Tracheal occlusions evoke respiratory load compensation and neural activation in anesthetized rats

Kathryn M. Pate and Paul W. Davenport
Physiological Sciences, University of Florida, Gainesville, Florida
Submitted 9 November 2010; accepted in final form 31 October 2011

Pate KM, Davenport PW. Tracheal occlusions evoke respiratory load compensation and neural activation in anesthetized rats. J Appl Physiol 112: 435–442, 2012. First published November 10, 2011; doi:10.1152/japplphysiol.01321.2010.—Airway obstruction in animals leads to compensation and avoidance behavior and elicits respiratory mechanosensation. The pattern of respiratory load compensation and neural activation in response to intrinsic, transient, tracheal occlusions (ITTO) via an inflatable tracheal cuff are unknown. We hypothesized that ITTO would cause increased diaphragm activity, decreased breathing frequency, and activation of neurons within the medullary and pontine respiratory centers without changing airway compliance. Obstructions were performed for 2–3 breaths followed by a minimum of 15 unobstructed breaths with an inflatable cuff sutured around the trachea in rats. The obstruction procedure was repeated for 10 min. The brains of obstructed and control animals were removed, fixed, sectioned, and stained for c-Fos. Respiratory pattern was measured from esophageal pressure (Pes) and diaphragm electromyography (EMGdia). The obstructed breaths resulted in a prolonged inspiratory and expiratory time, an increase in EMGdia amplitude, and a more negative Pes compared with control breaths. Neurons labeled with c-Fos were found in brain stem and suprapontine nuclei, with a significant increase in c-Fos expression for the occluded experimental group compared with the control groups in the nucleus ambiguus, nucleus of the solitary tract, lateral parabrachial nucleus, and periaqueductal gray matter. The results of this study demonstrate tracheal occlusion-elicited activation of neurons in brain stem respiratory nuclei and neural areas involved in stress responses and defensive behaviors, suggesting that these neurons mediate the load compensation breathing pattern response and may be part of the neural pathway for respiratory mechanosensation.

diaphragm; c-Fos; respiratory control; PAG; intrinsic occlusion

Load compensation has brain stem and suprapontine neural components. The primary non-invasive techniques for imaging brain activity in response to respiratory stimuli include functional magnetic resonance imaging (28, 44) and cortical evoked potentials (18, 20–22), but these methods are nonspecific and only provide information about regional neural activity. c-Fos immunohistochemical analysis of neural tissue is a way to obtain specific information about increases in activity at the individual neuronal level in response to a stimulus (25). However, this method requires removal of brain tissue and can therefore only be done in animals. c-Fos expression has been reported in the periaqueductal gray (PAG) in response to laryngeal afferent stimulation (2) and throughout the brain and spinal cord in response to phrenic nerve stimulation (38).

Determining mechanistically how increased resistances and occlusion during breathing affect the respiratory pattern, motor output, and neural response is vital to understanding the regulation of respiratory load compensation in respiratory obstructive diseases such as obstructive sleep apnea (OSA). The neural mechanisms of respiratory load compensation is also related to the sensations associated with mechanical challenges to breathing in conscious animals. Previous research has used two primary methods for increasing airway resistance: intrinsic changes in lung mechanics with bronchoconstriction and changes in respiratory mechanics with extrinsic respiratory loads. Bronchoconstriction, such as methacholine challenge, is used to elicit intrinsic, nonspecific, sustained mechanical loading. Bronchoconstriction can also reduce lung compliance, induce lung inflammation, and change blood gases (48), modulating the specific mechanical effects of bronchoconstriction loading. Alternatively, extrinsic loads such as a resistance (R) applied extrinsically at the mouth or via a tracheal tube, is specific to mechanical load compensation responses and the load duration and intensity can be controlled (13, 17, 51). Our laboratory has developed a model of mechanical load-specific intrinsic tracheal occlusions in anesthetized rats that produce increases in upper airway resistance without changing lung compliance. The intrinsic, transient tracheal occlusions (ITTO) elicit load compensation Vt-T and diaphragm activity changes. The neural substrates involved in mediating the load compensation response were investigated using c-Fos immunohistochemistry. It was hypothesized that ITTO in the rat would elicit a load compensation respiratory motor pattern response and corresponding neuronal activation, and the neurons activated by the ITTO will express c-Fos within brain stem and suprapontine brain nuclei. The results of this study suggest that this model of ITTO elicits respiratory motor and neural load compensation responses and has potential applications for respiratory studies in conscious animals.

Address for reprint requests and other correspondence: P. W. Davenport, Physiological Sciences, Univ. of Florida, Gainesville, FL 32603 (e-mail: pdavenpo@ufl.edu).

http://www.jappl.org

MATERIALS AND METHODS

These experiments were performed on male Sprague-Dawley rats (360.5 ± 77.4 g). The animals were housed two to a cage in the University of Florida animal care facility where they were exposed to a 12:12-h light/dark cycle. The experimental protocol was reviewed and approved by the Institutional Animal Care and Use Committee of the University of Florida.

Surgical Procedures

All animals were anesthetized with urethane (1.3 g/kg ip) and anesthesia was supplemented as needed (20 mg/ml). Anesthetic depth was verified by the absence of a withdrawal reflex to a rear paw pinch. Body temperature was measured with a rectal probe and maintained at 38°C using a heating pad. Animals were spontaneously breathing room air.

A saline-filled tube (PE-90) was passed through the mouth into the esophagus and connected to a second calibrated pressure transducer to measure esophageal pressure (P$_{es}$). The P$_{es}$ signal was amplified (Stoelting, Wood Dale, IL), digitized at 1 kHz (Cambridge Electronics Designs 1401 computer interface; Cambridge, UK), computer processed (Spike2, Cambridge Electronics Design), and stored for subsequent analysis. Pleural pressure changes were inferred from relative changes in P$_{es}$.

Diaphragm EMG (EMG$_{dia}$) was recorded with Teflon-coated wire electrodes, threaded through 25-gauge needles. The distal end of the wires were bare and bent to form a hook. With the animal in a supine position, the right ribcage was lifted and the needle was inserted in a rostral and dorsal direction through an incision in the skin. The needle was retracted and the hooked tip of the electrode remained in the costal portion of the right side of the diaphragm. Two electrodes were inserted for bipolar EMG$_{dia}$ recording. The external ends were bare and connected to a high-impedance probe. The signal was amplified (PS11, Grass Instruments) and band-pass filtered (0.3–300 Hz). Analog output was digitized at 1 kHz (Cambridge Electronics Designs 1401 computer interface), computer processed (Spike2, Cambridge Electronics Design), and stored for subsequent analysis.

The trachea was exposed by a ventral incision. The trachea was freed from surrounding tissue, and an inflatable cuff (Fine Science Tools) was sutured around the trachea, two cartilage rings caudal to the larynx. The actuator tube of the cuff was connected to an air-filled coverslip (Vectamount).

The occlusion was applied for 2–3 breaths and then removed for at least 15 unobstructed breaths (confirmed by P$_{es}$). This was repeated for the duration of the 10-min experimental trial. Animals in control groups 2 and 3 were maintained without obstructions during the 10-min period.

Following completion of the experimental trial, the animal was maintained under anesthesia for 90 min. Following this 90-min period the animal was killed with an overdose of anesthetic and transcardially perfused with 0.5 ml heparin into the left ventricle followed by 200 ml perfusion saline and 200 ml 4% paraformaldehyde in 0.4 M PBS. The brain was removed and placed in 4% paraformaldehyde for 24 h. The brain was then transferred to a solution of 30% sucrose in PBS. The fixed tissue was frozen, sectioned with a cryostat (Carl Zeiss, HM101) into coronal slices 40 μm thick, and placed in PBS for storage.

Immunohistochemical Analysis

For each brain, every third section of tissue was used for processing. Slices were incubated for 1 h in a 1:30 solution of goat serum in PBS + Triton X-100 (GS-PBS-T). The tissue then sat overnight in a solution of rabbit anti-c-Fos primary antibody (1:2000, sc-52r; Santa Cruz Biotechnology). The following day the tissue was washed for 1 h in a solution of 1% GS-PBS-T and then incubated for 2 h in a solution of goat anti-rabbit biotin (1:500, Jackson ImmunoResearch). Following another 1 h wash in 1% GS-PBS-T, all slices were treated with avidin-biotin peroxidase complex (ABC Vectastain Kit; Vector, Burlingame, CA) and washed again (1% GS-PBS-T for 1 h). To complete the staining process, a chromagen solution [0.05% diaminobenzidine hydrochloride, 2.5% ammonium sulfate, 0.033% hydrogen peroxide in 0.05 M Tris-HCl (DAB); Sigma] was applied for roughly 3 min until the tissue changed to a darker brown color, indicative of Fos-like immunoreactivity (FLI). The reaction was washed with distilled water, and then all tissue was washed three times in PBS. Following staining, slices of tissue were mounted on glass slides and were allowed to air-dry for 3 days. All slides were dehydrated with a series of washes in alcohol and CitraSolv, then placed under a coverslip (Vectamount).

Data Analysis

Breathing pattern. Breathing pattern analysis was performed with Spike 2 software on six experimental animals and four control group 1 animals. Raw EMG$_{dia}$ signal recordings were rectified and integrated (50 ms time constant). The amplitude of the integrated EMG$_{dia}$ signal was measured (Fig. 1) from baseline to peak amplitude (relative units). Inspiratory time (T$_i$) was the duration of EMG$_{dia}$ burst; expiratory time (T$_e$) was the duration between EMG$_{dia}$ bursts; total breath time (T$_{tot}$) was the sum of T$_i$ and T$_e$ (Fig. 1). P$_{es}$ was measured from end expiration to peak (Fig. 1). These parameters were analyzed for the complete unobstructed control breath (C) prior to occlusion, each occluded breath (O1-O3), and the complete unobstructed recovery breath (R) immediately following occlusion (Fig. 1). T$_i$, T$_e$, and T$_{tot}$ were averaged for each rat and for each group. P$_{es}$ and EMG$_{dia}$ amplitude were normalized by dividing O1-O3 and R by the control breath, C. The normalized P$_{es}$ and EMG$_{dia}$ values were compared for each rat and for each group.

c-Fos. Images of brain sections to be analyzed were viewed using light microscopy (Zeiss Axioskope) and captured with imaging software (AIS). Masks delineating areas to be counted were drawn on the images using Adobe Photoshop according to brain regions (41), and the masked images were saved. Neurons expressing c-Fos were identified by the round, dark staining of their nuclei (Fig. 2). Size and shape limits were set to exclude spots that were too lightly stained or were outside the diameter range of 7–10 μm. The number of c-Fos-labeled neurons in the area of interest was counted (MetaMorph). For
OCCLUSIONS EVOKE LOAD COMPENSATION AND NEURAL ACTIVATION

Fig. 1. Diaphragm electromyography (EMGdia) and esophageal pressure (Pes) traces during tracheal occlusion in intact (experimental) and tracheostomized (control group 1) animals. The broken horizontal line indicates end-expiratory Pes, which remained stable throughout the experiment. Inspiratory and expiratory time (Ti and Te, respectively) measurements are indicated by the horizontal arrows, and the shaded area indicates the time period the tracheal cuff was inflated.

each brain, three sections per nucleus were counted bilaterally. If no bilateral differences were seen, counts for that nucleus were averaged. These values were then combined according to group and used for between-group comparisons (Table 1).

Statistics. All breathing parameters for C, O1, O2, O3, and R were averaged within each rat. These values were used to conduct a two-way ANOVA for each parameter with breath number and group as factors. Differences were statistically significant if \( P < 0.05 \). A one-way ANOVA was performed comparing c-Fos expression in all four groups for each nucleus. If statistical significance was achieved \( P < 0.05 \), post hoc analysis was obtained via multiple \( t \)-tests with Bonferroni’s correction to determine specific group differences. All data are reported as means ± SE.

RESULTS

Breathing Pattern

In obstructed animals with an intact trachea, Ttot was significantly greater during O1-O3 compared with the preceding C and subsequent R breaths \(( P < 0.002 \); Fig. 3C). Prolongation of Ti \(( P < 0.03 \); Fig. 3A) and to a greater extent Te \(( P < 0.008 \) ) contributed to this Ttot difference. The Ttot of the R breath returned to pre-obstruction, C breath values. The rectified and integrated EMGdia signal progressively increased in amplitude over the course of the obstruction \(( P < 0.001 \) ), and remained significantly elevated above C values during the R breath \(( P < 0.02 \); Fig. 4A). Similarly, inspiratory Pes became increasingly negative during the obstruction \(( P < 0.001 \) ) and also remained significantly more negative during R compared with C \(( P < 0.004 \); Fig. 4B). During all obstructions in tracheostomized animals no changes in breath timing or EMGdia were observed (Figs. 1, 3, 4). Presurgical baseline recordings for Pes, EMGdia, and breath timing did not differ from end-experimental values. Implantation of the tracheal cuff had no effect on Pes, EMGdia, or breathing pattern when it was sutured in place and deflated.

Fos-Like Immunoreactivity

FLI was found in all nuclei analyzed for all groups (Table 1). In the medulla, the nucleus ambiguus (nA) and caudal nucleus of the solitary tract (cnTS; Fig. 2) had significantly greater c-Fos expression in the experimental group compared with all other groups \(( P < 0.002 \) ). The ANOVA resulted in significant difference between groups in the rostral nTS (rnTS, \( P < 0.04 \)) but Bonferroni corrected post hoc analysis revealed no differences.

In the locus ceruleus (LC) the experimental group had the greatest amount of FLI compared with controls. In parabrachial subnuclei the external lateral (elPBN) area showed the greatest number of Fos-expressing cells. In the elPBN, dorsal lateral (dlPBN), and central lateral subnuclei the experimental group had greater FLI compared with all control groups, and the difference was significant in the dlPBN \(( P < 0.03 \), comparing experimental and control group 3). The lateral crescent subnucleus was the only pontine region analyzed where the tracheostomized animals had the greatest c-Fos expression.

In the midbrain PAG, the greatest amounts of FLI were observed in the caudal ventral region (cvPAG). The tracheostomized control group 1 had the most stained cells in all PAG regions, and the difference in group means was significant in the caudal dorsal (cdPAG) and cvPAG for this group compared with control group 3 \(( P < 0.007 \) ). The experimental group had significantly more c-Fos expression in the cvPAG compared with control group 3 \(( P < 0.001 \) ). No differences between any groups were seen in the rostral dorsal PAG (rdPAG). In addition, the experimental group and control group 1 had significant \(( P < 0.05 \) ) rostral-caudal differences in the dorsal PAG; the experimental group showed significant dorsal-ventral \(( P < 0.05 \) ) differences in the PAG.

DISCUSSION

Our method of ITTO for 2–3 breaths in anesthetized rats elicited respiratory load compensation, including a prolongation of Ti and Te, increased EMGdia activity, and more negative inspiratory Pes. ITTO in anesthetized rats also induced neural activation in respiratory brain stem nuclei, and nuclei involved in cardiovascular, stress, and fear responses. End-expiratory Pes did not change with obstruction or recovery in any animals, indicating that ITTO does not change lung compliance. The short duration of occlusions ensured minimal changes in blood gases, supporting our hypothesis that ITTO elicits a load compensation respiratory motor pattern response and neuronal activation within respiratory related brain nuclei. In the tracheostomized control group 1, tracheostomy decreased baseline Pes to a value that remained stable during obstructions and recovery. Because Ti, Te, EMGdia, and Pes were not altered in control group 1 during ITTO, the changes in these parameters observed in the experimental group result from the load compensation response of the respiratory neural control system to a breathing effort against a closed airway. Tracheal compression alone was not sufficient to evoke altered breath timing and respiratory motor responses. The ITTO model, thus, can be used to study diseases characterized by repeated increases in upper airway resistance and occlusion due to upper airway obstruction or collapse such as OSA.

Pes and EMGdia follow a similar pattern of activity during occluded breaths, with Pes becoming more negative as EMGdia amplitude increases from O1 to O3. These values remain elevated above control breaths during the recovery breaths, although they are less than during obstructions. Forster and...
colleagues (27) found that sustained inspiratory resistive loading in conscious ponies caused an augmentation of Vt and respiratory motor drive, but not breath timing during recovery breaths. This behavior persisted even after pulmonary and diaphragmatic deafferentation. The augmentation of parameters during recovery breaths after ITTO is likely a result of the respiratory system acting to restore ventilation after sustained perturbation. The mechanisms underlying these responses remain unknown, but it is clear that consecutive obstructions to breathing necessitate a compensatory response present in both.

Table 1. c-Fos Expression in medullary and suprapontine nuclei

<table>
<thead>
<tr>
<th>Nucleus</th>
<th>Ctrl 3 (mean±SE)</th>
<th>Ctrl 2 (mean±SE)</th>
<th>Ctrl 1 (mean±SE)</th>
<th>Exp (mean±SE)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>cdPAG</td>
<td>40.8 (6.4)</td>
<td>70.3 (22.2)</td>
<td>131.0 (33.7)*</td>
<td>88.7 (17.6)</td>
<td>0.007</td>
</tr>
<tr>
<td>cvPAG</td>
<td>57.6 (17.0)</td>
<td>153.0 (31.4)</td>
<td>236.7 (46.7)*</td>
<td>176.5 (39.2)*</td>
<td>0.001</td>
</tr>
<tr>
<td>rdPAG</td>
<td>59.5 (18.9)</td>
<td>58.0 (9.8)</td>
<td>69.2 (19.4)</td>
<td>52.8 (39.2)</td>
<td>n/a</td>
</tr>
<tr>
<td>nA</td>
<td>1.0 (0.2)</td>
<td>0.5 (0.2)</td>
<td>1.4 (0.3)</td>
<td>4.9 (0.5)†</td>
<td>0.001</td>
</tr>
<tr>
<td>cnTS</td>
<td>61.9 (16.0)</td>
<td>37.6 (10.5)</td>
<td>66.6 (19.7)</td>
<td>169.5 (21.5)†</td>
<td>0.002</td>
</tr>
<tr>
<td>rnTS</td>
<td>44.0 (0)</td>
<td>79.7 (26.5)</td>
<td>53.3 (6.5)</td>
<td>203.3 (44.3)</td>
<td>0.040</td>
</tr>
<tr>
<td>LC</td>
<td>42.5 (12.3)</td>
<td>37.8 (13.8)</td>
<td>42.7 (16.2)</td>
<td>44.9 (10.2)</td>
<td>n/a</td>
</tr>
<tr>
<td>clPBN</td>
<td>13.2 (3.0)</td>
<td>26.8 (7.3)</td>
<td>15.1 (6.8)</td>
<td>29.8 (5.1)</td>
<td>n/a</td>
</tr>
<tr>
<td>elPBN</td>
<td>38.0 (4.9)</td>
<td>30.1 (7.0)</td>
<td>20.7 (7.8)</td>
<td>38.1 (3.9)</td>
<td>n/a</td>
</tr>
<tr>
<td>lcrPBN</td>
<td>17.1 (2.7)</td>
<td>17.9 (2.7)</td>
<td>18.1 (3.1)</td>
<td>16.4 (2.4)</td>
<td>n/a</td>
</tr>
<tr>
<td>dIPBN</td>
<td>6.1 (1.3)</td>
<td>6.7 (0.8)</td>
<td>9.1 (1.3)</td>
<td>12.4 (1.4)*</td>
<td>0.007</td>
</tr>
</tbody>
</table>

Values are the mean number of cells stained positively for c-Fos in each region of interest (SE). Exp is the experimental group that received occlusions with an intact trachea. Ctrl 1 is the control group 1 that received occlusions but was able to breathe through a tracheostomy. Ctrl 2 is the control group 2 that received surgical instrumentation but remained unoccluded, and Ctrl 3 is the control group 3 that was anesthetized but received no instrumentation. cdPAG, cvPAG, rdPAG, caudal dorsal, caudal ventral, and rostral dorsal periaquaductal gray, respectively; nA, nucleus ambiguus; cnTS and rnTS caudal and rostral nucleus of the solitary tract, respectively; LC, locus ceruleus, clPBN, elPBN, lcrPBN, and dIPBN, central lateral, external lateral, lateral crescent, and dorsal lateral parabrachial subnuclei, respectively. *Significantly different from the Ctrl 3 group; †significantly different from all other groups. Note that the SE for Ctrl 3 in the region of the rnTS was 0 because there was only one animal from this group with acceptable tissue for analysis.
the anesthetized and conscious state. Although the augmented recovery response in the conscious ponies has a reflexive component (40, 47), it cannot be ruled out that there may also be a behavioral response to the sensations associated with respiratory mechanical loading.

The sensation of difficult or uncomfortable breathing is associated with respiratory obstructive diseases and can be experimentally induced by increases in airway resistance (45, 46). Conscious humans can respond to respiratory resistive loading and occlusion similarly to conscious animals (1, 3, 14), and these responses may be due to sensations associated with respiratory load compensation (6, 45, 46). Significant variability in human responses exist, however, that can be attributed to perceptual differences between individuals (3, 14). Our model of ITTO may be a useful tool in future studies aimed at understanding the neural mechanisms mediating conscious animal behavioral responses specific to respiratory mechanical loads. The ITTO model has potential usefulness in future studies due to the level of placement of the cuff intrinsically, around the extrathoracic trachea, and its reversibility. Other models of tracheal occlusions include manipulating the flow of air through a tracheostomy, which acts as a reversible but extrinsic mechanical load, and tracheal banding, which is an intrinsic but relatively irreversible form of mechanical loading. Alternatively, inflatable balloons can be implanted in the interior of the trachea in some species; however, rodents have very small tracheal lumens and we were unable to find a balloon small enough not to obstruct normal breathing. The ITTO model allows us to study the responses to this type of occlusion in rodents, something not possible with the interior balloon model.

The decreased breathing frequency during ITTO in the experimental group was primarily due to an increase in the duration of Te. Because Te is normally longer than Ti for a given breath, there could be more potential for breath-timing modulation during Te than Ti. This is especially true in the anesthetized rat where Te is longer than in that of an awake rat. During ITTO at FRC in anesthetized rats, the occlusion prevents a change in lung volume. Lung inflation activates pulmonary stretch receptors (PSR), respiratory afferents that proj-

Fig. 3. Breath timing data during tracheal occlusions in animals with an intact trachea in (the experimental group, Exp) and in animals able to breathe through a tracheostomy (control group 1, Ctrl 1). A: Ti: obstructed breath (O1-O3) values were significantly different from control (C) for the Exp animal (P < 0.03); B: Te: O1-O3 values were significantly different from C for the Exp animal (P < 0.008); C: total breath duration (Ttot): O1-O3 values were significantly different from C for the Exp animal (P < 0.002).
activated afferents within the area of tracheal compression and afferents modulated by the increased respiratory effort associated with breathing against the occlusion. One of the limitations of using c-Fos immunohistochemistry is that sensory and motor neural activation cannot be differentiated. It is possibly that the enhanced activity in the nTS in the experimental group is a result of the motor output caused by other neural regions activated by ITTO. Considering there was no observable respiratory motor response in the control groups resulting from ITTO and the nTS is a major respiratory motor nucleus, the enhanced FLI in this region is likely a major contributor to the observed load compensation respiratory motor response. The nA, a nucleus within the ventral respiratory group (VRG), also showed significant increases in FLI in experimental animals compared with controls. Because the nA is also a respiratory motor nucleus, c-Fos expression in this area is likely a result of efferent load compensation activation in addition to activation by airway afferents and inputs from the nTS. Respiratory load compensation, thus, has a neural component that includes both sensory and motor activated medullary neurons.

ITTO also evoked significant neural activation in the dIPBN. The dlPBN responds to various respiratory stimuli including inspiratory occlusions (23) and is also an important part of the descending pathway from the PAG involved in cardiovascular (29, 30) and respiratory (30) control. Neurons in the nTS that are activated by vagal afferents send projections to pontine respiratory neurons, including the IPBN (26, 36). The increased FLI seen in the IPBN after ITTO could be a response to afferent activation or efferent projections from the IPBN to other neural areas such as the limbic system (42) and may play a role in an animal’s affective response to increased respiratory mechanical loads. No differences were seen between groups in the LC, a nucleus involved in stress responses and a source of norepinephrine in the brain (8). The lack of group differences in the LC appears to be a result of substantial neural activation across all group conditions, possibly resulting from global anesthetic-related activation or an elevated and sustained stress response from handling the animals. Neural activation seen in the PBN is likely the result of integrating ascending medullary and descending PAG projections into both respiratory and cardiovascular responses to ITTO. The PBN is also involved in defensive, fear, and limbic pathways, and although animals in this study were anesthetized, the neural responses related to ITTO induced threat of suffocation-induced avoidance may be present.

The caudal region of the midbrain PAG was activated by ITTO, with the ventral region more than the dorsal. The PAG is known to be involved in defensive behaviors (4, 9, 43), pain modulation (7, 39), respiratory activation (31, 52), and modulation of the respiratory load compensation response (51). The results of Zhang et al. (53) showed greater respiratory activation in response to cdPAG compared with rdPAG stimulation. The dPAG and vPAG have been implicated in panic and anxiety, respectively (5, 9, 10, 12, 43), and the vPAG appears to be involved in conditioned fear responses (43). It is possible that ITTO causes respiratory-specific activation in the cdPAG and cvPAG, but that ITTO also stimulates stress or fear pathways that additionally contribute to PAG activation. In addition, one cannot rule out the potential contribution of other sensory afferents to respiratory Vt-T, motor, and neural responses to ITTO.

Fig. 4. A: normalized peak amplitude of the integrated EMGdia trace during tracheal occlusions in animals with an intact trachea in (the experimental group, Exp) and in animals able to breathe through a tracheostomy (Ctrl 1). O1-O3 and recovery (R) values were significantly different (*) from C for the Exp animal (P < 0.001, O1-O3; P < 0.02, R). B: normalized peak negative esophageal pressure in the same animals. O1-O3 and recovery (R) values were significantly different (*) from C for the Exp animal (P < 0.001, O1-O3; P < 0.004; R).

OCCLUSIONS EVOKE LOAD COMPENSATION AND NEURAL ACTIVATION

The caudal region of the midbrain PAG was activated by ITTO, with the ventral region more than the dorsal. The PAG is known to be involved in defensive behaviors (4, 9, 43), pain modulation (7, 39), respiratory activation (31, 52), and modulation of the respiratory load compensation response (51). The results of Zhang et al. (53) showed greater respiratory activation in response to cdPAG compared with rdPAG stimulation. The dPAG and vPAG have been implicated in panic and anxiety, respectively (5, 9, 10, 12, 43), and the vPAG appears to be involved in conditioned fear responses (43). It is possible that ITTO causes respiratory-specific activation in the cdPAG and cvPAG, but that ITTO also stimulates stress or fear pathways that additionally contribute to PAG activation. In addition, one cannot rule out the potential contribution of other sensory afferents to respiratory Vt-T, motor, and neural responses to ITTO.

J Appl Physiol • doi:10.1152/japplphysiol.01321.2010 • www.jappl.org
Any lack of significant differences in FLI expression between control group 1 and the experimental group in certain nuclei may be due to tracheal compression as an afferent stimulus projecting to those specific areas. Although FLI is a useful indicator of neuronal activity in response to acute stimuli, many control groups are needed to ensure activation is not a result of handling, anesthesia, noise, scent, or other factors. Cells stained positively for the c-Fos cannot be divided into sensory or motor activation, and this method cannot provide an indication of inhibition. With appropriate control groups, however, c-Fos immunohistochemistry can identify activated neural substrates that should be investigated in future studies.

**Summary**

The results of this study suggest that airway resistance can be increased intrinsically without changing lung compliance, evoking respiratory load compensation indicated by breath timing, diaphragm activity, and esophageal pressure changes. C-Fos activity in dorsal respiratory group and VRG medullary nuclei suggest neuronal activation directly related to brainstem load compensation reflex. Activation of pontine and midbrain nuclei are consistent with a role for fear, anxiety, and defensive behaviors elicited by airway occlusion. c-Fos expression in the caudal PAG following ITTO is likely due to both respiratory- and fear-specific activation, and the caudal PAG may play an important influential role on the behavioral and physiological responses to ITTO via descending pontine projections. The neural mechanisms mediating the load compensation response to ITTO need further investigation, including differentiating between afferent and efferent activation via multiple neuronal electrophysiological and anatomical methods. This model has potential applications in studying OSA, a respiratory obstructive disease characterized by repeated, increased upper airway resistance and occlusion. There are also applications for ITTO as an animal model of respiratory load elicited sensations of breathing effort. Modifying the ITTO model for future studies has important implications for elucidating the neural mechanisms modulated by respiratory diseases such as OSA, behavioral load compensation, and respiratory sensations.

**DISCLOSURES**

No conflicts of interest, financial or otherwise, are declared by the authors.

**AUTHOR CONTRIBUTIONS**

Author contributions: K.M.P. and P.W.D. conception and design of research; K.M.P. and P.W.D. performed experiments; K.M.P. and P.W.D. analyzed data; K.M.P. and P.W.D. interpreted results of experiments; K.M.P. prepared figures; K.M.P. drafted manuscript; K.M.P. and P.W.D. edited and revised manuscript; K.M.P. and P.W.D. approved final version of manuscript.

**REFERENCES**