Potential switch from eupnea to fictive gasping after blockade of glycine transmission and potassium channels

WALTER M. ST.-JOHN,[1] ILYA A. RYBAK,[2] AND JULIAN F. R. PATON[3]
[1]*Department of Physiology, Dartmouth Medical School, Lebanon, New Hampshire 03756;*
[2]*School of Biomedical Engineering, Science and Health Systems, Drexel University,*
Philadelphia, Pennsylvania 19104; and [3]*Department of Physiology, School of*
Medical Sciences, University of Bristol, Bristol BS8 1TD, United Kingdom

St.-John, Walter M., Ilya A. Rybak, and Julian F. R. Paton. Potential switch from eupnea to fictive gasping after blockade of glycine transmission and potassium channels. *Am J Physiol Regul Integr Comp Physiol* 283: R721–R731, 2002. First published July 8, 2002; 10.1152/ ajpregu.00004.2002.—This study evaluated possible neuronal mechanisms responsible for the transition from normal breathing (eupnea) to gasping. We hypothesized that a blockade of both inhibitory glycinergic synaptic transmission and potassium channels, combined with an increase in extracellular concentration of potassium, would induce a switch from an eupneic respiratory pattern to gasping. Efferent activities of the phrenic, vagal, and hypoglossal nerves were recorded during eupnea and ischemia-induced gasping in a perfused in situ preparation of the juvenile rat (4–6 wk of age). To block potassium channels, 4-aminopyridine (4-AP, 1–10 μM) was administered. Strychnine (0.2–0.6 μM) was used to block glycinergic neurotransmission. After administrations of 4-AP, excess extracellular potassium (10.25–17.25 mM), and strychnine, the incrementing pattern of eupneic phrenic activity was altered to a decrementing discharge. Hypoglossal and vagal activities became concentrated to the period of the phrenic burst with expiratory activity being reduced or eliminated. These changes in neural activities were similar to those in ischemia-induced gasping. Results are consistent with the concept that the elicitation of gasping represents a switch from a network-based rhythmogenesis for eupnea to a pacemaker-driven mechanism.

neural control of breathing; respiratory pattern generation; blockade of inhibition; ionic channels; hypoxia

---

THE EUPNEIC VENTILATORY PATTERN of decerebrate in vivo preparations is altered to gasping after a brain stem transection at the pontomedullary junction (22). A similar transformation from eupnea to gasping follows exposure to severe hypoxia or ischemia (41–44). Exposure of intra-arterially perfused preparations of the newborn or juvenile rat to hypoxia or ischemia likewise alters the pattern of activity of the phrenic nerve from eupnea to gasping (7, 41, 45–47).

During eupneic breathing in decerebrate preparations, the firing frequency within inspiratory bursts, as assessed by activity of the phrenic nerve, increases in a "ramplike" manner (see, e.g., Refs. 31 and 32), whereas during gasping, the phrenic discharges have a decrementing pattern (e.g., Refs. 30, 42–44). In addition, activities of cranial and spinal nerves become concentrated to the period of the phrenic burst with the change from eupnea to gasping. This concentration of activities during neural inspiration is manifested by a reduction or elimination of discharges during neural expiration of both cranial and spinal nerves (42–44, 48, 50, 51).

Hypoxia or ischemia suppresses inhibitory synaptic transmission within the brain stem (33, 36). There is also evidence that hypoxia induces gasping through both a direct modulation of channel conductances and alteration of ionic homeostasis in the extracellular environment. Specifically, hypoxia suppresses several types of voltage-gated potassium channels (4, 9, 16, 20, 21, 49), activates persistent sodium channels (13, 15, 17), and induces an augmentation of the extracellular concentration of potassium ions (24).

Previous experimental and computational studies have demonstrated that an increase in the external potassium concentration may release endogenous pacemaker/bursting activity (12, 18, 23, 34, 35). An augmentation in the extracellular potassium concentration shifts the reversal potential for potassium to more positive values of voltage and hence reduces all potassium currents (34, 35). Our modeling studies have demonstrated that the reduction of these currents may change the balance between the potassium currents and persistent sodium current, allowing the latter to overcome the former. This, in turn, may release the persistent sodium-dependent intrinsic bursting activity in conditional pacemaker neurons (34, 35). Based on our modeling results, we have offered a switching concept of respiratory rhythmogenesis. This concept suggests that the eupneic rhythm in vivo is generated by a network mechanism. With the suppression of potassium currents, either directly and/or via an increase in extracellular potassium concentration, along with the suppression of synaptic inhibition, the venti-

---

latory rhythm is switched from eupnea to a pacemaker-driven gasping (34, 35).

We therefore hypothesize that a simultaneous blockade of inhibitory synaptic transmission, combined with an increase of the extracellular potassium concentration and suppression of potassium channels, would alter the pattern of respiratory motor outflow from the ramp-incrementing discharges of eupnea to the decrementing discharges of gasping.

METHODS

In Situ Preparation

The in situ perfused preparation of the juvenile rat was used. This preparation has the significant advantage over in vitro mammalian preparations in that cranial and spinal nerves have distinctly different discharge patterns, which are similar to those during eupnea, apneusis, and gasping in vivo (41, 45–47). Exclusive of one in vitro slice preparation (19), all mammalian en bloc and slice preparations typically exhibit a single decrementing pattern, with little variation, which is similar to the gasp in vivo (30, 43, 44). The in situ perfused preparation also allows for administration of pharmacological agents that would be incompatible with viability of in vivo preparations. Of course, it is recognized that ventilatory activity is not identical in vivo and in situ. Because all oxygenation of the in situ preparation is derived from solution, long-term steady-state evaluations require a reduced metabolic rate and, hence, hypothermia (26–28, 41, 45–47). The eupneic frequency is thus reduced compared with evaluations in vivo at normothermia (41). Nevertheless, as documented in previous studies and results herein, even in hypothermia, the different ventilatory patterns of eupnea, apneusis, and gasping, with their distinctive characteristics, are all exhibited by the arterially perfused in situ preparation (26–28, 41, 45–47).

Thirty-seven juvenile rats (80–120 g, approximately 4–6 wk of age) were used. The procedures were described in detail previously (25–28, 41, 45–47). Briefly, rats were deeply anesthetized with halothane and decerebrated at a precollicular level. Before decerebration, body temperature was reduced by immersion in artificial cerebrospinal fluid, which was cooled to a temperature between 5 and 10°C. The portion of the body caudal to the diaphragm was removed. In some studies, ribs on the left side and the superior lobe of the left lung were also removed. The phrenic nerves were sectioned in all studies. In some preparations, the vagal, hypoglossal, and brachial nerves were also sectioned.

A catheter was inserted into the descending aorta (double lumen, size 3.5 or 4.0 Fr), advanced rostrally, and tied in place. Perfusate (see below) was warmed to 31°C and pumped into the descending aorta at constant flow. From a reservoir, the perfusate passed through a roller pump, a filter (Millipore 45 μM), two "bubble traps," and then the cannula in the aorta. Perfusate leaked from the numerous sectioned vessels and collected in a reservoir surrounding the animal. The perfusate was recirculated and reequilibrated with 95% O_2-5% CO_2. Perfusion pressure was recorded from one lumen. Perfusion pressure was increased gradually until activity of the phrenic nerve returned with a ramplike pattern. This activity was monitored by a glass "suction" electrode, amplified, filtered (0.6–6.0 kHz), integrated (50-ms time constant), and recorded. A similar system was used to record activities of the vagal, hypoglossal, and brachial nerves.

The perfusate contained the following in distilled water (in mM): 1.25 $MgSO_4$, 1.25 KH_2PO_4, 5.0 KCl, 25 $NaHCO_3$, 125 NaCl, 2.5 $CaCl_2$, 10 dextrose, and 0.1785 Ficoll 70. Under control conditions, the perfusate was equilibrated with 95% O_2-5% CO_2. Measured at 31°C, the pH of the perfusate was 7.38. Vercuronium bromide (3 μg/ml) was added to the perfusate to eliminate motor movements.

Experimental Protocols

Further characterizations of eupnea and gasping in situ. In 13 preparations, phrenic activity was recorded during eupnea and ischemia-induced gasping. In five of these preparations, activity was recorded concomitantly from the vagus nerve. Efferent activity of the hypoglossal nerve was recorded in three other preparations. Five preparations in which phrenic activity was recorded during eupnea and gasping were also evaluated after administration of drugs (see below).

Phrenic activity was recorded in six other preparations after the precollicular decerebration and then after transections of the brain stem at the pontomedullary junction and at the level of the medullary obex. These preparations were not exposed to ischemia.

Eupnea and gasping in situ after administration of drugs. In 23 preparations, activity of the phrenic nerves was recorded during eupnea. Activities of the following nerves were also recorded in some of these preparations: vagus ($n = 5$), hypoglossal ($n = 4$), and brachial ($n = 2$). As noted above, in five experiments, ischemia was introduced temporarily and gasping was elicited. No ischemia was introduced in the remaining 18 experiments.

The following were administered: *1*) 4-aminopyridine (4-AP, 1–10 μM) to block potassium channels (including the transient potassium A channels), and *2*) strychnine sulfate (0.2–0.6 μM) to block inhibitory transmission by glycine. In addition, potassium chloride was added to the perfusate to raise the extracellular concentration of potassium ions (maxima of 10.25–17.25 mM). A minimum of 10 min was taken after additions of any substances before responses were evaluated.

After all drugs had been administered, fresh perfusate was introduced to five preparations. After drug administration in six other preparations, the brain stem was transected at the pontomedullary junction and then at the level of the medullary obex.

Data Analysis

Integrated activity of the phrenic nerve was quantified as to the duration of the burst (neural inspiration; T_I), period between bursts (neural expiration; T_E), the total duration of the respiratory cycle ($T_I + T_E$; and the reciprocal, the respiratory frequency *f*), and peak height. Changes in values of peak phrenic activity could only be compared for a subset of the preparations after some experimental perturbations. In these procedures, requiring extended times for completion, it was necessary to reposition the recording electrode periodically. Hence, changes in values of peak phrenic activity could not be analyzed for all preparations. As an index of the rate of rise of phrenic activity, the time at which peak phrenic activity was reached was normalized as a percentage of the duration of the burst. For other neural activities recorded concomitant with that of phrenic activity, peak activities during neural inspiration and expiration were defined. For hypoglossal activity, its time of onset relative to that of the phrenic burst was evaluated. After any experimental perturbation, variables during a minimum of five ventilatory cycles were defined and averaged. Statistical evaluations were by
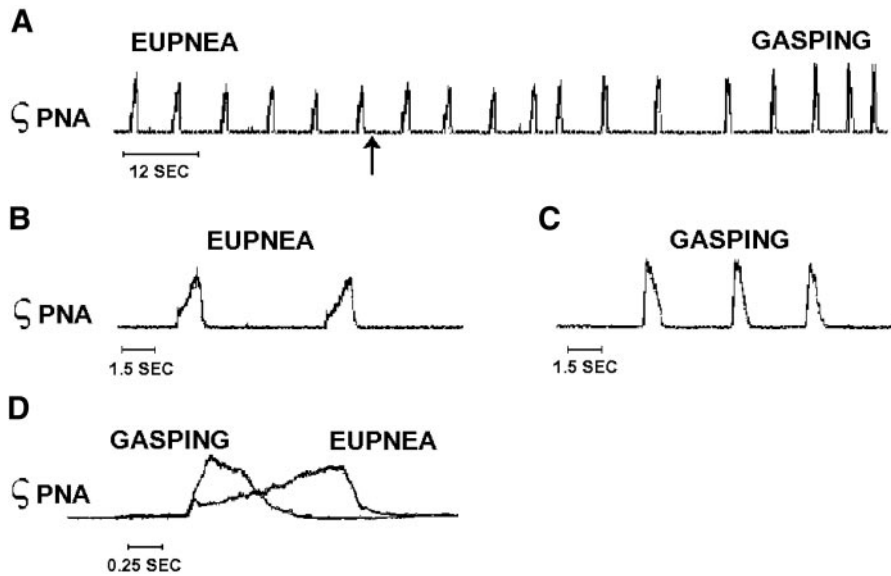
Fig. 1. Alteration in pattern of phrenic nerve activity in ischemia. Integrated records of activity of the phrenic nerve (δPNA) are shown during eupnea and during the changes that followed a termination of perfusion and ultimate establishment of gasping. *A*: arrow designates termination of perfusion. Last 5 cycles had the decrementing pattern of gasping. Middle panel shows cycles of eupnea (*B*) and gasping (*C*). *D*: a single eupneic and gasping cycle on an expanded time base. Note the incrementing eupneic discharge and decrementing gasp.

the nonparametric Wilcoxon test. $P < 0.05$ was considered as significant.

RESULTS

Characteristics of Neural Activities in Eupnea and Ischemia-Induced Gasping

The changes in the characteristics of phrenic activity with the alteration from eupnea to gasping are shown in Fig. 1. With the onset of ischemia, peak phrenic activity initially rose and was then succeeded by a period of variable duration in which the period between phrenic bursts was prolonged. This period was succeeded by a series of gasps (Fig. 1*A*). The time in ischemia before the onset of gasping varied from approximately 30 to 60 s in different preparations.

A primary distinction between eupnea and gasping was in the rate of rise of inspiratory activity. Hence, eupnea was characterized by a sudden onset of activity and then a ramplike rise, which achieved a peak near the end of the phrenic burst (Fig. 1, *B* and *D*). In gasping, peak phrenic activity was reached almost immediately after onset, with activity then declining during the remainder of the phrenic burst (Fig. 1, *C* and *D*, and Fig. 2*D*).

The duration of neural inspiration was less in gasping (0.79 ± 0.11 s) than in eupnea (1.17 ± 0.11 s), whereas neither the duration of neural expiration nor the burst frequency was significantly different (Fig. 2, *A* and *B*). Peak integrated phrenic activity was significantly greater in gasping than eupnea ($130 \pm 15\%$ of eupneic value; $P < 0.05$, Fig. 2*C*). Reflecting the greater rate of rise of inspiratory activity, the time to reach the peak of the phrenic burst was significantly less in gasping than eupnea. Thus, in eupnea, peak phrenic activity was achieved after $73 \pm 1.6\%$ of the burst was completed, whereas in gasping this period declined to $29 \pm 2.0\%$ of the burst (Fig. 2*D*).

The finding of similar frequencies of eupnea and gasping confirms previous results for examinations at 31°C. At higher temperatures, the frequency of eupnea is significantly greater than that of gasping (41).

In addition to alterations in phrenic activity, alterations in activities of the hypoglossal and vagal nerves with the change from eupnea to gasping were similar to those that have been described in detail for in vivo preparations (e.g., 42, 43, 48, 50, 51). Hence, as shown in Fig. 3*A*, the onset of hypoglossal activity preceded that of the phrenic in eupnea. The difference in onset varied from 300 to 981 ms in various preparations; the mean difference was 591 ± 199 ms. In gasping, this
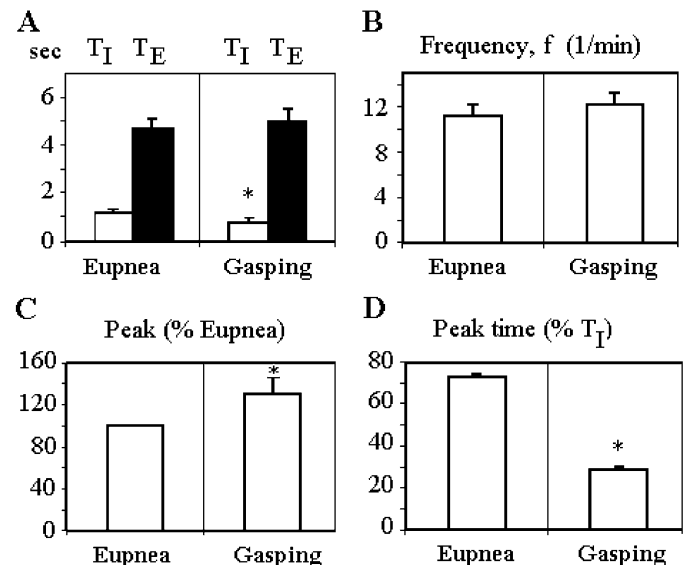

Fig. 2. Alterations in the duration of the phrenic burst (T_I; *A*), period between bursts (T_E; *A*), frequency f (*B*), peak height (*C*), and time to reach peak activity (peak time; *D*) with a change from eupnea to ischemia-induced gasping. Left bars are mean values ($\pm$SE) in eupnea; right bars are values in ischemia. *$P < 0.05$ compared with value in eupnea. Note that T_I was significantly less and peak height significantly greater in gasping. Time to reach peak height was significantly reduced in gasping.

Fig. 3. Activities of the phrenic nerve and hypoglossal and vagal nerves in eupnea (*A* and *C*) and ischemia-induced gasping (*B* and *D*). *A* and *B* show integrated activities of the phrenic nerve and hypoglossal nerve (δXII). There is a change in pattern of nerves to decrementing in gasping. Note also that activity of the hypoglossal nerve, which commences in late neural expiration in eupnea, commences at the same time as the phrenic nerve in gasping (see also Fig. 6). Small excursions in records of neural activities represent the electrocardiogram. *C* and *D* show integrated activities of the phrenic nerve and vagus nerve (δVNA). Note that in eupnea, vagus nerve has a large burst of activity at the beginning of neural expiration. Such expiratory activity is eliminated in gasping, and the vagus nerve discharges along with the phrenic nerve (see also Fig. 8).
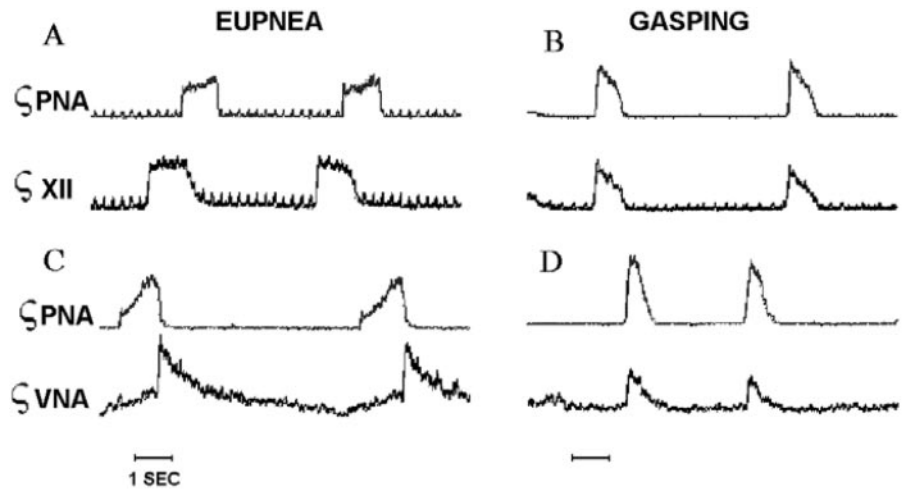
mean difference declined to 84 ± 31 ms (range 51–148 ms) (Fig. 3*B*). As in Fig. 3, *A* and *B*, peak hypoglossal activity declined in each preparation with the change from eupnea to gasping.

For vagal activity during eupnea, the burst of inspiratory activity was succeeded by an early expiratory burst of greater amplitude in four of five preparations (Fig. 3*C*): inspiratory activity was slightly higher in the remaining preparation. With the change to gasping, the expiratory activity was reduced or totally eliminated, whereas inspiratory activity persisted but showed a change in pattern from incrementing to decrementing (Fig. 3*D*). Thus peak expiratory activity in gasping averaged 59 ± 14% of the eupneic value, whereas inspiratory activity was 92 ± 19% of eupnea.

Brain Stem Transections

In six preparations exhibiting eupnea, the brain stem was transected at the pontomedullary junction. After a transection through approximately the dorsal half of the brain stem, the pattern of phrenic activity in three preparations was changed to a sustained discharge typical of apneusis (Fig. 4*B*). In the other preparations, eupnea continued. In the three preparations exhibiting apneusis, the duration of the phrenic burst increased from a mean of 1.08 ± 0.2 to 2.16 ± 0.2 s, and the period between bursts also increased from a mean of 4.18 ± 0.18 to 9.26 ± 4.6 s. After a complete transection, the pattern was changed from eupnea or apneusis to a decrementing pattern of gasping in five of six preparations (Fig. 4*C*). All phrenic activity ceased in the remaining preparation.

The duration of the phrenic burst was shorter during gasping than eupnea in all five preparations, with this duration falling significantly from a mean of 1.0 ± 0.2 s in eupnea to 0.6 ± 0.09 s in gasping. Other variables were not systematically altered. Hence, as opposed to ischemia-induced gasping, peak height did not rise in every preparation after a brain stem transection between pons and medulla.

Alterations in Eupnea and Gasping After Administrations of 4-AP

Changes in activity of the phrenic nerve. In 15 preparations, the concentration of 4-AP in the perfusate was progressively augmented before either strychnine or excess potassium chloride was introduced. The concentrations varied from 1 to 10 μM, with differing concentrations being applied to different preparations. For purposes of statistical evaluations, data have been evaluated after additions of 2, 4, and 6 μM (Fig. 5*A*). Because it was necessary to reposition the recording electrode periodically in some preparations, values of peak phrenic activity could only be defined for a subset of preparations (see legend of Fig. 5).
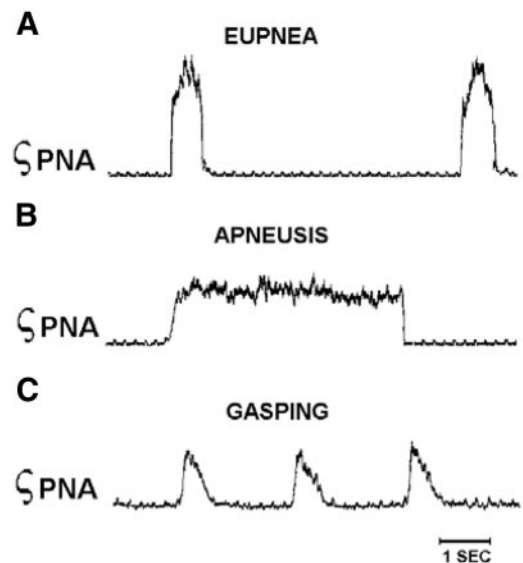

Fig. 4. Alterations in pattern of phrenic activity after brain stem transections between pons and medulla. Eupnea (*A*) was recorded in preparation having an intact mesencephalon, pons, and medulla. Apneusis (*B*) was observed after a transection through the dorsal half of brain stem at the pontomedullary junction. Gasping (*C*) followed completion of the brain stem transection.
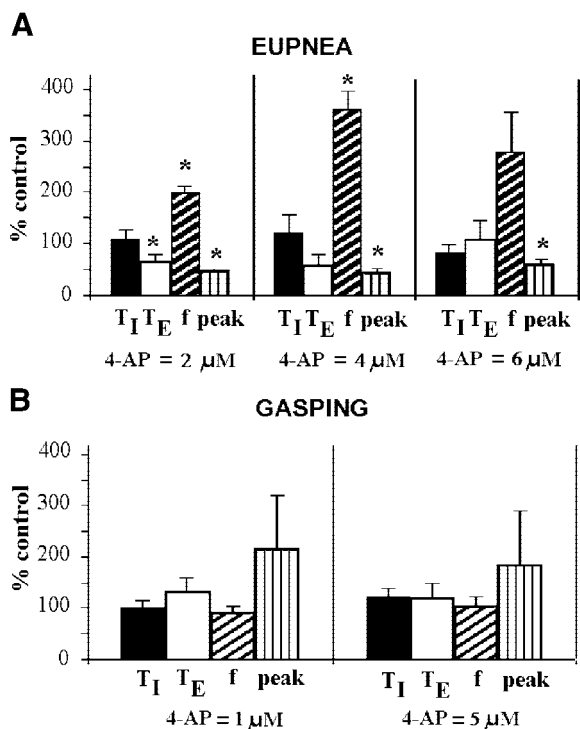
Fig. 5. Alterations of eupnea and ischemia-induced gasping after administrations of 4-aminopyridine (4-AP). A: mean values (±SE) of phrenic T_I, T_E, f, and peak height (peak) after administration of the designated concentrations of 4-AP. Values have been expressed as percentage of control values before administrations of 4-AP. *$P <$ 0.05 compared with control values. Note increases in f, except at 6 μM of 4-AP, and fall in peak height at all levels of 4-AP. B: comparable variables for ischemia-induced gasping. No variable was significantly altered after 4-AP. Number of preparations examined: eupnea, 2 μM 4-AP, $n = 15$; 4 μM 4-AP, $n = 10$; 6 μM 4-AP, $n = 8$; gasping, 1 and 5 μM 4-AP, $n = 5$. Peak height could be only defined in a subset of preparations: eupnea, 2 μM 4-AP, $n = 14$; 4 μM 4-AP, $n = 10$; 6 μM 4-AP, $n = 6$; gasping, 1 and 5 μM 4-AP, $n = 5$.

Control values during eupnea for the 15 animals were very similar to those for the entire group (Fig. 2). Mean values ± SEs were T_I, 0.98 ± 0.06 s; T_E, 4.27 ± 0.33 s; and f, 12.4 ± 0.96 bursts/min. Peak integrated phrenic activity fell at all levels of 4-AP, whereas frequency significantly increased after additions of 2 and 4 μM of 4-AP (Figs. 5A and 6B). The augmentation in frequency was due to reductions in the duration of neural expiration (Fig. 5A). At concentrations of 4-AP of 6 μM, the duration of neural expiration was prolonged in three of nine preparations, compared with control values. Such prolongations accounted for the absence of a significant change in T_E and frequency at these higher levels of 4-AP (Fig. 5A).

The absence of a significant change in the duration of neural inspiration reflected the finding that this duration underwent variable changes in different preparations and/or at different concentrations of 4-AP. In eight preparations, the duration of the phrenic burst remained constant or declined at various concentrations of 4-AP, whereas in the other seven preparations, the duration of the phrenic burst was greatly augmented at one or more concentrations of 4-AP (Fig. 7B). Hence,

in these seven preparations, a pattern of ventilatory activity was recorded, which was typical of apneusis (Fig. 7B). There was no consistency as to the dose of 4-AP, which resulted in the induction of apneusis.

Five preparations were exposed to ischemia after various concentrations of 4-AP had been added to the perfusate. As opposed to eupnea, T_I, T_E, frequency, and peak height in ischemia-induced gasping were not significantly altered from control levels at any concentration of 4-AP (Fig. 5B).

Changes in activities of the vagal, hypoglossal, and brachial nerves. Activities of the vagal ($n = 5$), hypoglossal ($n = 4$), and brachial ($n = 2$) nerves were recorded before and after additions of 4-AP. The control discharges in eupnea were as described above for vagal and hypoglossal motor nerves. Hence, vagal nerve discharged during neural inspiration and expiration with the discharge in the latter being greater than the former in all but one preparation. For all preparations, peak activity in expiration averaged 145% of the inspiratory value. Hypoglossal activity commenced before the phrenic burst in eupnea and then discharged throughout neural inspiration (Fig. 6A). Activity of the brachial nerve had only a low level of tonic discharge, with no discernible bursting or respiratory modulation in eupnea.

After the additions of 4-AP, changes in activity of the vagal nerve paralleled that of the phrenic nerve in that
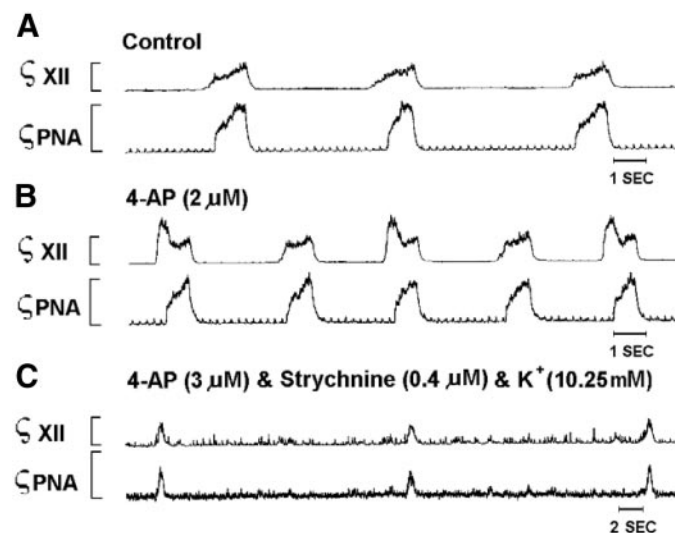

Fig. 6. Activity of the hypoglossal nerve in eupnea and fictive gasping. A: integrated activities of the hypoglossal and phrenic nerves in eupnea. B: changes induced by 4-AP alone. Note that hypoglossal activity rises quickly at the start of the burst in cycles after addition of 4-AP alone. C: hypoglossal and phrenic activities in fictive gasping elicited by administrations of 4-AP, strychnine, and excess extracellular potassium chloride. (Value given represents total potassium in the perfusate). In this and Figs. 7, 8, and 10, brackets at *left* of tracings indicate relative amplitude of integrated neural activities. Note that activity of the hypoglossal nerve, which started before that of the phrenic in eupnea (A), starts at the same time in fictive gasping (C). Also, in this preparation, peak activities of phrenic and hypoglossal nerves were reduced and tonic activity increased in fictive gasping compared with control levels, before drugs were administered.
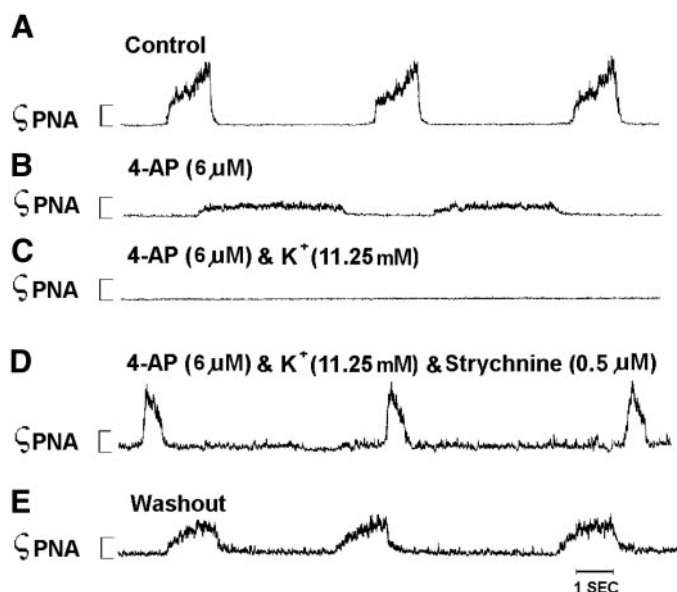
Fig. 7. Alteration of phrenic nerve activity from incrementing pattern of eupnea to decrementing pattern of fictive gasping after administrations of 4-AP, strychnine, and excess extracellular potassium chloride. *A*: integrated phrenic nerve activity (δPNA) in eupnea. *B*: changes induced by 4-AP alone. After an addition of 4-AP, phrenic discharge has acquired a discharge similar to apneusis. *C*: changes induced by 4-AP and excess extracellular potassium. Value is for total potassium in perfusate. *D*: fictive gasping. Phrenic discharge has converted to the decrementing gasplike pattern after addition of strychnine. *E* shows partial recovery of the incrementing pattern associated with eupnea after substitution of fresh perfusate.

quency was initially recorded. We refer to the latter as "fictive" gasping. Over the next 20 min, a purely decrementing pattern was established in some preparations (e.g., Fig. 7*D*) although in others, a low-amplitude discharge also persisted (Fig. 8*B*).

The average concentrations at which fictive gasping was elicited were as follows: 4-AP, 5.06 μm (range 3–9 μm); total extracellular potassium (phosphate + chloride), 11.85 mM (range 10.25–17.25 mM); strychnine, 0.413 μm (range 0.2–0.6 μm).

In Fig. 9, variables of fictive gasping are compared with those in the same preparations during eupnea, before drugs were administered. A similar comparison, but of eupnea and ischemia-induced gasping, had been presented in Fig. 2. For both ischemia-induced and fictive gasping, the durations of neural inspiration fell significantly and the rate of rise of phrenic activity increased significantly compared with eupnea. As opposed to ischemia-induced gasping, peak phrenic activity was significantly reduced in fictive gasping. However, as shown in Fig. 5*A*, administrations of 4-AP alone caused a significant reduction in peak phrenic activity. Indeed, in one-half of the preparations, peak phrenic activity in fictive gasping was above that after administrations of 4-AP alone. In addition to differences in the levels of peak phrenic activity, fictive gasping differed from ischemia-induced gasping in that the duration of neural inspiration was slightly, but significantly, less during the former.
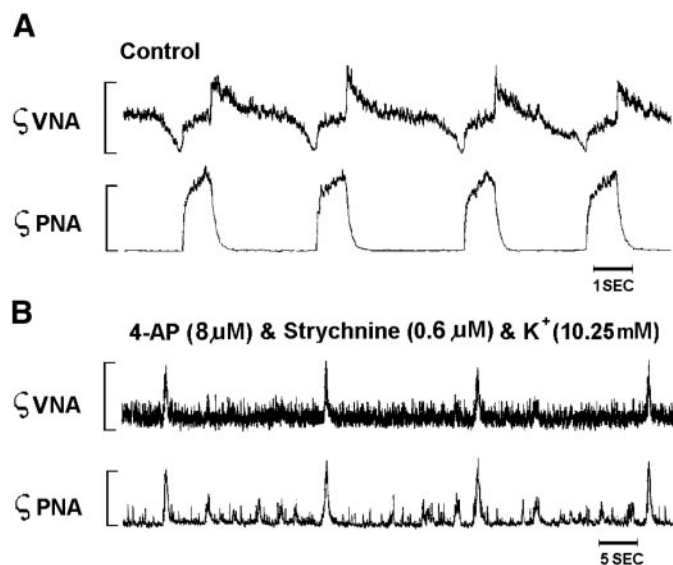
peak activities in neural inspiration and expiration declined. Hypoglossal activity likewise declined, but only in a limited proportion of the respiratory cycles. Hence, as shown in Fig. 6*B*, after administrations of 4-AP, hypoglossal activity in some cycles commenced suddenly and immediately rose to a higher peak value than that achieved in cycles in which hypoglossal activity rose in a ramplike fashion. In both types of cycle, which was observed in all four preparations, hypoglossal activity still commenced before the phrenic discharge. Activity of the brachial nerve was unaltered at any concentration of 4-AP.

Fictive Gasping After 4-AP, Excess Potassium Chloride, and Strychnine

Alterations in activity of the phrenic nerve. In 15 of 18 preparations, the combination of 4-AP, excess potassium chloride, and strychnine resulted in a change in the pattern of phrenic activity from the incrementing pattern of eupnea to the decrementing pattern of gasping (Figs. 6*C*, 7*D*, and 8*B*). For the other three preparations, only a tonic phrenic discharge was recorded.

The change in pattern of phrenic activity from incrementing to decrementing occurred gradually. The order in which these substances were administered did not influence the response. Within 5 min of completion of the additions of 4-AP, excess potassium chloride, and strychnine, a pattern of incrementing bursts at high frequency and decrementing bursts at a lower fre-

Fig. 8. Activity of the vagus nerve in eupnea and fictive gasping. *A*: integrated activities of the vagal (δVNA) and phrenic (δPNA) nerves in eupnea. For vagal activity, note discharges during both neural inspiration and expiration. *B*: integrated activities of the vagal and phrenic nerves in fictive gasping elicited by administrations of 4-AP, strychnine, and excess extracellular potassium chloride (value given is for total potassium in the perfusate). Note that activity of the vagus nerve during neural expiration has been greatly reduced, compared with the discharge in neural inspiration (see also Fig. 3). In this preparation, peak heights of phrenic and hypoglossal activities were reduced and tonic levels of activity increased in fictive gasping compared with control levels.
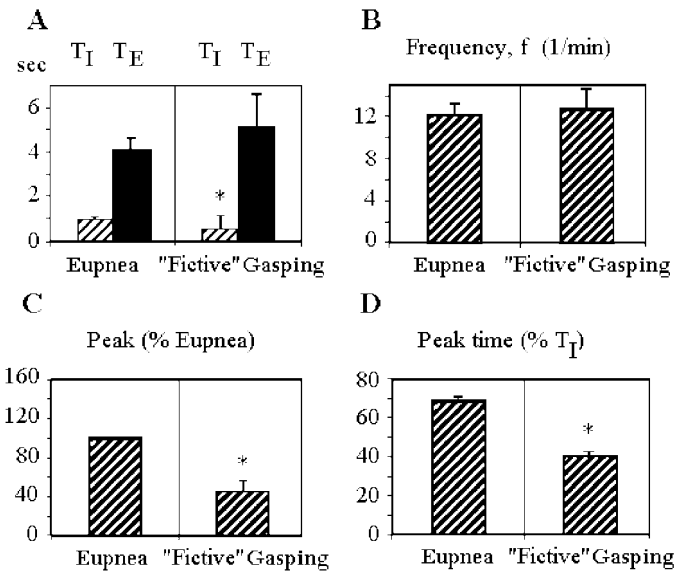
Fig. 9. Alterations in the duration of the phrenic T_I and T_E (A), f (B), peak height (C), and time to reach peak activity (peak time; D) with a change from eupnea to fictive gasping. Fictive gasping was induced by administrations of 4-AP, strychnine, and excess potassium. Left bars are mean values (±SE) in eupnea; right bars are values in fictive gasping. *$P < 0.05$ compared with value in eupnea. Note that T_I was significantly less and peak height was also significantly reduced in fictive gasp. Time to reach peak height was significantly reduced in gasping. Values of peak height are only given for a subset of data, and the phrenic nerve was repositioned in some of these. See text for discussion.

On introduction of fresh perfusate, the decrementing pattern of gasping was replaced by an incrementing pattern as in control (Fig. 7E).

Alterations in activities of the hypoglossal and vagal nerves. Changes in activities of the hypoglossal and vagal nerves were similar in fictive and ischemia-induced gasping. For hypoglossal activity, its time of onset before phrenic activity was reduced in all preparations with the onset of gasping. Specifically, the onset of hypoglossal discharge preceded that of phrenic by a mean of 591 ± 199 ms in eupnea. This time was reduced to 84 ± 31 ms in fictive gasping (see Figs. 3B and 6C). Compared with values in eupnea, peak height of hypoglossal activity in fictive gasp was reduced to a mean of 87% of the eupneic value in the three preparations in which recordings had been maintained.

Vagal activity was altered with the onset of fictive gasping in that activity during neural expiration was greatly reduced or eliminated (Fig. 8B). Hence for three preparations in which continuous recordings had been maintained, peak vagal activity in early neural expiration was 54, 204, and 207% of the value during the eupneic inspiration. In fictive gasping, these values fell to 0, 0, and 107% of that during neural inspiration, respectively. The peak vagal activity in neural inspiration fell in one of three preparations with the change from eupnea to fictive gasping.

Alterations in fictive gasping after brain stem transections. Six preparations had received 4-AP, strychnine, and excess potassium chloride and exhibited fictive gasping. The pattern of this fictive gasping was not markedly altered after a transection at the pontomedullary junction (Fig. 10). The frequency of gasping underwent variable changes. All rhythmic phrenic activity was eliminated after a subsequent medullary section through the medullary obex.

DISCUSSION

Characteristics of Gasping In Vivo and In Situ

The hallmark of gasping of in vivo preparations is the extremely rapid rise of inspiratory activity compared with eupnea (Fig. 1; Refs. 42–44). In decerebrate rodent preparations in vivo, this rapid rise of inspiratory activity is such that the incrementing pattern of phrenic discharge of eupnea is altered to a decrementing pattern in gasping (8, 30, 42, 43, 51). This pattern of phrenic activity is very similar whether gasping results from exposure to severe hypoxia, ischemia, or transection of the brain stem at the pontomedullary
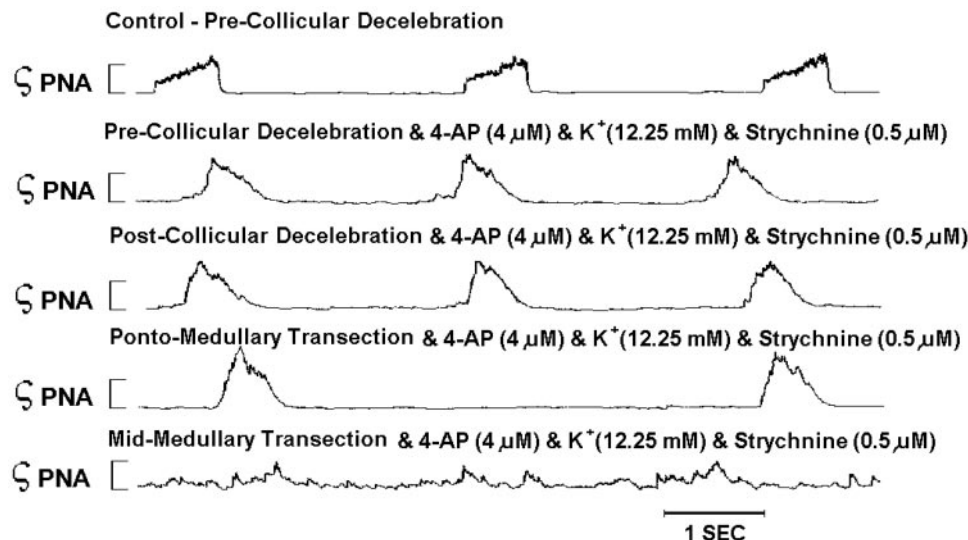
Fig. 10. Comparison of fictive gasping pattern of phrenic activity after administrations of 4-AP, excess potassium chloride, and strychnine and after transection of the brain stem at various levels. Note that decrementing discharge pattern of integrated phrenic activity was maintained after removal of the mesencephalon (postcollicular transection) and pons (pontomedullary transection) but was eliminated after removal of the rostral medulla. For comparison, records of the incrementing pattern of phrenic activity during eupnea are shown in top panel.

junction. Concerning the latter, the present in situ results were identical to those in vivo. The pattern of phrenic activity was altered from eupnea to apneusis after a partial separation of pons from medulla and to gasping after a complete transection at the pontomedullary junction (42–44). Similar experimental perturbations cause similar alterations from eupnea to gasping in neonatal, juvenile, and adult in vivo and in situ perfused preparations (7, 41–47, 51).

Also consistent across experimental preparations is the observation that with the alteration from eupnea to gasping, regardless of how induced, expiratory activities of cranial and spinal nerves become reduced, and discharge is concentrated to the period of the phrenic burst (42–48, 50). Such a concentration to the period of the phrenic burst is also observed for activity of the hypoglossal nerve. As demonstrated in a number of in vivo studies, hypoglossal activity in eupnea may commence much before that of the phrenic nerve. In gasping, such activities commence at essentially the same time (see, e.g., Ref. 50). A similar change in the onset of hypoglossal activity has been observed in situ, as reported herein.

Characteristics of Fictive Gasping

The alterations in phrenic activity that followed the combined administrations of 4-AP, excess potassium chloride, and strychnine were the same as changes after exposure to either ischemia or transection of the brain stem at the pontomedullary junction. Specifically, in fictive gasping, the rate of rise of integrated phrenic activity increased significantly and was not different from that of ischemia-induced gasping. The pattern of phrenic activity changed from incrementing to decrementing. However, in fictive gasping, the duration of the phrenic burst was significantly less than in ischemic-induced gasping. Moreover, as opposed to the latter, peak phrenic activity did not increase significantly above the eupneic level, but actually declined in fictive gasping.

Concerning these differences in phrenic activity, the drugs administered to elicit fictive gasping would be distributed throughout the central nervous system. Hence, actions at regions other than the medullary region responsible for the neurogenesis of gasping would seem inevitable. In this context, as noted above, peak phrenic activity in eupnea fell in all preparations after administrations of 4-AP alone. These actions of drugs at diverse regions of the central nervous system could account for the difference in some aspects of phrenic activity between fictive gasping and gasping induced by hypoxia, ischemia, or brain stem transections.

Further supporting the conclusion that fictive gasping represents activation of the medullary mechanisms for gasping are changes in hypoglossal and vagal activities. Specifically, in ischemia-induced gasping and fictive gasping, hypoglossal and vagal activities acquired a similar inspiratory discharge pattern, and expiratory vagal activities were greatly reduced or eliminated. The lag between the onsets of hypoglossal and phrenic activities was likewise greatly reduced.

In addition to changes in neural activities, per se, the pattern of fictive gasping was not markedly altered after transections of the brain stem at the pontomedullary junction. Because gasping is generated by mechanisms inherent to the medulla (42–44), the absence of change in fictive gasping after the transections supports the concept that these pharmacological and chemical interventions had produced alterations of the brain stem ventilatory control system that are similar to those induced by ischemia, hypoxia, or the brain stem transections in intact preparations.

Relative Sensitivities of Eupnea and Gasping to Blockade of Inhibitory Synaptic Transmission and Potassium Channels

Eupnea was severely distorted after administrations of 4-AP and strychnine. Such a distortion of eupnea was also observed after a blockade of receptors for either glycine or GABA$_A$ (46). These observations in the perfused juvenile rat confirm those of Hayashi and Lipski (14) in a perfused preparation of the adult rat.

As opposed to eupnea, ischemia-induced gasping was not altered after the administrations of 4-AP. Similarly, gasping persisted after administrations of doses of bicuculline or strychnine much in excess of those that greatly disrupted the eupneic rhythm (46). These antagonists of receptors for GABA and glycine or, in the present study, blockers of the potassium channels were added to the perfusate and, hence, distributed throughout the central nervous system. Hence, the distortions of eupnea after administration of these antagonists may also reflect alterations of numerous circuits that influence the pattern of the eupneic ventilatory cycle. However, the alterations of eupnea, but not gasping, after these experimental perturbations are consistent with the concept that different neurophysiological mechanisms underlie the neurogenesis of these two patterns of automatic ventilatory activity. We have proposed that a pontomedullary circuit underlies the neurogenesis of eupnea, whereas gasping results from the discharge of pacemaker neurons of the rostral medullary gasping center-pre-Bötzinger complex (42–44, 46).

Switching Concept for Respiratory Rhythmogenesis

Results of the present study provide support for a switching concept of respiratory rhythmogenesis. This concept suggests that respiratory rhythm may be generated by either a network or a hybrid mechanism, depending on the conditions (34, 35). It is proposed that hypoxia may produce a switch in the respiratory rhythm generation from a pontomedullary network mechanism for eupnea to a medullary pacemaker-driven mechanism for gasping (34, 35).

A hybrid pacemaker-network concept suggests that a subpopulation of conditional pacemaker neurons in pre-Bötzinger complex comprises a necessary rhythm-generating kernel. The pacemaker bursting discharges

of these neurons, which are obligatory for generation of the respiratory rhythm, drive a wider pattern-forming network (see, e.g., Ref. 3, 5, 29). The existence of a kernel of pacemaker/bursting neurons may explain the resistance of in vitro rhythms to a blockade of inhibitory synaptic transmission (1, 37). However, in contrast to the network theories and models, the hybrid pacemaker-network concept does not provide explanation for various systems-level phenomena, including independent regulation of respiratory phases, respiratory reflexes, and phase resetting produced by stimulation of afferent nerves.

The switching concept (34, 35) is compatible with both the network and hybrid pacemaker-network theories. This compatibility arises because the different respiratory rhythms of eupnea and gasping may be generated by different mechanisms. Importantly, in the most recent version of the hybrid pacemaker-network model (38), the respiratory rhythm can be generated in two different modes. One mode includes a pacemaker kernel and the other a network in which "none of the kernel neurons exhibit intrinsic pacemaker-like oscillations" (38).

The seemingly now common conclusion that the respiratory network can generate a rhythmic output by either a pure network or a hybrid pacemaker-network raises several important questions. First, what are the conditions that define each of these mechanisms for rhythmogenesis? A related question concerns the conditions that may induce switching from one mechanism to another. Finally, which mechanism is responsible for the neurogenesis of eupnea?

The hybrid pacemaker-network model (38) considers neither the differences between eupneic and gasping rhythms nor the possible differences in the mechanisms for generation of these different breathing patterns. In contrast, the switching model (34, 35) explicitly suggests that the eupneic rhythm is generated by a network mechanism, while pacemaker-driven oscillations underlie the neurogenesis of gasping. Our modeling studies have demonstrated that the release of pacemaker-driven oscillations may be induced by an imbalance between the potassium and persistent sodium currents, combined with a suppression of phasic inhibition (34, 35). We thus proposed that in hypoxia or ischemia, phasic inhibition within the pontomedullary network, including that impinging on neuronal activities within the pre-Bötzinger complex, is suppressed. In addition, the persistent sodium current, which is a major source of endogenous bursting (see Refs. 3, 5, and 38), is activated (13, 15, 17). At the same time, the potassium currents are inhibited through both a direct effect of hypoxia on channel conductances (4, 9, 16, 20, 21, 49) and via the hypoxia-induced elevation of extracellular potassium concentration (24). Therefore, during hypoxia or ischemia, the suppressed phasic inhibition and reduced potassium currents may not be able to restrain endogenous, persistent sodium-dependent pacemaker mechanisms. Release of the latter provides a switch from a network-based eupneic pattern to the pacemaker-driven gasping. Respiratory rhythmogenesis may also be switched from a pontomedullary network mechanism to a medullary pacemaker-driven mechanism after a brain stem transection at the pontomedullary junction. The mechanisms by which this transection causes a release of medullary mechanisms for gasping have not been defined.

Neurogenesis of Eupnea: Pontomedullary or Medullary Circuits

An obligatory component in the hybrid pacemaker-network model for respiratory rhythm generations is an endogenous bursting activity of a population of conditional pacemaker neurons in the rostral medullary pre-Bötzinger complex. The importance of such pacemaker neurons for the neurogenesis of eupnea was hypothesized on the basis of results from in vitro studies in which neuronal activities in this region are critical for the neurogenesis of the in vitro rhythm. Yet, until recently, only a single rhythm has been exhibited by all mammalian in vitro en bloc or slice preparations, and this rhythm appears identical to gasping in vivo (30, 43, 44). In this context, the in vitro rhythm persists after a blockade of inhibitory synaptic transmission (1, 37). As shown herein and in previous studies (46), gasping but not the eupneic rhythm also persists after this blockade. Yet, some investigators maintain that this in vitro rhythm is a variant of eupnea and, indeed, that this pre-Bötzinger complex represents the "noeud vital" for breathing (1, 29, 39).

The concept of the pre-Bötzinger complex as a noeud vital for gasping but not eupnea appears to be supported by the recent results of Gray et al. (11). These investigators have reported that the eupneic ventilatory pattern of unanesthetized rats is altered to one of ataxia after destruction of neurons in the pre-Bötzinger complex. Eupnea continued until almost all of these neurons had been destroyed bilaterally. While the ataxic ventilatory pattern was sufficient to sustain viability, exposure to hypoxia resulted in terminal apnea with no gasps being reported. The irreversible elimination of gasping after ablation of neurons in their study is not considered by Gray et al. (11). At the same time, there is evidence that activation of neuronal activities within the pre-Bötzinger complex in vivo can release gasping (40).

The concept that a pontomedullary neuronal circuit is essential for the neurogenesis and expression of eupnea is supported by observations that, from the day of birth, ablations of the rostral pontile pneumotaxic center cause an alteration of the eupneic rhythm (43, 44). Moreover, in vivo or in situ, no respiratory rhythm except gasping has been reproducibly recorded after the isolation of pons from medulla (42). In this context, however, Lieske et al. (19) have reported that different "breathing patterns," which are considered comparable to eupnea, sighs, and gasps, can be recorded from a medullary slice preparation.

These results of Lieske et al. (19) raise the possibility that eupnea can be generated by a neuronal circuit that is inherent to the medulla. Lieske et al. (19) point

out that, in their reduced preparation, the pattern of the different rhythms cannot be expected to be identical to those in vivo. However, some of their findings do appear in conflict with those in vivo. Hence, sighs are eliminated in vivo after sectioning of the carotid sinus nerves and vagi (2, 10). Both of these afferents are obviously lacking in the slice. Moreover, whereas the eupneic rhythm in vivo or in situ is severely distorted by administration of strychnine, the eupneic rhythm of the slice persists after these administrations. Finally, and most importantly, the rhythm that Lieske et al. (19) designate as "gasping" appears to be identical to the rhythm that has previously been preponderant in all mammalian in vitro en bloc and slice preparations.

A Reassessment of Pacemaker Neurons In Vitro

As considered in detail above, much evidence has been obtained from in vitro preparations in support of the hypothesis that the discharge of pacemaker neurons underlies the generation of respiratory rhythms in vitro. The persistent sodium current has been attributed to the genesis of discharge of pacemaker neurons (3, 5, 18, 29, 38). However, in vitro rhythmic activities have recently been reported to continue in a medullary slice preparation after a pharmacological blockade of persistent sodium channels (6). This observation led to the conclusion that "voltage-dependent bursting in pacemaker neurons is not essential for respiratory rhythmogenesis" (6). This conclusion is in direct opposition to all previous concepts from this same laboratory (e.g., Refs. 29, 37, 39).

It is enigmatic how the in vitro rhythm could be generated in the absence of both pacemaker neurons and synaptic inhibition in neuronal circuits (6). However, rejection of an essential role for voltage-dependent bursting in respiratory rhythm generation requires a demonstration that rhythmic activity continues after a blockade of persistent sodium channels in all pacemaker neurons regardless of their location within the slice. While it is argued that the blockade of persistent sodium channels was ubiquitous, neurons were not tested throughout the entire slice (6). It cannot be discounted definitively that, due to a lack of penetration of the antagonist, the discharge of pacemaker neurons might still occur in deeper regions of medullary tissue.

Summary and Conclusions

Results of the present study, combined with those of previous investigations, support the conclusion that gasping is generated by mechanism intrinsic to the gasping center-pre-Bötzinger complex of the rostral medulla (30, 34, 35, 40, 42–44). As gasping, but not eupnea, is resistant to a blockade of inhibitory synaptic transmission (46), the discharge of medullary pacemaker neurons is proposed to generate the gasp. The persistent sodium current is critical for the pacemaker discharge (3, 5, 34, 35, 38). In eupnea, this persistent sodium-dependent pacemaker activity is suppressed, and the respiratory rhythm is generated by neural

mechanisms within the pontomedullary neuronal circuit, which is necessary for the generation of eupnea (34, 35).

Hypoxia or ischemia causes a suppression of inhibitory transmission on medullary neurons (33, 36). In addition, potassium currents are inhibited both through direct influences of hypoxia on channel conductances and through an elevation of extracellular potassium concentration (4, 9, 16, 20, 21, 24, 49). The net result of these hypoxia-induced changes is that the latent bursting pacemaker activity of medullary neurons is released and gasping is generated.

In studies herein, we have reproduced these influences of hypoxia on the brain stem respiratory control system. By a combination of a blockade of glycinergic transmission, a pharmacological suppression of potassium channels, and an elevation of extracellular potassium concentration, the pattern of ventilatory activity has been switched from eupnea to fictive gasping. This fictive gasping has many of the same characteristics in terms of activities of cranial and spinal nerves as does hypoxia or ischemia-induced gasping or gasping resulting from transection of the brain stem at the pontomedullary junction.

REFERENCES

1. **Ballanyi K, Onimaru H, and Homma I.** Respiratory network function in the isolated brainstem-spinal cord of newborn rat. *Prog Neurobiol* 59: 583–634, 1999.
2. **Bartlett D.** Origin and regulation of spontaneous deep breaths. *Respir Physiol* 12: 230–238, 1971.
3. **Butera RJ, Rinzel JR, and Smith JC.** Models of respiratory rhythm generation in the pre-Bötzinger complex. I. Bursting pacemaker neurons. *J Neurophysiol* 82: 382–397, 1999.
4. **Conforti L and Millhorn DE.** Selective inhibition of a slow-inactivating voltage-dependent K^+ channels in rat PC12 cells by hypoxia. *J Physiol* 502: 293–305, 1997.
5. **Del Negro CA, Johnson SM, Butera RJ, and Smith JC.** Models of respiratory rhythm generation in the pre-Bötzinger complex. III. Experimental tests of model predictions. *J Neurophysiol* 86: 59–74, 2001.
6. **Del Negro CA, Morgado-Valle C, and Feldman JL.** Respiratory rhythm: an emergent network property? *Neuron* 34: 821–830, 2002.
7. **Dutschmann M, Wilson RJA, and Paton JFR.** Respiratory activity in neonatal rats. *Auton Neurosci* 84: 19–29, 2000.
8. **Fung ML and St.-John WM.** Separation of multiple functions in ventilatory control of pneumotaxic mechanisms. *Respir Physiol* 96: 83–98, 1994.
9. **Gebhardt C and Heinemann U.** Anoxic decrease in potassium outward currents of hippocampal cultured neurons in absence and presence of dithionite. *Brain Res* 837: 270–276, 1999.
10. **Glogowska M, Richardson PS, Widdicombe JG, and Winning AJ.** The role of the vagus nerves, peripheral chemoreceptors and other afferent pathways in the genesis of augmented breaths in cats and rabbits. *Respir Physiol* 16: 179–196, 1972.
11. **Gray PA, Janczewski WA, Mellen N, McCrimmon DR, and Feldman JA.** Normal breathing requires pre-Bötzinger complex

neurokinin-1 receptor-expressing neurons. *Nature Neurosci* 4: 927–930, 2001.

12. **Hahn PJ and Durand DM.** Bistability dynamics in simulations of neural activity in high-extracellular-potassium conditions. *J Comput Neurosci* 11: 5–18, 2001.

13. **Hammarström AK and Gage PW.** Inhibition of oxidative metabolism increases persistent sodium current in rat CA1 hippocampal neurons. *J Physiol* 510: 735–741, 1998.

14. **Hayashi F and Lipski J.** The role of inhibitory amino acids in control of respiratory motor output in an arterially perfused rat. *Respir Physiol* 89: 47–63, 1992.

15. **Horn EM and Waldrop TG.** Hypoxic augmentation of fast-inactivating and persistent sodium currents in rat caudal hypothalamic neurons. *J Neurophysiol* 84: 2572–2581, 2000.

16. **Jiang C and Haddad GG.** A direct mechanism for sensing low oxygen levels by central neurons. *Proc Natl Acad Sci USA* 91: 7198–7201, 1994.

17. **Kawai Y, Qi J, Comer AH, Gibbons H, Win J, and Lipski J.** Effects of cyanide and hypoxia on membrane currents in neurons acutely dissociated from the rostral ventrolateral medulla of the rat. *Brain Res* 830: 246–257, 1999.

18. **Koshiya N and Smith JC.** Neuronal pacemaker for breathing visualized in vitro. *Nature* 400: 360–363, 1999.

19. **Lieske SP, Thoby-Brisson M, Telgkamp P, and Ramirez JM.** Reconfiguration of the neural network controlling multiple breathing patterns: eupnea, sighs and gasps. *Nature Neurosci* 3: 600–607, 2000.

20. **Liu H, Moczydlowski E, and Haddad GG.** O_2 deprivation inhibits Ca^{2+}-activated K^+ channels via cytosolic factors in mice neocortical neurons. *J Clin Invest* 104: 577–588, 1999.

21. **Lopez-Barneo J, Pardal R, and Ortega-Saenz P.** Cellular mechanisms of oxygen sensing. *Annu Rev Physiol* 63: 259–287, 2001.

22. **Lumsden T.** Observations on the respiratory centres in the cat. *J Physiol* 57: 153–160, 1923.

23. **Marchetti C, Beato M, and Nistri A.** Evidence for increased extracellular $[K^+]$ as an important mechanism for dorsal root induced alternating rhythmic activity in the neonatal rat spinal cord in vitro. *Neurosci Lett* 304: 77–80, 2001.

24. **Melton JE, Kadia SC, Yu QP, Neubauer JA, and Edelman NH.** Respiratory and sympathetic activity during recovery from hypoxic depression and gasping in cats. *J Appl Physiol* 80: 1940–1948, 1996.

25. **Paton JFR.** Rhythmic bursting of pre- and post-inspiratory neurones during central apnoea in mature mice. *J Physiol* 502: 623–639, 1997.

26. **Paton JFR.** A working heart-brainstem preparation of the mouse. *J Neurosci Methods* 65: 63–68, 1996.

27. **Paton JFR.** The ventral medullary respiratory network of the mature mouse studied in a working heart-brainstem preparation. *J Physiol* 493: 819–831, 1996.

28. **Paton JFR, Li YW, and Kasparov S.** Reflex response and convergence of pharyngoesophageal and peripheral chemoreceptors in the nucleus of the solitary tract. *Neuroscience* 93: 143–154, 1999.

29. **Rekling JC and Feldman JL.** Pre-Bötzinger complex and pacemaker neurons: hypothesized site and kernel for respiratory rhythm generation. *Annu Rev Physiol* 60: 385–405, 1998.

30. **Remmers JE.** *Control of Breathing in Health and Disease*, edited by Altose M and Kawakami Y. New York: Dekker, 1998, p. 1–40.

31. **Richter DW.** *Comprehensive Human Physiology*. Berlin, Germany: Springer-Verlag, 1996, vol. II, p. 2079–2095.

32. **Richter DW and Ballantyne D.** *Central Neurone Environment*. Berlin, Germany: Springer, 1983, p. 164–174.

33. **Richter DW, Bischoff A, Anders K, Bellingham M, and Windhorst U.** Response of the medullary respiratory network of the cat to hypoxia. *J Physiol* 443: 231–256, 1991.

34. **Rybak IA, Paton JFR, Rogers RF, and St.-John WM.** Generation of the respiratory rhythm: state-dependency and switching. *Neurocomputing* 44–46: 605–614, 2002.

35. **Rybak IA, St.-John WM, and Paton JFR.** *Frontiers in Modeling and Control of Breathing: Integration at Molecular, Cellular, and Systems Levels.* New York: Plenum/Kluwer, 2001, p. 159–164.

36. **Schmidt C, Bellingham MC, and Richter DW.** Adenosinergic modulation of respiratory neurones and hypoxic responses in the anaesthetized cat. *J Physiol* 483: 769–781, 1995.

37. **Shao XM and Feldman JL.** Respiratory rhythm generation and synaptic inhibition of expiratory neurons in pre-Bötzinger complex: differential roles of glycinergic and GABAergic neural transmission. *J Neurophysiol* 77: 1853–1860, 1997.

38. **Smith JC, Butera RJ, Koshiya N, Del Negro C, Wilson CG, and Johnson SM.** Respiratory rhythm generation in neonatal and adult mammals: the hybrid pacemaker-network model. *Respir Physiol* 122: 131–147, 2000.

39. **Smith JC, Ellenberger HH, Ballanyi K, Richter DW, and Feldman. JL.** Pre-Bötzinger complex: a brainstem region that may generate respiratory rhythm in mammals. *Science* 254: 726–729, 1991.

40. **Solomon IC, Edelman NH, and Neubauer JA.** Patterns of phrenic motor output evoked by chemical stimulation of neurons located in the pre-Bötzinger complex in vivo. *J Neurophysiol* 81: 1150–1161, 1999.

41. **St.-Jacques R and St.-John WM.** Sensitivities of eupnea and gasping to alterations in temperature of in vivo and perfused rat preparations. *Respir Physiol* 123: 215–224, 2000.

42. **St.-John WM.** Neurogenesis, control and functional significance of gasping. *J Appl Physiol* 68: 1305–1315, 1990.

43. **St.-John WM.** Medullary regions for neurogenesis of gasping: noeud vital or noeuds vitals? *J Appl Physiol* 81: 1865–1877, 1996.

44. **St.-John WM.** Neurogenesis of patterns of automatic ventilatory activity. *Prog Neurobiol* 56: 97–117, 1998.

45. **St.-John WM and Paton JFR.** Characterizations of eupnea, apneusis and gasping in a perfused rat preparation. *Respir Physiol* 123: 201–213, 2000.

46. **St.-John WM and Paton JFR.** Neurogenesis of gasping does not require inhibitory transmission using $GABA_A$ or glycine receptors. *Respir Physiol Neurobiol* In press.

47. **St.-John WM and Rybak IA.** Influence of levels of carbon dioxide and oxygen upon gasping in perfused rat preparation. *Respir Physiol* 129: 279–287, 2002.

48. **St.-John WM, Zhou D, and Fregosi RF.** Expiratory neural activities in gasping. *J Appl Physiol* 66: 223–231, 1989.

49. **Thompson RJ and Nurse CA.** Anoxia differentially modulates multiple K^+ currents and depolarizes neonatal rat adrenal chromaffin cells. *J Physiol* 512: 421–434, 1998.

50. **Tomori Z, Fung ML, Donic Donicova VV, and St.-John WM.** Power spectral analysis of respiratory responses to pharyngeal stimulation in cats: comparisons with eupnoea and gasping. *J Physiol* 485: 551–559, 1995.

51. **Wang W, Fung ML, Darnall RA, and St.-John WM.** Characterizations and comparisons of eupnea and gasping in neonatal rats. *J Physiol* 490: 277–292, 1996.