Carotid body denervation alters ventilatory responses to ibotenic acid injections or focal acidosis in the medullary raphe


Hodges, M. R., C. Opansky, B. Qian, S. Davis, J. M. Bonis, K. Krause, L. G. Pan, and H. V. Forster. Carotid body denervation alters ventilatory responses to ibotenic acid injections or focal acidosis in the medullary raphe. J Appl Physiol 98: 1234–1242, 2005. First published December 3, 2004; doi:10.1152/japplphysiol.01011.2004.—Our aim was to determine the effects of carotid body denervation (CBD) on the ventilatory responses to focal acidosis and ibotenic acid (IA) injections into the medullary raphe area of awake, adult goats. Multiple microtubules were chronically implanted into the midline raphe area nuclei either before or after CBD. For up to 15 days after bilateral CBD, arterial PCO2 (Paco2) (13.3 ± 1.9 Torr) was increased (P < 0.001), and CO2 sensitivity (−53.0 ± 6.4%) was decreased (P < 0.001). Thereafter, resting Paco2 and CO2 sensitivity returned (P < 0.01) toward control, but Paco2 remained elevated (4.8 ± 1.9 Torr) and CO2 sensitivity reduced (−24.7 ± 6.0%) ≥40 days after CBD. Focal acidosis (FA) at multiple medullary raphe area sites 23–44 days post-CBD with 50 or 80% CO2 increased inspiratory flow (Vt), tidal volume (Vt), metabolic rate (Vo2), and heart rate (HR) (P < 0.05). The effects of FA with 50% CO2 after CBD did not differ from intact goats. However, CBD attenuated (P < 0.05) the increase in Vt, Vt, and HR with 80% CO2, but it had no effect on the increase in VO2. Rostral but not caudal raphe area IA injections increased Vt, BP, and HR (P < 0.05), and these responses were accentuated (P < 0.001) after CBD. CO2 sensitivity was attenuated (−20%; P < 0.05) <7 days after IA injection, but thereafter it returned to prelesion values in CBD goats. We conclude the following: (1) the attenuated response to IA after CBD provides further evidence that the carotid bodies provide a tonic facilitory input into respiratory control centers, (2) the plasticity after CBD is not due to increased raphe chemoreceptor sensitivity, and (3) the “error-sensing” function of the carotid body blunts the effect of strong stimulation of the raphe.

control of breathing; CO2 chemoreception; peripheral chemoreception

ONE ROLE OF THE PERIPHERAL chemoreceptors, or carotid bodies, relates to the ability to respond to changes in arterial O2 and CO2, providing error feedback to the ventilatory control system (5, 13, 23). Additionally, the carotid bodies appear to provide a tonic excitatory input into the ventilatory control system, as evidenced by the effects of carotid body denervation (CBD) studies (7, 12, 19). CBD leads to hypoventilation during eupnea and exercise and to a reduction in CO2 sensitivity. However, in most species resting arterial Paco2 (Paco2) and CO2 sensitivity return to pre-CBD levels within weeks after CBD, indicating that there is plasticity within this system (7).

The mechanism of the plasticity in breathing after CBD is unknown, but intact aortic chemoreceptors are not required (22). Serotonin has been implicated in other forms of plasticity, but it remains to be determined whether the serotonergic or other neurons of the medullary raphe are involved in the plasticity after CBD (15). It is, however, conceivable that the mechanism of plasticity is due to a “resetting of gain” of the intracraniol chemoreceptors, whereby central chemoreceptors (including the medullary raphe) become more sensitive to changes in CO2/H+.

The medullary raphe nuclei have traditionally been defined by a population of serotonergic neurons (11, 24). Recent studies suggest that an additional characteristic of these neurons is that they are chemosensitive (21, 25) and that they are capable of influencing breathing in intact animals, as demonstrated by microdialysis-induced focal acidification and by selective lesioning of serotonergic neurons in rats (8, 9, 16–18).

In addition to serotonin, recent evidence also suggests both neurokinin-1 receptor (NK1R)- and glutamate receptor-expressing neurons play a role in the raphe influence on breathing and chemoreception (1, 3, 10, 18). The toxin saporin conjugated to substance P (10, 18) and ibotenic acid (IA; 3, 10) injections into the raphe attenuated CO2 sensitivity. The lesion effects on breathing in goats seem specific to hypercapnia, with no effect on breathing at rest, breathing during sleep, or the exercise hyperpnea (10). However, the effects on CO2 sensitivity were transient, returning to prelesion levels after >7 days, indicating that there is recovery or plasticity in the system (10).

Therefore, we hypothesize that, during the initial hyperventilation phase after CBD, the ventilatory responses to focal acidosis in the raphe area will be attenuated but that once plasticity has occurred the response to focal acidosis will be accentuated. Moreover, we hypothesize that this plasticity will result in a greater than normal acute and chronic response to IA injections into the raphe area.

METHODS

Data were obtained from nine female and one castrated male adult goats weighing 55.5 ± 5.3 kg. The goats were housed and studied in an environmental chamber with a fixed ambient temperature and photoperiod. All goats were allowed free access to hay and water, except for periods of study. All aspects of the study were reviewed and approved by the Medical College of Wisconsin Animal Care Committee before the studies were initiated.

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EFFECTS OF CAROTID BODY DENERVATION ON RAPHE STIMULATION

Experimental Design

Three surgeries were performed: an initial surgery for subcutaneous elevation of the carotid arteries and for placement of electrodes into the diaphragm, a second for medullary raphe microtubule (MT) implantation and a third for sham, unilateral or bilateral CBD. The order of the latter two surgeries was reversed in some cases to control for potential effects of MT implantation on CBD. Assessments of resting breathing $P_{aCO_2}$ and ventilatory response to $CO_2$ began $\geq$3 days before CBD, or $\geq$2 wk after MT implantation surgery, and were performed nearly daily throughout the protocol. In seven of these goats, responses to focal acidosis in the medullary raphe were measured between 23 and 40 days after CBD in six goats. Thereafter, we injected 500 nl and/or 10 µl of IA (a nonspecific glutamate receptor excitoxotoxin) in the awake state. The IA injections (when more than one was made) were separated by a minimum of 7 days. After completion of these protocols each animal was euthanized, the head perfused and fixed, and the medulla harvested. The medullary tissues were then frozen sectioned and stained with hematoxylin and eosin and an antibody against tryptophan hydroxylase (TPOH) to assess MT placement and lesion size and severity.

Surgical Procedures

Instrumentation surgery. An initial surgery was performed to elevate a 5-cm segment of the carotid arteries, and for electrode implantation into the diaphragm, as described previously (26). Briefly, the goats were anesthetized with a cocktail of ketamine and xylazine (24:1 vol:vol), and intubated, mechanically ventilated, and anesthesia was maintained with 1–1.5% halothane in $O_2$. Under sterile conditions, the carotid arteries were isolated from the vagi, elevated superficial to the muscle, and the skin sutured. A left lateral thoracotomy permitted access to the costal diaphragm into which were sewn Teflon-coated silver wire electromyographic (EMG) electrodes (Cooner Wire). After surgery, the goats received cefitufur sodium (2 mg/kg) daily as an antibiotic for 1 wk.

Brain implantation surgery. After $\geq$3 wk, a second surgery was performed to chronically implant two ($n=8$) or three ($n=2$) MTs into the medullary raphe nuclei. Through an occipital craniotomy, and with daily injections thereafter of cefitufur sodium (2 mg/kg) for 1 wk after surgery.

Physiological Measurements

For all studies, a fitted mask was taped firmly to the snout, and a two-way breathing valve was attached to the mask. The inspired port of the valve was connected to a pneumotachograph and computerized data acquisition system to measure inspiratory flow ($V_i\dot{}$). The expired port was connected to a spirometer (Trisst) for collection of expired air and analysis of $O_2$ and $CO_2$ concentrations [O$_2$ consumption (metabolic rate) ($V_O_2$) and $CO_2$ production ($V_{CO_2}$), respectively]. A chronically placed catheter in the elevated carotid artery was used to monitor arterial blood pressure (BP) and heart rate (HR) and for arterial blood sampling to obtain pH, arterial $P_02$, ($PaO_2$) and $P_{aCO_2}$ values (model 278, Ciba-Corning). Rectal temperature of the animal was measured at regular intervals. Proximal ends of the diaphragm EMG wires were connected via microclips to a Grass recorder for signal processing and paper recording during all studies. The EMG signals were filtered at a band pass of 3–500 Hz, and sampled at 250 Hz. The EMG signals were then full-wave rectified and passed through a moving time averager (time constant of 0.1 s) to obtain the integrated diaphragm (Dia$_i\dot{}$) signal as previously reported (26).

Assessment of $CO_2$ sensitivity. $CO_2$ responses were obtained nearly every day beginning $\geq$3 days before CBD surgery, or $\geq$2 wk after brain implantation surgery and throughout the entire protocol. Room air (RA) breathing, BP, and HR were measured for 30 min before exposure to three levels of elevated inspired $CO_2$ (2.5, 5.0, and 7.5% $CO_2$ in RA). Arterial blood samples were drawn during the control period and during the fourth and fifth minute of each $CO_2$ exposure level. The change in expired ventilation ($V_e\dot{}$) and $P_{aCO_2}$, from RA breathing to all levels of $CO_2$ was used determine the relationship between $V_e\dot{}$ and $P_{aCO_2}$, and was used as an index of $CO_2$ sensitivity. These studies were also performed before and for $\geq$7 days after IA injections.

Assessment of CBD. The ventilatory response to intravenous and intra-arterial bolus injections of sodium cyanide (NaCN) was used to assess the peripheral (carotid) chemoreflex before and after sham, unilateral or bilateral CBD surgery. While the goats were breathing RA, 100 µg/kg of NaCN were rapidly infused into a catheter in the jugular vein, followed by an injection of an equal volume of saline and then by a second injection of NaCN at 5-min intervals. Similarly, 10 µg/kg of NaCN was rapidly infused into a catheter in one or both carotid arteries, followed by saline and a second NaCN injection. The ventilatory response to intravenous NaCN injections was then expressed as inspiratory flow ($V_i\dot{}$) from 10 to 30 s after venous, or from one to five breaths after carotid, NaCN injection divided by $V_i\dot{}$ 30 s before the injection [ventilatory response ratio (VRR)].

Physiological response to focal acidosis after CBD. The CMA Microdialysis, Solna, Sweden) 12 microdialysis probes (20-kDa molecular mass cutoff) had a 70-µm shaft length, a 2-µm membrane length, and a 0.5-µm membrane diameter. Identical microdialysis probes were used in our initial sets of experiments, when we established that microdialysis with 50% $CO_2$ and 80% $CO_2$ generated an extracellular fluid acidosis slightly greater than, or three times greater than that observed with 7.5% inspired $CO_2$ in RA (8, 9). The contents of the dialysate (mock cerebral spinal fluid) have been previously described (8, 9).
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V̇I, HR, BP, VO₂, and V̇O₂ were measured continuously or at regular intervals during a 15-min control period, during 45 min of microdialysis (flow rate = 50 μl/min), and for 15 min after termination of the dialysate flow. Arterial blood was drawn during the final 5-min period of the control, microdialysis and recovery periods. Three different dialysate pH and PCO₂ conditions were tested: 1) 6.4% CO₂ (pH = 7.31–7.36, PCO₂ = 41–47 Torr), 2) 50% CO₂ (pH = 6.5–6.6, PCO₂ = 250 Torr), and 3) 80% CO₂ (pH = 6.3–6.4, PCO₂ > 500 Torr).

**Injections of IA**

**Acute effects.** V̇I, BP, and HR were measured for a 15-min control period, during and for up to 5 h after 500-nl and/or 10-μl IA injections. V̇I, BP, and HR data were averaged over 5-min epochs and were expressed as a percentage of the control value for each individual animal to reconcile differences in absolute values among animals of varying size. PaCO₂ data were collected at the beginning and end of the control period and at 30 and 60 min after IA injection.

**Chronic effects.** On the basis of previous data, the time course of the neurotoxic effects of IA in the MRN is 3–7 days postinjection (10). Therefore, CO₂ sensitivity and eupneic PaCO₂ values were obtained for at least 3 days before IA injections, and for at least 7 days post-IA injections, to assess the chronic effects of each neurotoxin.

**Altered breathing periods.** As in a previous study, we assessed the presence of altered breathing periods, including breaths with prolonged expiratory time (PTE) greater than two times the normal expiratory time (Tr), obstructive apneas, and fractionated breaths [FBBr; 3 or more complete respiratory cycles within a breath (10)]. The PTE events were quantified by dividing the duration of the PTE divided by the normal (eupneic) Tr (PTE/Tr ratio), and we counted the occurrence of these events, as well as the frequency of FBBr (events/h).

**Histological Studies**

All aspects of histology studies were performed exactly as done in previous studies (10). Medullary tissues from experimental goats were harvested 0–8 days post-IA injection, placed in a 4% paraformaldehyde solution for 24–48 h, and then placed in a 30% sucrose solution for an additional 48 h. The medullas were then frozen and serial sectioned (20–25 μm) in a transverse plane, and the sections adhered to chrom alum-coated slides. The tissue was then stained with hematoxylin and eosin and an antibody against tryptophan hydroxylase (TPOH) and coverslipped for microscopic examination. The protocols for both hematoxylin and eosin and TPOH staining have been previously described (10).

**Location of the MTs.** The site of implantation was identifiable by visualization of an area of absent or disrupted tissue, which extended over a finite rostrocaudal distance and was related to the size of the implanted MT. The implantation site was defined as being at the tip (ventral-most aspect) and middle of the MT-induced tissue disruption.

**Neuronal count regions.** The medullary raphe area count region was in an area that included (but was not restricted to) the traditional location(s) of serotonergic neurons, beginning ventral to the dorsal motor nucleus of the vagus and 1.0 mm lateral to the midline bilaterally, and to the ventral surface of the medulla. We defined the count region to include an area (volume) of tissue slightly greater than that used in previous investigations due to the presence of TPOH-expressing neurons at a distance ≤1.0 mm lateral to the midline in the goat. Neurons in the inferior olivary nuclei were excluded from all midline neuron counts. A second region near the ventral lateral medullary surface (VLM) was counted bilaterally in TPOH-stained tissue to serve as a control, non-lesion-count region.

**Lesion quantification.** The lesion was quantified two ways: 1) by neuron counts from the raphe area and VLM region, and 2) volumetrically. Midline raphe and VLM neuron counts were made every 100–250 μm and averaged every 500 μm from −5.0 mm caudal to and +10.5 mm rostral to obex in unoperated control tissues from four goats [data reported previously (10)] and greater than −1.5 mm caudal to and +1.5 mm rostral to the lesion site in experimental tissues (n = 9). Total living, dead, and TPOH-expressing neurons were counted in the raphe-area, and TPOH-expressing neurons counted in the VLM. With the use of hematoxylin and eosin staining, living neurons stain purple, whereas dead neurons stain pink and are circular in shape [described previously (10)]. Direct comparisons of midline raphe counts at 0.5-mm intervals between unoperated control and experimental goats were used to determine the lesion effect in TPOH-expressing-stained tissues (n = 4), with VLM counts serving as an internal control site. Each 0.5-mm midline TPOH-expressing count region in each experimental animal was designated either lesion or nonlesion on the basis of the presence or absence of dead neurons (ascertained by hematoxylin and eosin stain) in adjacent tissue sections.

We have previously attempted to quantify the extent of the lesion on the basis of the reduction in total living neurons remaining at the lesion site compared with the expected number of living neurons from unoperated control animals (10). However, we found considerable variation in number of living neurons in the control goats. Accordingly, we determined that the reduction in living neurons in the lesion goats was not a suitable measure to quantify the lesion.

**Volumetric quantification of the lesion was partitioned into two major volumes (in mm³): 1) total medullary volume and 2) total raphe area volume. The total raphe area volume was further partitioned into three subvolumes: 1) volume containing dead neurons, 2) volume devoid of neurons, and 3) volume of tissue displaced by the inserted MT(s).**

**Data and Statistical Analyses**

**Focal acidosis studies.** We calculated V̇I, VT, frequency, VO₂, mean arterial blood pressure (BP) and heart rate (HR). Calculated variables were binned into 5-min periods, and these were means divided by the 15-min control period to yield a percent control value. These values were then subjected to a two-way ANOVA with repeated measures (treatment and time) to compare microdialysis with 6.4% to either 50 or 80% CO₂ and a two-way ANOVA (treatment and time) to compare cardio body-intact data with CBD data. The threshold for significance set to P < 0.05.

**Acute effects of neurotoxin injections.** We calculated V̇I, BP, and HR for each of the time-control and neurotoxin injection studies. V̇I, BP and HR were binned in 30-s intervals, and then they were averaged into 5-min mean values for each injection study. Each goat served as its own control, where raw values for all parameters were divided by the 15-min control period mean and then further binned to obtain 5-min means as a percent of the control value. For each parameter (V̇I, BP, and HR), the individual mean values for each time period for each goat were averaged and statistically analyzed by using a one-way ANOVA with repeated measures. Caudal and rostral, as well as pre- and post-CBD, data were compared with a two-way ANOVA, or a two-way ANOVA with repeated measures.

**Chronic effects of the neurotoxin injections.** The CO₂ sensitivity and PaCO₂ values from 3 days before IA injection were averaged to obtain a preinjection control value for each individual animal. An average for 2 or more consecutive days from 3 to 7 days post-IA injection (nadir) was taken as the representative effect of each neurotoxin. A third mean was calculated from 2 or more consecutive days at 8 or more days after the neurotoxin injection(s) to reflect the chronic (final) effects. A t-test was performed on the changes between pre- and post-IA.

**RESULTS**

**Anatomy**

**Location of implanted MTs.** The midline locations of the implanted MTs are shown in Fig. 1. The letter symbols denote the ventral-most aspect of each implanted MT in each animal.
is near 1.0, indicating that volume injections had no effect on V\textsubscript{t} (Fig. 3, A and B). The VRR with bolus intravenous injections of NaCN was significantly attenuated after bilateral CBD (\(P < 0.001\)), whereas the VRR was not different from saline injections (\(P = 0.534\); Fig. 3A). Similarly, the VRR with intra-arterial NaCN injections was significantly attenuated after bilateral CBD (\(P < 0.001\)), whereas the saline and post-CBD VRR were not different from one another (\(P = 1.0\); Fig. 3B).

**Effects of CBD.** Before sham, unilateral or bilateral CBD surgery, all goats exhibited resting \(\text{Paco}_2\), \(\text{Pao}_2\), arterial pH, and \(\text{CO}_2\) sensitivities within the normal range for goats (19, 26; Fig. 4). However, the goats exhibited increased resting \(\text{Paco}_2\) (\(13.3 \pm 1.9\) Torr) and decreased \(\text{Pao}_2\) (\(-16.7 \pm 5.1\) Torr), arterial pH (\(-0.026 \pm 0.007\)), and \(\text{CO}_2\) sensitivity (\(-53.0 \pm 6.4\%\)) up to 15 days after bilateral CBD (\(P < 0.001\); Fig. 4). However, 15 days after CBD, these values began to return toward normal, but even up to 40 days \(\text{Paco}_2\) and \(\text{CO}_2\) sensitivity (\(-24.7 \pm 6.0\%\)) remained above and below control respectively. Unilateral CBD tended to increase \(\text{Paco}_2\) (4.4 Torr), and decrease \(\text{CO}_2\) sensitivity (\(-22.5\%\)), but had no effect on arterial pH or \(\text{Pao}_2\). Sham denervation \((n = 1)\) did not have any effect on resting variables or \(\text{CO}_2\) sensitivity at all time points after sham-CBD surgery.

**Physiological response to focal acidosis after CBD.** The data describing the results of focal acidosis before CBD have been reported previously (9), but they are included for comparison. Data from unilateral (\(n = 1\)) and bilateral (\(n = 5\)) CBD goats were pooled. Microdialysis with 6.4% \(\text{CO}_2\) at multiple (2–3) rathe area sites had no effects on all variables after sham and/or bilateral CBD (\(P = 0.148\)). Microdialysis with either 50 or 80% \(\text{CO}_2\) at multiple rathe area sites increased \(\text{Vt}, \text{Vr}, \text{V02},\) and HR (\(P < 0.05\)), but it had no effect on respiratory frequency or BP (\(P > 0.05\)) 23 or more days after CBD.

CBD had no effect on all calculated variables with microdialysis with 6.4% \(\text{CO}_2\) (\(P > 0.05\)). Additionally, there were no effects of CBD on the physiological response to microdialysis with 50% \(\text{CO}_2\) (\(\geq 23\) days post-CBD), with the exception of a significantly greater increase in HR after CBD (\(P = 0.017\)). However, \(\text{Vt}, \text{Vr},\) and HR were significantly attenuated (\(P \leq 0.007\)) during microdialysis at 80% \(\text{CO}_2\) after CBD, but CBD had no effect on respiratory frequency or \(\text{V02}\) (\(P > 0.05\); Fig. 5). The ventilatory response to microdialysis with 50% (\(+9.1 \pm 4.4\%\); \(n = 1\)) and 80% \(\text{CO}_2\) (\(+17.2 \pm 4.8\%\); \(n = 2\)) <23 days after bilateral CBD was also less than that observed pre-CBD (Fig. 6). The attenuated ventilatory response to focal acidosis pre- and post-CBD was further reflected in the \(\text{Vt}\) and \(\text{Dia}_{\text{a}}\) activities, whereas the increase in \(\text{Dia}_{\text{a}}\) activity appeared to also be attenuated (Fig. 6). Sham denervation \((n = 1)\) had no effect on the physiological response to focal acidosis with 6.4, 50, or 80% \(\text{CO}_2\) (data not shown).

**Verification of CBD.** The VRRs for intravenous saline injections in bilateral-, unilateral-, and sham-denervated goats

![Diagram](image-url)
Acute responses of mCSF and IA injections. The 10-μl injections of mCSF in both caudal and rostral medullary raphe-areas had no effects on V′I, HR, or BP (P > 0.05) up to 5 h after injection. Similarly, 500-nl (data not shown) or 10-μl IA injections in caudal raphe areas had no effects on V′I, HR, or BP (Fig. 7; P > 0.05; n = 12). However, both 500-nl and 10-μl IA injections in rostral raphe regions increased V′I, HR, and BP in carotid body-intact goats (P = 0.013, n = 4). These responses, particularly with 10-μl injections, were accentuated in CBD goats, exhibiting greater increases in V′I, HR and BP (P < 0.001, n = 7). The acute response to rostral raphe area IA injections in CBD animals was so robust in some cases that the goats had to be euthanized within 24–48 h after injection (see also asterisks in Fig. 1). These animals often exhibited excessive salivation, reduced swallowing, airway obstruction, nystagmus, failure to stand and/or maintain sternal posture, and in some cases what appeared to be hypotonia. Some of these symptoms persisted, despite normalization of resting breathing 6–10 h post-IA.

Chronic effects of IA injections. Because of the robust acute response to more rostral IA injections, we were unable to assess the chronic effects in all goats. Resting Paco2, (−0.2 ± 0.6 Torr) and CO2 sensitivity (−6.0 ± 1.0%) were unaltered up to 7 days after 500-μl IA injections (P > 0.05, n = 4). However, CO2 sensitivity was attenuated (−20.0 ± 8.0%; P < 0.05), but resting Paco2 was unaltered (+1.34 ± 0.4 Torr; P > 0.05) up to 7 days after 10-μl IA injections in either the caudal or rostral raphe areas (n = 4). CO2 sensitivity returned to control levels >7 days post IA injection (P > 0.05).

Altered breathing periods. Altered breathing periods, including PTe (Fig. 8) and FBr events were observed in seven of nine goats. The PTE/TE ratio >2 wk after MT implantation was 2.19 ± 0.05, and was unaltered by unilateral or bilateral CBD (2.37 ± 0.16) or >3 days after IA injections (2.10 ± 0.09; P = 0.109; n = 6). The frequency of occurrence of the PTE events was 8.4 ± 1.3/h after MT implantation, and it was unaffected after CBD (6.4 ± 1.3/h) or IA injections (9.0 ± 1.5/h; P = 0.504, n = 6). FBr were observed in one goat, whereas the
frequency of FBr after MT implantation and subsequent unilateral CBD was 15.0/11006 0.6/h and 17.7/11006 0.4/h, respectively.

**DISCUSSION**

The major findings of the present study were the following:
1) CBD led to hypoventilation (14 Torr) and a 53% reduction in CO2 sensitivity for up to 15 days after denervation, but thereafter there was a significant recovery in resting PaCO2 and CO2 sensitivity; 2) physiological responses to focal acidosis in the medullary raphe nuclei were not different or were significantly attenuated after CBD relative to carotid body-intact state; 3) the acute effects of IA injections were greater in more rostral regions of the raphe than the caudal raphe, and these effects were accentuated after CBD; and 4) the chronic effects of neurotoxic lesions after CBD on CO2 sensitivity were similar to those previously reported in carotid body-intact goats.

**Physiological Effects of CBD**

There are strong data supporting the hypotheses that the carotid bodies both 1) directly sense arterial O2 and CO2 levels, providing afferent feedback to the ventilatory control centers (5, 23), and 2) provide a tonic excitatory input into breathing centers (12, 19, 20). The reduced hypoxic and CO2 responses and the hypoventilation at rest and during steady-state exercise after CBD support the hypothesis of a general loss of excitatory input to breathing centers, rather than a specific CO2 chemoreception effect (19). Additionally, creating reversible dysfunction of the rostral ventrolateral medulla by thermode cooling reduces breathing more in awake CBD goats than in intact goats (20). Finally, Fatemian et al. (4) found that the magnitude

Fig. 3. Confirmation of carotid body denervation (CBD). Ventilatory response to intravenous (A) and intra-arterial (carotid; B) injections of sodium cyanide (NaCN) or saline 1–24 days after bilateral (n = 7), unilateral (n = 2), or sham (n = 1) denervation is shown. The response is expressed as the ventilatory response ratio, where Vt from 10–30 s (intravenous) or 1–5 breaths (carotid) after bolus NaCN or saline injection divided by Vt from 30 s before injection. Note that bolus saline injections yielded a ventilatory response ratio of ~1.0, indicating no ventilatory response. Note also that the ventilatory response ratio to intravenous and carotid bolus NaCN injections was significantly attenuated after bilateral CBD (*P < 0.001, 1-way repeated-measures ANOVA).

Fig. 4. Physiological effects of CBD. Resting arterial PCO2 (PaCO2), CO2 sensitivity [change in expired ventilation/change in PaCO2 (ΔVt/ΔPaCO2)], and resting arterial PO2 (PaO2) and arterial pH in bilateral (n = 6), unilateral (n = 2), and sham (n = 1)-denervated goats at three time points: control (>3 days before denervation or sham denervation), peak (maximum effect 2 or more days 16 days postdenervation), and recovery (2 or more days <16 days postdenervation). Note that PaCO2 increased (*P < 0.001) and CO2 sensitivity, PaO2, and arterial pH decreased (*P ≤ 0.015, 1-way repeated-measures ANOVA) during the peak effect in bilateral CBD goats. Also note that <16 days after bilateral CBD PaCO2 remained elevated (***P = 0.046) but was significantly less than the peak effect (P = 0.001), and CO2 sensitivity remained attenuated (***P = 0.011) but was significantly greater than the peak effect (P = 0.019, 1-way repeated-measures ANOVA), indicating significant but not complete recovery post-CBD.
of the slow (central) phase of the CO2 chemoreflex in humans was attenuated after carotid body resection.

In the present study, we observed no difference or a significant attenuation of the breathing response to focal acidosis with 50 and 80% CO2 at multiple raphe sites after CBD, respectively, compared with that observed before denervation. This attenuation persisted even after significant plasticity that returned PaCO2 and CO2 sensitivity toward control values. CBD, however, did not attenuate the increase in metabolic rate associated with focal acidosis in the medullary raphe, indicating that the attenuation was specific to respiratory control. These and previous findings after CBD support the hypothesis that under physiological conditions central respiratory neurons rely on tonic facilitory input from the carotid bodies. Because of the general attenuation in breathing, we postulate that the tonic facilitation must provide drive at the level of premotor or motoneurons and that it likely does not selectively provide tonic drive to central chemoreceptors per se. An alternative explanation of the attenuated response to focal acidosis after CBD is that because of the chronic hypoventilation and hypercapnia after CBD, brain pH regulatory mechanisms may have been upregulated; thus, dialysis with high CO2 may have caused less of a change in brain pH after compared with before CBD.

**Plasticity After CBD**

Recovery of function after a perturbation to a control mechanism can be “due to restoration of the same mechanism or substitution of another mechanism” (7). In this context, plasticity refers to the mechanism or process by which the observed recovery occurs. We observed ~66 and 50% recovery (P < 0.01) in resting PaCO2 and CO2 sensitivity, respectively, ≥15 days after bilateral CBD. The finding that the ventilatory response to focal acidosis in the intact goats has been previously reported (9), and the unilateral and bilateral CBD data were pooled. Note that focal acidosis with 80% CO2 led to a significant increase in 

![Fig. 5. Physiological effects of focal acidosis after CBD. Vt, tidal volume (VT), breathing frequency (f), and metabolic rate (VO2) are expressed as a percentage of control during control, microdialysis of 80% CO2 (dotted line), and recovery periods before (Pre-CBD, n = 7) and after (Post-CBD, unilateral (n = 1) and bilateral (n = 6)] CBD. The data for the intact goats have been previously reported (9), and the unilateral and bilateral CBD data were pooled. Note that focal acidosis with 80% CO2 led to a significant increase in Vt, VT, and VO2 in both carotid body-intact (P < 0.05) and -denervated goats (P ≤ 0.033, 1-way repeated-measures ANOVA), but the increases in Vt and VT (but not VO2) were significantly attenuated >23 days post-CBD (*P < 0.001, 2-way ANOVA for treatment and time).

![Fig. 6. Vt and integrated diaphragm activity (DiaI) during focal acidosis is attenuated after CBD. Data tracings of Vt and DiaI from one goat during room air breathing (A) and during microdialysis with 80% CO2 (B) pre-CBD, and breathing room air (C) and during microdialysis with 80% CO2 (D) 10 days and 23 days (E and F, respectively) after bilateral CBD. Note that focal acidosis at multiple midline raphe sites increases both Vt (+30%) and DiaI activity before CBD, but the increase in Vt (+10% and +20%) and DiaI with microdialysis with 80% CO2 is attenuated 10 and 23 days after CBD, respectively.

A

B

C

D

E

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plasticity restoring eupneic PaCO₂ and CO₂ sensitivity after CBD remains unclear. The incomplete recovery of PaCO₂ and CO₂ sensitivity up to 40 days after CBD differs from our previous findings in goats of complete recovery 15 days after CBD (19). The ventilatory response to NaCN of the current bilateral CBD goats was 1.26 ± 0.07, which is less than the 1.56 ± 0.02 in our previous study (19); thus less or no residual peripheral chemosensitivity may have contributed to the observed reduced plasticity in this compared with our previous studies. A second potential contributor to the reduced recovery was that the prior raphe lesion (when CBD was performed after MT implantation) may have sufficiently attenuated tonic raphe modulation of respiratory neurons or attenuated a serotonin-mediated mechanism of plasticity. The importance of tonic modulation was evident from the recovery time from the anesthesia after the CBD surgery. Post-CBD anesthesia recovery time was longer (3–7 h) than normal (<1 h) in most goats. Extreme examples were two goats that had previously undergone multiple MT implantations into the raphe that we were unable to recover from anesthesia after CBD surgery.

Our laboratory has previously presented findings that emphasized the importance of tonic raphe modulation of respiratory neurons, evidenced by the observations of altered breathing periods after raphe lesions (10). Our laboratory had noted that after raphe MT implantation (and thereafter) all 13 goats recurrently had breaths with prolonged Tₑ relative to normal Tₑ (central apnea), obstructive apneas, and/or FBr. For example, 9 of 13 goats had PTE/Tₑ ratios of 2.53 ± 0.15 that occurred 8.2 ± 1.4 times/h. Similarly, in the present study, we observed PTE events in six of nine goats, where the PTE/Tₑ ratio was 2.19 ± 0.05 that occurred 8.4 ± 1.4 times/h. Additionally, we previously reported that injections of saporin conjugated to substance P or IA had no effect on the frequency or other characteristics of these events (10). Similarly, we found no effects of CBD or IA injections on these events in the present study. It appears that the frequency and duration of the PTE is independent of carotid tonic facilitation or chemoreceptor related “error sensing.” As previously, we conclude that these events were due to imbalances of excitation and/or inhibition of respiratory rhythm-generating neurons as a result of either a destruction of raphe neuromodulatory neurons, and/or physical destruction of fibers of passage from the raphe to other respiratory nuclei such as the pre-Bötzinger complex or destruction of fibers of passage between the bilaterally located pre-Bötzinger complex neurons.

**Acute and Chronic Effects of IA**

Previously, our laboratory reported that 10-μl injections of IA in rostral, but not caudal, medullary raphe regions increased breathing, BP, and HR (10). Similarly, in this study, we found

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**Fig. 7.** Acute ventilatory response to caudal and rostral ibotenic acid injections. Vᵢ for 15 min before and up to 5 h after 10-μl injections of ibotenic acid in either the caudal (before and after CBD, n = 12), rostral (carotid body intact (CB-intact), n = 4), or rostral (CBD, n = 7) midline raphe areas during wakefulness is shown. The site-specific, acute ventilatory response to injections in the caudal raphe area were not different between intact and CBD goats (data not shown), and therefore the data were pooled. Vᵢ increased (P < 0.001) after 10-μl rostral raphe area ibotenic acid injections in CB-intact and CBD goats, but not after caudal injections (P > 0.05, 1-way repeated-measures ANOVA). Vᵢ was greater (P < 0.001) with rostral ibotenic acid injections after CBD than that observed in the CB-intact goats, and the CB-intact response was greater (P < 0.001, 2-way ANOVA for treatment and time) than the caudal injections, indicating both CBD- and site-specific responses.

**Fig. 8.** Altered breathing periods were unaltered by CBD. Data tracings of Vᵢ and Diaᵢ from 1 goat (goat I) 14 days after microtubule implantation (A) and 9 days after bilateral carotid body denervation (B). Note that the goat has periods of prolonged expiratory time where Diaᵢ and Vᵢ stop. Also note that the duration of the prolonged expiratory time is similar before and after bilateral CBD relative to the normal expiratory time, indicating that CBD had no effect on the prolonged expiratory time.
that 10-μl injections of IA in CBD goats also increased Vt, HR, and BP when injected in the rostral, but not caudal, medullary raphe regions, where CBD accentuated these responses. Interestingly, CBD in goats, ponies, and dogs has also been previously shown to accentuate the ventilatory response at the onset of exercise, resulting in an accentuated hyperventilation at the onset of exercise (2, 6, 19). Therefore, it may be that the accentuation of the acute IA response and the accentuated ventilatory response at the onset of exercise after CBD is due to the loss of both carotid chemoreceptor- and baroreceptor-related error feedback control, effectively removing the “brake” on the IA- or exercise-induced hyperpnea.

The site-specific results of the acute IA response are more intriguing considering that the largest numbers of TPOH- and NKIR-expressing raphe area neurons overlap with these rostral injection sites (10). Both cell types have been previously implicated in the role of the medullary raphe as a central CO2/H+ chemoreceptor, where NKIR-expressing cell-specific and IA-induced lesions both transiently reduced CO2 sensitivity by 23 and 27% (10). In this study, we also report similar losses of TPOH-expressing neurons, lesion volumes, and numbers of dead neurons within the lesion site, and a similar transient reduction in CO2 sensitivity in CBD goats.

The more severe responses to IA after CBD extended beyond the first hour and affected many physiological systems. After 10-μl IA injections in the rostral raphe areas, five goats were unable to maintain sternal recumbent posture, had nystagmus, and had an acute hyperpnea up to 5 h postinjection. The postural effects persisted for 24–48 h in some cases, and as a result they were euthanized. In other goats, arterial hypertension, excessive salivation, absence of swallowing, and/or severe airway constriction were observed acutely. The severity of the effects have not been previously seen with 10-μl IA injections in caudal or rostral raphe regions in carotid body intact goats or in caudal regions in CBD goats. These findings seem to suggest that the carotid bodies influence not only brain stem respiratory neurons but also other neurons or neural circuits coordinating breathing with other physiological functions.

Conclusions

It appears that the carotid body J functions to minimize disruption of blood gas homeostasis by sensing an error caused by a strong stimulus, which it then attenuates or inhibits, and 2) also provides tonic excitation needed for the full expression of responses to other respiratory stimuli.

GRANTS

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