evans rats (Youngentob et al. 1991). In addition, a pilot study using 2 Hilltop Sprague Dawley rats (Hilltop Laboratory, Scottsdale, PA) found CO_2 detection thresholds of 0.21% and 0.84%. The results from experiments using Hilltop, Long–Evans, and Zucker rats show a striking similarity in the ability to detect and discriminate low concentrations of CO_2 from air. In all 3 rat strains, the detection threshold was around 0.5% CO_2, which is well below the end-tidal CO_2 concentrations of 4–5% for rats.

It should be noted, however, that the actual detection CO_2 detection thresholds may be even lower than 0.5% CO_2. In the present study and in the study by Youngentob et al. (1991), the CO_2 concentrations were sampled with a CO_2 analyzer prior to the CO_2 entering the testing chamber, which may have led to an overestimation of the CO_2 concentrations rats were detecting. We found that CO_2 diffusion from the sampling nozzle resulted in a 50% drop in the CO_2 concentration approximately 1 cm away from the surface of the nozzle. Observations of the rats during training sessions revealed that most rats sniffed within 1 cm of the sampling nozzle and were therefore likely sampling a CO_2 concentration that was close to the CO_2 analyzer output.

Inspired and expired CO_2

An obvious question that arises from these results is how can rats detect 0.5% CO_2 when receptors in their nasal cavities are exposed to 4–5% CO_2 with each expiration? The answer to this question may be related to the nature of the CO_2 stimulus pattern. In the present study and the studies cited above, the CO_2 was delivered in a constant (tonic) pattern so that the CO_2 could be sniffed or inhaled at any time during the CO_2 presentation. In contrast, expired CO_2 concentrations appear to receptors in the nasal cavity as a phasic wave of CO_2 that alternates between 4% and 5% during expiration and 0% during inspiration. A possible explanation is that the olfactory receptor output is gated such that the signal during the inspiratory phase is detected, whereas the signal occurring during the expiratory phase is not. Previous studies show support for this type of respiratory synchronization at the olfactory epithelium (Chaput 2000) and at bulbar and cortical levels (Buonviso et al. 2006). In the study by Chaput (2000), EOG recordings in anesthetized freely breathing rats were found to be synchronized with the respiratory cycle so that the maximum EOG response to a 10- or 60-s stimulus of odorant occurred late in the inspiratory phase. In this case, the phasic decrease in EOG amplitude during each expiratory phase is likely due to desorption and washout of the odorants with the expiratory air. In the present study, however, both the inspiratory and expiratory air contained CO_2 so washout would only occur when the inspired CO_2 concentration was above the rats' end-tidal CO_2 concentrations of 4–5%. For inspired CO_2 concentrations below 5%, the CO_2 in the expired air would add to the inhaled stimulus concentration. In addition, the phasic nature of the stimulus would be lost as the inspired CO_2 concentration approached the end-expiratory CO_2 concentration. Interestingly, we found that 3 rats (Z4, Z5, and Z7) exhibited a decrease in discrimination of CO_2 concentrations around 3–5% (Figure 2), suggesting that some of the rats had difficulty discriminating inspired CO_2 from air when the concentration was near the end-tidal CO_2 concentration.

Nasal CA inhibition

The second objective of this study was to determine whether topical application of a CA inhibitor to the nasal cavity would affect the baseline CO_2 detection thresholds. We found that after nasal CA inhibition, none of the rats were able to detect CO_2 concentrations of 3.5% or lower and 4 of 7 rats were not able to detect 5% CO_2. In contrast, all the rats could still detect 12.5% CO_2 after nasal CA inhibition. These results show that CA is required to detect CO_2 concentrations below approximately 5% and that CA does not seem to play a role or is not required for the detection of CO_2 concentrations above 5%. These results support the hypothesis that the olfactory receptors in the nasal epithelium exhibiting CA activity are CO_2-sensitive chemoreceptors that respond to physiological concentrations of CO_2. In previous experiments on rats, EOGs were recorded in response to CO_2 as low as 0.5% (Coates 2001), similar to the detection threshold reported here and in the study by Youngentob et al. (1991).

Furthermore, the EOG response to CO_2 in rats exhibited a dose-dependent increase in amplitude until the EOG response reached a maximum around 14% CO_2, indicating that there is a limit to which CO_2-sensitive olfactory receptors can respond. The results of the current study and the EOG study using rats (Coates 2001) are consistent with studies using frogs (Coates and Ballam 1990) and salamanders (Getchell and Shephard 1978; Getchell et al. 1980) where it was shown that these amphibians possess CO_2-sensitive olfactory receptors that are stimulated by CO_2 ranging from 0.5% to 10%.

The control experiments show that perfusion of the nasal cavity with mammalian Ringers caused an increase in CO_2 detection thresholds in 6 of the 7 rats (Table 1) but that the average detection threshold 90 min after mammalian Ringers perfusion (1.41 ± 0.30) was not significantly different ($P = 0.15$) from average baseline threshold values (0.48 ± 0.07). This increase in detection threshold may be due to the application of fluid to the nasal cavity, which may have increased the thickness of the nasal mucosa, creating a larger diffusion barrier (Getchell et al. 1980). We tested rats 90 min after the administration of mammalian Ringers or methazolamide so this interval likely minimized the physical effects of the nasal perfusions. The 90-min interval also allowed time for the methazolamide to diffuse to CA sites in the nasal epithelium and fully inhibit the nasal CA.

Methazolamide is membrane permeable and therefore inhibits CA in both extracellular and intracellular locations (Maren 1977). Using histochemical or immunocytochemical techniques, CA has been shown to be present in olfactory receptor neurons in frogs (Coates et al. 1998), rats (Brown et al. 1984; Coates 2001), mice (Kimoto et al. 2004), and guinea pigs (Okamura et al. 1996, 1999). In addition, nasal gland cells (Okamura et al. 1996, 1999; Kimoto et al. 2004), cells in the respiratory epithelium (Okamura et al. 1996; Coates et al. 1998), and the nasal mucosa (Kimoto et al. 2004) are known to contain CA. The CA in the nasal mucosa and olfactory receptors of the mouse was identified as CA isoenzymes VI and II, respectively (Kimoto et al. 2004). Using mRNA levels to assess gene expression of CA isoenzymes, Tarun et al. (2003) found expression of CA XII, II, VB, IV, IX, III, XIV, I, VI, VII, listed in order of relative abundance, in scrapings from the respiratory epithelium of humans.

Because the methazolamide used in the present study is membrane permeable, CA in any of locations cited above would have been inhibited. The increase in CO_2 detection threshold after nasal CA inhibition indicates that the putative CO_2-sensitive olfactory receptors, which exhibit CA activity, were inhibited and this decreased the ability of rats to detect CO_2. Experiments using mice (Kimoto et al. 2004) show that CA II appears to be the main isoenzyme in the subset of olfactory receptor neurons exhibiting CA activity. Given that CA II is an intracellular and cytoplasmic form of the enzyme, it is likely that the methazolamide used in the present study inhibited the formation of intracellular H+ when CO_2 was presented as a stimulus. This mechanism of CO_2 transduction is similar to that proposed for respiratory CO_2 chemoreceptors where an increase in intracellular H+ with CO_2 stimulation is thought to affect H+-sensitive channels leading to a depolarization of the chemoreceptor and initiation of a physiological response (Putnam 2001). It should be noted, however, that to the best of our knowledge, specific H+-sensitive channels have not yet been identified in olfactory receptor neurons.

In addition to inhibiting the intracellular CA, methazolamide should have also inhibited extracellular CA in the nasal mucosa. However, it is not clear how this would affect the CO_2 concentration in the nasal mucosa. If the role of the mucosal CA is to buffer the acid load that occurs with expiratory CO_2 (Kimoto et al. 2004), then inhibition of this CA would have decreased the formation of extracellular H+ and increased the CO_2 concentration in the mucosa. This is supported by studies on humans showing that systemic CA inhibition caused an increase in nasal mucosa pH 30, 60, and 90 min following the administration of the CA inhibitor, dichlorphenamide (Cavaliere et al. 1996).

The effect of CA inhibition with methazolamide appears to last many hours. One rat that was tested 90 min and a second time 6 h after the initial methazolamide administration could still not detect CO_2 concentrations of 3.5% or below. However, 24 h later, this same rat was again able to detect low concentrations of CO_2, indicating that the effects of topical methazolamide administration are long lasting yet fully reversible within 24 h. In the present study, we used a relatively high concentration of methazolamide (10 mM) to improve the chance that the drug would inhibit intracellular CA. It is possible that lower concentrations of methazolamide would still adequately inhibit nasal CA but would allow a faster recovery from inhibition.

Olfactory and trigeminal CO_2 sensitivity

The results show that although CA inhibition caused a significant increase in CO_2 detection thresholds, CA inhibition did not affect the ability of the rats to discriminate 12.5% CO_2 from air. This was unexpected given that EOG responses to CO_2 in rats have been shown to plateau around 14% CO_2 (Coates 2001). A possible explanation is that at this high CO_2 concentration and long stimulus duration (multiple respiratory cycles), the uncatalyzed hydration of CO_2 took place at a rate sufficient to generate intracellular H+ in olfactory CO_2 receptors. Alternatively, it is possible that the detection of 12.5% CO_2 is due to stimulation of trigeminal nerve endings in the nasal mucosa. Although it is well established that high concentrations of CO_2 stimulate the trigeminal system (Bryant and Silver 2000; Hummel and Livermore 2002; Shusterman 2002), it has not been reported whether 12.5% CO_2 can stimulate trigeminal nerves in the nasal mucosa of the rat. In studies on humans, detection thresholds have been reported for CO_2 as low as 10% when the stimulus

duration was at least 2 s (Wise et al. 2004), indicating that the trigeminal system may be more sensitive to CO_2 than usually reported.

The observation that CA inhibition did not affect the ability of the rats to discriminate 12.5% CO_2 from air, when it has been shown that CA plays a role in nociceptive responses to CO_2 (Steen et al. 1992; Komai and Bryant 1993; Bryant 2000), may be due to the method of CA inhibition used in the present study. Topical administration of methazolamide should have inhibited any CA present within the nasal cavity of the rat. This suggests that 1) rats are detecting the 12.5% CO_2 via olfactory or trigeminal receptors without the aid of the catalyzed hydration of CO_2, 2) methazolamide in not completely inhibiting nasal CA, or 3) trigeminal CA is not inhibited to the same degree that olfactory receptor CA is inhibited, which may be due to differences in the CA isoforms or CA locations within the nasal epithelium. It should be noted that although there is strong evidence showing CA plays a role in the detection of CO_2 by trigeminal afferents (Bryant and Silver 2000; Hummel and Livermore 2002; Shusterman 2002), to date, there have been no studies showing that CA is present within the nasal trigeminal nerve endings (Bryant 2000).

Conclusions

The results of this study show that Zucker rats are capable of discriminating CO_2 concentrations as low as 0.5% from control air (0% CO_2). Given that the average CO_2 detection threshold is much lower than the end-tidal CO_2 concentration that occurs with each expiration, it appears that rats have ability to discriminate low concentrations of inhaled CO_2 from expiratory CO_2. This ability may be due to the different phases in the respiratory cycle when the inspired CO_2 is sampled by nasal CO_2 receptors or differences in the way that tonic and phasic stimuli are processed by the olfactory system.

The increase in CO_2 detection threshold that occurred after topical inhibition of nasal CO_2 with methazolamide indicates that CA located in the nasal epithelium is required for the discrimination of low concentrations of CO_2. The results of this study provide further evidence that olfactory neurons exhibiting CA activity are CO_2 chemoreceptors sensitive to physiological concentrations of CO_2. For a discussion of the possible functions of CO_2-sensitive olfactory receptors, see the reviews by Coates (2001) and Milsom et al. (2004).

Acknowledgements

The authors thank Jennifer L. Surace and Elizabeth L. Chiota for their contribution to this project and Bruce P. Bryant for his suggestions during the preparation of this manuscript. The methazolamide was a gift from Lederle Laboratories, Division of American Cyamamid Company, Pearl River, NY. This project was funded, in part, by a Russell Pearce and Elizabeth Crimian Heuer Foundation award given to the Allegheny Neuroscience Program by the Council of Independent Colleges.

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Accepted December 10, 2006