A mapping study of cardiorespiratory responses to chemical stimulation of the midline medulla oblongata in ventilated and freely breathing rats

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Verner, Todd A., Ann K. Goodchild, and Paul M. Pilowsky. A mapping study of cardiorespiratory responses to chemical stimulation of the midline medulla oblongata in ventilated and freely breathing rats. Am J Physiol Regul Integr Comp Physiol 287: R411–R421, 2004.—The aim of this study was to examine the cardiorespiratory effects of chemically stimulating neurons in the midline medulla oblongata (MM) of artificially ventilated and freely breathing anesthetized rats. Earlier studies reported that stimulation of the MM elicits increases or decreases in mean arterial pressure (MAP) and phrenic nerve activity, depending on the mode and site of stimulation, anesthetic, and species. In the first series of experiments, rats were anesthetized with urethane, artificially ventilated, paralyzed, and bilaterally vagotomized. The rostrocaudal extent of the MM was mapped by microinjections of DL-homocysteic acid or L-glutamate (both 100 mM, 100 nl), and, in line with previous studies, most injections produced only small responses in MAP, heart rate, and splanchnic sympathetic nerve activity. Increases in respiratory parameters were evoked in caudal regions. However, activation of a discrete region of the MM at the level of the caudal pole of the facial nucleus (CP7) consistently caused a dramatic reduction in phrenic nerve amplitude and/or frequency and, in six rats, produced a prolonged apnea. The second series of experiments was carried out on freely breathing pentobarbitone sodium-anesthetized rats, with a diaphragmatic electromyogram used to monitor respiratory activity. Respiratory activity could again be abolished at CP7 after microinjections of glutamate (100 mM, 50 nl); however, these responses were accompanied by large decreases in MAP and moderate reductions in heart rate. This depression of respiratory activity may be due to activation of propriobulbar inhibitory neurons that project to known respiratory centers in the brain stem.

THE MIDLINE MEDULLA OBLONGATA (MM) of the brain stem contains the medullary raphe nuclei (MRN): the obscurus (ROb), pallidus (RPa), and magnus (RMa) (60). These nuclei provide the main source of serotonin (5-HT) to the brain stem and spinal cord (10). Many neurochemicals, including glutamate, γ-aminobutyric acid (GABA), somatostatin, thyrotropin-releasing hormone, substance P, and enkephalin, are colocalized with serotonergic neurons (13, 18, 36, 37, 46). The raphe nuclei have been implicated in various physiological functions, including cardiovascular responses to hemorrhage (27, 28), respiration and thermoregulation (7, 9, 61), and nociception (for reviews see Refs. 44 and 55). Neurons with respiratory-related firing patterns have been identified in this region in cats and rats (23, 33), and some neurons in this region are chemosensitive (see Ref. 56 for review).

Stimulation or inhibition of the MRN can produce differential effects on arterial pressure and/or heart rate (HR), depending on the site and mode of stimulation/inhibition, the anesthetic, and the species. It has been suggested that these contradictory results are due to stimulation of a mixed population of sympathoexcitatory and sympathoinhibitory neurons in the MRN (17). Coleman and Dampney (15) microinjected glutamate into the MM of pentobarbitone sodium (pentobarbitone)-anesthetized rabbits and described a powerful depressor and bradycardic site within a highly restricted portion of the RPa and ROb, caudal to the obex. A similar depressor region was identified with microinjections of excitatory amino acids in halothane (27)- and pentobarbitone (63)-anesthetized rats that extends from the obex rostrally for ~1.5 mm. In rats anesthetized with urethane, however, microinjections of excitatory amino acids into the same region of the MM predominantly produces small increases in arterial pressure accompanied by small tachycardic responses (28).

Similar contradictory responses have been found in the MM with regard to its effects on respiratory function. In cats anesthetized with α-chloralose, electrical or chemical stimulation of the ROb and RPa produced increases in phrenic nerve activity (PNA) (29). Similarly, in cats anesthetized with a mixture of α-chloralose and urethane, electrical stimulation of the ROb increased PNA, while stimulation of the RPa produced inconsistent effects on PNA (31). Conversely, electrical stimulation of the ROb and RMa in cats decerebrated or anesthetized with a variety of anesthetics depressed or inhibited PNA (40, 41). In rats anesthetized with urethane or an α-chloralose-urethane mixture, electrical or chemical stimulation of various sites within the MM produced no consistent effects (21) or increases in respiratory activity (3, 8).

As a result of these conflicting results and the fact that electrical stimulation, which has been favored in respiratory studies, excites cell bodies and fibers of passage, we sought to provide a comprehensive map of the cardiorespiratory effects produced by chemical stimulation of the rostrocaudal extent of the MM in rats. The MM was stimulated with DL-homocysteic acid (DLH) or L-glutamate (Glu) in artificially ventilated urethane-anesthetized and freely breathing pentobarbitone-anesthetized Sprague-Dawley rats.

METHODS

All experiments were carried out in accordance with the Australian Code of Practice for the Care and Use of Animals for Scientific Purposes and were approved by the Animal Care and Ethics Com-
mittee of the Royal North Shore Hospital. Experiments were carried out on 35 adult male Sprague-Dawley rats (350–500 g; Gore Hill Research Laboratories, Sydney, Australia). Animals were housed in small groups in temperature-controlled conditions with a fixed 12:12-h light-dark cycle, and food and water were available ad libitum. Two separate series of experiments were carried out in this study: the first on artificially ventilated rats and the second on freely breathing rats. Rectal temperature of the rats was monitored and thermostatically maintained throughout the experiments at 37.0 ± 1°C by means of a heat blanket and infrared lamp.

**Ventilated Rats**

In ventilated rats (n = 17), anesthesia was induced with halothane (4.0–5.0% in O2; Fluothane, Zeneca) followed by administration of urethane [1.3 g/kg ip, 10% (wt/vol) in saline, ethyl carbamate; Sigma-Aldrich]. Additional doses of urethane (0.13 g/kg iv) were administered when required, evidenced by large fluctuations in blood pressure (20–30 mmHg) in response to noxious stimuli. A tracheostomy was performed to permit artificial ventilation, and the right common carotid artery and jugular vein were cannulated for the recording of arterial pressure and the intravenous administration of drugs and fluids, respectively. Arterial pressure was measured via the arterial cannula connected to a pressure transducer (Abbott Critical Care Systems). Rats were artificially ventilated (70–80 cycles/min, 3.0–4.0 ml/cycle) with O2-enriched room air via a respirator (UGO Basile, Biological Research Apparatus). Bilateral vagotomies were performed on 14 of 17 rats. All rats were paralyzed with pancuronium (1.6 mg/kg iv; pancuronium bromide; AstraZeneca), and additional doses (0.8 mg/kg iv) were administered hourly or when required, as indicated by fluctuations in expired CO2. Expired CO2 was measured using a CO2 analyzer (Capstar-100, CWE) and maintained between 3.5 and 5% of expired gases by adjustment of respiratory rate or volume.

The left greater splanchnic nerve was exposed using a retroperitoneal approach. The phrenic nerve was exposed in the neck via a dorsal parafascial approach. The two nerves were cut distally, immersed in pools of paraffin oil, and recorded with bipolar electrodes to permit recording of preganglionic sympathetic nerve activity (ssNA) and PNA. Nerve activity was filtered between 30 Hz and 1 kHz, amplified (CWE) and sampled at 1.000 Hz (model 1401 PLUS, CED), and displayed as a waveform using Spike2 software (version 5, CED).

**Freely Breathing Rats**

In freely breathing rats (n = 18), anesthesia was induced with halothane as described above, pentobarbitone sodium (60 mg/kg ip) was injected, and atropine sulfate (0.1 ml ip, 500 µg/ml; Astra Pharmaceuticals) was administered to reduce mucus secretions in the airways. Adequate levels of anesthesia were maintained by additional injections of pentobarbitone (3–6 mg/kg ip) diluted in saline. Rats were intubated and allowed to freely breathe O2-enriched room air for the duration of the experiment. The right superficial femoral artery was cannulated for the recording of arterial pressure, and respiratory activity was recorded using bipolar electrodes inserted into the diaphragm or intercostal muscles and sampled as described above.

**Microinjections**

In all experiments, rats were placed in a prone position with the head secured in a stereotoxic frame (Kopf Instruments), ear bars placed in the auditory canals, and the head angled at −15°. An occipital craniotomy was performed, with the cut edges of bone sealed using bone wax, and the dural and arachnoid membranes were removed to expose the dorsal surface of the medulla oblongata. The calamus scriptorius was the landmark on the dorsal surface of the medulla oblongata used as the stereotaxic zero point in experiments.

In ventilated rats, the rostrocaudal extent of the MM was stimulated using 100-nl microinjections of 100 mM DLH (n = 113; ICN Biochemicals) or Glu (n = 103; Sigma). In freely breathing rats, microinjections of Glu (n = 129, 50 nl, 100 mM) were made in the MM concentrated at the level around the CP7. The volume of Glu used in this group was smaller to reduce the spread of the drug and allow a more concise map of this region of the MM. DLH and Glu were dissolved in phosphate-buffered saline (PBS), and pH was adjusted to 7.4. In each experiment, injection sites located at least two different rostrocaudal levels on the ventral levels were marked with microinjections of methylene blue [2% (wt/vol) in PBS, 200 nl; Sigma] or biotinylated albumin [0.1% (wt/vol) in PBS, 100 nl; Sigma]. Microinjections were made at least 5 min apart by pressure injection with a 5-ml syringe. The volume of each injection was monitored by the movement of the meniscus of the drug against gradations on a calibrated scale.

**Immunohistochemistry**

At the conclusion of the experiment, rats were killed by intravenous injection of KCl (3.0 M, 0.3 ml) or transcardially perfused with PBS followed by formaldehyde (4% in 0.1 M sodium phosphate-buffered solution (NaPB), pH 7.4). The brain was removed and fixed in formaldehyde (4% in 0.1 M NaPB) overnight. The brain stem was then isolated, and the meninges were removed and washed in 0.1 M NaPB and then sectioned using a vibrating microtome (50 µm; Leica). Sections were washed in 50% ethanol for 30 min and then washed three times in Tris-phosphate-buffered solution (10 mM Tris·HCl, 10 mM NaPB, 0.9% NaCl, pH 7.4). In experiments where methylene blue was used, the injection sites were visualized by a nickel-based diaminobenzidine reaction (64). Injection sites marked with biotinylated albumin were visualized by incubation of the sections in ExtrAvidin peroxidase (1:1000; Sigma) for 4–16 h, washed three times in Tris-phosphate-buffered solution, and then exposed to diaminobenzidine (64).

Sections were mounted serially onto gelatinized slides, dehydrated with ethanol, and counterstained with cresyl violet, and coverslips were applied. Injection sites were verified using a microscope (Zeiss), with ventral landmarks used to determine the location of the injections. The response to each injection was mapped on coronal sections adapted from Paxinos and Watson (53).

**Controls**

Microinjections of 10 mM Glu and PBS were made in four of the freely breathing experiments at sites where microinjections of 100 mM Glu produced a profound effect.
Analysis

Mean arterial pressure (MAP) and HR were derived from arterial pressure, nerve activities were rectified, and a moving average was applied (1.0 s for sSNA and 100 ms for PNA). The control values were taken as the mean level of each variable 30 s before each injection, and the response was taken as the peak response after each microinjection. All responses were calculated as a percentage of preinjection control. Where applicable, values are means ± SE. The cardiorespiratory responses were grouped according to their rostro-caudal level and graphed. Paired, two-tailed t-tests were applied to assess the significance of the responses compared with the preinjection control levels.

Within the artificially ventilated group, analysis was performed to determine whether there was any difference between the cardiovascular responses to DLH and Glu and between the responses in intact and vagotomized rats. A Bartlett’s test for homogeneity of variances was applied, and it was found that the variance between responses was heteroscedastic. As a result, a square root transformation was applied to the responses before one-way ANOVA (59).

Further analysis of PNA was carried out after microinjections of DLH or Glu into a restricted region of the rostral MM (n = 29 sites) to determine whether the reductions in phrenic nerve frequency were due to a change in the inspiratory or expiratory phase of the respiratory cycle. The following criteria were used: 1) sites were immunohistologically verified to be within a 500-μm radius of a restricted region that consistently produced apneic responses, and 2) microinjection of DLH or Glu (100 mM, 100 nl) produced a reduction in phrenic nerve frequency without apnea. A waveform average of PNA was created over a 15-s period immediately before microinjection and 15 s during the peak response on PNA. From this, the durations of the inspiratory and expiratory phases were calculated, and the response was expressed as a percentage of the preinjection control. These results were then grouped and given as means ± SE, and paired, two-tailed t-tests were performed to assess significance.

RESULTS

Ventilated Rats

Cardiovascular responses. In urethane-anesthetized rats, the cardiovascular responses to microinjections of DLH (100 mM, 100 nl) or Glu (100 mM, 100 nl) throughout the MM were of the same direction and magnitude in nonvagotomized and vagotomized preparations. A one-way ANOVA was carried out between the intact (DLH) and vagotomized (DLH or Glu) rats, and no significant difference (P = 0.2) was found in the arterial pressure responses between the three groups. Microinjections of DLH or Glu made throughout the rostrocaudal extent of the MM predominantly produced small pressor responses (Fig. 1, see Fig. 3A), with 182 of 216 injections producing pressor responses <20 mmHg. These pressor responses were generally accompanied by small increases in sSNA (Figs. 2B and 3B), with 199 of 216 injections producing...

Fig. 2. Responses in artificially ventilated, urethane-anesthetized rats. A: increases in PNA. Glu (100 mM, 100 nl) was microinjected into a site in the MM ~12.3 mm caudal to bregma and 3.5 mm ventral from the dorsal surface (~12.3 mm/3.5 mm). Arterial pressure (AP) increased by 31% (from 108 to 142 mmHg), heart rate (HR) increased by 4% (from 472 to 489 beats/min), splanchnic sympathetic nerve activity (sSNA) increased by 5%, phrenic nerve frequency increased by 51% (from 0.76 to 1.15 Hz), and phrenic nerve amplitude increased by 23%.

B: reductions in PNA. Microinjection of DLH (100 mM, 100 nl) into the MM (~11.8 mm/3.5 mm) caused a 23% reduction in phrenic nerve frequency (from 1.02 to 0.79 Hz) and a 28% reduction in phrenic nerve amplitude, accompanied by a 22% pressor response (from 86 to 105 mmHg), a 2% increase in HR (from 461 to 470 beats/min), and a 16% increase in sSNA.

C: abolition of PNA. Microinjection of DLH (100 mM, 100 nl) at the level of CP7 (~11.3 mm/3.5 mm) caused an abolition of PNA that lasted for 254 s, accompanied by a small pressor response (12%) and an increase in sSNA (5%) with no effect on HR. Solid circles in the medullary section at top right in A–C show location of each microinjection. Arrowhead, time of microinjection.
a <20% increase in sSNA. Microinjections of DLH or Glu produced little effect on HR, with responses >10% seen after only 2 of 216 injections.

Phrenic nerve responses. Microinjections of DLH and Glu into the MM had no effect on PNA (n = 55) or produced one of three responses: 1) an increase in phrenic nerve frequency and amplitude (n = 60; Figs. 2A and 3, C and D), 2) a reduction in phrenic nerve frequency and/or amplitude (n = 93; Figs. 2B and 3, C and D), or 3) an abolition of PNA (n = 9; Fig. 2C). All three responses were obtained in the intact and vagotomized rats with microinjections of DLH or Glu.

Increases in PNA. Sites that produced increases in PNA (Figs. 1 and 3, C and D) were largely restricted to levels ranging from 13.8 to 12.3 mm caudal from bregma. All sites producing an increase in PNA were accompanied by small increases in MAP and sympathetic nerve activity, with little effect on HR (Figs. 2A and 3).

Reductions in PNA. Sites that reduced PNA were typically accompanied by small pressor responses and small increases in sSNA, with little effect on HR (Figs. 2B and 3), and were restricted to more rostral levels of the medulla oblongata ranging from 11.8 to 10.8 mm caudal to bregma (Figs. 1 and 3).

Abolition of PNA. Apneic responses were largely restricted to the level of CP7 (11.3 mm caudal to bregma) and were evoked most commonly at a depth of 3.5 mm from the dorsal surface (Fig. 1) and accompanied by small increases in MAP and sSNA, with little or no effect on HR (Figs. 2C and 3). At these sites, the duration of apnea ranged from 51.3 to 281.6 s, with a mean duration of 137.3 ± 35.4 s (n = 6 sites). In one experiment, all four injections made at this level produced apneic responses ranging from 93.1 to 254.3 s; in another experiment, one apneic response with a duration of 5.4 s was also seen at a site 1 mm more caudally at a depth of 3.5 mm. This site corresponds approximately to the level of the rostral extent of the ROb, the middle of the RPa, and the caudal end of the RMa.

Sites within a 500-µm radius of this restricted apneic region that produced a reduction in phrenic nerve frequency without a <20% increase in sSNA. Microinjections of DLH or Glu produced little effect on HR, with responses >10% seen after only 2 of 216 injections.

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in RF (47 sites) or apnea (53 sites), with only 10 sites producing an increase in RF and 19 sites having no effect on respiratory activity.

Brief apneic responses (Fig. 5A) were concentrated at the level of CP7 (Fig. 4A). At this level, 37 injections made at depths from 2.0 to 3.5 mm ventral produced an apneic response with an average duration of 4.12 ± 0.42 s and were accompa-

ried by depressor and bradycardic responses. Other injections at this level mainly produced reductions in RF (n = 17), with only three injections having no effect and three increasing RF.

The group data for the cardiorespiratory responses in pentobarbitone-anesthetized, freely breathing rats are shown in Fig. 6. Microinjections of Glu at the levels examined produced moderate (~20%) and significant reductions in MAP and small (~10%) but significant changes in HR (Fig. 6, A and B). RF was reduced at all levels, reaching highly significant reductions at the level of CP7 (Fig. 6C). As seen in the urethane-anesthetized group of rats, apneic responses were frequently elicited at this level.

Control Experiments

In four experiments carried out in freely breathing, pentobarbitone-anesthetized rats, PBS (50 nl, pH 7.4) was microinjected into 23 sites where 100 mM Glu microinjections produced profound effects on MAP or RF. PBS had no effect on MAP, HR, or RF at any of these sites (results not shown). Microinjections of 10 mM Glu were also made at 27 sites in these experiments (Figs. 4B and 5B). At the more caudal level (bregma −11.8 mm), microinjections of 10 mM Glu evoked little response in MAP, HR, or RF. More rostrally (bregma −11.3 to −10.8 mm), the cardiorespiratory responses to 10 mM Glu were less than the 100 mM responses. The effects on RF, however, were similar in magnitude to the 100 mM Glu responses, with apnea being produced at six sites.

DISCUSSION

The most significant and novel finding is that respiration was consistently depressed after chemical activation of neurons in the MM between 10.6 and 11.6 mm caudal to bregma. In particular, apnea was elicited in urethane- and pentobarbitone-anesthetized rats at sites concentrated at the level of CP7. This study also provides the first comprehensive maps of the cardiorespiratory effects of chemically stimulating the MM in anesthetized artificially ventilated and freely breathing rats. Microinjecting the excitatory amino acids DLH and Glu into the MM between 10.6 and 11.6 mm caudal to bregma, produces depressor responses and bradycardia. At rostral levels, respiratory activity was depressed, with apnea occurring at a majority of sites at the level of CP7.

Methodological Considerations

Urethane and pentobarbitone are two commonly used anesthetics and were chosen in the present study because of their opposing cardiovascular effects with respect to MRN stimula-
Both anesthetics have been used and neuronal ACh receptors (25). Pentobarbitone is shorter acting and exerts its anesthetic effects largely through actions on GABA<sub>A</sub> receptors (25). Both anesthetics have been used extensively in previous studies investigating cardiorespiratory responses with preinjection control levels: *<i>P</i> < 0.05, ***<i>P</i> < 0.005. Each brain stem level represents midpoint of a 0.5-mm section, as adapted from Paxinos and Watson (53).

Fig. 6. Group responses to stimulation of the MM from 12.0 to 10.6 mm caudal from bregma in freely breathing, pentobarbitone-anesthetized rats. A: MAP was decreased by highly significant amounts at all rostrocaudal levels examined. B: HR was decreased significantly at all levels, reaching highly significant levels at the more rostral levels (bregma −11.3 and −10.8 mm). C: respiratory frequency was decreased at all levels, reaching highly significant reductions at more rostral levels (bregma −11.3 and −10.8 mm). Values are means ± SE. Paired, 2-tailed <i>t</i>-tests were used to compare peak responses with preinjection control levels: *<i>P</i> < 0.05. ***<i>P</i> < 0.005. Each brain stem level represents midpoint of a 0.5-mm section, as adapted from Paxinos and Watson (53).

The dose of drug used and the volume injected were based on previous recommendations (24, 43). The spread of the Glu concentration (28, 63). Urethane is a long-lasting general anesthetic that has modest effects on multiple neurotransmitter-gated ion channels, including GABA<sub>A</sub>, glycine, N-methyl-d-aspartate, and neuronal ACh receptors (25). Pentobarbitone is shorter acting and exerts its anesthetic effects largely through actions on GABA<sub>A</sub> receptors (25). Both anesthetics have been used extensively in previous studies investigating cardiorespiratory reflexes (47, 48, 63).

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Atropine sulfate was administered to rats in the pentobarbitone-anesthetized group, but not in the urethane-anesthetized group. Although we cannot rule out that atropine may have had an effect, we find it unlikely, because the direction of the respiratory responses was the same in rats that did and those that did not receive atropine. Furthermore, the cardiovascular responses found in the pentobarbitone-anesthetized group are in the same direction and of a similar magnitude to a previous study where atropine was not administered (63).

### Cardiovascular Responses

Small pressor responses were evoked throughout the rostrocaudal extent of the medulla oblongata. This result is in agreement with a previous study by Heslop et al. (28) carried out in urethane-anesthetized rats. Neurons in the MRN provide a major source of input to sympathetic preganglionic neurons in the intermediolateral cell column of the thoracic and upper lumbar spinal cord (1). However, the pressor and excitatory response to electrical stimulation of the ROb is abolished by bilateral destruction of, and attenuated by microinjection of glycine into, the rostral ventrolateral medulla (RVLM) (12). Therefore, it is likely that the pressor responses are mediated via a direct projection from the MRN to sympathoexcitatory neurons in the RVLM (62, 63).

The depressor and bradycardic responses found in pentobarbitone-anesthetized rats in the present study are in agreement with a previous study (63). These depressor responses were accompanied by a reduction in the firing rate of presympathetic neurons in the RVLM (63), and bilateral blockade of GABA<sub>A</sub> receptors in the RVLM abolishes the depressor responses in rabbits (16). It is therefore likely that the depressor responses seen in the present study are due to inhibition of sympathoexcitatory neurons in the RVLM.

Depressor responses can also be evoked in halothane (26)- and chloralose-urethane-anesthetized rats (3), in contrast to the pressor responses evoked in urethane-anesthetized animals. Hence, cardiovascular responses evoked by stimulating the MM are anesthetic dependent. The depressor responses evoked...
in pentobarbitone-anesthetized preparations may be due to the GABA-mimetic effects of pentobarbitone (35, 39), but this possibility awaits further study.

Respiratory Effects

Chemical stimulation of the rostral MM in urethane- and pentobarbitone-anesthetized rats consistently inhibited respiratory activity in the present study. This indicates that the respiratory effects are independent of the anesthetic used and are therefore likely to be mediated by a separate population of neurons, other than those responsible for the cardiovascular responses. This is not surprising, inasmuch as the MRN is a heterogeneous population of neurons that are implicated in many physiological functions, including thermoregulation (7, 9, 61) and nociception (44, 55), as well as cardiovascular and respiratory regulation.

Previous studies have reported that chemical or electrical stimulation of the MRN increased PNA in rats (3, 7) and cats (29–31). The increases in PNA were ameliorated by intravenous administration of 5-HT antagonists (32). The increases in PNA in the present study in urethane-anesthetized rats were restricted to the caudal levels of the medulla, where many serotonergic neurons are found (60), and may also be mediated by 5-HT.

This is the first study to find that respiratory activity can be abolished by chemical stimulation of the MM in rats at the level of CP7 and that this effect can be elicited under two different anesthetics. The difference in the duration of apnea under the two different anesthetics may be due to the fact that the urethane-anesthetized group was artificially ventilated, with the end-tidal CO₂ kept constant. Conversely, rats in the pentobarbitone-anesthetized group were allowed to breathe freely. Reductions in respiratory activity, especially apnea, would have resulted in an increased blood concentration of CO₂ activating peripheral and central chemoreceptors, leading to an increase in respiratory drive (41).

Lalley (40, 41) found that electrical stimulation, which activates cell bodies and fibers of passage, of the ROb and RMa depresses PNA in cats, with stimulation at the highest intensity producing apnea that lasts for the duration of the stimulus. Interestingly, PNA was consistently depressed after electrical stimulation of the RMa at the level of the facial nucleus, a region that consistently produced apnea in the present study after chemical stimulation in rats. Nevertheless, intravenous administration of 5-HT antagonists partially reduced the depression of PNA, and inhibition of 5-HT reuptake with intravenously administered fluoxetine enhanced depression of PNA, indicating that 5-HT may be partly responsible (41). However, the same study found that depletion of 5-HT with a pretreatment of reserpine had no effect on the depression of PNA (40, 41), indicating that the role of 5-HT is unclear.

Two possible sites where inhibition of respiratory activity could occur include the phrenic motor nucleus (PMN) and the VRG. In the rat, tracing (19) and electrophysiological (34) studies have shown that neurons in the three MRN project to the PMN and, in the cat, that 5-HT neurons synapse with the PMN (54). However, the most responsive area found in the present study corresponds to a population of “third-order” neurons that affect phrenic premotor neurons revealed by the viral tract-tracing work of Dobbins and Feldman (19). Iontophoretic application of 5-HT to the PMN has weak stimulatory effects (40, 41), indicating that the depression of respiratory activity is not likely to be caused by the release of 5-HT at the PMN but may be due to nonserotonergic actions. Iontophoresis of GABA and glycine onto phrenic motor neurons reduces their activity; however, intravenous administration of GABA or glycine antagonists did not block the depression of PNA in response to MRN stimulation (41, 42), indicating that the depression of PNA is not due to GABA or glycine. Therefore, it appears that the reduction in respiratory activity in the present study is not due to direct actions in the PMN, in agreement with Lalley (41).

The VRG in the ventrolateral medulla oblongata includes inspiratory and expiratory neurons and is another possible site at which the depression of respiratory activity may be mediated. Neurons from all three nuclei of the MRN send projections to all regions of the VRG (58). Microinjection of the 5-HT₁A receptor agonist 8-hydroxy-2-(di-n-propylamino)tetralin into the pre-Bötzinger complex of the VRG produces apnea (57). Furthermore, blockade of 5-HT₁A receptors with intravenously administered NAN-190 antagonized, but did not block, the depression of PNA after electrical stimulation of the ROb in cats (42). It is therefore possible that the respiratory depression and apnea seen in the present study may be partly due to release of 5-HT in the VRG, which in turn acts on 5-HT₁A receptors. On the other hand, excitation of expiratory neurons in the Bötzinger complex and caudal VRG results in a depression of respiratory activity (14). Conceivably, the depression of respiratory activity in the present study is due to the release of 5-HT, Glu, or some other neurotransmitter acting pre- or postsynaptically in respiratory nuclei, such as the Bötzinger complex.

Chemosensitivity and the Raphe

Several studies have shown the presence of chemosensitive neurons in the medullary raphe nuclei (see Ref. 56 for review). Exposure of adult (51) and juvenile and neonatal rats (2) to moderate hypercapnia increases Fos labeling in MRN neurons that are predominantly in close apposition to surface arteries (51), and are serotonergic (11). In tissue cultures derived from rat MM, acidosis-stimulated neurons were found to be serotonergic, whereas all acidosis-inhibited neurons were nonserotonergic (66). Injection of acetazolamide, a chemical that produced focal acidosis, increases the amplitude of PNA at several sites in the MRN (4). As a result, it is not surprising that neurons in the MRN play a major role in the respiratory response to hypercapnia. In piglets, blockade of neurons in the MRN by microinjection of muscimol (45) and lidocaine and ibotenic acid (20) reduces the increase in respiratory activity in response to increased CO₂ but has no effect on respiratory activity under resting conditions.

Exposure of juvenile (6) and adult rats (5) to hypoxic conditions also increases Fos expression in neurons in the MRN, especially at the level of the facial nucleus, and many of these neurons are serotonergic (22). Interestingly, after exposure to acute hypoxia, there is an increase in 5-HT levels in the VRG of cats that coincides with the onset of hypoxic depression (57).
Conclusions

This is the first study to show that chemical stimulation of a discrete region of the MM at the level of CP7 can consistently produce apnea in rats. Our data also confirm the data of others (26, 28, 63) that the cardiovascular effects of chemically stimulating the MM in rats are highly anesthetic dependent (increases with urethane but decreases with pentobarbital).

The apneic responses were not anesthetic dependent, indicating that the neurons responsible for the depression of respiratory activity are separate from those responsible for the cardiovascular effects. We hypothesize that the apneic and respiratory-depressive effects may be due, at least in part, to the activation of neurons, some of which may be serotonergic, that project to respiratory nuclei in the brain stem, possibly the Bötzing complex, where they release Glu and/or 5-HT. This results in the activation of a potent inhibitory nucleus. Alternatively, 5-HT acts on inhibitory 5-HT1A receptors to inhibit neurons at some other sites that are responsible for the generation of respiratory activity.

Perspectives

Neurons in the MRN are functionally heterogeneous and play an integral role in the homeostatic regulation of thermoregulation and nociceptive modulation (for reviews see Ref. 49 and Refs. 44 and 55, respectively). Although neurons in these nuclei do not play a tonic role in the regulation of arterial pressure (26) or respiration (45), their activation can produce increases or decreases in these parameters depending on the experimental protocol used (3, 28, 42, 63).

Importantly, some neurons in the MRN are chemosensitive to pH (see Ref. 56 for review) and are activated in response to hypercapnia and hypoxia (5, 6). The integrity of neurons in this region is necessary for the full respiratory response to hypercapnia (45).

Recently, sudden infant death syndrome (SIDS) or a subset of SIDS cases has been attributed to a developmental abnormality in the ventral medulla oblongata, termed “the medullary serotonergic network deficiency hypothesis” (38). It is believed that this abnormality or deficiency results in a failure of homeostatic responses to life-threatening challenges such as hypoxia and hypercapnia during sleep (38, 52). This therefore raises the possibility that the apneic responses in the present study may be involved in the altered response to hypoxia/hypercapnia that is implicated in SIDS.

REFERENCES

CARDIORESPIRATORY RESPONSES FROM THE MIDLINE MEDULLA

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