Chronic hypoxia increases the gain of the hypoxic ventilatory response by a mechanism in the central nervous system

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VENTILATORY ACCLIMATIZATION to hypoxia (VAH) is a time-dependent increase in ventilation during sustained exposure to hypoxia, which is observed in humans and other mammals (20). VAH is expressed in two ways. First, ventilatory sensitivity to O2 is increased so the slope of the isocapnic hypoxic ventilatory response (HVR) increases (18). Second, ventilatory drive increases so hyperventilation persists after normoxia is restored and arterial PCO2 (PacO2) is regulated at a lower level (20, 22).

The physiological mechanisms of VAH are not completely known, but they involve, at least, increased O2 sensitivity of the carotid body chemoreceptors (reviewed in Ref. 17) and plasticity in the integration of this afferent input in the central nervous system respiratory centers (6). Increased carotid body afferent activity for a given arterial PO2 (Pao2) has been observed in rats, cats, and goats exposed to sustained hypoxia for 8 h to 2 wk (3, 4, 13). This sensory plasticity can explain VAH during early exposure to chronic hypoxia (CH). However, after longer periods of hypoxia, rats also show evidence of plasticity in the central nervous system (19). The “central gain of the HVR,” quantified as the phrenic nerve response to fixed levels of electrical stimulation of the carotid sinus nerve, increases after 7 days of chronic sustained hypoxia (6).

The idea of an increased central gain of the HVR with CH was suggested earlier in a study on the effects of the chemoreceptor stimulant doxapram in humans acclimatized to high altitude (10). The authors measured responses to a variety of ventilatory stimuli and postulated that CH increases ventilatory drive by increased facilitation of the medullary ventilatory control center. However, it was not known whether CH changed the sensitivity of carotid body chemoreceptors to doxapram or nonspecific central effects of the drug, which also could explain the results. Hence, we designed experiments to test for such changes and to evaluate the potential of doxapram as an experimental tool to study changes in the central gain of the HVR during CH in humans and conscious animals. The results support this by finding an increased ventilatory response to maximum carotid body stimulation with doxapram after CH but no change in the sensitivity of carotid bodies to a maximum dose of doxapram or noncarotid body effects of doxapram on ventilation.

METHODS

Experimental animals. Male Sprague-Dawley rats (300–550 g for ventilation and phrenic nerve studies; 150–180 g for carotid sinus nerve studies; Charles River) were housed in standard rat cages in a vivarium with a 12:12-h light-dark cycle and were fed ad libitum a standard rat diet. All experiments were approved by the Animal Care and Use Committees of the University of California, San Diego, and the University of Utah. The experiments conformed to national standards for the care and use of experimental animals and the American Physiological Society’s “Guiding Principles in the Care and Use of Animals.” Animals were divided into two experimental groups: one was exposed to CH and the other was maintained under control normoxic (CN) conditions in room air.

Chronic hypoxia. Rats were exposed to CH in a hypobaric chamber maintained at 0.5 atm (380 Torr), which approximates exposure to 10% inspired O2 at sea level and mimics the conditions of CH encountered at ~6,000 m above sea level. Animals were placed in the hypobaric chamber in individual cages, and the pressure was lowered over a 5-min period. The chamber was opened once daily (~10 min) for regular cage maintenance or when it was necessary to remove animals for experimentation. The hypobaric chamber was maintained in the same vivarium that housed the CN animals. Two days of CH were used for experiments involving ventilatory and isolated carotid body responses to doxapram, and 7 days of CH were used for experiments involving the phrenic nerve responses to doxapram. We used different durations of CH to accommodate changes in animal husbandry procedures implemented during the completion of different experimental protocols reported here. However, the essential features
of VAH are constant over 2–14 days (19, 15), justifying the comparison of data between groups.

**Measurement of ventilatory responses in awake rats.** Two days before an experiment, catheters were placed in the femoral artery and vein under halothane or isoflurane anesthesia as described previously (1). At the same time, a 25-gauge thermocouple (IT-18, Physitemp) was placed under the dorsal skin next to the catheters. During the experiment, arterial blood samples (0.2 ml) were analyzed for PaO₂, PaCO₂, and arterial pH (system 1306, Instrumentation Laboratory), and doxapram was infused intravenously with the use of a peristaltic pump (P720, Instech).

For the experiments, individual rats were placed in a Plexiglas chamber (7 liters), and inspired ventilation (VI) was measured by continuous flow barometric plethysmography as described previously (1). An electronic gas mixer (MFC-4, Sable Systems) mixed O₂ and N₂ to the desired inspired gas concentrations, and the resultant chamber gas concentration was measured with a mass spectrometer (MGA 1100, Perkin-Elmer). The inflowing gas (3 l/min) entered the chamber through a tube (7 cm long, 1 cm internal diameter) that was filled with similar lengths of PE-20 tubing, which provided high impedance and reduced the loss of pressure signals. Gas was removed from the chamber at the same rate as it entered through a vacuum valve (M series, Nupro) attached to a vacuum pump (model 25, Precision Scientific). We did not use a reference chamber and compensated for changes in the pressure signal baseline by adjusting the vacuum valve to maintain a chamber pressure near the atmospheric level, monitored by water manometer after each change of inspired gases. Air temperature was measured with a thermometer, and relative humidity was monitored with a digital hygrometer, both of which were sealed in the plethysmograph. Respiratory frequency (f) was calculated directly from the ventilation-induced pressure swings. Tidal volume (VT) was calculated from the ventilation-induced pressure changes, which were measured by a differential pressure transducer referenced to atmosphere (MP45, Validyne). Output from the transducer demodulator was recorded on a digital data-acquisition system (DT-2801, National Instruments; 200-Hz sample rate) and a transducer demodulator was recorded on a digital data-acquisition system referenced to atmosphere (MP45, Validyne). Output from the transducer demodulator was recorded on a digital data-acquisition system (DT-2801, National Instruments; 200-Hz sample rate) and a chart recorder (Gould). Calibration pulses (1 ml) were injected from a gas-tight syringe into the chamber at a rate similar to the rats’ inspiratory duration.

**Protocol for ventilatory responses.** CN rats (n = 21) or rats acclimatized to 2 days of CH (n = 13) were weighed and placed into the plethysmograph for a 30-min acclimation period. During this period, the plethysmograph was ventilated with the animals’ chronic O₂ level, i.e., 21% O₂ for CN rats and 10% O₂ for CH rats. Once animals were acclimatized to the chamber, we collected 1 min of ventilatory data for analysis and sampled arterial blood. Then the acute ventilatory response to O₂ was measured by changing from 21% to 10% O₂ in CN rats and vice versa for CH rats. Between 10 and 15 min after an acute change in inspired O₂ fraction (FI₀₂), we collected 1 min of ventilatory data for analysis and sampled arterial blood.

After we obtained control measurements, the animals were returned to their chronic O₂ level for at least 15 min. We repeated this protocol while doxapram was intravenously infused (4.6 mg·kg⁻¹·min⁻¹). Ventilatory data were collected and analyzed for 1 min during a 4-min infusion of doxapram when breathing was stable, which was typically between 3 and 4 min of infusion. After stopping the infusion, we allowed the animals to recover so breathing returned toward control levels and then measured the ventilatory response to doxapram at a different O₂ level, i.e., 10% O₂ for CN rats and 21% for CH rats. FI₀₂ was changed for 15 min, and doxapram was infused for 4 of the last 5 min. Ventilatory data and arterial blood samples were collected during 1 min of the doxapram infusion when breathing was stable.

In early experiments, we used 30% O₂ instead of 21% O₂ to reduce any normoxic ventilatory drive from arterial chemoreceptors. However, there was no significant difference in the responses to doxapram or acute hypoxia starting with 30% (n = 13) vs. 21% O₂ (n = 12), so the results were pooled.

**Data analysis for ventilatory responses.** Student’s t-test was used to compare the effects of chronic O₂ level (CH vs. CN) on the change in ventilatory variables in response to doxapram (ΔVₚₒₓ). The measurements were designed to test the hypothesis that CH changes the ventilatory response to a maximal stimulation of the carotid bodies with doxapram. Hence, the acute O₂ level is not as important as an arterial chemoreceptor stimulus if doxapram truly provides a maximum stimulation of the carotid body (as confirmed in the results, see below). However, the acute O₂ level will affect the magnitude of the ventilatory response to doxapram by affecting the initial (predoxapram) VT. The acute O₂ level could also affect ΔVₚₒₓ by nonarterial chemoreceptor mechanisms such as general effects of O₂ on excitability in the brain. Hence, we analyzed ventilatory variables (VT, V̇E, and f) separately under high O₂ conditions (breathing 21% or 30% O₂) and hypoxic conditions (breathing 10% O₂). A two-way ANOVA was used to assess main effects of chronic O₂ level (CN vs. CH) and treatment (control vs. doxapram) and interactions between chronic O₂ and treatment for all ventilatory variables. All averages are expressed ± SE, and P < 0.05 was considered significant.

**Measurement of phrenic nerve response to doxapram after carotid body denervation.** To quantify the ventilatory effects of doxapram occurring independent of carotid body stimulation, we measured the phrenic nerve response to doxapram infusion after carotid body denervation in anesthetized, paralyzed, vagotomized male rats. Animals were divided randomly into CN (n = 5) and CH (n = 7) groups. CH rats were exposed to hypoxia for 7–8 days. Anesthesia was induced with isoflurane in 50% O₂ and maintained with urethane (1.6 g·kg⁻¹·iv). Rats were then tracheotomized and artificially ventilated (model 683, Harvard Instruments) with 50% O₂, which is a standard procedure with this anesthesia preparation to prolong viability. End-tidal PCO₂ (Capnogard 1265, Novametic Medical Systems) and tracheal pressure (P23, Gould) were continuously monitored. Both carotid sinus nerves were sectioned to remove carotid body chemoreceptor input to the central nervous system. Both vagus nerves were transected to remove afferent feedback from pulmonary stretch receptors and vagal arterial chemoreceptors, and the animals were paralyzed (pancuronium bromide, 3.5–4.0 mg·kg⁻¹·iv). The femoral artery...
was catheterized to monitor blood pressure (P23, Gould) and measure arterial blood gases (Synthesis GEM Premier 3000). The femoral vein was catheterized for administration of fluids and experimental drugs.

The left phrenic nerve was isolated dorsally, cut distally, and desheathed. Nerve activity was recorded with a bipolar silver electrode in a mineral oil pool, amplified (P511 with HiZ probe, Grass), rectified, and integrated with a moving averager (MA-821, CWE with 50-ms time constant). Maximum moving average burst amplitude and the frequency of phrenic bursts were analyzed with an A/D analysis system (MP100A, BioPac).

Protocol for phrenic nerve response. After placement of the phrenic nerve on the recording electrode, nerve activity was allowed to stabilize for 60 min in hyperoxia (FiO\textsubscript{2} = 0.50). The apneic threshold was determined by slowly increasing ventilator rate and/or volume until phrenic activity ceased. Baseline nerve activity was then established by increasing end-tidal PCO\textsubscript{2} to 3 Torr above the value measured at the apneic threshold and allowed to stabilize for at least 10 min. Maximum phrenic response to 2 min of 10% CO\textsubscript{2} and phrenic response to 1 min of isocapnic hypoxia (FiO\textsubscript{2} = 0.10, balance N\textsubscript{2}) were measured, with a return to baseline for 20 min in between. After the hypercapnic and hypoxic challenges, phrenic activity was allowed to return to baseline over a 30-min period. Doxapram was infused for 4 min at 4.6 mg·kg\textsuperscript{-1}·min\textsuperscript{-1} during hyperoxia and phrenic activity measured at four time points during the infusion (30, 90, 150, and 210 s postinfusion).

Data analysis for phrenic response. Phrenic burst amplitude (\textit{f}Phr) and \textit{f} were averaged over 10 bursts at all time points of interest. \textit{f}Phr was normalized to the baseline value and neural ventilation (\textit{f}Phr·\textit{f}) was calculated for each time point. We analyzed the phrenic response to doxapram with a two-way ANOVA: (experimental groups = CN and CH) × (time after doxapram infusion = 0, 5, 15, 30, and 60 min). All averages are expressed ± SE, and \textit{P} < 0.05 was considered significant.

RESULTS

Effects of CH on ventilatory responses to doxapram. VAH was detected as significant main effects of chronic O\textsubscript{2} level on V\textsubscript{T} under acute normoxic and hypoxic conditions (Fig. 1, A and B, respectively). This was due to a significant main effect of chronic O\textsubscript{2} level on f in both acute normoxia and hypoxia conditions (Fig. 1, E and F), but the main effect of chronic O\textsubscript{2}
level was significant for VT only in acute normoxia (Fig. 1D).

CH also increased the effect of doxapram on Vt; there was a significant interaction between chronic O2 level and doxapram in both acute normoxia and hypoxia for Vt (Fig. 1, A and B). The only component of ventilation for which there was a significant interaction between chronic O2 level and doxapram was f in acute hypoxia (Fig. 1F).

Although baseline Vt increased with CH, Vt after doxapram increased even more in the CH rats. Hence, ΔVt was significantly greater in CH than in CN rats. For rats breathing normoxic air, ΔVt was 1,399 ± 132 ml·min⁻¹·kg⁻¹ in CH vs. 776 ± 125 ml·min⁻¹·kg⁻¹ in CN rats. The significantly greater ΔVt in CH rats during normoxia was due to increases in both VR and f in response to doxapram, but, individually, these changes were not significant. For rats that breathed hypoxic gas, ΔVt was 1,201 ± 178 ml·min⁻¹·kg⁻¹ in CH vs. 506 ± 81 ml·min⁻¹·kg⁻¹ in CN rats. In acute hypoxia, changes in Vt with doxapram were similar in the two groups, and f increased with doxapram more in the CH rats than in the CN rats (Fig. 1). In both CN and CH rats, the change in Vt was greater during normoxia than hypoxia because hypoxia elevates the baseline Vt measured before doxapram infusion.

Table 1 summarizes the blood-gas data during doxapram infusion in normoxia and hypoxia. The number of animals in Table 1 is less than the total number studied in the plethysmograph because the catheters did not always remain patent, and only data for 21% O2 but not 30% O2 breathing are reported.

Effects of doxapram on isolated carotid bodies. Adding 150 μM of doxapram to the normoxic superfusate caused large increases in carotid sinus nerve discharge (Fig. 2). This response to doxapram was larger than the response to acute hypoxia, which is <200 impulses/s before CH and <400 impulses/s after CH (4). However, there was no significant difference between the averaged or peak response to doxapram between CN and CH rats (Fig. 3). There were some differences in the time course of the response to doxapram after CH so the rise to maximum discharge was faster and sustained longer in CH vs. CN rats (Fig. 2). This resulted in more total discharge during a trial of doxapram stimulation after CH (424,680 ± 33,559 impulses) than with CN (186,513 ± 46,256 impulses) because of the prolonged tail-off in nerve activity (Fig. 2). However, the average or peak carotid sinus nerve response to doxapram did not change with CH (Fig. 3), and these are the relevant variables to consider for judging the ventilatory response to carotid body stimulation by doxapram infusion.

The peak and average carotid sinus nerve responses to smaller doses of doxapram (15 μM) did show some differences with CH (not shown). The response to 15 μM was significantly less than the response to 150 μM, indicating that the smaller dose did not produce a maximum response from the carotid body. These differences in response to submaximal stimulation with doxapram are similar to the differences reported for submaximal stimulation with hypoxia (4).

### Table 1. Arterial blood gases in CN and CH rats breathing room air at sea level without (control) and with doxapram infusion

<table>
<thead>
<tr>
<th></th>
<th>Arterial Po2</th>
<th>Arterial PCO2</th>
<th>Arterial pH</th>
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<tbody>
<tr>
<td>FIO2 = 0.21</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>CN control (n = 5)</td>
<td>81.8 ± 3.0</td>
<td>36.3 ± 1.8</td>
<td>7.44 ± 0.01</td>
</tr>
<tr>
<td>CN doxapram (n = 5)</td>
<td>106.2 ± 1.0</td>
<td>23.7 ± 0.9</td>
<td>7.56 ± 0.02</td>
</tr>
<tr>
<td>CH control (n = 4)</td>
<td>91.0 ± 3.9</td>
<td>26.6 ± 1.5</td>
<td>7.43 ± 0.02</td>
</tr>
<tr>
<td>CH doxapram (n = 4)</td>
<td>103.3 ± 8.1</td>
<td>15.2 ± 2.1</td>
<td>7.57 ± 0.01</td>
</tr>
<tr>
<td>FIO2 = 0.10</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CN control (n = 9)</td>
<td>35.2 ± 1.4</td>
<td>25.8 ± 0.7</td>
<td>7.58 ± 0.01</td>
</tr>
<tr>
<td>CN doxapram (n = 6)</td>
<td>44.7 ± 2.4</td>
<td>20.9 ± 1.4</td>
<td>7.63 ± 0.05</td>
</tr>
<tr>
<td>CH control (n = 5)</td>
<td>46.6 ± 4.2</td>
<td>22.2 ± 0.9</td>
<td>7.50 ± 0.02</td>
</tr>
<tr>
<td>CH doxapram (n = 3)</td>
<td>58.0 ± 5.0</td>
<td>16.6 ± 1.6</td>
<td>7.46 ± 0.12</td>
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Values are means ± SE for number of samples in parentheses. FIO2, inspired O2 fraction; CN, control, normoxic; CH, chronically hypoxic.

Fig. 2. Exposure to 150 μM doxapram for 150 s evokes a large and prolonged increase in carotid sinus nerve (CSN) activity in both normal (normoxic) and CH in vitro preparations (bathed at Po2 = 450 Torr).

Fig. 3. Averaged and peak CSN activities evoked by 150 μM doxapram in normal vs. CH preparations are not significantly different.
Effects of doxapram on phrenic nerve response after carotid body denervation. In anesthetized rats, CH significantly decreased end-tidal Pco2 at the apneic threshold as expected for acclimatization and reported previously (6). For CN rats, the apneic threshold was 37.6 ± 1.2 Torr vs. 28.5 ± 1.2 Torr for CH rats. Carotid body denervation was considered successful if the phrenic nerve response to 1 min of hypoxia (Fio2 = 0.10) was <30% of control (16).

Doxapram caused an increase in phrenic activity that was apparent by 30 s in both CN and CH rats and remained elevated for at least 240 s (Fig. 4). The increase in fPhr also persisted for all time points in both groups, although only remained elevated through 90 s in CH rats vs. 150 s in CN rats. No significant differences between CN and CH rats were observed at any time point in fPhr, f, or their product.

DISCUSSION

This study shows that the respiratory stimulant doxapram increases ventilation more in rats that have been acclimatized to CH than in the CN rats. This result cannot be explained by either a larger response to doxapram in isolated carotid bodies after CH or a larger noncarotid body effect of doxapram on ventilation after CH. The simplest explanation for the results is that CH mediates plasticity in central nervous system respiratory centers, increasing the ventilatory output for a given increase in afferent input from arterial chemoreceptors. This can be described as an increase in the central gain of the HVR, which has been demonstrated previously as an increased phrenic response to electrical stimulation of the carotid sinus nerve in CH anesthetized rats (6).

Effects of doxapram on carotid bodies. Nishino et al. (14) found that doxapram produced a simple additive effect on carotid body discharge frequency with hypoxic or hypercapnic stimuli. This is consistent with our results that found a similar increase in carotid body discharge with a high dose of doxapram in both chronically normoxic and hypoxic animals, despite increases in carotid body baseline discharge after CH. Our findings support the idea that doxapram can be a useful experimental tool to study the physiological response to a constant and high level of afferent input from the carotid bodies under CN and CH conditions.

Doxapram increases carotid body activity in the type I cells by inhibiting K+ current through TWIK-related acid-sensitive K+ (TASK)-1 and TASK-3 channels (5). Our results demonstrate that CH does not change the effect of a maximum dose of doxapram. However, this does not preclude a role for TASK channels in submaximal increases in carotid body activity in CH animals. Doxapram also induces the release of dopamine in the carotid body (2), and this is associated with increased activity in the carotid sinus nerve, although dopamine may not be excitatory in the carotid body (8). Changes in dopamine and its effects in the carotid body with CH primarily maintain O2 sensitivity in a normal range despite long-term changes in stimulation and baseline activity levels (reviewed in Ref. 17). The changes in the dynamic response of carotid bodies to doxapram that we observed may involve dopaminergic mechanisms, as well as TASK channels.

Previous work has shown significant increases in both normoxic baseline and hypoxic-stimulated levels of carotid body discharge between 0 and 16 days of CH (4). Although baseline discharge increases during acute normoxia after CH (4), we observed no change in doxapram-stimulated maximum discharge after CH. Consequently, changes in carotid body discharge with doxapram should be less after CH than before. Hence, our approach based on the ventilatory response to doxapram tends to underestimate the increase in central gain of the HVR with CH.

Effects of doxapram on ventilation. Doxapram is described as both a ventilatory stimulant and analeptic (central nervous system stimulant), which increases ventilation by multiple mechanisms depending on the dose. At low doses, doxapram stimulates ventilation primarily through its effects on the carotid body; however, at higher doses, it has independent effects on ventilation, for example, through direct stimulation of respiratory neurons in the central nervous system (12). We had to use high doses because our goal was to produce a comparable level of carotid body stimulation in CN and CH rats, and our isolated carotid body studies showed this was only possible with high doses. For our ventilatory response studies, we used the maximum clinical dose of doxapram (1.0 mg/kg bolus or a total of 4 mg/kg by infusion) but adjusted it for the higher metabolic rate in rats using the general relationship for metabolism vs. body mass (O2 consumption = a M0.75, where M is body mass). Larger doses resulted in inconsistent ventilatory responses and, in some cases, convulsions.

We observed increased ventilatory responses to doxapram after CH. However, direct effects of high doses of doxapram on respiratory neurons could be sensitized by CH. Hence, it was essential that we quantify and control for potential changes in the effects of doxapram on ventilation with CH that are independent of arterial chemoreceptor input. To this end, we measured the phrenic nerve response to doxapram after carotid body denervation. The vagus nerve was sectioned in these experiments to remove pulmonary stretch receptor afferents that can couple phrenic activity with the ventilator. This was also useful for our experiments because it removed potential input from vagal arterial chemoreceptors, which have been reported in rats (11). We estimate the magnitude of nonarterial chemoreceptor effects of doxapram as 29% of the intact response. Pilot experiments (n = 5) showed that carotid sinus

Fig. 4. Increased neural ventilation (phrenic activity) in anesthetized, paralyzed, vagotomized, carotid body-denervated and artificially ventilated rats during a 4-min infusion of doxapram. Values are averages over 1-min intervals. *P < 0.05 compared with baseline value. No differences were observed between CN and CH rats.
nerve section decreased the phrenic response to doxapram an average of 71%. However, these are only rough estimates because we could not ensure a stable baseline in phrenic activity before and after denervation in these pilot experiments. We used a nonrepeated measures design to eliminate this problem for the data presented in the Results.

We found that the increase in neural ventilation during the last minute of a 4-min doxapram infusion was the same in both control and CH rats (Fig. 4). Therefore, we conclude that changes in ventilatory responses to doxapram with CH are not explained by changes in nonarterial chemoreceptor effects of doxapram with two assumptions. First, the nonarterial chemoreceptor effects in anesthetized animals are assumed to be the same in awake animals. We chose the anesthetized preparation to facilitate elimination of all arterial chemoreceptor input in both CN and CH rats. The alternative of studying awake rats with carotid body denervation requires significant time for recovery, and the operation is known to block normal VAH (16). Hence, we could not match the initial conditions for measuring responses to doxapram in intact and denervated CH animals. Additionally, procedures for denervating vagal arterial chemoreceptors and subsequent recovery in rats have not been established. Therefore, we used anesthetized animals to test for the absence of nonarterial chemoreceptor doxapram effects after CH.

Our second assumption is that the nonarterial chemoreceptor effects of doxapram on ventilation are the same after 2 or 7 days of CH. We did not observe any differences in these effects between 0 and 7 days, so this seems unlikely. Additionally, the fundamental features of VAH, such as absolute levels of ventilation and arterial blood gases, are constant in rats acclimatized to this level of hypoxia between 2 and 7 days (19). If the nonarterial chemoreceptor effects of doxapram decreased between 2 and 7 days of CH, then we would have underestimated their effects at 2 days and overestimated the increase in central gain of the HVR with CH. However, the ventilatory response to doxapram almost doubled after 2 days of CH. Therefore, we would take a very dramatic spike in the nonarterial chemoreceptor effects of doxapram at 2 (but not 7) days of CH to negate our conclusions.

The simplest explanation for the increased ventilatory response to doxapram that we observed with CH is an enhanced translation of carotid body afferent input into ventilatory motor output by respiratory centers in the central nervous system, i.e., increased central gain of the HVR. The result cannot be explained by changes in carotid body sensitivity to doxapram (Fig. 3) or noncarotid body effects of doxapram with CH (Fig. 4). This is consistent with previous studies on humans and ponies, which also found significant increases in ventilatory responses to doxapram after 3 days to 3 wk of hypoxic acclimatization (9, 10). All of these results support changes in the central nervous system with CH, assuming that the direct effects of doxapram on carotid bodies and respiratory centers are unchanged with CH as we found in rats.

As discussed previously (19), there is no strong experimental evidence disproving an increased central gain of the HVR with long-term CH. However, there is strong experimental evidence that, during early exposure to CH, plasticity within the carotid bodies alone can completely explain VAH, as well as any changes in the HVR. For example, increased carotid body sensitivity to hypoxia can completely account for the increased ventilation during 6-h hypoxic exposures in goats (7). Vizek et al. (21) did not find a significant increase in the central gain of the HVR (i.e., their “translation index”) in cats after 48 h of hypoxia. This difference from our results with rats could reflect the well-known species differences in the speed of VAH. In general, it appears that plasticity in the carotid body explains early phases of VAH while plasticity in the central nervous system contributes to increases in the HVR after 2 days or more of CH (19).

Summarizing, in both CN and CH rats, high doses of doxapram produce the same effect on 1) the carotid body and 2) noncarotid body effects on ventilation. However, the ventilatory response to doxapram is increased by CH, and the simplest explanation is plasticity in central mechanisms that increase the translation of chemoreceptor afferent information into a ventilatory efferent response. Previously, the best experimental evidence for plasticity in the central nervous system with CH contributing to an increased HVR came from neural recordings in anesthetized animals (6). Hence, doxapram provides a promising tool to study the time course of changes in the central gain of the HVR during CH in awake animals and humans.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

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