Persistence of eupnea and gasping following blockade of both serotonin type 1 and 2 receptors in the in situ juvenile rat preparation

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Persistence of eupnea and gasping following blockade of both serotonin type 1 and 2 receptors in the in situ juvenile rat preparation. J Appl Physiol 103: 220–227, 2007. First published April 5, 2007; doi:10.1152/japplphysiol.00071.2007.—In severe hypoxia or ischemia, normal eupneic breathing is replaced by gasping, which can serve as a powerful mechanism for “autoresuscitation.” We have proposed that gasping is generated by medullary neurons having intrinsic pacemaker bursting properties dependent on a persistent sodium current. A number of neuromodulators, including serotonin, influence persistent sodium currents. Thus we hypothesized that endogenous serotonin is essential for gasping to be generated. To assess such a critical role for serotonin, a preparation of the perfused, juvenile in situ rat was used. Activities of the phrenic, hypoglossal, and vagal nerves were recorded. We added blockers of type 1 and/or type 2 classes of serotonergic receptors to the perfusate delivered to the preparation. Eupnea continued following additions of any of the blockers. Changes were limited to an increase in the frequency of phrenic bursts and a decline in peak heights of all neural activities. In ischemia, gasping was induced following any of the blockers. Few statistically significant changes in parameters of gasping were found. We thus did not find a differential suppression of gasping, compared with eupnea, following blockers of serotonin receptors. Such a differential suppression had been proposed based on findings using an in vitro preparation. We hypothesize that multiple neurotransmitters/neuromodulators influence medullary mechanisms underlying the neurogenesis of gasping. In greatly reduced in vitro preparations, the importance of any individual neuromodulator, such as serotonin, may be exaggerated compared with its role in more intact preparations.

EUPNEA AND GASPING ARE TWO fundamental patterns of automatic ventilatory activity that differ in multiple aspects. Eupnea is characterized by a sequential activation of muscles innervated by cranial and spinal nerves during inspiration and expiration (26). As opposed to eupnea, cranial and spinal nerves in gasping have a stereotypical, decrementing, synchronous inspiratory pattern with minimal expiratory activity (21, 22). The pattern of automatic ventilatory activity can be altered from eupnea to gasping by removal of pons and/or exposure to severe hypoxia or ischemia (9, 10, 14, 21, 22, 24).

Our laboratory has long maintained that different physiological mechanisms are responsible for generating eupnea and gasping. Eupnea is hypothesized to result from a complex interaction of cellular and synaptic properties within a pontomedullary neuronal network, whereas gasping depends on intrinsic cellular mechanisms localized in the medulla (14, 21, 22). Within the medulla, gasping is irreversibly eliminated following ablation of neurons within a ventrolateral “pre-Botzinger” complex and adjoining “gassing center” (4, 8, 20–22). Neuronal activities within this region have the proper discharge pattern for generating the gasp in that their discharge commences just before or concomitant with the onset of the phrenic burst (14, 25). Some of these “preinspiratory” or “inspiratory” medullary neuronal activities acquire an intrinsic burster discharge following a blockade of fast excitatory and inhibitory synaptic transmission. This burster discharge is dependent on conductance through persistent sodium channels because blockers of this conductance eliminate both the burster discharge and also gasping of in situ or in vivo preparations (14). The same concentration of blockers causes little alteration of eupnea of in situ preparations having an intact pontomedullary brain stem or of unanesthetized in vivo preparations (27).

Our laboratory has proposed that gasping is generated by the synchronized activity of excitatory neurons some of which may have intrinsic pacemaker bursting properties dependent on a persistent sodium current (14). Conductances through persistent sodium channels may be markedly influenced by exogenous levels of serotonin (6, 7, 30). Given this linkage of persistent sodium channel, serotonin, and gasping, it is hypothesized that endogenous serotonin is essential for gasping to be generated. Such a critical linkage of gasping and serotonin has been proposed based on results from an in vitro slice preparation in which a blockade of serotonin 2A receptors eliminated one type of rhythmic activity, which is considered to be analogous to gasping (30).

The decerebrate in situ preparations of the juvenile and neonatal rat exhibit patterns of automatic ventilatory activity that are comparable to eupnea and gasping of in vivo preparations (14, 24–27). Using this preparation, we have evaluated the hypothesis that activation of serotonin 5-HT2A subtype is essential for the neurogenesis of gasping. Gasping was still generated and little altered following a blockade of both 5-HT3 and also 5-HT1 receptor subtypes. Eupnea could similarly still be generated. We conclude that serotonin is but one of many neurotransmitters that can influence mechanisms underlying the neurogenesis of gasping.

METHODS

Experimental Preparations

Seventy-four perfused preparations of the decerebrate juvenile rat were used. The preparation was identical to that described previously (24), with surgical procedures being performed under deep halothane anesthesia, as assessed by an absence of a withdrawal response to page charges. The article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
noxious pinching of a paw. Anesthesia was discontinued following decerebration. Gallamine triethiodide was added to the perfusate to eliminate spontaneous respiratory movements. These procedures were approved by the Institutional Care and Use Committee of Dartmouth College and Dartmouth Medical School.

The descending aorta was cannulated, and perfusion was commenced. The perfusate contained the following in distilled water (in mM): 1.25 MgSO4, 1.25 H3PO4, 5.0 KCl, 25 NaHCO3, 125 NaCl, 2.5 CaCl2, 10 dextrose, and 0.1785 Ficoll 70. The temperature of the perfusate as it entered the aorta was 31°C. Under control conditions, the perfusate was equilibrated with a gas mixture of 95% O2-5% CO2.

Efferent activity of the phrenic nerve was recorded in all preparations. Efferent activity was also recorded from the central cut ends of the vagus nerve in 22 preparations, and the hypoglossal nerve was recorded in 29 preparations. Recordings were obtained during eupnea, with the perfusate equilibrated with 95% O2-5% CO2. To alter the pattern to gasping, the perfusion was terminated for a maximum of 70 s, thus producing a condition of ischemia.

Blockade of Serotonin Receptors

We blocked activities of serotonergic neurons using antagonists and agonists of the 5-HT1 and/or 5-HT2 class of receptors. As a mixed 5-HT1 and 5-HT2 receptor antagonist, methysergide maleate was used in concentrations of 5–40 μM. To block activities of neurons having 5-HT1A receptors, the agonist (R)-(+)-8-hydroxy-2-(di-n-propylamino) tetralin (8-OH-DPAT) was added to the perfuse in concentrations of 1–3 μM. Activation of the 5-HT1A receptor, which is an autoreceptor located on serotonergic neurons, by 8-OH-DPAT results in hyperpolarization and a decrease in cell firing. Finally, as an antagonist of the 5-HT2 class of receptors, ketanserin tartrate (ketanserin; 5–30 μM) was used. These concentrations of methysergide, 8-OH-DPAT and ketanserin were chosen based on preliminary experiments in which increasing concentrations of drugs were added to the perfusate until a clear alteration of phrenic discharge in eupnea was obtained. Concentrations below and slightly above this level were then used during eupnea and gasping. All drugs were from Tocris and Sigma.

Experimental Protocol

Activities of the phrenic, hypoglossal and vagal nerves were recorded for a minimum of 30 min under control conditions, with the perfusate equilibrated with 95% O2-5% CO2. Neural discharges during this period had an eupneic pattern (14, 24). In one subset of experiments, perfusion was terminated for a maximum of 70 s, and neural discharges of gasping were recorded. Perfusion was then recommenced, and the eupneic pattern of neural discharges was reestablished. One of the blockers of serotonergic receptors was then added to the perfusate, and neural activities recorded for a minimum of 10 min. Perfusion was again terminated for 70 s. A second round of recovery from ischemia was then performed in some experiments.

In a second subset of experiments, blockers of serotonergic receptors were introduced during the initial phase of eupnea, with no ischemia before the introduction of drugs. Ten minutes after drug administration, ischemia was produced for 70 s followed by a 30-min recovery with normal perfusion. This second set of experiments was performed to preclude a prior influence of brain stem hypoxia/ischemia on the responses to drugs.

Analyses of Data

Integrated phrenic activity was analyzed as to the duration of the burst [neural inspiratory (Ti)], period between bursts [neural expiratory (Te)], peak height, and time to reach peak height, expressed as a percentage of Ti. Integrated activities of the hypoglossal and vagal nerves were analyzed as to their peak heights in neural inspiration and expiration. We also defined the difference in time of onset of hypoglossal and vagal activities compared with that of the phrenic nerve. All of these variables were defined in both eupnea and gasping. Statistical evaluations were via an ANOVA with a Bonferroni correction for multiple comparisons. For a few comparisons involving pooled data, a Wilcoxon test was used.

RESULTS

Eupnea and Gasping

The patterns of phrenic, hypoglossal and vagal activities during eupnea and gasping were as described previously (14, 23–27). In eupnea, integrated phrenic discharge had a sudden onset and then a rise to reach a peak level at a time close to the termination of the burst (Fig. 1). Vagal discharge commenced before that of the phrenic [mean of 471 ± 82 (SE) ms] and had a burst, of equal or greater amplitude, in neural expiration (Fig. 1). Hypoglossal discharge also commenced before the phrenic [mean = 352 ± 46 (SE) ms], but it was largely limited to neural inspiration (Fig. 2).

Following a termination of perfusion, neural activities initially increased and then ceased. Activities returned with a pattern of gasping in which all nerves had a decrementing discharge, with peak activity being reached soon after onset. The difference in the time of onset of vagal and hypoglossal activities compared with phrenic was significantly reduced in gasping (vagus = 12 ± 7.5 ms, hypoglossal = 9 ± 9 ms; P < 0.05), and expiratory vagal discharge was reduced or eliminated (Fig. 1). For 21 preparations in which phrenic discharge was measured in both eupnea and gasping, the duration of the phrenic bursts was significantly less in gasping (0.60 ± 0.02 s) than eupnea (0.87 ± 0.08 s; P < 0.001). Peak integrated phrenic discharge was reached earlier in the phrenic burst in gasping (34.8 ± 1.8% of neural inspiration) than eupnea (59.0 ± 3.8%; P < 0.001). Other variables of phrenic discharge were very similar in eupnea and gasping.

Methysergide: Mixed 5-HT1 and 5-HT2 Receptor Antagonist

Alterations in eupnea. Eupnea continued following administrations of 2.5–40 μM of methysergide in 42 of 47 preparations.
Hyperplopy, or apnea, was recorded in 41 of 47 preparations that had received no drugs with few statistically significant changes being obtained (Fig. 4). Of interest is that hypoglossal discharge could become uncoupled from the phrenic burst in gasping following methysergide. This separation, evidenced by extra bursts of hypoglossal discharge (Fig. 2) or an absence of hypoglossal discharge, was frequently but not systematically observed.

Preparations that had received 30 μM (n = 3) or 40 μM (n = 1) methysergide also exhibited gasping, but, as in eupnea, peak phrenic height was greatly reduced compared with values before the drug.

**8-OH-DPAT: 5-HT1A Receptor Agonist**

As for methysergide, eupnea and gasping persisted following administrations of 8-OH-DPAT to 13 preparations. Changes were limited solely to increases in frequency of the phrenic burst in eupnea following 1.5 and 3.0 μM and of gasping following 1.5 μM. These changes were due to diminutions in the interval between bursts (Ts) (Fig. 5).

**Ketanserin: 5-HT2A Receptor Antagonist**

Alterations in eupnea. Ketanserin, at concentrations of 5–30 μM was administered to a total of 21 rats. Again, much variability in responses was observed between preparations, and, whereas, the frequency of phrenic bursts generally increased and the peak phrenic height generally declined (Figs. 6 and 7), no changes were statistically significant (Fig. 8). No preparation exhibited apnea.

Peak integrated hypoglossal discharge also declined, especially at the highest concentrations of ketanserin. Indeed, as shown in Fig. 7, peak activities of the phrenic and hypoglossal nerves declined together and were barely discernible at the highest concentrations of ketanserin. Also, the time of onset of hypoglossal compared with phrenic discharge was reduced in four of the five preparations in which hypoglossal activity was limited solely to increases in frequency of the phrenic burst in eupnea.

Comparison of Figs. 2 and 3 with Fig. 1 reveals that gasping following methysergide was similar to that of preparations that had received no drugs with few statistically significant changes being obtained (Fig. 4). Of interest is that hypoglossal discharge could become uncoupled from the phrenic burst in gasping following methysergide. This separation, evidenced by extra bursts of hypoglossal discharge (Fig. 2) or an absence of hypoglossal discharge, was frequently but not systematically observed.
recorded (504 ± 90 ms before the phrenic before ketanserin and 157 ± 130 ms after ketanserin).

Alterations in Gasping

In ischemia, multiple gasps were observed in all but two preparations in which only a single gasp, following by a tonic discharge was recorded. Both of these preparations had received 10 µM of ketanserin.

The duration of the phrenic burst (Ti), interval between bursts (Te), and frequency of gasping were not significantly different in preparations that received ketanserin compared with control preparation. However, as is shown in Figs. 6 and 7, by comparison with peak height of phrenic discharge in eupnea before administration of ketanserin, this peak height was reduced in gasping after the drug had been administered. Also reduced was the peak height of hypoglossal discharge and, indeed, hypoglossal and phrenic discharges became uncoupled in many preparations following ketanserin (Fig. 7).

DISCUSSION

Although endogenous serotonin may modulate activities of spinal and cranial nerves in eupnea and gasping, activation of serotonin receptors is not essential for the neurogenesis of either eupnea or gasping. A modulation by serotonin, but continuation of eupneic ventilatory activity in the absence of this modulation, is in agreement with many previous studies using in vivo preparations (2, 16, 18, 19). For gasping, a critical role for serotonin receptors in its neurogenesis has been proposed based on results from one type of thick, medullary slice in vitro preparation (30). In this preparation, a differential influence of serotonin on its two rhythmic discharges was reported with the decrementing, “gasplike” discharge being eliminated following
a blockade of serotonin receptors while the “eupneic-type” rhythm continued. Yet, a similar decrementing rhythmic activity of a number of other in vitro preparations continued unabated following a blockade of serotonin receptors (e.g., Refs. 1, 2, 5, 11). Finally, in an earlier report with the thick slice preparation, the eupneic-type discharge was reported to be critically dependent on endogenous serotonin for its expression (15).

Differences Between in Vitro and in Situ Findings

We believe that the relationship between rhythmic activities of various in vitro preparations with eupnea and gasping in vivo or in situ continues to be vague (see, e.g., 14, 26, 27). Yet, it is clear that in one type of medullary slice preparation, its rhythmic decrementing discharge and the discharge of one group of burster neurons were both eliminated following a blockade of receptors for serotonin (30). These burster neurons, which are dependent on conductances through persistent sodium channels, are located in the medullary region, which is critical for gasping (14).

Our finding that gasping continued following administration of blockers of serotonin might mean that these blockers did not reach medullary neurons that are critical for the neurogenesis of gasping and/or did not reach these neurons in sufficient concentration. Although changes in peak heights of cranial and spinal nerves and respiratory frequency did occur following administration of blockers, these changes might reflect actions upon multiple components of the pontomedullary and spinal respiratory network (1, 2, 11, 13, 16).

Another explanation for differences between findings from in vitro and in situ preparations is that the importance of neuromodulators such as serotonin may be greatly exaggerated in substantially reduced in vitro preparations. In both the in vitro medullary respiratory system and in many other components of the central nervous system, conductances through persistent sodium channels may be markedly influenced by exogenous levels of serotonin (6, 7, 30). Yet, in addition to serotonin, multiple neuromodulators influence the conductance through persistent sodium channels (e.g., Refs. 6, 7, 30, 31).
in vitro slice preparations, in which neural pathways and endogenous neurotransmitters may be substantially reduced, an alteration in sensitivity to remaining neurotransmitters may be established (6, 7). We submit that in a more intact preparation, norepinephrine and other transmitters that modulate conductivity through persistent sodium channels would render endogenous levels of serotonin as of minor importance for the neurogenesis of gasping.

Eupnea and Gasping in Situ: Confirmation of Previous Results

The alterations in the discharge patterns of vagal, hypoglossal and phrenic nerves on exposure to ischemia confirm findings reported in detail previously. Hence, the incrementing pattern of phrenic discharge was altered to a decrementing discharge as were the integrated discharge patterns of the vagus and hypoglossal nerves (21, 24–26). Moreover, the earlier onset of hypoglossal and vagal, compared with phrenic, discharge was eliminated with the change from eupnea to gasping. Also eliminated was the marked discharge of the vagus nerve in early neural expiration (21, 24–26).

Another observation that confirmed previous findings was the uncoupling of hypoglossal from phrenic discharge following administration of blockers of serotonin (23). This uncoupling reinforces the concept, established in many publications, of a differential control of respiratory-modulated activities of cranial compared with spinal neural activities (e.g., Refs. 18, 19, 23). Moreover, this uncoupling is also compatible with the conclusion that serotonin may modulate and coordinate different rhythms but that it is not essential for the generation of any particular rhythm.

Endogenous Serotonin Modulates Eupnea and Gasping

The serotonin system is widespread in the brain stem, and changes in release of serotonin result in multiple alterations in
homeostatic functions (1, 2, 11, 13, 16). For the respiratory system, influences on activities of premotor and both cranial and spinal motoneurons are well described. In this context, our results indicate that serotonergic mechanisms may be of importance for the earlier onset of discharge of hypoglossal and vagal than phrenic discharge, because this earlier onset was lessened or eliminated following blockade of serotonin receptors. Yet, the concomitant change in respiratory frequency obscures the specificity of this change in onset of neural activation. Thus serotonin increased the respiratory frequency, which may shorten the lead time between hypoglossal and vagal activities and the onset of phrenic discharge. Finally, an importance of serotonin for “central chemoreception” has also been advanced (17), but this importance is unclear (12, 16).

Given these widespread influences of serotonin, the specific factors cannot be defined that are responsible for the changes in both frequency and peak height of activities of cranial and spinal nerves that were observed in some preparations following administration of blockers of the various types of serotonin receptors.

Serotonin, Gasping, and Sudden Infant Death Syndrome

Recently, widespread abnormalities in the serotonergic system within the brain stems of victims of the “sudden infant death syndrome” (SIDS) have been reported (13). Yet, numerous attempts to link an interruption of the serotonergic system with a major failure of the cardiorespiratory system, as would be expected in SIDS, have been unsuccessful (see discussion in Ref. 16). It has long been hypothesized that the SIDS reflects a failure of gasping and/or a failure of gasping to be an effective mechanism of autoresuscitation (3). The importance of endogenous serotonin for the neurogenesis of a rhythm in vitro led to the supposition that serotonin might also be critical for gasping in vivo and, by extension, establish a link between serotonin and SIDS (30). Results reported herein do not confirm that serotonin is an exclusive and necessary factor for the neurogenesis of gasping. Perhaps the lack of significant changes in cardiorespiratory function following interruption of the serotonergic system in vivo points to the multiplicity of neurotransmitter and neuromodulators that define this function. Hence, interruption of any single neuromodulator/neurotransmitter system alone would not lead to SIDS.

REFERENCES


