Selected Contribution: Chronic intermittent hypoxia enhances respiratory long-term facilitation in geriatric female rats

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Zabka, A. G., G. S. Mitchell, E. B. Olson, Jr, and M. Behan. Selected Contribution: Chronic intermittent hypoxia enhances respiratory long-term facilitation in geriatric female rats. J Appl Physiol 95: 2614–2623, 2003.—Age and the estrus cycle affect time-dependent respiratory responses to episodic hypoxia in female rats. Respiratory long-term facilitation (LTF) is enhanced in middle-aged vs. young female rats (72). We tested the hypothesis that phrenic and hypoglossal (XII) LTF are diminished in acyclic geriatric rats when fluctuating sex hormone levels no longer establish conditions that enhance LTF. Chronic intermittent hypoxia (CIH) enhances LTF (41); thus we further predicted that CIH would restore LTF in geriatric female rats. LTF was measured in young (3–4 mo) and geriatric (20–22 mo) female Sasco Sprague-Dawley rats and in a group of geriatric rats exposed to 1 wk of nocturnal CIH (11 vs. 21% O2 at 5-min intervals, 12 h/night). In anesthetized, paralyzed, vagotomized, and ventilated rats, time-dependent hypoxic phrenic and XII responses were assessed. The short-term hypoxic response was measured during the first of three 5-min episodes of isocapnic hypoxia (arterial PO2 35–45 Torr). LTF was assessed 15, 30, and 60 min postepisodic hypoxia. Phrenic and XII short-term hypoxic response was not different among groups, regardless of CIH treatment (P > 0.05). LTF in geriatric female rats was smaller than previously reported for middle-aged rats but comparable to that in young female rats. CIH augmented phrenic and XII LTF to levels similar to those of middle-aged female rats without CIH (P < 0.05). The magnitude of phrenic and XII LTF in all groups was inversely related to the ratio of progesterone to estradiol serum levels (P < 0.05). Thus CIH and sex hormones influence the magnitude of LTF in geriatric female rats.

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Sex hormones alter with age. Aging female rats stop cycling with advanced age and, therefore, are not exposed to cycle-dependent fluctuations of female sex hormones. However, instead of menopause, as seen in aged women, female rats enter a stage of persistent estrus or diestrus (26).

Because LTF is 5-HT dependent, and 5-HT levels are augmented by pretreatment with 1 wk of CIH, similar to young male rats.

**METHODS**

**Experimental Groups**

Experiments were performed on intact female Sasco Sprague-Dawley rats (Charles River Laboratories, Wilmington, MA; rat colony K-62, Kingston, NY). Two age groups were studied: young rats in estrus and diestrus (3–4 mo; Young) and geriatric rats in persistent estrus and diestrus (20–22 mo; Ger). In addition, one group of geriatric rats was treated with 7 days of CIH (Ger/CIH). All experimental procedures were approved by the School of Veterinary Medicine Animal Care and Use Committee at the University of Wisconsin-Madison.

**Food Restriction**

Geriatric rats received 75% of ad libitum intake from age 3–4 mo to increase lifespan and reduce tissue tumor development according to guidelines by Weindruch (69). Moderate reduction of caloric intake has been shown to extend longevity, to reduce the formation of neoplasms especially of the pituitary and mammary glands, and to decrease the incidence of diseases that are associated with obesity (69). Although the caloric restriction was mild and only thought to prevent obesity without having an impact on normal, physiological weight, the effects of such dietary restriction on LTF are unknown. Water intake was ad libitum.

**CIH**

One group of geriatric rats (n = 8) was exposed to seven consecutive nights of CIH as described previously (41). Rats were placed in a 112-liter Plexiglas chamber and received an alternating gas mixture of air, O2, and N2 to adjust inspired O2 to 21% or 11% in 5-min intervals between 6 PM and 6 AM. The rate of gas flow was sufficient to achieve steady-state O2 concentrations within 1 min and to maintain CO2 levels below 0.5%. Between 6 AM and 6 PM, the chamber was continuously flushed with room air (21% O2).

**Determination of the Estrus Cycle**

Rats were briefly anesthetized with isoflurane daily to assess the progression of the estrus cycle. Epithelial cells were collected by vaginal swab for at least one complete estrus cycle as assessed under light microscopy (31) or for at least eight consecutive days in geriatric rats. Only young animals that were clearly in estrus or diestrus were studied. All geriatric rats were acyclic, exhibiting either persistent estrus or diestrus.

**Experimental Preparation**

Anesthesia was initiated with isoflurane in an induction chamber and maintained with a nose cone that was subsequently replaced by a tracheal cannula (3.5–3.5% in 50% O2, balance N2). The tracheal cannula was placed to allow artificial pump ventilation (model 683, Harvard Apparatus, Holliston, MA) and for monitoring tracheal pressure. Rats were slowly converted to urethane anesthesia (1.6 g/kg in distilled water) through an intravenous catheter placed in the right femoral vein. Adequacy of anesthesia was tested regularly by assessing blood pressure responses to toe pinch. Supplemental urethane was administered intravenously when necessary to minimize blood pressure responses to toe pinch. Blood pressure was monitored through a catheter in the right femoral artery, and discrete blood samples (0.2 ml in a 0.5-ml heparinized glass syringe) were collected to determine arterial blood gases (PaO2 and PaCO2), pH, and base excess (ABL 500; Radiometer, Copenhagen, Denmark). Values were corrected to the rectal temperature. Body temperature was maintained between 37 and 38°C by using a heated table.

To prevent spontaneous breathing efforts and entrainment of respiratory motor output with the ventilator, rats were bilaterally vagotomized and paralyzed (pancuronium bromide, 2.5 mg/kg iv, supplemented as necessary). End-tidal CO2 was measured with a flow-through capnograph (Cannogard, model 1265, Novametrix; Wallingford, CT), with sufficient response time to measure end-expiratory gases in rats.

The left phrenic and XII nerves were isolated via a dorsal approach, cut distally, desheathed, submersed in mineral oil, and placed on bipolar silver wire electrodes. Nerve activities were amplified (∼10,000), band-pass filtered (100 Hz to 10 kHz) (model 1700, A-M Systems; Carlsborg, WA), and integrated (time constant = 50 ms, model MA-821RSP, CWE, Ardmore, PA). Integrated nerve signals were digitized and processed with commercial computer software (WINDAQ, Akron, OH). To terminate an experiment, rats were euthanized with an intravenous overdose of urethane.

**Experimental Protocol**

Nerve signals were allowed to stabilize for ~60 min after surgical procedures under hypoxic [inspired O2 fraction (FiO2) = 0.5; PaO2 > 150 Torr] and normocapnic conditions. The CO2 apneic threshold was determined by monitoring end-tidal Pco2 while increasing the ventilation pump rate to lower Paco2 until phrenic nerve activity ceased. Pump rate was gradually decreased until rhythmic phrenic nerve activity resumed. The end-tidal Paco2 at the resumption of phrenic activity was designated as the apneic threshold. Baseline nerve activities were established at end-tidal Pco2 levels 2–3 Torr above this CO2 apneic threshold by increasing inspired CO2 and/or decreasing respiratory pump rate. After phrenic and XII nerve discharge had become stable, baseline conditions of blood gases, pH, and base excess were assessed by an initial blood sample. All subsequent blood samples were compared with this initial baseline value. Strict isocapnic conditions (~1 Torr from baseline Paco2) were maintained throughout an experiment by monitoring end-tidal CO2 and making adjustments in ventilation rate and/or inspired CO2 as necessary. To minimize alveolar atelectasis, the lungs were hyperinflated approximately every 60 min.

A schematic presentation of a protocol is shown in Fig. 1. The protocol started with three episodes of isocapnic hypoxia of 5-min duration (FiO2 = 0.11–0.12, target PaO2 = 35–45 Torr), separated by 5 min of hyperoxia (FiO2 = 0.5). The rats were then monitored for 60 min while isocapnic conditions in
hyperoxia were maintained. Arterial blood samples were drawn at 15, 30, and 60 min after the final hypoxic episode to confirm isocapnic conditions. A protocol ended with 5 min of hypercapnia (end-tidal P\textsubscript{CO\textsubscript{2}} 80–90 Torr) to assess maximal CO\textsubscript{2}-stimulated nerve activity. Subsequently, 5-min hypoxia was administered to compare nerve activity to that during the first hypoxic episode.

Rats with deviations in P\textsubscript{aCO\textsubscript{2}} greater than 1 Torr from the baseline value were excluded from analysis. Therefore, changes in P\textsubscript{aCO\textsubscript{2}} had minimal impact on the results of this study. Also excluded were animals with a blood pressure decrease of more than 30 mmHg from baseline conditions at 60 min posthypoxia.

**Data Analysis**

Phrenic and XII nerve activities were recorded throughout the protocol. Peak integrated amplitude (Δ|\text{Phr}| and Δ|\text{XII}|), burst frequency (bursts/min), and mean arterial blood pressure (MAP) were measured at the following time points: baseline; last minute of first hypoxic episode (STHR); 15, 30, and 60 min after the final hypoxic episode; and during the final minute of the hypercapnic response. Nerve activities were averaged over a 60-s period for each condition. Changes in amplitude from baseline were normalized as a percentage of baseline nerve activity (% baseline) and as a percentage of the hypercapnic response (% maximum). Changes in burst frequency were expressed as a difference from baseline in bursts per minute.

**Sex Hormone Levels**

Arterial blood samples (1 ml) were taken as soon as the arterial catheter was placed and additionally before euthanasia of the animal at the end of the protocol. Subsequently, blood samples were centrifuged to collect serum. Serum was immediately frozen at −70°C. After collection of all serum samples, estradiol and progesterone levels were analyzed by using RIA (Estradiol Coat-A-Count, Progesterone Coat-A-Count; Diagnostic Products, Los Angeles, CA). Before analysis of the samples, the assays were validated with pooled serum from 10 rats.

Depending on the variable, either a one-way or a two-way ANOVA with a repeated-measures design (SigmaStat Version 2.0, Jandel, San Rafael, CA) was performed, followed by a least significant difference post hoc test for individual comparisons. Differences were considered significant if P < 0.05. All data reported are means ± SE. Serum levels of estradiol and progesterone and the progesterone-to-estradiol (P/\text{E}) ratio of individual rats were related to the magnitude of phrenic and XII LTF via multiple linear regression. A variable was considered to significantly contribute to the model if P < 0.05.

**RESULTS**

**Experimental Animals**

Because of closure of colony K62 of Sasco Sprague-Dawley rats, young female rats were not available to study after CIH treatment. Thus we present data for Young, Ger, and Ger/CIH rats only. Because there is evidence for strain differences in respiratory control (20, 21), it was not appropriate to substitute rats from another colony.

**Estrus Cycle**

The time-dependent phrenic and XII responses measured (STHR, LTF, etc.) did not significantly differ between estrus and diestrus within an age group (all P > 0.05). Therefore, data measured at both stages of the estrus cycle were combined in each age group of rats.

**Apneic Threshold and CO\textsubscript{2} Regulation**

The CO\textsubscript{2} apneic threshold was significantly higher in untreated geriatric rats compared with young and CIH-treated geriatric rats (50 ± 1 vs. 44 ± 1 and 44 ± 1 Torr, respectively; P < 0.05). Within each group, mean P\textsubscript{aCO\textsubscript{2}} remained nearly isocapnic to the baseline value throughout the protocol (Table 1).

**Baseline Respiratory Activity**

**Amplitude.** The ratio of baseline to maximal CO\textsubscript{2} response amplitude for phrenic and XII nerve activity measured in volts did not differ among Young, Ger, and Ger/CIH rats (Phrenic: Young = 0.5 ± 0.1; Ger = 0.4 ±
Geriatric rats treated with chronic intermittent hypoxia. PaCO$_2$ (2–3 Torr above CO$_2$ apneic threshold) was significantly higher in geriatric untreated rats.

Thus differences in the range from baseline to maximal PaCO$_2$ measured at different time points were not significant among groups (Young, Ger, Ger/CIH). Comparing individual groups, only the Ger/CIH group showed significant XII LTF vs. baseline, at 60 min postepisodic hypoxia (Fig. 3B; $P < 0.001$). At 60 min postepisodic hypoxia, LTF in the Ger/CIH group ($\Delta$/XII) was significantly greater than in Ger rats (Fig. 3B; $P = 0.038$).

**Burst Frequency**

There were significant time ($P < 0.001$) and time $\times$ treatment interactions ($P < 0.001$), indicating differences in frequency LTF among groups that increase with time posthypoxia. Young and Ger rats had a significant frequency increase vs. baseline at 30 and 60 min postepisodic hypoxia ($P < 0.05$). Furthermore, both Young and Ger rats had a significantly greater

**Phrenic LTF**

There were significant effects of time ($P < 0.001$) and the time $\times$ treatment interaction ($P = 0.019$), indicating differences in LTF among groups that increase with time posthypoxia. Phrenic amplitude increased progressively in all rat groups postepisodic hypoxia, indicating significant LTF ($P < 0.001$). Comparing individual groups, LTF was significant vs. baseline at 30 ($P < 0.001$) and 60 ($P = 0.004$) min postepisodic hypoxia in CIH-treated geriatric rats; LTF was significant only at 60 min in Young and Ger rats ($P < 0.006$). At 60 min postepisodic hypoxia, LTF in the Ger/CIH group was significantly greater compared with Young and Ger rats (Young = 53 ± 20; Ger = 44 ± 11; Ger/CIH = 119 ± 41% baseline, Fig. 3A; $P < 0.05$). However, the two-way ANOVA for repeated measures revealed an unequal variance, which did not become equal after different transformations were attempted (log, square root, square). Therefore, a two-way ANOVA on ranks was performed. With this statistical approach, only the time effect was significant ($P < 0.001$) but not time $\times$ treatment interaction. No individual group difference was significant at any time postepisodic hypoxia.

### Table 1. PaCO$_2$ measured at different time points

<table>
<thead>
<tr>
<th>PaCO$_2$</th>
<th>Baseline</th>
<th>Hypoxia</th>
<th>15 min</th>
<th>30 min</th>
<th>60 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Young</td>
<td>47 ± 1</td>
<td>49 ± 1</td>
<td>47 ± 1</td>
<td>47 ± 1</td>
<td>47 ± 1</td>
</tr>
<tr>
<td>Geriatric</td>
<td>53 ± 1</td>
<td>56 ± 1</td>
<td>54 ± 1</td>
<td>54 ± 1</td>
<td>53 ± 1</td>
</tr>
<tr>
<td>Ger/CIH</td>
<td>47 ± 1</td>
<td>48 ± 1</td>
<td>48 ± 1</td>
<td>47 ± 1</td>
<td>47 ± 1</td>
</tr>
</tbody>
</table>

Values are means ± SE for arterial PaCO$_2$ (PaCO$_2$), in Torr. Ger/CIH, geriatric rats treated with chronic intermittent hypoxia. PaCO$_2$ (2–3 Torr above CO$_2$ apneic threshold) was significantly higher in geriatric untreated rats.
increase in frequency than the Ger/CIH group at 30 min postepisodic hypoxia (Fig. 3C; \( P < 0.05 \)), whereas only the young rats exhibited a greater response at 60 min postepisodic hypoxia.

**Sex Hormone Levels**

Levels of estradiol (pg/ml) and progesterone (ng/ml), measured at the beginning of surgery and the end of experiments, did not differ among groups (separated in estrus and diestrus, Figs. 4). Progesterone levels increased substantially in all animals during experiments (Fig. 4B).

**Sex Hormone Levels and LTF**

Hormone levels measured at the beginning of surgery were correlated with the magnitude of LTF. Because sex hormones influence the serotonergic system and 5-HT receptor activation during intermittent hypoxia is necessary to elicit LTF, baseline (preintermittent hypoxia) sex hormone levels or their ratio may influence LTF. There was a significant correlation between phrenic and XII LTF vs. the progesterone-to-estradiol ratio, when estradiol was included in the

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**Fig. 3.** LTF in Young, Ger, and Ger/CIH female rats. LTF was measured as an increase from baseline in peak amplitude of integrated phrenic (A) and XII (B) neurograms. Increases in amplitude and burst frequency (C) are shown at 15, 30, and 60 min after the final hypoxic episode. Phrenic LTF was not different between Young and Ger female rats but was significantly greater in Ger/CIH rats relative to Young and Ger rats at 60 min postepisodic hypoxia \( (P < 0.05) \). XII LTF was not different between Young and Ger female rats but was significantly greater in Ger/CIH than in Ger rats at 60 min postepisodic hypoxia \( (P < 0.038) \). Only Young and Ger rats developed LTF in burst frequency at 30 and 60 min postepisodic hypoxia \( (P < 0.05) \). Values are means ± SE. *Significantly different from baseline. **Significantly different among groups.

**Fig. 4.** Serum levels of estradiol and progesterone measured at the beginning and at the end of anesthesia. Estradiol (A) levels (pg/ml) did not differ among groups (estrus, diestrus) and did not change during anesthesia. Progesterone (B) levels (ng/ml) did not differ among groups (estrus, diestrus) but increased significantly from beginning (presurgery) to the end of an experimental protocol \( (P < 0.05) \). Values are means ± SE.
model as a nonsignificant variable. Thus the P/E ratio is a significant determinant of LTF, but variability associated with estradiol must be accounted for to reveal this effect (P < 0.05; Fig. 5, A and B).

The multiple linear model using a backward step-wise elimination procedure between phrenic LTF, treatment group (Trt = CIH-treated), the P/E ratio, and estradiol (E) was

\[
\text{Phrenic LTF} = 20.550 + (75.558 \times \text{Trt}) - (10.257 \times \text{P/E}) - (0.884 \times E)
\]

\[ P = 0.014; \quad R^2 = 0.319 \]

The regression for XII LTF was

\[
\text{XII LTF} = -7.529 + (44.608 \times \text{Trt}) - (5.274 \times \text{P/E}) - (0.273 \times E)
\]

\[ P = 0.015; \quad R^2 = 0.338 \]

MAP

During hypoxic episodes, MAP decreased significantly, but it was not different from baseline in any group at any time postepisodic hypoxia. Ger female rats had a significantly lower MAP than Young and Ger/CIH groups at all time points measured except during hypoxia (Fig. 6; P < 0.05).

DISCUSSION

In this study, we demonstrated that hypoxic phrenic and XII responses are similar in geriatric and young female rats. However, the expression of both phrenic and XII LTF was decreased from responses previously observed in middle-aged female rats (71). We also demonstrated for the first time that, similar to young male rats, XII LTF is significantly enhanced by CIH in geriatric female rats; the effects of CIH on phrenic LTF are less clear.

Estrus Cycle in Geriatric Rats

In contrast to young and middle-aged rats, geriatric female rats stop cycling regularly. At first, the duration of one cycle (4–5 days; Ref. 17) gradually extends until the rats become acyclic, exhibiting persistent estrus or diestrus (26). This pattern is similar to women in their transition to menopause, making the rodent a good model to investigate changes that occur during and after menopause (70). In a previous study (72), we hypothesized that the P/E ratio at different stages of the estrus cycle influences respiratory control. Thus we analyzed serum levels of estradiol and progesterone in young cycling and geriatric acyclic female rats to relate them to time-dependent hypoxic responses. We found that the magnitude of LTF in both neurograms is inversely related to the P/E ratio.
Apneic Threshold and CO₂ Regulation

The significantly higher CO₂ apneic threshold in untreated geriatric vs. young female rats indicates diminished respiratory drive under the conditions of this study. The basis of this difference in drive is unclear. One possible explanation is a higher sensitivity in aged animals to general anesthesia (33). In contrast, CIH-treated geriatric rats had a significantly lowered apneic threshold that was closer to values observed in young female rats. A decreased CO₂ apneic threshold is consistent with reported effects of CIH on young male rats (23). Because baseline PaCO₂ was established 2–3 Torr above the CO₂ apneic threshold in each group and was regulated close to this baseline value throughout the protocol, similar levels of respiratory drive typified the baseline conditions in each group. We felt it was important to match respiratory drive rather than the level of PaCO₂ per se because of the nonlinear relationship between PaCO₂ and phrenic motor output (15). CIH-treated geriatric rats revealed a significantly higher baseline frequency compared with the other two groups, a phenomenon commonly seen animals treated with CIH or sustained hypoxia (14, 41).

STHR

Amplitudes of phrenic and XII motor output were significantly increased during hypoxia in all three group of rats but were not different among groups. In contrast to studies reporting reduced ventilatory responses to hypoxia in geriatric individuals (19, 63), the geriatric female rats of this study expressed the same hypoxic response as young rats, similar to a previous report of phrenic responses in geriatric male rats from our laboratory (22). In our previous study on middle-aged female rats, greater hypoxic phrenic and XII responses were observed compared with young female rats (72). This observation suggests a peak responsiveness to respiratory challenges in middle age. On the other hand, Schlenker and Goldman (62) demonstrated the greatest hypoxic ventilatory response in geriatric female rats compared with middle-aged and young female rats, a pattern opposite to aging male rats in their study. However, it is somewhat difficult to compare these studies because ventilatory responses reflect neuromechanical interactions whereas only neural drive was assessed in our study.

All groups of rats showed increases of burst frequency during hypoxia. In the CIH-treated group of geriatric rats, however, this increase was significantly smaller compared with both untreated groups. Because CIH increases baseline frequency in male rats (23), the range available to increase frequency during hypoxia in the Ger/CIH group may have been limited.

LTF

LTF is a form of plasticity evoked by episodic hypoxia and is expressed as a persistent augmentation of respiratory motor output. In anesthetized and vagotomized rats, the augmentation is mainly in amplitude with a lesser frequency response (4, 20, 30). Additional hypoxic episodes over a period of time (CIH) evoke a form of metaplasticity in male rats expressed as enhanced LTF (23, 59). In this study, we investigated the effects of gender and age on CIH-enhanced LTF and whether the response is unique to phrenic motor output. To the best of our knowledge, this is the first study reporting the effect of CIH on XII motor output, and the first in female rats. Because the magnitude of phrenic LTF in young rats was similar to that in our previous study on young female rats (72), we combined these data sets, yielding information on young, middle-aged, and old female rats. Although the magnitude of phrenic LTF did not differ between young and geriatric rats in this study, middle-aged rats exhibited greater LTF than either group (72). Thus 5-HT-dependent plasticity in phrenic motor output seems to be strongest at an age when the reproductive system reaches a stable plateau of circulating sex hormones in rats, i.e., middle age. Although young and middle-aged rats have a regular estrus cycle, middle-aged rats have more follicles and a higher concentration of estradiol (39). Some middle-aged female rodents also have elevated levels of circulating estradiol when they approach the transition to an acyclic stage (70).

Estradiol can influence the serotonergic system by a number of different mechanisms, enhancing the serotonergic function during cycle stages with higher estradiol levels such as proestrus and late diestrus (8, 9, 29, 45, 54, 56, 65). Thus middle-aged female rats at their reproductive peak might have a greater capacity for serotonergic neuroplasticity (expressed as phrenic LTF) than young rats with an immature reproductive system or geriatric rats with a degraded reproductive system.

The mechanism whereby a decreased LTF in geriatric female rats is restored to levels characteristic of middle-aged female rats is unclear. Unfortunately, because colony K62 of Sasco Sprague-Dawley rats was discontinued by the supplier, we could not evaluate the effects of CIH on young female rats of this strain. In young male rats, CIH-enhanced LTF is attenuated by the selective 5-HT₂ antagonist ketanserin and blocked by the broad-spectrum 5-HT antagonist methysergide (23). Whether similar results with 5-HT receptor antagonists are seen in female rats remains to be determined.

Another possible contributing factor in the mechanism of enhanced respiratory LTF in geriatric CIH-treated rats is an indirect effect via changes in sex hormones. However, circulating levels of estradiol and progesterone were not different among groups, indicating that CIH did not influence hormone levels per se. Caution is necessary when speaking about serum hormone levels, because they do not reflect levels and receptor expression in relevant CNS areas. Estrogen and progesterone receptors have been reported in serotonergic raphe neurons (2, 9) and have been localized in phrenic and XII motoneurons (Behan M, unpublished observations). Future studies taking into ac-
count receptor expression in respiration-related neurons are necessary to further our understanding of aging, sex hormones, and respiratory LTF.

In contrast to phrenic, XII motor output revealed LTF in geriatric female rats only when pretreated with CIH, thus indicating a relative lack of 5-HT-dependent plasticity in XII motor output in both Young and Ger rats. This is the first report demonstrating the existence of CIH-induced metaplasticity in XII nerve activity. At this time, it is difficult to conclude where or through which cellular and/or synaptic mechanisms CIH enhanced XII LTF. The XII nuclei receive serotonergic input from the caudal raphe nuclei, but the relative capacity for serotonergic influence may be more limited (43, 44).

Young and Ger rats exhibited frequency LTF, whereas Ger/CIH rats did not. In contrast to Ger/CIH rats, both groups had low baseline frequencies. Recently, in an extensive meta-analysis of rats, there was an inverse correlation between baseline frequency and frequency LTF, regardless of rat strain, age, gender, or pharmacological manipulation (Baker-Herman TL, and Mitchell GS, unpublished observations). Thus the absence of frequency LTF in Ger/CIH rats may be the nonspecific result of their elevated baseline frequency.

Sex Hormone Levels

The standard error bars for estradiol and progesterone were large, possibly reflecting the time of the day at which blood samples were drawn for each individual rat; the stage of the estrus cycle may have progressed. The estrus cycle of mature rats is very short (4–5 days), and a difference of hours can change hormone levels substantially. Geriatric rats stay in one stage of the estrus cycle and do not undergo major fluctuations of blood hormone levels, but there is still variability among animals. Despite (or because of) this variability, there was an inverse correlation between sex hormone levels and the magnitude of LTF measured at 60 min. Because P/E ratios were significantly correlated to the magnitude of both phrenic and XII LTF, sex hormone balance appears to be a critical determinant of LTF. However, serum levels of sex hormones do not reflect their levels in the brain. Further investigation is needed to determine whether serum levels of gonadal hormones are reflected in respiratory related areas of the CNS.

Estradiol increases 5-HT levels in the central nervous system by increasing tryptophan hydroxylase, decreasing 5-HT reuptake transporter mRNA, inhibiting monoamine oxidase, and altering 5-HT receptor expression (8, 9, 29, 42, 45, 54, 56, 65). Progesterone increases the firing rate of serotonergic neurons in the medullary raphe nucleus (36). Progesterone receptors are upregulated by estradiol and downregulated by progesterone (8, 24, 46). Conversely, estrogen receptors are upregulated by low levels and downregulated by high levels of estrogens (2, 64). Thus a certain P/E ratio may optimize estrogen and progesterone receptor expression, thereby enhancing respiratory LTF.

Because sex hormone levels correlated with LTF were measured at the beginning of surgery, progesterone increases during the experiment were not taken into account. We speculate that progesterone levels increased during experiments in parallel to increasing corticosteroid levels because progesterone is an intermediate metabolite during the transformation from cholesterol to corticosteroids elicited by stress. The effect of anesthesia on sex hormones parallels increases in corticosterone and progesterone in both male and female rats (Zabka AG, Mitchell GS, and Behan M, unpublished observations).

MAP

Geriatric rats had lower MAP than young rats. After CIH treatment, blood pressure in geriatric female rats was increased to levels comparable to those of young female rats. CIH has been shown to elevate MAP in young male rats (23). Hypoxic episodes given over a period of time stimulate peripheral chemoreceptors, increasing sympathetic outflow and upregulating the renin-angiotensin system, which in turn would cause sustained elevation of systemic blood pressure (16, 25, 28, 40, 47, 59). The decrease of blood pressure during hypoxia is commonly seen in this experimental preparation and did not differ as a percent change from baseline among groups. Furthermore, because the behavior of MAP after the hypoxic episodes did not differ among groups (i.e., no change from baseline prehypoxia values), MAP should not have influenced the outcome of this study.

Critique of Methods

All rats were anesthetized, vagotomized, paralyzed, and pump ventilated. This reduced in vivo preparation allows us to study neural activity without influence from lung mechanics or vagal feedback while maintaining precise control of arterial blood-gas composition. Furthermore, respiratory LTF has been extensively characterized in male rats by use of this model, thereby facilitating comparisons with female rats (4, 5, 23, 21, 34, 71).

Serum levels of estradiol were unaffected by anesthesia. In contrast, progesterone levels increased progressively during anesthesia (Zabka AG, unpublished observations). This increase in progesterone concentration may parallel an increase in corticosterone levels that was also observed (data not shown), possibly because of anesthetic stress. Although we cannot exclude direct or indirect effects of changes in the levels of progesterone due to general anesthesia on the outcome of this study, all groups of rats showed an identical pattern of progesterone elevation during experiments. Thus differences among groups relate to factors independent of the increase in progesterone.

Physiological Significance

Respiratory LTF provides an excellent model with which to study the effects of age and gender on neuroplasticity in general and 5-HT-dependent plasticity in
specific. From another perspective, respiratory LTF may provide unique insights into the etiology of important age- and gender-related breathing disorders, such as obstructive sleep apnea (OSA) (11, 13, 38, 68). OSA occurs mainly in middle-aged men and in women after menopause when not on hormone replacement therapy (11, 32, 68). This pattern strongly correlates with age-related changes in sex hormones, such as a constant decline of testosterone in men and a more abrupt decrease of ovarian sex hormones in women when they reach menopause. The observation that CIH treatment restored LTF in the acyclic geriatric rats may suggest that intermittent hypoxia associated with the onset of OSA may elicit compensatory mechanisms that offset the tendency for increased apneic events. Furthermore, our observations may suggest that diurnal CIH may be developed as a therapeutic tool for increasing upper airway tone and ventilatory stability during sleep.

DISCLOSURES

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