Effects of baclofen on the Hering-Breuer inspiratory-inhibitory and deflation reflexes in rats

Erin Seifert and Teresa Trippenbach. Effects of baclofen on the Hering-Breuer inspiratory-inhibitory and deflation reflexes in rats. Am. J. Physiol. 274 (Regulatory Integrative Comp. Physiol. 43): R462–R468, 1998.—The objective of this study was to evaluate effects of baclofen, a γ-aminobutyric acid type B (GABAB) receptor agonist, injected into the nucleus of the solitary tract, on the Hering-Breuer inspiratory-inhibitory (TI-inhibitory) and deflation reflexes in urethane-anesthetized adult Wistar rats (n = 7). The TI-inhibitory reflex was estimated from changes in peak amplitude of the integrated diaphragmatic electromyogram and inspiratory time (Ti) provoked by airway occlusion at end expiration. The deflation reflex was evaluated from changes in Ti and expiratory time (Te) of the first two breaths (Ti-1, Te-1 and Ti-2, Te-2) immediately after a decrease in tracheal pressure (Ptr). Under control conditions, airway occlusion at end-Te prolonged Ti (66 ± 5% mean ± SE) and the following Te (54 ± 11%). Decreases inPtr, from 2 to ~5 cmH2O, evoked an increase in Ti and shortening of Te of both breaths. Both effects were Ptr dependent, and Ti-1 and Te-1 differed from Ti-2 and Te-2, suggesting a rapid adaptation to the stimulus. At Ptr of ~5 cmH2O, Ti-1 and Ti-2 increased by 30 ± 2 and 43 ± 6%, respectively, and Te-1 and Te-2 decreased by 53 ± 4 and 33 ± 7%, respectively. During unloaded breathing, 60 pmol baclofen prolonged Ti by 120 ± 11% and left Te unaffected. Baclofen abolished vagally mediated changes in Te. On the other hand, the Ti increases caused by either airway occlusion (24 ± 8%) or Ptr of ~5 cmH2O (Ti-1; 16 ± 5%) were still significant, but Ti-1 and Ti-2 were not different. A GABAB receptor antagonist, CGP-35348 (2.8 nM), reversed these effects of baclofen. These results imply that stimulation of GABAB receptors attenuates but does abolish vagally mediated control of Ti. The difference in effects of baclofen on the central and vagal control of Ti and Te suggests different distribution of GABAB receptors in neuronal networks controlling each of these respiratory phases.

ACTIVATION OF Γ-AMINOBUTYRIC ACID (GABA) TYPE B RECEPTORS INHIBITS NEURAL ACTIVITY IN A NUMBER OF AREAS OF THE CENTRAL NERVOUS SYSTEM (1, 5, 6, 24, 46). Baclofen, a GABAB receptor agonist, may provoke this inhibition via activation of postsynaptic GABAB receptors and hyperpolarization of the postsynaptic membrane or via activation of presynaptic GABAB receptors and inhibition of synaptic transmission due to a block of a transmitter release from presynaptic terminals (5, 6, 46). GABAB receptors are present within the medullary structures involved in control of ventilation (14, 17, 27, 38). In the respiratory neuronal networks, activation of GABAB receptors by baclofen results in a prolongation of inspiration without simultaneous changes in expiration in rats (36, 40, 43) and cats (24, 32).

Vagal afferent fibers from the lungs and other visceral organs terminate in the nucleus of the solitary tract (NTS; 4, 8, 18, 23, 26). In the NTS, baclofen inhibits postsynaptic potentials evoked in NTS neurons by stimulation of the solitary tract in brain stem slices in vitro (6) and stimulation of vagus nerve in a neonatal rat brain stem-spinal cord preparation (41, 42). Consistent with the inhibitory action of baclofen, the Hering-Breuer expiratory-promoting (TE promoting) reflex was abolished after NTS injection of baclofen (40). The respiratory and cardiovascular components of the pulmonary chemoreflex, mediated by pulmonary C fibers, were attenuated by baclofen injections (36). The effects of baclofen were reversed by CGP-35348, a GABAB receptor antagonist (30). These results suggest that GABAB receptors are located within the medullary pathways of these two vagally mediated reflexes and that they may modulate responses of the cardiorespiratory system to slowly adapting stretch receptor and vagal C fiber activities (36, 40).

The present study was undertaken to further explore the involvement of GABAB receptors in modulation of the pulmonary vagal reflexes by examining the Hering-Breuer inspiratory-inhibitory (Ti inhibitory) and deflation reflexes. The baclofen-evoked block of the Te-promoting reflex may suggest that baclofen will have a similar effect on the Ti-inhibitory reflex. However, the medullary pathways for the Ti-inhibitory and Te-promoting reflexes are different (9), and distribution of GABAB receptors within these pathways may vary also. Different roles of GABAB receptors in the vagal control of the respiratory timing phases are suggested by an observation that intravenous injections of baclofen in paralyzed and artificially ventilated cats did not eliminate the ability of vagal volume-related activity to induce an inspiratory off-switch (32). Withholding lung inflation prolonged inspiratory activity, whereas inflations during inspiration terminated this inspiration. In this study, quantitative evaluation of effects of baclofen on the Ti-inhibitory reflex is missing (32). Also, because effects of baclofen strongly depend on the dose and the route of application (24, 32), the above observation may only be specific for the peripheral application of large doses of baclofen. Nevertheless, a possibility exists that baclofen does not affect the Ti-inhibitory reflex. Some data of the present study have been published in abstract form (37).

METHODS

Experiments were done on seven spontaneously breathing male Wistar rats weighing 210–290 g. The experimental protocol was approved by the University Animal Care Committee and followed the principles of the Canada Council on Animal Care. Animals were anesthetized with urethan (1.2 g/kg body wt ip) and treated with atropine (0.02 mg/kg im) to diminish tracheal secretion. A supplemental dose of urethan...
(0.2 g/kg) was given when the animal responded to a noxious stimulus (a tail pinch) with increased heart rate or frequency of respiration. The maximal cumulative dose of urethane did not exceed 1.6 g/kg. Animals were tracheotomized and cannulated with a custom-made stainless steel pneumotachograph.

To obtain tidal volume, the airflow signal derived from the pneumotachograph was amplified (Hewlett-Packard 8805, Andover, MA) and electronically integrated by means of a volume integrator (Hewlett-Packard 8815A). Tracheal pressure (Ptr) was measured from a side arm of the pneumotachograph connected, via a differential pressure transducer (MP 45–871; Validyne, Northridge, CA), to a carrier amplifier (Hewlett-Packard 8805A). A T-shaped cannula was attached to the distal end of the pneumotachograph. One end of the horizontal arm of this cannula was connected to a three-way stopcock to apply either pure oxygen or vacuum flow. The other end of this cannula was open to room air. The flow of oxygen of 1.5 l/min through the open cannula did not affect the Ptr. Before experiments, the vacuum flow was calibrated with a water manometer for changes in pressure from −2 to −5 cm H2O in 1-cm H2O steps. The range of negative pressures was based on our preliminary study, in which Ptr of −2 cm H2O was the threshold stimulus necessary to trigger the Hering-Breuer deflation reflex, and the response attenuated at Ptr lower than −4 cm H2O (unpublished data). The time constant of the deflation system was 40 ms.

A cannula filled with heparinized saline was inserted into the tail artery for recording arterial blood pressure. The tail cannula was connected, via a three-way stopcock, to a liquid-filled pressure transducer (Hewlett-Packard 1290C) and to a syringe pump (Razel Scientific Instruments, Stanford, CA) for continuous injection of 6% dextran (1.2 ml/h). The signal was fed into a carrier amplifier (Hewlett-Packard 8805C) for signal conditioning. Rectal temperature was monitored by means of a tungsten-constantan thermocouple (Omega DP 30; Omega Technology, Stanford, CA) and maintained at 36–38°C using an infrared lamp.

The skin and abdominal muscles were cut in the midline, and two Teflon-coated, seven-stranded, stainless steel wires with a 1-mm exposed end were sewn into the diaphragm immediately beneath the xiphoïd process. The muscles and the skin were sewn, and the electrode leads, free of insulation, were connected to an amplifier (Grass Instruments PS11, Quincy, MA). The diaphragmatic electromyogram (DiEMG) was rectified and processed by a modified Paynter filter with an averaging time of 100 ms.

Rats were studied in the prone position with the head mounted in a stereotaxic apparatus (David Kopf Instruments, Tujunga, CA) with the dorsal surface of the brain stem in the horizontal plane. The medulla was exposed after minimal craniotomy and removal of dura and arachnoid membranes. Injections of saline and drugs into the NTS were made using single-barrel glass micropipettes with an outer tip diameter of 3 µm. A micromanipulator was used to position the micropipette tip at different sites between 0.2 and 0.6 mm caudal to the obex, 0.5 mm lateral from the midline, and 0.3 mm below the dorsal surface of the medulla. The NTS injection procedure has been previously described in detail (36, 40). Saline solutions of baclofen and CGP-35348 were prepared daily from stock solutions. The concentration of baclofen in 110 nl of injection volume was 0.5 mM and that of CGP-35348 was 25 mM. The injection volumes thus contained 60 pmol baclofen and 2.8 nmol CGP-35348. These doses were based on the previous study describing inhibitory effects of baclofen on the Hering-Breuer Te-promoting reflex (40). The osmolality of the saline and drug solutions was 283 mosmol, and pH was adjusted to 7.4 with bicarbonate. Both drugs were gifts from Ciba-Geigy.

At the end of experiments, 110 nl of Fast Green FCF was injected at the drug injection sites. Rats were killed with an overdose of urethane. The brain stem was removed, immersed in 10% Formalin at 4°C for 48 h, and then transferred to 30% sucrose and maintained at 4°C in that solution until sectioning with a cryostat. A series of 25-µm sections was cut through the region of interest. Preparation of sections was previously described (36, 40). The injection sites, illustrated in Fig. 1, were verified histologically with the aid of a microdissection guide of the rat brain (31).

Experimental protocol. After surgery, animals were allowed 20 min for stabilization. The integrated DiEMG, Ptr, tidal volume (VT), and arterial blood pressure were continuously recorded on a six-channel pen recorder (Brush 260; Gould, Cleveland, OH) at a paper speed of 10 mm/s. Records for data analysis were taken at a paper speed of 25 mm/s. After three to five control breaths, the airway was occluded either at VT, for the purpose of measuring the compliance of the total respiratory system (Crs), or at the end-Te, for the purpose of estimating the strength of the Hering-Breuer inspiratory-inhibitory reflex (44). At least 10 breaths were allowed between the occlusions. The vacuum flow was adjusted to generate a selected subatmospheric Ptr. Three breaths were recorded, and the vacuum line was opened during the last 50% of expiration of the fourth breath. The whole range of changes in Ptr was tested in a random order. Lung deflation was maintained for three breaths. Immediately after return to atmospheric Ptr, lungs were overinflated by application of a pulse of positive pressure to the open end of the T-shaped cannula. To ensure the return of the respiratory system to the steady state after changes in Ptr, 20 breaths were allowed and measurements of Crs were repeated before the next deflation. The occlusions and lung deflations to a given Ptr were performed three times. The above protocol was repeated 10 min after bilateral NTS injection of saline, 10 min after baclofen injected to the saline-injection sites, and 5 min after CGP-35348 injected to the same NTS location.
Data analyses. Records of DiEMG were digitized using a graphics tablet (Hewlett Packard P11A) connected to a microcomputer. Ti was defined as the time interval between the onset and the rapid decline of the DiEMG. Te was measured from the end of Ti to the onset of the next DiEMG. At each experimental step, changes in the peak amplitude of DiEMG (Di), Ti, and Te caused by either airway occlusion at end-Te or lung deflation were evaluated as percent of their values during unloaded breaths immediately preceding either of the maneuvers. The ratio between VT and Ptr recorded during occlusions at VT was used to evaluate Crs. Mean arterial blood pressure (MAP) was measured manually from the records, and the heart rate was derived from systole-diastole fluctuations of the blood pressure record.

One-way analysis of variance (ANOVA) for repeated measures was used to test the stability of the baseline cardiorespiratory variables recorded between airway occlusions and deflations and to evaluate effects of the NTS injections of baclofen and CGP-35348 on control variables. Preliminary analyses using a two-tailed paired t-test showed no differences in the baseline variables and the reflex responses before and after the NTS injection of saline. Therefore, all values for these two conditions were pooled and presented as control. The same analysis was used to compare percent changes in respiratory variables evoked by airway occlusion at end-Te and Crs before and after lung deflations at each experimental step. The response to lung deflation was evaluated from the first two respiratory cycles (breath 1 and breath 2) after the onset of a decrease in Ptr. The response to the step decreases in Ptr and the difference between variables of breath 1 and breath 2 at control, after baclofen, and after CGP-35348 was evaluated with a two-way ANOVA for repeated measures. ANOVA tests were followed by the Tukey-Kramer post hoc test. All values are presented as group means ± SE. A probability of 0.05 or less was accepted as significant.

RESULTS

Effects of baclofen and CGP-35348 on the cardiorespiratory variables. Table 1 shows group mean values of cardiorespiratory variables recorded at control, 10 min after the NTS injection of 60 pmol baclofen, and 5 min after 2.8 nmol CGP-35348. Baclofen prolonged Ti (P < 0.001) and had no effects on Te and Di. Compliance of the respiratory system decreased (P < 0.0001). Increases in MAP and HR were observed during the first 5 min after baclofen injection. However, 10 min after the injection, both values returned to their controls. Effects of baclofen were reversed by CGP-35348 (Table 1).

Effects of airway occlusion at end expiration. During control conditions, airway occlusion at end expiration increased Di by 12 ± 3% (P = 0.02) and prolonged Ti and Te by 66 ± 5 and 54 ± 11%, respectively (P = 0.0001 and P = 0.002). After baclofen, Ti prolongation was diminished in comparison with that before baclofen (P = 0.003), although the increase (24 ± 8%) from the immediate control value was still significant (P = 0.02). Te and Di were not affected and were significantly different from pre-baclofen responses (P = 0.05 and P = 0.001, respectively; Fig. 2). The effects of airway occlusion on the respiratory phases returned to normal after injection of CGP-35348: Ti and Te increased by 71 ± 7 and 59 ± 7%, respectively (P = 0.0001 and P = 0.0002, respectively). Effects of the NTS injections of baclofen were site independent.

Effects of lung deflation. Lung deflation elicited a prolongation of Ti, a shortening of Te, and a small increase in Di (Fig. 3, top traces). The effects increased with decreasing Ptr (Fig. 4). The Di increase of 12% reached significance (P = 0.05) only at Ptr of −5 cmH2O. Because of these small Di responses, further analyses were exclusive to effects of lung deflation on the respiratory timing phases. A decrease in Ptr to −2 cmH2O evoked significant changes during breath 1 in Ti and Te (P = 0.02 and 0.0001, respectively). At Ptr of −5 cmH2O, Ti of first two breaths (TI-1 and TI-2) increased by 30 ± 2 and 43 ± 6%, respectively (P < 0.0001 for both breaths), and Te of first two breaths (TE-1 and TE-2) decreased by 53 ± 4 and 33 ± 7%, respectively (P < 0.0001 for both breaths). Ti-1 and Ti-2 differed (P = 0.0003) at Ptr between −3 and −5 cmH2O, and TE-1 was shorter than TE-2 (P = 0.0002) at all step reductions in Ptr.

Baclofen prevented the shortening of TE and decreased the Ti response of both deflation breaths (Fig. 3, middle traces, and Fig. 5). A decrease in Ptr to −4 cmH2O was necessary to provoke a significant prolongation of Ti. At Ptr of −5 cmH2O, the Ti-1 and Ti-2 prolongations were 16 ± 5% (P = 0.002) and 17 ± 4% (P = 0.001), respectively, and were less than those at pre-baclofen conditions (P = 0.002 and P = 0.0001 for Ti-1 and Ti-2 comparisons, respectively). At all changes in Ptr, there were no significant differences between Ti-1 and Ti-2. CGP-35348 reversed effects of baclofen on the Ti and Te responses during breath 1 and breath 2 (Fig. 3, bottom traces, and Fig. 5). The effects of baclofen on the deflation reflex were independent of the NTS injection site. At all experimental steps between lung deflations, Crs remained constant.

Table 1. Cardiorespiratory variables recorded at each experimental step

<table>
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<tr>
<th></th>
<th>Di, arbitrary units</th>
<th>Ti, s</th>
<th>Te, s</th>
<th>VT, ml</th>
<th>Crs, ml/cmH2O</th>
<th>MAP, mmHg</th>
<th>HR, beats/min</th>
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<tr>
<td>Control</td>
<td>10</td>
<td>0.21±0.01</td>
<td>0.38±0.02</td>
<td>1.5±0.2</td>
<td>0.22±0.05</td>
<td>97±4</td>
<td>450±18</td>
</tr>
<tr>
<td>Baclofen</td>
<td>9.8±0.9</td>
<td>0.46±0.05</td>
<td>0.37±0.04</td>
<td>1.5±0.2</td>
<td>0.17±0.04*</td>
<td>97±10</td>
<td>461±24</td>
</tr>
<tr>
<td>CGP-35348</td>
<td>9±0.1</td>
<td>0.21±0.01</td>
<td>0.35±0.03</td>
<td>1.7±0.2</td>
<td>0.23±0.06</td>
<td>81±6*</td>
<td>446±22</td>
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Values are means ± SE for before (control) and after nucleus of the solitary tract injection of baclofen (60 pmol) followed by injection of CGP-35348 (2.8 nmol) in 7 rats. Di, peak amplitude of integrated diaphragmatic electromyogram; Ti, inspiratory time; Te, expiratory time; VT, tidal volume; Crs, compliance of total respiratory system; MAP, mean arterial blood pressure; HR, heart rate. *Significantly different from control (before baclofen).
DISCUSSION

In agreement with our earlier studies on rats (36, 40, 43) and others on cats (24, 32), baclofen, a GABA_B receptor agonist, prolonged Ti and had no effect on Te. In contrast to the baclofen-evoked block of the Te-promoting reflex, the Ti-inhibitory reflex after baclofen and the Ti component of the deflation reflex were attenuated but remained significant. In contrast, baclofen abolished changes in Te produced by either airway occlusion at end expiration or lung deflation. During lung deflation before baclofen, Ti increased and Te decreased. Prolongation of Ti was smaller and shortening of Te was greater during breath 1 than breath 2. After baclofen, the differences between these two breaths were no longer present. CGP-35348 reversed effects of baclofen on the breathing pattern and the Hering-Breuer reflexes.

An additional observation in this study was a decrease in Crs after baclofen and its recovery after CGP-35348. The mechanisms of this baclofen effect are not clear. Changes in the breathing pattern per se cannot give the explanation (28). Baclofen, spreading from the site of injection, could cause disinhibition on neurons of the vagus motor nuclei. As a consequence, the airway smooth muscle tension and the functional residual volume would increase as the result of air trapping. However, because rats were pretreated with atropine, this interpretation is unlikely. The absence of augmented breaths after baclofen and their abundance after CGP-35348 (43) may be a possible reason for the baclofen-evoked decrease in Crs.

The medullary pathways involved in the reflex and central control of Ti and Te are distinct (2, 9). The Ti-inhibitory reflex depends exclusively on activation of high-threshold receptors, and the response has all-or-nothing properties (9). The Te-promoting reflex is mediated by both high- and low-threshold slowly adapting vagal receptors (11). On the basis of studies of steady-state conditions in rats, the deflation reflex is considered to be mediated by two subgroups of slowly adapting vagal receptors: “deflation sensitive” and “inflation sensitive” (3, 34, 35, 45). In the present study, the differences between breath 1 and breath 2 suggest that vagal rapidly adapting receptors may contribute in transient effects of lung deflation. Slowly and rapidly adapting receptors converge on specific groups of the NTS neurons (2, 4, 8, 23, 26), and the target neurons for vagal control of Ti and Te may be scattered within the respiratory network. The different effects of baclofen on Ti and Te and the Ti and Te components of the Hering-Breuer reflexes may depend on diverse distribution of GABA_B receptors within the neuronal circuitry controlling either of these phases and/or the reflex pathways. Possible different effects of baclofen on different types of respiratory medullary neurons may support the above suggestion. In a study on cats, intravenous injections of low doses of baclofen increased the frequency of inspiratory neuron activity in the dorsal and ventral respiratory groups and abolished activity of a late inspiratory neuron (24). The diverse distribution of GABA_B receptors may be further suggested by predominant location of the mechanisms for medullary inspiratory and late expiratory inhibitions on different areas of the dendritic tree (22).
The baclofen-evoked prolongation of \( T_I \) and unaffected \( T_E \) resemble effects of suppression of the pontine pneumotaxic mechanism input. In animals with intact vagus nerves, elimination of the pontine input produces prolongation of \( T_I \) without changes in \( T_E \) (10, 13). A pharmacological blockade of the glutamate N-methyl-D-aspartate (NMDA)-receptor subgroup has similar to the above effects on the respiratory timing (12, 13, 20, 21, 33). Within the pneumotaxic mechanism, NMDA receptors may contribute to \( T_I \) termination by activa-

**Fig. 3.** Effects of a drop in tracheal pressure (Ptr) to 5 cmH\(_2\)O below atmospheric on integrated diaphragmatic electromyogram (DiEMG) in a representative rat before baclofen (top traces), after bilateral NTS injection of baclofen (middle traces), and after CGP-35348 injected to the same NTS location (bottom traces). Ptr calibration and time scale are the same for all. Horizontal lines indicate duration of lung deflation. Instantaneous effects of lung deflation were evaluated from the first 2 breaths of lung deflation.

**Fig. 4.** Group mean effects of decreasing Ptr between \(-2\) and \(-5\) cmH\(_2\)O before baclofen on \( T_I \) (A) and \( T_E \) (B) of first (breath 1) and second (breath 2) breaths during lung deflation in 7 rats. Changes in \( T_I \) and \( T_E \) are expressed in percent changes from their immediate pre-deflation values presented in the graphs at Ptr of 0 cmH\(_2\)O as 100%. Changes in \( T_I-1 \) and \( T_E-1 \) were always significant (\( P = 0.02-0.0001 \) for \( T_I \), and \( P = 0.0001 \) for all \( T_E \) values). ††Significant differences between \( T_I-1 \) and \( T_I-2 \) (\( P = 0.0003 \)). †Significant differences between \( T_E-1 \) and \( T_E-2 \) (\( P = 0.0002 \)). Bars show SE.
tion of the medullary late-inspiratory neurons, also identified as the Ti off-switch neurons (12, 13). Like baclofen, lesions in the site of the pneumotaxic mechanism (10, 11) and blockade of NMDA receptors (12, 13) increase the threshold but do not abolish the Ti-inhibitory reflex. In contrast to effects of baclofen, the Te-promoting reflex is effective after either of the interventions (12, 13). Therefore, baclofen may affect signal transmission between neurons controlling Ti and afferent pathways of vagal slowly adapting receptors affecting Te but not Ti.

The baclofen-induced attenuation of the Ti-inhibitory reflex and the Ti component of the deflation reflex may be due to activation of postsynaptic GABA \(_B\) receptors, and hyperpolarization of inspiratory neurons, and/or activation of presynaptic GABA \(_B\) receptors and disfacilitation of the Ti off-switch neurons receiving input from slowly adapting vagal receptors (16). Either site of baclofen action would decrease excitability of the Ti off-switch. We would expect both sites to play a role because our administration of baclofen would not selectively affect one location over another, and in vitro studies show baclofen-induced changes at both pre- and postsynaptic locaions (6, 41, 42). Hyperpolarization or disfacilitation of inspiratory neurons may also explain the lack of Di increase during airway occlusion at end expiration. The presence of the Ti-inhibitory reflex after baclofen agrees with the observation of preserved ability of vagal slowly adapting receptors to induce Ti off-switch following a systemic application of large (8–12 mg/kg) doses of baclofen in artificially ventilated anesthetized or decerebrated cats (32). However, in the latter study, effects of baclofen on the intensity of the Ti-inhibitory reflex have not been evaluated. The involvement of GABA \(_B\) receptors in modulation of respiratory reflexes mediated by rapidly adapting vagal receptors was suggested in this study by the lack of the difference between breath 1 and breath 2, and in this and previous studies by the absence of augmented breaths after baclofen and their abundance after CGP-35348 in baclofen-treated rats (43). These observations imply that besides the medullary pathways of the Hering-Breuer inflation reflexes, GABA \(_B\) receptors are present on the medullary pathway of vagal fibers mediating instantaneous reflex effects of lung deflation in rats.

Evaluation of the Hering-Breuer deflation reflex. In the majority of mammals, Ti prolongation and Te shortening during lung deflation depend on a decreased slowly adapting receptor activity and activation of rapidly adapting receptors (7). In the rat, examination of pulmonary reflexes mediated by rapidly adapting vagal receptors is difficult. These receptors have low sensitivity to a steady-state deflation (3, 34, 35, 45). Histamine, described as a specific stimulus for rapidly adapting receptors, leading to a rapid and shallow respiration in other species (19), in rats produced apnea as the primary effect (unpublished observation). Thus the effect of histamine was similar to that provoked by application of phenylbiguanide in rats (36), a chemical used to stimulate pulmonary C endings (7, 19). Therefore, we could not use histamine as a tool for evaluation of respiratory reflexes mediated by rapidly adapting vagal receptors. On the basis of the studies of the steady-state conditions in rats, we know that lung deflation affects two subgroups of slowly adapting vagal fibers: activity of “deflation-sensitive” receptors increases and that of “inflation sensitive” receptors decreases (3, 34, 35, 45). The transient effects of lung deflation have not yet been well defined (45). We propose that the greater shortening of Te during breath 1 than breath 2 of deflation is likely to reflect the short-lasting activation of rapidly adapting vagal receptors during breath 1. On the other hand, a greater prolongation of Ti during breath 2 than breath 1 may depend on a decrease in “inflation-sensitive” receptors combined with activation of “deflation-sensitive” receptors. The differences in breath 1 and breath 2 could not depend on increased chemoreceptor activity. The rats breathed oxygen. Therefore, during the time of the first two deflation breaths, \(P_{O_2}\) in arterial blood could not decrease to levels stimulating peripheral chemoreceptors. In addition, increased \(CO_2\) levels following breath 1 would contribute to Te shortening during breath 2 but not breath 1. Finally, small and a similar increase in Di of both breaths argues against any detectable deflation-related changes in chemical drive.
Limited effects of method of injection. A relatively large volume of the NTS injections could cause tissue damage, transient changes in the extracellular pressure, alterations in the concentration of ions in the extracellular fluid, and large gradients in concentration of baclofen in the surrounding brain tissues (15, 25, 29). Despite the obvious limitations of the injection method, it is unlikely that effects of baclofen on Ti and the vagal control of Ti and Te rely on volume- or pressure-induced artifacts. To avoid rapid changes in the interstitial pressure, the injections of volumes of 110 nl were slow and lasted up to 30 s. Injection of the same volume of saline had no effects on the breathing pattern nor the Hering-Breuer reflexes. After CGP-35348, a GABA_\text{B}_2 receptor antagonist, injected into the baclofen-injection sites, Ti and the Ti-inhibitory and deflation reflexes returned to normal, demonstrating that respiratory effects of baclofen were related to activation of GABA_\text{B}_2 receptors rather than to tissue damage. Finally, in a study of Sved and Tsukamoto (39), describing reversible effects of baclofen on the baroreflex in rats, the volume of the NTS injections was similar to that in the present study.

In summary, these results extend our earlier observations of inhibitory effects of baclofen on the pulmonary chemoreflex and the Hering-Breuer Te-promoting reflex and imply that GABA_\text{B}_2 receptors may also modulate the Hering-Breuer Ti-inhibitory and deflation reflexes. The difference in effects of baclofen on the central control of Ti and Te suggests different involvement of GABA_\text{B}_2 receptors in control of each of these respiratory phases. The effective Ti-inhibitory reflex and the absence of Te component of the deflation reflex suggest that GABA_\text{B}_2 receptors may affect the synaptic transmission of vagal activity converging on medullary neurons controlling Te but not Ti. We propose that the prolongation of unloaded Ti and decreased effects of airway occlusion and lung deflation on Ti may depend on 1) a delayed Ti off-switch due to activation of postsynaptic GABA_\text{B}_2 receptors and hyperpolarization of neurons responsible for Ti termination and/or 2) disfacilitation of these neurons due to presynaptic action of baclofen.

Perspectives

Diverse effects of baclofen on the central and reflex control of Ti and Te imply a complex role of GABA_\text{B}_2 receptors in control of respiration in the rat. We discuss possible involvement of GABA_\text{B}_2 receptors located within the site of the pneumotaxic mechanism in the baclofen-evoked prolongation of Ti and attenuation of the Ti-inhibitory reflex. This site of baclofen action could be examined by evaluating the Hering-Breuer inflation and deflation reflexes following microinjections of baclofen into the specific pontile areas. Also we propose the baclofen-evoked inhibition of the Ti off-switch neurons as a possible explanation of a decreased excitability of the Ti off-switch. Indeed, inhibition of late-inspiratory neuron activity by the same dose of baclofen that produced increased frequency of firing in inspiratory neurons in cats, reported by Lalley (24), suggests that baclofen may cause hyperpolarization of the Ti off-switch neurons and disfacilitation on inspiratory neurons. However, because effects of baclofen on different types of respiratory neurons are based on records from a limited number of cells (1 late-inspiratory neuron and 8 inspiratory neurons), more studies are necessary to confirm these effects. Because rats only recently became the most popular animals in the respiratory laboratory, control of respiration in the rat is not yet well understood. The results of the present study allow us to speculate on organization of the central control of breathing and add to our knowledge of the Hering-Breuer reflexes in this species.

The authors thank Ciba-Geigy, Basel, Switzerland, for the generous gift of (−)-baclofen and CGP-35348. This study was supported by the Quebec Lung Association. E. Seifert was a recipient of an award from Fonds Pour la Formation de Rechercheurs et l’Aide a la Recherche.

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Received 14 July 1997; accepted in final form 13 October 1997.

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