Neonatal maternal separation enhances phrenic responses to hypoxia and carotid sinus nerve stimulation in the adult anesthetized rat

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Neonatal maternal separation enhances phrenic responses to hypoxia and carotid sinus nerve stimulation in the adult anesthetized rat. J Appl Physiol 99: 189–196, 2005. First published March 24, 2005; doi:10.1152/japplphysiol.00070.2005.—In awake animals, our laboratory recently showed that the hypoxic ventilatory response of adult male (but not female) rats previously subjected to neonatal maternal separation (NMS) is 25% greater than controls (Genest SE, Gulemetova R, Laforest S, Drolet G, and Kinkead R. J Physiol 554: 543–557, 2004). To begin mechanistic investigations of the effects of this neonatal stress on respiratory control development, we tested the hypothesis that, in male rats, NMS enhances central integration of carotid body chemoafferent signals. Experiments were performed on two groups of adult male rats. Pups subjected to NMS were placed in a temperature-controlled incubator 3 h/day from postnatal day 3 to postnatal day 12. Control pups were undisturbed. At adulthood (8–10 wk), rats were anesthetized (urethane; 1.6 g/kg), paralyzed, and ventilated with a hyperoxic gas mixture [inspired O2 fraction (FiO2) = 0.5], and phrenic nerve activity was recorded. The first series of experiments aimed to demonstrate that NMS-related enhancement of the inspiratory motor output (phrenic) response to hypoxia occurs in anesthetized animals also. In this series, rats were exposed to moderate, followed by severe, isocapnic hypoxia (FiO2 = 0.12 and 0.08, respectively, 5 min each). NMS enhanced both the frequency and amplitude components of the phrenic response to hypoxia relative to controls, thereby validating the use of this approach. In a second series of experiments, NMS increased the amplitude (but not the frequency) response to unilateral carotid sinus nerve stimulation (stimulation frequency range: 0.5–33 Hz). We conclude that enhancement of central integration of carotid body afferent signal contributes to the larger hypoxic ventilatory response observed in NMS rats.

control of breathing; development; plasticity

THE NEURAL CIRCUITS GENERATING and regulating respiratory activity are functional at birth; however, the way in which this vital homeostatic system develops and performs throughout life is strongly influenced by the environment (5). As it has been shown for several other neural circuits (19), adequate sensory stimulation is essential to proper development of the respiratory control system. For instance, rat pups maintained under hypoxic conditions [inspired O2 fraction (FiO2) = 0.6] grow into adults that cannot develop a full ventilatory response to a moderate (but not severe) hypoxic challenge (2, 24–27, 34). Detailed mechanistic studies have shown that dysfunction of the hypoxic ventilatory response in these rats is due to their reduced sensitivity to hypoxia (3, 25), owing to carotid body hypoplasia (9) and disruption of dopaminergic neurotransmission (34). These results show that exposure to “normal” arterial PO2 (PaO2) fluctuations is essential to proper development of the neural circuits that regulate breathing (5, 25, 29). However, several studies indicate that environmental factors influencing early life programming of the respiratory control system are not limited to respiratory stimuli or exposure to pharmacological agents, such as nicotine (38) or cocaine (31).

The tactile, olfactory, and auditory stimuli that the dam provides her pups during the first 2 wk of life are among the most potent stimuli affecting central nervous system development (4, 20). While the lifelong consequences of disrupting mother-pup interactions during this critical period have commonly been associated with neuroendocrine and behavioral disorders (1, 21), our laboratory recently showed that adult male (but not female) rats subjected to maternal separation during early life are hypertensive and show a hypoxic ventilatory response 25% greater than that of controls (12). These results raise intriguing questions regarding the etiology of respiratory disorders, because infants born prematurely often require special neonatal care that can interfere with natural mother-infant interactions (35).

Although the mechanisms underlying the persistent and gender-specific effects of neonatal maternal separation (NMS) on respiratory control development are still poorly understood, the more robust increase in breathing frequency observed in male (but not female) rats shortly after the onset of hypoxia suggest that carotid body function is enhanced (12, 17). Mechanistic investigations of neurophysiological aspects of respiratory control can require procedures that are difficult to perform in awake animals. To circumvent these limitations, we wished to pursue our studies on the effects of NMS on respiratory control development using an anesthetized, paralyzed, and artificially ventilated rat preparation. Because it is essential to determine whether this experimental approach is adequate for such studies, we first showed that NMS-related enhancement of the hypoxic ventilatory response occurs in the anesthetized rat preparation. Our results indicating that NMS exerts similar effects on the hypoxic ventilatory response in both awake and anesthetized rats allowed us to proceed with our second objective, which was to test the hypothesis that, in male rats, NMS enhances central integration of carotid body chemoafferent signal. To this aim, we compared inspiratory (phrenic) motor output responses to carotid sinus nerve stimulation. The latter procedure mimics the essential features of hypoxic activation of the carotid bodies, while bypassing chemoafferent transduction mechanisms that could be affected by NMS (14).

Parts of this work have been reported in abstract form (17).

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MATERIALS AND METHODS

Experiments were performed on 26 adult male Sprague-Dawley rats (Charles River Canada, St.-Constant, Québec, Canada). Rats were supplied with food and water ad libitum and maintained in standard laboratory and animal care conditions (21°C, 12:12-h dark-light cycle: lights on at 0600 and off at 1800). Laval University Animal Care Committee approved the experimental procedures described in this manuscript, and the protocols were in accordance with the guidelines detailed by the Canadian Council on Animal Care.

Mating and NMS procedures. Virgin females were mated and delivered 10–15 pups. Two days after delivery, litters were culled to 12 pups, when necessary, with a roughly equal number of males and females. The NMS protocol was identical to the one used in our laboratory’s previous studies (12, 18) and was inspired from that of Wigger and Neumann (39). Briefly, the entire litter was separated daily from their mother 3 h/day (0900–1200) from day 3 to 12. Separated pups were placed in a temperature (35°C)- and humidity (45%)-controlled incubator and isolated from each other by a cardboard partition. On day 21, rats were weaned and housed under standard animal care conditions until adulthood (8–10 wk old), at which time phrenic nerve recordings were performed. The phrenic activity measured from this experimental group was then compared with that of animals in which the nest was not disturbed during the first 2 wk postpartum and therefore not subjected to the NMS procedure. These animals are the most desirable control group for investigations of the effects of maternal separation (22). In both series of experiments, each group of rats was composed of animals originating from at least three different litters to ensure that any difference was due to the experimental treatment (NMS) rather than a litter-specific effect.

Surgical preparation. Phrenic nerve activity was recorded to compare the neural correlate of inspiratory motor output between controls and rats subjected to NMS. Anesthesia was induced with isoflurane in a closed chamber and then maintained via nose cone (3.5% isoflurane, \( F_{IO2} = 0.5 \), balance N\(_2\)). The rat was placed on a homeothermic blanket (Harvard Instruments), and rectal temperature was maintained between 37 and 38°C. The trachea was cannulated, and the rat was ventilated mechanically (model 683, Harvard Instruments) while maintaining the inspired isoflurane level constant (3.5%). Lungs were hyperinflated roughly once per hour to prevent alveolar atelectasis. Bilateral vagotomy was performed at the midcervical level to prevent entrainment of inspiratory motor output with the ventilator. A venous femoral catheter was inserted for anesthetic and fluid administration. An arterial femoral catheter was inserted for blood pressure monitoring (Transbridge TBM4M-B, World Precision Instruments) and with an arterial femoral catheter was inserted for anesthetic and fluid administration. A venous femoral catheter was inserted for blood pressure monitoring (Transbridge TBM4M-B, World Precision Instruments) and withdrawal of blood samples for arterial blood-gas analysis. Rats were slowly converted from isoflurane to urethane anesthesia (1.6 g/kg iv in distilled water). The isoflurane level within the inspired gas mixture was decreased progressively while urethane was infused slowly (−0.25 ml/min) to minimize the effects of urethane injection on arterial blood pressure. This procedure took, on average, 20 min. The adequacy of the plane of anesthesia was ensured by monitoring blood pressure responses to toe pinch. Once this was completed, rats were paralyzed with pancuronium bromide (2.5 mg/kg iv). When necessary, a constant intravenous infusion of a sodium bicarbonate (5%) and lactated Ringer solution (50:50, 1.7 ml/kg/h) was initiated to maintain fluid and acid-base balance. PaO\(_2\), arterial PCO\(_2\) (Paco\(_2\)), and arterial pH (pH) in blood were measured with a blood-gas analyzer (model ABL-5, Radiometer, Copenhagen, Denmark) by withdrawing arterial blood in a heparinized capillary. Blood gases and pH values were corrected to the measured rectal temperature of the rat for each sample. The end-tidal PCO\(_2\) (PET\(_{CO2}\)) was measured in the expired line of the ventilatory circuit using an in-line CO\(_2\) analyzer (model 1265, Novametrix, Wallingford, CT) with sufficient response time (<75 ms). Values obtained from this capnograph closely approximated PaCO\(_2\) (difference between PaCO\(_2\) and PET\(_{CO2}\) ranging between 0 and 6 Torr). At the end of the experiment, euthanasia was performed by urethane overdose.

Phrenic nerve recording. Phrenic nerve was isolated unilaterally, using a left dorsal approach, cut distally, and desheathed. The nerve was submerged in mineral oil and placed on a bipolar silver recording electrode. Nerve activity was amplified (gain = 10,000; model 1700, AM-Systems, Everett, WA), band-pass filtered (100 Hz to 10 kHz), and fed to a moving averager (model MA-821, CWE) before being digitized and recorded with a data acquisition system (model DI-720, Dataq Instruments, Akron, OH).

Experimental protocol. Once the rat preparation was ready, 60 min were allowed for the electroneurogram and arterial blood pressure to stabilize under hyperoxic conditions (\( F_{IO2} = 0.5 \)) before the onset of the experiments. The CO\(_2\) apneic threshold for inspiratory (phrenic) activity was then determined by mechanically hyperventilating the rats until phrenic nerve activity ceased, at which point a first blood sample was taken for blood-gas measurements. Both Pa\(_{CO2}\) and PET\(_{CO2}\) were noted, and the rate of the ventilator was decreased progressively until phrenic activity returned and Pa\(_{CO2}\) was set 2–3 Torr above the apneic threshold (see Table 1) by adjusting the ventilatory rate and/or inspired CO\(_2\), as necessary. Because NMS decreased the apneic threshold (see RESULTS), this procedure served to standardize baseline phrenic activity relative to its threshold rather than an arbitrarily predetermined Pa\(_{CO2}\) level.

Series I: phrenic response to hypoxia. Experiments were performed on seven controls and nine NMS rats. The protocol began by taking a baseline arterial blood sample. All subsequent blood-gas data were compared with this initial value. Baseline phrenic activity was recorded, and rats were exposed to moderate hypoxia (\( F_{IO2} = 0.12 \)) for 5 min. The intensity of the hypoxic stimulus was then increased to \( F_{IO2} = 0.08 \); this level of severe stimulation was maintained for 5 min also. Relative isocapnia was maintained throughout the entire protocol.

Table 1. Comparison of arterial blood gases and selected cardiovascular variables between undisturbed (control) rats and rats previously subjected to neonatal maternal separation under baseline conditions (\( F_{IO2} = 0.5 \)) and during moderate \( (F_{IO2} = 0.12) \) followed by severe \( (F_{IO2} = 0.08) \) hypoxia

<table>
<thead>
<tr>
<th>Variable</th>
<th>Control Baseline</th>
<th>NMS Baseline</th>
<th>( F_{IO2} = 0.12 ) Control</th>
<th>NMS</th>
<th>( F_{IO2} = 0.08 ) Control</th>
<th>NMS</th>
</tr>
</thead>
<tbody>
<tr>
<td>PaO(_2), Torr</td>
<td>220±7</td>
<td>233±9</td>
<td>44±4*</td>
<td>46±2*</td>
<td>39±4*</td>
<td>39±3*</td>
</tr>
<tr>
<td>PaCO(_2), Torr</td>
<td>46.3±1.3</td>
<td>41.8±0.8†</td>
<td>43.8±0.9</td>
<td>40.9±0.6†</td>
<td>45.0±1.8</td>
<td>40.2±0.7†</td>
</tr>
<tr>
<td>pH</td>
<td>7.35±0.008</td>
<td>7.39±0.01†</td>
<td>7.32±0.02*</td>
<td>7.39±0.01†</td>
<td>7.26±0.003*</td>
<td>7.32±0.02*</td>
</tr>
<tr>
<td>Mean arterial blood pressure, mmHg</td>
<td>103±8</td>
<td>108±5</td>
<td>59±7*</td>
<td>69±2*</td>
<td>57±6*</td>
<td>53±2*</td>
</tr>
<tr>
<td>Heart rate, beats/min</td>
<td>462±7</td>
<td>438±8†</td>
<td>477±9</td>
<td>464±7*</td>
<td>358±25*</td>
<td>428±10‡</td>
</tr>
</tbody>
</table>

Values are means ± SE. NMS, neonatal maternal separation; \( F_{IO2} \), inspired O\(_2\) fraction; PaO\(_2\), arterial \( Po2\); PaCO\(_2\), arterial \( Pco2\); pH, arterial pH. *Significantly different from baseline, \( P < 0.05 \). †Significantly different from corresponding control value, \( P < 0.05 \). ‡Significantly different from corresponding control value, \( P < 0.1 \).
by monitoring end-tidal CO₂ and adjusting the ventilator rate and/or inspired CO₂ accordingly. A blood sample was drawn at the end of each level of hypoxia. Table 1 reports pH, Pao₂, and PacO₂ values obtained under each condition. A hypercapnic response test (PetCO₂ ~ 90 Torr; 3 min) was done at the end of each experiment to obtain a measure of maximal (or at least a standardized hypercapnic control) nerve burst amplitude. This level of hypercapnia is commonly used for the hypercapnic response test (e.g., Ref. 3) because increasing PetCO₂ beyond this level does not increase phrenic burst amplitude further.

**Series II: phrenic response to carotid sinus nerve stimulation.** To determine whether NMS elicits functional plasticity at the level of central chemosensory integration, the left carotid sinus nerve was isolated via a dorsal approach, cut distally, and placed on a fine bipolar silver-wire electrode for electrical stimulation (NMS: n = 5; control: n = 5). The stimulation protocol and parameters were similar to those used by Ling et al. (23). Using an isolated pulse stimulator (model 2100, AM-systems), the current threshold was established in each experiment by determining the minimum current necessary to elicit a repeatable response in phrenic nerve activity (20 Hz, 0.2-ms duration). The stimulation current was then set at three times this threshold current for the duration of the experiment. Thus the carotid sinus nerve was stimulated with constant currents (30–450 μA) at increasing frequencies (0.5–33 Hz; 0.2-ms pulse duration). Stimulus frequency was changed sequentially in ten, 45-s stimulation episodes: 0.5, 1, 2, 5, 8, 11, 14, 17, 20, and 33 Hz. To test whether responses to carotid sinus nerve stimulation were secondary to current spread, we crushed the proximal end of the carotid sinus nerve at the conclusion of four experiments and determined that phrenic responses could no longer be evoked by stimulation. After the final stimulus episode, a hypercapnic phrenic response was determined (PetCO₂ ~ 90 Torr).

**Data analysis.** Mean “steady-state” values for peak integrated phrenic nerve activity (PPhr), phrenic burst frequency, mean arterial blood pressure, and heart rate were obtained by averaging data over a 1-min period immediately before the onset of stimulation (baseline), and at the end of each level of stimulation (hypoxia or carotid sinus nerve stimulation). Temporal dynamics of the acute responses to hypoxia was assessed by averaging phrenic burst frequency and mean arterial blood pressure in 20-s “bins” over the course of the first hypoxic period (FIO₂ = 0.12). Maximum PPhr was obtained by averaging the values obtained at the conclusion of the hypercapnic episode.

Changes in PPhr during hypoxia or carotid sinus nerve stimulation were expressed relative to PPhr during both baseline and hypercapnic condition (i.e., maximal activity). Expressing changes in PPhr relative to baseline and to a standardized maximum (hypercapnic) level of activity addresses concerns regarding potential normalization artifacts that may occur when comparing neurograms within and between rats. Because all responses (and conclusions) were identical regardless of the normalization used, all PPhr data are presented as percent change from baseline.

**Statistical analysis.** Data were analyzed by using a two-way ANOVA (stimulus × treatment) using a repeated-measures design when appropriate (Statview 5.0 SAS Institute, Cary, NC). These analyses were followed by a post hoc Fisher’s test when appropriate. Statistical inferences concerning differences between experimental groups with only one variable (e.g., CO₂ apneic threshold) were made with an unpaired Student’s t-test. Differences were considered significant at P < 0.05. All data are presented as means ± SE.

**RESULTS**

**NMS reduces the apneic threshold.** The lower PacO₂ values reported in Table 1 for the NMS group reflect the fact that the apneic threshold for these animals (39 ± 1 Torr) was, on average, 3–4 Torr lower than in control rats (43 ± 1 Torr).

**Neonatal maternal separation enhances the acute response to moderate hypoxia.** The blood pressure [PPhr recordings presented in Fig. 1 illustrate the dynamics of the acute cardiorespiratory responses to moderate hypoxia (FIO₂ = 0.12) observed in adult rats previously subjected to NMS and in control rats. Under baseline conditions, phrenic burst frequency and mean arterial blood pressure were similar in both groups (Figs. 1 and 2A); however, heart rate was slower in NMS rats (treatment: P < 0.0001; Table 1). On initiation of moderate hypoxia, both groups of rats showed a rapid increase in phrenic burst frequency (stimulus: P < 0.0001); however, the frequency observed in NMS rats was greater than in controls (treatment: P = 0.0004), and the response varied according to treatment (stimulus × treatment: P < 0.0001; Fig. 2A). Mean arterial blood pressure decreased during hypoxia (stimulus: P < 0.0001). While the pressure response varied according to treatment also (stimulus × treatment: P < 0.001), these treatment-related differences became apparent after the second minute of hypoxic exposure (Figs. 1 and 2B).

**NMS enhances the steady-state responses to moderate and severe hypoxia.** Analysis of phrenic activity over the last minute of each hypoxic period showed that the phrenic burst frequency response was augmented by NMS (stimulus × treatment: P < 0.03; Fig. 3A). Both groups showed a significant frequency increase during moderate hypoxia, but the frequency measured in NMS rats was greater than in controls (Fig. 3A). Subsequent exposure to severe hypoxia (FIO₂ = 0.08) had a depressant effect on phrenic burst frequency, but unlike...
controls, rats subjected to NMS were able to maintain a frequency above baseline (Fig. 3A).

Hypoxic exposure increased Phr in an intensity-dependent manner (stimulus: P < 0.0001; Fig. 3B), and overall, the amplitude measured in NMS rats was greater than in controls (treatment: P = 0.04; Fig. 3B). Measurement of minute phrenic activity (Phr × phrenic burst frequency) as an index of inspiratory motor output showed that NMS augmented the hypoxic response of this variable also (stimulus × treatment: P < 0.04; Fig. 3C).

Mean arterial blood pressure decreased during both hypoxic exposures (stimulus: P < 0.0001); however, analysis of the steady-state measurements obtained failed to reveal a significant effect of NMS on this aspect of the hypoxic response (treatment effect: P = 0.88; stimulus × treatment: P = 0.12; Table 1). Heart rate was affected by hypoxia also (P < 0.0001). During moderate hypoxia, NMS rats showed a tachycardia, whereas the heart rate of controls was unchanged. During severe hypoxia, a bradycardia was observed in control rats only; heart rate of NMS rats remained near baseline level and was greater than in controls (Table 1).

Fig. 2. Time course of the phrenic burst frequency (A) and arterial blood pressure (B) responses to moderate isocapnic hypoxia (FIO2 = 0.12) measured in controls and rats subjected to NMS. Values are means ± SE; n, no. of animals. Note that time scale of the blood pressure data is truncated at 240 s due to the blood sampling procedure. *Significantly different from baseline, P < 0.05. †Significantly different from the corresponding control value, P > 0.05. ‡Significantly different from the corresponding control value, P > 0.1.

Fig. 3. Comparison of phrenic burst frequency (A), integrated phrenic burst amplitude (B), and minute phrenic activity (C; frequency × amplitude) recorded in controls and rats previously subjected to neonatal maternal separation under baseline conditions and at the end of a 5-min exposure to moderate (12%), followed by severe hypoxia (8%). Integrated phrenic burst amplitude is expressed as a percent change from baseline. Values are means ± SE. *Significantly different from baseline, P < 0.05. †Significantly different from the corresponding control value, P > 0.05. ‡Significantly different from the corresponding control value, P > 0.1.
NMS enhances the amplitude but not the phrenic burst frequency response to carotid sinus nerve stimulation. Under baseline conditions, phrenic burst frequency and mean arterial blood-gas measurements obtained in this series of experiments were comparable to those obtained in the hypoxic series (series effect: \( P > 0.05 \) for frequency, \( \text{pH} \), \( \text{PaO}_2 \), and \( \text{PaCO}_2 \)). Carotid sinus nerve stimulation increased both phrenic burst frequency and \( \text{ICP} \) (stimulus: \( P < 0.0001 \) for both; Fig. 4). Unlike burst frequency (Fig. 4A), the \( \text{ICP} \) response to carotid sinus nerve stimulation was enhanced in rats previously subjected to NMS (stimulus \( \times \) treatment: \( P < 0.0001 \); Fig. 4B). Although direct comparisons are difficult, it is interesting to note that, for the control group, the magnitude of the frequency increase during carotid sinus nerve stimulation was comparable to the one reported during moderate hypoxia (Fig. 2A vs. 4A). With respect to the amplitude component of the response, the \( \text{ICP} \) increase observed in both groups of rats during carotid sinus nerve stimulation compared well with the changes observed during hypoxic stimulation (Fig. 3B vs. 4B).

NMS enhances the blood pressure response to carotid sinus nerve stimulation. Carotid sinus nerve stimulation increased mean arterial blood pressure (stimulus effect: \( P < 0.0001 \)). In NMS rats, blood pressure values increased above baseline when the carotid sinus nerve was stimulated at frequencies between 5 and 17 Hz in NMS rats (Fig. 5A). These results, combined with the fact that mean arterial blood pressure did not increase in controls suggest that NMS enhanced the blood pressure response to carotid sinus nerve stimulation (stimulus \( \times \) treatment: \( P = 0.061 \); Fig. 5A). Heart rate of NMS rats was slower in this series also (treatment: \( P = 0.041 \)); however, the effects of carotid sinus nerve stimulation on this variable were marginal (\( P = 0.07 \)).

**DISCUSSION**

The results of the present study show that, in adult male rats, NMS-related enhancement of the hypoxic ventilatory response can be observed in the anesthetized rat preparation. These results further support the notion that disruption of mother-pup interactions during early life has long-lasting consequences on respiratory control development, and the similarity of the
results between the two studies (awake vs. anesthetized rats) validate the use of this preparation for more detailed and better controlled neurophysiological experiments. Carotid sinus nerve stimulation is a good example of such an experimental procedure. This approach enabled us to demonstrate that, in NMS rats, enhancement of central integration of carotid body chemosensory signal that transits via the carotid sinus nerve contributes (at least in part) to the increased responsiveness to hypoxia observed in this group.

**Awake vs. anesthetized rats: “baseline” conditions and hypoxia.** Carotid bodies play an important role in setting sympathetic outflow, and because potentiation of the peripheral chemoreflex contributes to conditions such as hypertension (for reviews, see Refs. 11, 16), our laboratory recently proposed that, in awake animals, enhancement of carotid body chemosensitivity contributes to the hypertension observed in male NMS rats (12, 18). The absence of hypertension in anesthetized animals previously subjected to NMS under baseline conditions is consistent with this hypothesis because these rats were ventilated with a hyperoxic gas mixture to keep carotid body activation at a minimum level. The attenuation of the hypertensive response during moderate hypoxia in NMS vs. control rats supports this interpretation; however, these data should be interpreted with care because anesthesia can alter cardiovascular regulation.

The use of artificial ventilation enabled us standardize baseline phrenic activity by maintaining rats 2–3 Torr above the apneic threshold. This procedure showed that NMS rats have an apneic threshold lower than controls; consequently, baseline PaCO₂ was set at different levels for NMS and control rats. This situation differs from what has been reported previously in awake animals where, in males, baseline PaCO₂ was not significantly different between control and NMS rats (12). While the reason(s) underlying these discrepancies have not been addressed, the use of anesthesia, vagotomy, and hyperoxia are important distinctions between the two studies that may contribute to these differences.

Acute exposure to hypoxia triggers an increase in ventilatory activity that varies over the course of the hypoxic stimulus. The different time domains of the hypoxic ventilatory response are well described, and each time-dependent change in ventilatory activity reflects different neural mechanisms (33). For instance, the rapid increase in breathing frequency at the onset of hypoxia reflects carotid body-mediated activation of the hypoxic chemoreflex, and the rapidity and magnitude of this increase are hypothesized to reflect the responsiveness of this sensory organ to a rapid decrease in PaO₂. In awake animals, initiation of hypoxia triggered a greater breathing frequency increase in male NMS rats than in controls (12), and the present results show that NMS-related differences in the acute frequency response occur in the anesthetized rat preparation also. Together, these data suggest that NMS alters carotid body function, and our laboratory’s recent demonstration that NMS enhances tyrosine hydroxylase mRNA expression and dopamine D₂-receptor expression levels in the rat carotid body is consistent with this interpretation (18).

As hypoxia continues, breathing frequency reaches a steady state that persists over the next minutes to an hour, depending on the severity of the hypoxic stimulus (33). As our data show, this component of the response is slightly different in anesthetized animals in which the acute or immediate response is typically followed by a “short-term depression” of breathing frequency, a phenomenon unique to this preparation (33). In awake animals, the breathing frequency measured during the late phase of hypoxia (i.e., after 10 min of hypoxic exposure) revealed no difference between control and NMS rats (12). NMS-related enhancement of the breathing frequency measured after 5 min of hypoxia in the anesthetized rat preparation is the main difference between the results obtained in both studies. Despite the numerous differences between the awake animal and the anesthetized preparation, we originally anticipated that this aspect of the response would be similar between studies given that the experimental procedures were comparable for both groups of rats and that the PaCO₂ levels achieved during moderate hypoxia were the same in both awake and anesthetized animals. However, this prediction did not consider the fact that awake animals were not maintained isocapnic during hypoxia, such that PaCO₂ in the anesthetized rat were roughly 10 Torr above the levels measured in our previous study on awake animals (12). Since PaO₂ and PaCO₂ interact in a multiplicative fashion at the level of the carotid body (for reviews, see Refs. 7, 13), we propose that the differences in PaCO₂ between preparations augment the NMS-related enhancement of chemoafferent signal and its effects on respiratory motor output. Our results showing that carotid sinus nerve activation elicits identical phrenic burst frequency increases between groups support this interpretation.

In the awake rat, enhancement of the inspiratory flow response to hypoxia was the main factor contributing to the increased ventilatory response during the “late phase” of the hypoxic challenge (12). However, the limitations inherent to tidal volume measurements by whole body plethysmography brought us to interpret these data with care (8, 30). In that context, the results obtained in the anesthetized rat preparation are important in that they not only substantiate our previous findings but also further validate the use of this preparation in subsequent mechanistic studies.

**NMS enhances responsiveness to carotid sinus nerve stimulation.** Investigating the neural mechanisms regulating the hypoxic chemoreflex using hypoxia as a stimulus can impose certain limitations. Even in reduced preparations, severe hypoxia elicits respiratory depression, thereby making it difficult to construct intensity-response curves. For instance, progression to severe hypoxia decreases phrenic burst frequency. While the mechanisms underlying this phenomenon are complex, the fact that NMS rats can sustain severe hypoxia better than controls suggest that the overall degree of chemoreflex activation is greater in these animals. The use of carotid sinus nerve stimulation allowed us to address this treatment-related difference in the input-output relationship without hypoxia and/or changes in PaCO₂ as confounding factors while bypassing differences in carotid body sensitivity.

On the basis of the results obtained in *series I*, the similarity of the phrenic burst frequency responses to carotid sinus nerve stimulation obtained for both groups was unexpected, considering thatafferent fibers project not only to the NTS but to neurons involved in respiratory rhythm generation also [e.g., nucleus ambiguus and retroambiguus (10, 15)]. Several explanations can be put forward to explain this intriguing result; however, in light of the evidence indicating that medullary neurons involved in respiratory rhythm generation contain chemosensitive elements that are directly excited by hypoxia.
within the same rat strain adds perspective to the recent life can produce such different respiratory control phenotypes ever, the fact that environmental manipulations during early greater risk to subsequent disease development. Diversity in development of physiological phenotypes that may be at tant questions concerning the impact of neonatal care on the ders such as sleep-related breathing disorders (6, 16, 36).

function are key features of human cardiorespiratory disor-

neuroendocrine factors play an important role in the programming of the respiratory control system. The proximal mechanisms accounting for these differences in physiological phenotypes are still unclear but the increased “basal” corticosterone levels that characterize male rats subjected to NMS could contribute to shaping this physiological phenotype because this hormone modulates the expression of several genes. Nonetheless, our results suggest that neuroen-


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