Small reduction of neurokinin-1 receptor-expressing neurons in the pre-Bötzinger complex area induces abnormal breathing periods in awake goats

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The basic neural substrate required for respiratory rhythmogenesis is hypothesized to be the pre-Bötzinger complex (pre-BöC). In 1991, Smith et al. (26) demonstrated, in the isolated neonatal rat brain stem-spinal cord preparation, that elimination of this area eliminated respiratory-related rhythm. Histological investigation of the pre-BöC has demonstrated an increase in the substance P receptor neurokinin-1 (NK1R) density in this area, which presumably provides an anatomic marker for the pre-BöC (10, 12, 32, 34). Additionally, in vivo injection of the glutamate analog DL-homocysteine in anesthetized cats or rats (3, 19, 21, 27) uniquely increases (tonic and/or phasic) phrenic nerve discharge and thus provides a physiological definition of the pre-BöC.

Despite these advances, it is only recently that the role of this hypothesized respiratory “kernel” has been tested in an awake animal. Effects on breathing in awake rats from lesioning this region were reported in 2001 by Gray et al. (10), who injected into the pre-BöC a slow-acting neurotoxin [saporin conjugated to substance P (SP-SAP)] specific for NK1R-expressing neurons. Six days after the injection, in the awake state, an “ataxic” breathing pattern was evident, the rats were hypoxic and hypercapnic, and they had a reduced CO2 sensitivity. These effects only occurred with >80% reduction of NK1R-expressing neurons in the pre-BöC. Gray et al. (10) concluded that “…normal breathing in awake animals requires an intact pre-Bötzinger complex…”

The present study was designed to gain further insight into the function of the pre-BöC under various physiological conditions. Accordingly, because rhythm generation is the presumed role of the pre-BöC, we tested the hypothesis that the ataxic breathing pattern with pre-BöC lesioning reflects changes in rhythm with no change in pattern. To achieve this objective, we monitored breathing and respiratory muscle activity on a breath-by-breath basis in awake and asleep goats inhaling room air and in awake goats while inhaling CO2-enriched gas mixtures before and after SP-SAP-induced lesions in the pre-BöC area. Studies were completed during hypercapnia to determine whether irregular breathing with pre-BöC lesions would be eliminated, as usually occurs with other types of irregular breathing (1, 9, 14). Studies were completed during sleep because of the postulate by others that neural mechanisms responsible for generation of the respiratory rhythm are state dependent (24, 25).

MATERIALS AND METHODS

All physiological studies were completed during wakefulness or sleep on nine female and one castrated male adult goats (35–55 kg), in accordance with protocols approved by the Medical College of Wisconsin Animal Care Committee guidelines. The goats were housed, and physiological studies were completed in an environmental chamber with free access to food and water, except for periods of the studies. The goats were trained to stand or lay sternally in a stanchion. In six goats, no physiological studies were completed, but these goats

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were killed, and the medulla was extracted and preserved for histological analysis.

**Experimental Design**

After initial instrumentation surgery, eupneic breathing, blood gases, and CO₂ sensitivity measurements established the normal respiratory characteristics of the goats. The goats then underwent surgery for chronic implantation of microtubules bilaterally into the medulla. Two weeks later, after again completing the aforementioned measurements, a 5.5-h time control study was completed. On a subsequent day, the neurotoxin (SP-SAP) was bilaterally injected, and the acute (within the first 5 h, see procedures below) effects were recorded. Over the next 2 wk, the chronic effects of neurotoxin injection were assessed daily by measurements listed above.

**Surgical Procedures**

Animals were anesthetized initially with xylazine-ketaset (1:24) 1.4 ml iv, and, after intubation, anesthesia was maintained with 1.5% halothane in 100% O₂. Under sterile conditions, initial surgery was performed to elevate carotid arteries to just below the skin and to insert electrodes into the diaphragm and three upper airway muscles (thyroarytnoid, posterior cicoidarytnoid, and inferior pharyngeal constrictor), as well as placement of electrooculogram and midline electroencephalogram (EEG) electrodes (33). Chronic implantation of microtubules (stainless steel, 70 mm in length) was completed under stereotaxic guidance (4). Briefly, an occipital craniotomy was performed to expose the cerebellum and dorsal medullary surface. The midline, dorsal surface, and obex served as reference points in the medial-lateral, dorsal-ventral, and rostral-caudal directions, respectively. Because of the consistency of medullary anatomic landmarks among rats, cats, and goats (2, 4, 10, 27), we presumed that the pre-BötzC was located just ventral to the rostral pole of nucleus ambiguous (NA) and caudal to the retrofacial nucleus. Indeed, as in rat (10, 32), in this area of the goat, there is an increase in density of NK1R-immunoreactivity (ir) (Fig. 1). Thus, in nine goats, the target for placement of the microtubule tip was the dorsal portion of the rostral NA. To gain insight into whether physiological responses to NK1R lesions were unique to the pre-BötzC, microtubules were placed in one goat in the caudal NA on one side and into the vestibular nucleus on the other side. After implantation, microtubules were secured with dental acrylic and cranial screws.

Because of initial findings, in four goats, a tracheostomy was created 8–10 days after injection of SP-SAP, and, in two goats, electrodes were placed into three abdominal (Abd) muscles on the day the microtubules were implanted. For the tracheostomy, a 5-cm longitudinal midline incision was made just caudal to the cricoid cartilage. Following cauterization of four consecutive intercartilage rings, a midline incision through three to four cartilage rings was made, and, to prevent closure of the tracheostomy, the skin was sutured to the cartilage edges with Vicryl sutures. To place electrodes into the Abd muscles, a 5- to 8-cm lateral incision was made ventral and caudal to the 12th rib. Three distinct layers of Abd muscle were identified, and electrode wires were individually sutured into the muscle, secured, and externalized.

After the initial instrumentation surgery, goats were medicated with ceftiofur sodium (6 mg/kg im, every day) to minimize infection. After brain implantation surgery, dexamethasone was given (internal jugular, 3 times a day for 7 days, starting dose of 4.0 ml and decreasing by 0.5 ml per day) to minimize brain swelling. Antibiotics [ceftiofur sodium (every day) and chloramphenicol (20 mg/kg iv, 3 times a day) for the first 3 days and gentamycin (6 mg/kg, every day) thereafter] were administered for the duration of the studies.

**Procedures and Protocols**

**Daily measurements.** To monitor inspiratory flow (Vₐ), a pneumotachograph was attached to the inspired port of a breathing valve. The valve was attached to either a muzzle mask (airway intact) or endotracheal tube (tracheostomized). The pneumotachograph was connected to a Grass recorder, which was connected to a Citus 436 computer. Diaphragm activity and (when instrumented) upper airway and Abd muscle activities were also recorded. The elevated carotid artery...
was chronically catheterized to monitor arterial blood pressure and to obtain blood samples for pH, arterial \( \text{P}CO_2 \) (\( \text{PaCO}_2 \)), and arterial \( \text{P}O_2 \) determination. Rectal temperature was also measured after each blood sample was taken. For most studies during the day, \( \text{Vt} \) and respiratory muscle activity were continuously monitored over a 45-min period. During the first 30 min, the goats were inhaling room air, but then, to assess \( CO_2 \) sensitivity, the goats were exposed to three consecutive 5-min periods of 3, 5, and 7% \( CO_2 \). Arterial blood was sampled during the last 2 min of exposure at each level.

**SP-SAP or time control study.** On one day, to assess the normal variation on physiological functions, breathing and respiratory muscle activities, arterial blood pressure, and heart rate were monitored continuously during eupnea over 5.5 h with arterial blood sampled every hour. To assess the acute effects of injecting the neurotoxin, the same physiological functions were monitored for 5.5 h on another day. After a 0.5-h control period in eight goats with microtubules implanted bilaterally into the pre-Bo\( ^tZ \)C, a unilateral injection (50 \( \text{pM} \) in 10 \( \mu\)l) of the neurotoxin SP-SAP was made into one microtubule, and 1 h later an identical injection was made into the contralateral microtubule. This SP-SAP dose is larger than those injected in rats (10, 33) and was chosen because of the larger size of the goat medulla and because we wanted to maximize the lesion size. As a vehicle control, \( \text{SAP} \) (50 \( \text{pM} \) in 10 \( \mu\)l) was similarly microinjected bilaterally into the pre-Bo\( ^tZ \)C of a 9th goat, and, as a site control, SP-SAP was injected into nuclei outside of the pre-Bo\( ^tZ \)C in a 10th goat.

To assess the effects of SP-SAP injection on sleep, goats were studied from 2000 until 0300 during the night before and during the night 7 days after SP-SAP injections. Heart rate, arterial blood pressure, respiratory muscle activity, EEG, and electrooculogram activities were recorded continuously. Articular blood samples were drawn periodically during wakefulness and various sleep stages. Sleep analysis has been described previously (18). The awake state was defined as low-voltage, mixed-frequency EEG. Non-rapid eye movement (NREM) was defined as a synchronized low-frequency EEG (\( \approx2\) Hz) with amplitude two to three times greater than found while awake with concurrent absence of rapid eye movements (REMs). REM sleep was defined as a desynchronization of the EEG with relatively low-voltage, mixed-frequency EEG with frequent REMs. REMs were distinguished from eye blinks as they were defined as sharp waves with an amplitude of \( >30\,\text{mV} \) change from baseline.

Computerized computations were made of pulmonary ventilation (\( \text{Vt} \)), tidal volume, and breathing frequency. For statistical testing, individual goat data were grouped. Linear regression analysis was used to calculate ventilatory \( CO_2 \) sensitivity [change in (\( \Delta \)\( \text{Vt} \)/\( \Delta \text{P}CO_2 \), \( \text{l-min}^{-1} \cdot \text{Torr}^{-1} \)]]. One-way or two-way ANOVA was used to determine differences in mean data, which, when \( P \) value was \( <0.05 \), a Bonferroni post hoc test was used to detect specific differences.

**Histology.** On termination of the goat, the brain was flushed (10% sucrose in saline) and perfusion fixed (4% paraformaldehyde in saline). The brain stem was removed, postperfusion fixed (24 h, 4% paraformaldehyde), cryoprotected (30% glucose, 2–10 days), frozen, and sectioned (25 \( \mu\)m) into four series onto gelatin-coated slides. One series was stained with hematoxylin and eosin (H&E) for identification of dead or dying neurons (pink and altered morphology) used as one estimate of the area and extent of a lesion (33). In another series, tissue slides were rehydrated, stained with hematoxylin (3 min), tap water rinsed, dipped in 0.5% acid alcohol, tap water rinsed, exposed to eosin (30 s), and dehydrated in ascending alcohols to xylene before coverslipping.

Another series was used for immunohistochemistry. These slides were rinsed with Tris buffer, avidin and biotin blocked (30 min each), and protein digested (pepsin, 7 min). After peroxidase and protein block (20 min each), slides were exposed to rabbit anti-NK1R antibody (Chemicon, 1:3,000) for 2 h at room temperature with an identical application for another 2 h immediately following. Slides were rinsed, exposed to biotinylated anti-rabbit antibody, rinsed, reacted with diaminobenzidine (10 min), rinsed with Tris buffer, dehydrated (alcohol series to xylene), and coverslipped.

To establish NK1R expression in the rostral part of the ventrolateral respiratory column (VLRC) of normal goats, NK1R-expressing neurons were bilaterally counted at 200-\( \mu \)m intervals in a 1.2 \( \times \) 1.8 mm area just ventral to NA and medial to spinal trigeminal nucleus, beginning 2.0 mm rostral to the obex, and extending to 4.0 mm rostral to the obex. Similarly, to determine NK1R-expressing neurons lesioned in SP-SAP-injected goats, counts of NK1R-expressing neurons were bilaterally made over the same area in these goats. Percent NK1R-expressing neurons were calculated from total neurons divided by NK1R-expressing neurons times 100.

**RESULTS**

**Histology**

We found immunoreactivity for NK1R in the VLRC, the raphe nuclei, and the nucleus tractus solitarius and in some other nuclei. As in the rat (11, 34), in the goat there is an increase (\( P < 0.001 \)) in the percentage of NK1R-expressing neurons within the VLRC, starting at the rostral pole of NA and medial to spinal trigeminal nucleus, beginning 2.0 mm rostral to the obex, and extending to 4.0 mm rostral to the obex. Similarly, to determine NK1R-expressing neurons lesioned in SP-SAP-injected goats, counts of NK1R-expressing neurons were bilaterally made over the same area in these goats. Percent NK1R-expressing neurons were calculated from total neurons divided by NK1R-expressing neurons times 100.

**Effects of Microtubule Implantation Surgery**

By 14 days after microtubule implantation, eupneic \( \text{P}CO_2 \), \( \text{CO}_2 \) sensitivity did not differ from preimplant values (39.2 \( \pm \) 1.0 and 39.6 \( \pm \) 1.3 Torr, 1.9 \( \pm \) 0.3 and 2.0 \( \pm \) 0.3 \( \text{l-min}^{-1} \cdot \text{Torr}^{-1} \) pre- and postimplant, respectively). Because
SP-SAP injections were not made until after complete surgical recovery, our studies provide a valid assessment of the acute and chronic effects of SP-SAP injections.

**Acute Effects of SP-SAP**

The major acute (within the first 5 h) effects of SP-SAP injection were as follows: 1) an increased frequency of augmented breaths and 2) an increased frequency of abnormal breathing periods (ABPs). The ABPs consisted of a rapid series of small, complete inspiratory-expiratory cycles with coordinated respiratory muscle activities (Figs. 3 and 4, B and C) identical to the fractionated breaths previously reported (8). For each small breath within the period, respiratory time and volume values were 40–60% less than those of normal breaths ($P < 0.01$). These periods were not associated with other behaviors that elicit changes in breathing pattern, such as movement, regurgitation, coughing, swallowing, vocalizations, panting, or sniffing.

**Effects of SP-SAP 10–14 Days After Injection**

Ten to fourteen days after bilateral injection of SP-SAP into the pre-BötzC, the frequency of ABPs was increased sixfold ($P < 0.001$, Table 1). These ABPs exhibited one of two characteristics: 1) rapid, small, complete breaths with coordinated inspiratory-expiratory cycles (fractionated breaths, described above) or 2) breaths of varying duration and volume.

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*Fig. 2. Living, dying, and NK1R-expressing neurons in the pre-BötzC area in a control and lesioned goat. Presented are examples in an unoperated (control) goat (A, C, and E) and an SP-SAP-lesioned goat (B, D, and F) of hematoxylin and eosin (H&E) staining (A and B) and immunohistochemistry for NK1R (C, D, and F). Note in the H&E-stained slides the presence of living (arrows) or dead neurons (arrowheads) are present only in the lesioned goat. Additionally, NK1R-expressing neurons, characterized by punctate extracellular staining on the soma and dendrites (arrowheads) compared with the non-NK1R-expressing neurons (arrows) characterized by diffuse exclusively intracellular staining, are more abundant in the control goat than in the SP-SAP lesioned goat (C and D, respectively). Differences between NK1R-expressing and non-NK1R-expressing neurons shown in D are more clearly shown at higher magnification (F, ×20 magnification). E: in the H&E-stained medullary hemisection, the rectangle depicts the area of histological analysis.*
with an altered respiratory muscle activation pattern. In three goats, the ABPs were exclusively of the former type, whereas, in two goats, they were exclusively of the later type (Table 1). In the remaining three goats, ABPs were of both types (Table 1). ABPs were recorded in airway-intact as well as tracheostomized goats.

A prominent feature of the altered muscle activation patterns was a change from normal in the intensity and timing of Abd muscle activity. Often there was brief Abd muscle activity during diaphragm activity that interrupted $V_I$ (Fig. 5B). At other times, there was strong, prolonged Abd muscle activity simultaneous with diaphragm activity, resulting in an apnea ($13.3 \pm 2.9/h$, Fig. 5C). Frequently, there was stronger than normal Abd activity during expiration, and the subsequent $V_I$ either was augmented (Fig. 5A) or preceded diaphragm activity, thus indicating a partially passive inspiration ($14.6 \pm 3.7\%$ of all breaths, Fig. 6). Altered activation patterns of airway dilator and constrictor muscles were also occasionally observed (data not shown).

The frequency and characteristics of altered breathing patterns were not altered by increasing inspired CO₂ to 3, 5, or 7% (Fig. 7, $P > 0.10$). In addition, these events were the same in the awake state during the day and during night studies. However, pathological breathing patterns were eliminated during NREM sleep in seven of eight goats, but they frequently occurred on arousal from NREM sleep (Fig. 8).

Daytime eupneic $V_I$ and PaCO₂, and CO₂ sensitivity were not altered by the SP-SAP lesions ($P > 0.10$, Table 2). In addition, PaCO₂ at night was not elevated after the lesions during awake or sleep states (Table 2, $P > 0.10$). However, after the SP-SAP lesions, the goats were awake for a larger percentage of the night period compared with before the injections (pre vs. post, $61.0 \pm 2.8$ vs. $73 \pm 2.3\%$, $P < 0.01$), and there was less time in NREM sleep state ($35.5 \pm 1.8$ vs. $24.2\pm3.0\%$, $P < 0.01$), with an insignificant reduction in REM time.

Control Goats

There was no evidence of ABPs or increased number of augmented breaths in site control or vehicle control goats (data not shown).

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**Table 1.** Small bilateral reduction of NK1R-expressing neurons in the pre-Bötzer complex increases incidence of abnormal breathing periods, breaths with prolonged expiratory duration, and partially passive inspiratory flow

<table>
<thead>
<tr>
<th>Goat No.</th>
<th>NK1R, % Prelesion</th>
<th>NK1R, % Post-SP-SAP lesion</th>
<th>ABP, no./h Prelesion</th>
<th>ABP, no./h Post-SP-SAP lesion</th>
<th>FBr, no./h Prelesion</th>
<th>FBr, no./h Post-SP-SAP lesion</th>
<th>PTE, no./h Prelesion</th>
<th>PTE, no./h Post-SP-SAP lesion</th>
<th>PPIF, %total breaths Prelesion</th>
<th>PPIF, %total breaths Post-SP-SAP lesion</th>
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ABP, abnormal breathing periods; SP-SAP, saporin conjugated to substance P; NK1R, neurokinin-1 receptor (% total pre-Bötzer complex neurons that are neurokinin-1 receptor expressing); FBr, fractionated breaths; AMAP, altered muscle activation pattern (see text for details); PTE, prolonged expiratory duration; PPIF, partially passive inspiratory flow.
DISCUSSION

Major Findings

The major findings of this study were a 29% reduction in NK1R-expressing neurons in the pre-BötC bilaterally and a sixfold increase in ABPs 10 to 14 days after injection of SP-SAP into the pre-BötC area of awake goats. The characteristics of these events are consistent with transient changes in respiratory rhythm and/or pattern generation.

What Anatomic Sites Were Lesioned in the Present Study?

Like the rat (11, 32), in the goat, there is an increase in the percentage of NK1R-expressing neurons within the VLRC just

Fig. 4. Bilateral injection of SP-SAP into the pre-BötC area of awake goats elicits augmented breaths (B) and transient changes in respiratory rhythm (C). Shown for 1 goat are inspiratory flow (Vi) and raw diaphragm (Dia) and abdominal (Abd) muscle activities before (A) and +2 h (B and C) after SP-SAP injections in the pre-BötC area. These data are representative of all goats.

Fig. 5. Large expiratory muscle and simultaneous inspiratory muscle activities result in transient changes in respiratory rhythm. A: shown are representative tracings of Vi and raw Dia and Abd muscle activity during simultaneous Dia and Abd muscle activity, resulting in no Vi. B: when Abd activity stopped before Dia activity, the subsequent Vi was larger than normal. C: when Abd activity continued after cessation of Dia activity, then the subsequent inspiratory airflow began before Dia activity, indicating a partial passive inspiration.
ventral to the rostral pole of NA. In the goats that had bilateral injection of SP-SAP into this area (n = 8), NK1R-ir was reduced by 29% 2.6 to 3.6 mm rostral to obex. For reasons explained in the companion paper (35), 6 h before medullary harvest, ibotenic acid was injected into the same site as SP-SAP. During this 6-h period, some neurons might have lost their NK1R-ir solely because of the neurotoxic effect of the ibotenic acid. If this is the case, we have overestimated the effect of the SP-SAP toxin. We consider this possibility unlikely because, in the one goat that received only SP-SAP injection, the reduction in NK1R-ir was similar to that in the goats that received both SP-SAP and ibotenic acid.

Several studies have established unique physiological responses when receptor agonists were injected into the pre-
BötzC area (3, 11, 15, 19, 21, 27, 34), thus providing physiological identification of the pre-BötzC. For example, substance P, which binds to the NK1R uniquely, increases the frequency of augmented breaths (15) or increases hypoglossal nerve discharge frequency when injected into the pre-BötzC area (11). Moreover, glutamate receptor agonists uniquely increase phrenic and/or hypoglossal nerve discharge frequency when injected into the pre-BötzC area (3, 19, 21, 27, 33). In the present study, we found an increase in the frequency of augmented breaths for the first few hours after injection of SP-SAP, which supports the postulate of Lieske et al. (15) that the pre-BötzC is involved in the generation of these breaths. In addition, in these goats, the glutamate receptor agonist ibotenic acid induced a marked tachypnea for >1 h after the injection (35). Accordingly, these physiological responses demonstrate that the injections were made into the pre-BötzC-area.

The histological analysis indicated a relatively small number of dead neurons beyond the pre-BötzC area. These lesions in goats are thus comparable to the lesions in rats (10, 34). Pathological patterns of breathing that we have observed after SP-SAP injection could be, at least in part, due to destruction of some neurons adjacent to the pre-BötzC area. However, as summarized above, stimulation of neurons in these adjacent areas has minimal effect on breathing; thus destruction of these adjacent neurons would also have minimal effect on breathing.

**Physiological Status of Goats Before SP-SAP Injection**

Before the injection, measured variables were within the normal range for goats, which normally have a regular, eupneic breathing pattern with augmenting diaphragm activity throughout inspiration and Abd muscle activity during the last two-thirds of expiration (Fig. 3). One to two augmented breaths occur each hour, and fractionated breaths occur even less frequently (Fig. 1, Table 1). Sleep architecture in goats is typical of farm animals in that it is more fractionated and there is less NREM and REM sleep than in humans (18).

**What Insights Did We Gain Into the pre-BötzC Function?**

We hypothesized that an ataxic or irregular breathing pattern resulting from pre-BötzC area lesions (>6 days after SP-SAP injections) would manifest as transient changes in rhythm with no change in respiratory pattern. Indeed, 10–14 days after the SP-SAP injection, transient changes in rhythm increased (pre- vs. post-SP-SAP, 17.3 ± 7.4 vs. 47.6 ± 22.2/h) (Table 1) indicating transient changes in respiratory rhythm-generating mechanisms. However, another effect of the lesions were ABPs associated with altered respiratory muscle activation patterns, resulting in elimination of the eupneic breathing pattern (60.1 ± 22.6/h). These periods were characterized by highly irregular breaths within the period, and these were not observed before the SP-SAP injections. Breaths with expiratory durations greater than twice normal (13.3 ± 2.9/h), and breaths with a passive contribution to Vt (14.6 ± 3.7% of breaths) were examples of observations resulting from changes in the activation pattern of respiratory muscles.

The characteristics and frequency of the ABPs were not altered by increasing inspired CO₂. Our laboratory had previously found that inspired CO₂ did not eliminate transient changes in respiratory rhythm caused by lesions in nuclei rostral to the pre-BötzC (11). However, irregular breathing associated with either above or below normal chemoreceptor sensitivity is eliminated by increasing inspired CO₂ (1, 9, 14). Thus it seems that the present changes are unique and may reflect a disturbance in a basic neural circuit.

ABPs occurred at night during wakefulness but not during NREM sleep in seven of eight goats. Additionally, these events were almost always associated with an arousal from NREM sleep. Excitatory inputs to respiratory neurons are decreased during NREM sleep, and such inputs increase with arousals (5, 22, 23). Of interest was that the SP-SAP lesions resulted in a significant (P < 0.01) reduction of NREM sleep time and an increase in awake time that were beyond the normal values for goats (18). This finding is not unique to pre-BötzC lesions (18). The relationship (if any) between these findings and the absence of ABPs in most goats during NREM sleep is not apparent.

It has generally been assumed that the proper sequential activation of respiratory muscles is a function of a hypothesized respiratory pattern generator that is separate from the respiratory rhythm generator (7, 19, 21). Attempts to locate the site of pattern generation have been unsuccessful (19, 21). As a result, it has been postulated that the pre-BötzC area is a site important to both rhythm and pattern generation (25). Indeed, one possible explanation for the observed altered breathing patterns is that the SP-SAP injections lesioned pattern-generating neurons within the pre-BötzC area. Other possible explanations include the following: 1) destruction of interneurons between pre-BötzC area rhythm-generating neurons and pattern-generating neurons at another site, 2) disruption of the normal coordination between the pre-BötzC inspiratory rhythm generator and the recently discovered, more rostrally located expiratory muscle rhythm generator (6, 13, 20), and 3) disruption of a medullary-pontine network postulated necessary for an eupneic breathing pattern (16, 17, 28–31). Supposedly, the pre-BötzC inspiratory rhythm generator is normally dominant, and it inhibits the expiratory generator (6, 13, 20). Thus the pre-BötzC neuronal destruction after the SP-SAP injection may have attenuated this inhibition, resulting in inappropriate temporal activity of the rostral rhythm generator and expiratory muscles.

The present data do not permit distinguishing between the above alternative explanations. It seems clear, however, that a
modest reduction in NK1R-expressing neurons disrupted a neuronal circuit involved in rhythm and/or pattern generation. That the transient changes were greatly attenuated during NREM sleep may suggest that excitatory inputs associated with wakefulness have a differential effect on components of this neuronal circuit. Alternatively, with reduced excitatory inputs during NREM sleep, the compromised neuronal circuit may be capable of generating a stable rhythm and provide for proper sequential activation of respiratory muscles. Finally, others have postulated that rhythm-generating mechanisms are state dependent (24, 25); thus it is conceivable that, during NREM sleep, respiratory rhythm is generated by a more “rudimentary” mechanism that is not dependent on a full complement of pre-Bötzinger NK1R-expressing neurons (25).

In summary, the major new insight into pre-Bötzinger function in the awake state is that even a moderate reduction in NK1R-expressing neurons in the pre-Bötzinger area transiently disrupts the normal eupneic respiratory rhythm and/or pattern-generating mechanism(s). Additionally, our findings may support the concept of the existence of dual respiratory rhythm generators normally dominated by the pre-Bötzinger (inspiratory) rhythm generator. Finally, our data appear to support the concept of state dependency of respiratory rhythm- and pattern-generating mechanisms.

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