Ventilatory responses to acute and chronic hypoxia in mice: effects of dopamine D$_2$ receptors


1Department of Medicine and 2White Mountain Research Station, University of California, San Diego, La Jolla, California 92039-0623; and 3Vollum Institute, Oregon Health Sciences Center, Portland, Oregon 97201

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Huey, K. A., M. J. Low, M. A. Kelly, R. Juarez, J. M. Szewczak, and F. L. Powell. Ventilatory responses to acute and chronic hypoxia in mice: effects of dopamine D$_2$ receptors. J Appl Physiol 89: 1142–1150, 2000.—We used genetically engineered D$_2$ receptor-deficient [D$_2$-(-/-)] and wild-type [D$_2$- (+/ +)] mice to test the hypothesis that dopamine D$_2$ receptors modulate the ventilatory response to acute hypoxia [hypoxic ventilatory response (HVR)] and hypercapnia [hypercapnic ventilatory response (HCVR)] and time-dependent changes in ventilation during chronic hypoxia. HVR was independent of gender in D$_2$-(+/+) mice and significantly greater in D$_2$-(-/-) than in D$_2$-(+/+) female mice. HCVR was significantly greater in female D$_2$-(+/+) mice than in male D$_2$-(+/+) and was greater in D$_2$-(-/-) male mice than in D$_2$-(+/+) male mice. Exposure to hypoxia for 2–8 days was studied in male mice only. D$_2$-(+/+) mice showed time-dependent changes in “baseline” ventilation (inspired PO$_2$ = 214 Torr) and hypoxic stimulated ventilation (inspired PO$_2$ = 70 Torr) after 8 days of acclimatization to hypoxia, but D$_2$-(-/-) mice did not. Hence, dopamine D$_2$ receptors modulate the acute HVR and HCVR in mice in a gender-specific manner and contribute to time-dependent changes in ventilation and the acute HVR during acclimatization to hypoxia.

hypoxic ventilatory response; hypercapnic ventilatory response; acclimatization to hypoxia; carotid body; transgenic mice

VENTILATORY ACCLIMATIZATION to hypoxia produces a time-dependent increase in ventilation, a decrease in arterial PCO$_2$ (PaCO$_2$), and an increase in the hypoxic ventilatory response (HVR). This process has been well characterized in humans (10, 30, 39) and several animal models, such as goats (7, 32), ponies (8), cats (36, 38), and rats (23). Although all species studied exhibit ventilatory acclimatization to hypoxia, differences exist in the time course and mechanisms of acclimatization (4). The time course of acclimatization to hypoxia can range from only 6 h in the goat to weeks in the rat, which exhibits the most humanlike ventilatory acclimatization to hypoxia (23). However, the time course of acclimatization in the mouse has not been fully char-
acterized. This is important, because recent advances in molecular biology have produced many genetically manipulated mice that can be used to test physiological mechanisms of ventilatory acclimatization to hypoxia. Knowledge about the time course of ventilatory acclimatization to hypoxia in mice will provide a framework for interpreting results in genetically manipulated mice.

Changes in dopaminergic pathways are hypothesized to contribute to ventilatory acclimatization to hypoxia. Dopamine D$_2$ receptors modulate the HVR peripherally in the carotid body and in the central nervous system (CNS). During exposure to hypoxia, dopamine (DA) is released from carotid body glomus cells (13) and chemoafferent fibers in the nucleus tractus solitarius (NTS) (12), which is the primary synapse for afferent fibers from the carotid body (15, 37). At the carotid body, DA activates pre- and postsynaptic D$_2$ receptors (5, 22) and tonically inhibits carotid body neural output (16, 18, 36) and ventilation (2). Chronic hypoxia modifies the levels of carotid body DA and reduces D$_2$ receptor inhibition of carotid body neural output in rats and cats (2, 17). In contrast to the carotid body, D$_3$ receptors in the CNS tonically stimulate ventilation in rats (14), mice (25), and cats (16, 31). Chronic hypoxia also increases DA levels in the CNS (24). It is unclear whether D$_3$ receptors are involved in the increased CNS gain of the HVR with chronic hypoxia, but increased dopaminergic transmission in the NTS could increase D$_3$ receptor facilitation of the HVR.

In these experiments we measured acute HVR, hypercapnic ventilatory response (HCVR), and ventilatory acclimatization to hypoxia in D$_3$ receptor-deficient [D$_3$-(-/-)] and wild-type [D$_3$-(+/+)] mice. Using the D$_3$-(-/-) mice allowed us to characterize acute ventilatory responses and ventilatory acclimatization to hypoxia in the absence of D$_3$ receptors and without the complications of pharmacological blockade. This study was designed to test two hypotheses: 1) acute HVR and HCVR in D$_3$-(-/-) mice are different from those in D$_3$-(+/+) mice because of the involvement of D$_3$ recep-

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tors in the arterial chemoreflex pathway, and 2) the
time course of ventilatory acclimatization in \( \text{D}_2(+/−) \) mice is different from that in \( \text{D}_2(+/+) \) mice because of
time-dependent changes in \( \text{D}_2 \) receptor modulation of the HVR with chronic hypoxia.

**METHODS**

**Ventilatory Responses to Acute Hypoxia and/or Hypercapnia**

Ventilatory responses were quantified in restrained, awake, adult (16- to 26-wk-old) male and female \( \text{D}_2(+/+) \) (8 males and 8 females) and \( \text{D}_2(−/−) \) mice (7 males and 8 females, with 7 females exposed to hypoxic hypercapnia) with use of a head-out, dual-chamber plethysmograph (Buxco). The \( \text{D}_2(+/+) \) and \( \text{D}_2(−/−) \) mice were age-matched siblings derived from the breeding of \( \text{D}_2(+/−) \) parents and have been described previously (20). The mutant allele had been backcrossed onto the C57BL/6J genetic background for five successive generations.

**Protocol.** Male and female \( \text{D}_2(+/+) \) and \( \text{D}_2(−/−) \) mice were tested under the following conditions: 1) hypoxia (8% \( \text{O}_2 \), 2) hypercapnia (10% \( \text{CO}_2 \) and 21% \( \text{O}_2 \)), and 3) hypoxic hypercapnia (10% \( \text{CO}_2 \) and 8% \( \text{O}_2 \)). At the start of an experiment, a mouse was removed from its cage, weighed, and then placed into the plethysmograph. The mouse was allowed to acclimatize to the plethysmograph for 30 min while it breathed room air before exposure to each experimental gas mixture in a randomized order. Between experimental conditions, ventilation was allowed to return to baseline values during a 15- to 30-min washout period. BioSystem XA software (Buxco) was used to acquire tidal volume (\( V_t \)), respiratory frequency (\( f \)), and minute ventilation (\( V_{\text{I}} \)) every 30 s during the 5-min exposure to hypoxia or hypercapnia and the 10-min exposure to hypoxic hypercapnia.

**Data analysis.** To determine the effects of gender, genotype, and time-dependent ventilatory responses, a two-between-factor (gender and genotype), one-within-factor (time) ANOVA (Statview version 5.0.1) was used to determine significant differences in ventilatory responses to hypoxia, hypercapnia, or hypoxic hypercapnia. A two-way ANOVA was also used to determine significant effects of gender and genotype on steady-state ventilatory responses after 5 min of exposure to a given gas mixture. Significance was set at \( P < 0.05 \). Values are means ± SE.

**Ventilatory Responses to Chronic Hypoxia**

Acute ventilatory responses were measured in awake, unrestrained, adult (36- to 40-wk-old) male mice. \( \text{D}_2(+/+) \) and \( \text{D}_2(−/−) \) (\( n = 10 \)) mice were studied before (day 0) and after 2 and 8 days of exposure to normobaric hypoxia (inspiratory \( \text{Po}_2(\text{B}_{\text{O}_2}) = 70 \) Torr). The acclimatization chamber consisted of a clear plastic box that continuously received a hypoxic gas mixture at flow rates sufficient to prevent \( \text{CO}_2 \) buildup (fraction of inspired \( \text{CO}_2 < 0.005 \)). Food and water were provided ad libitum.

A small-volume (5-cm-diameter, 10-cm-long) flow-through plethysmograph was used for simultaneous measurements of \( \text{O}_2 \) uptake (\( V_{\text{O}_2} \)) and ventilation. Side ports of the metabolic chamber were connected to opposite sides of a differential pressure transducer (model DP103-18, Validyne). The reference chamber isolated the ventilation signal from ambient thermal and pressure noise. Total resistance in each circuit and between the chambers was balanced with flow resistors. The pressure transducer signal was demodulated (model CD-15, Validyne), sent to a chart recorder (model 2400S, Gould) for amplification and analog low-pass filtering, and then sampled at 250 Hz for real-time monitoring and data storage by computer (Apple Quadra 700 with PowerPC 601 CPU; National Instruments Lab-NB A/D board and Labview software).

\( V_{\text{r}} \) values were determined from the differential pressure data according to the principles described by Drorbaugh and Fenn (6) and adapted to a flow-through system by Jacky (19). Humidity data were acquired with a humidity sensor (model HH-3602, Hy-Cal Engineering, El Monte, CA) fitted just downstream of the chamber. To calibrate \( V_{\text{r}} \), 0.1-ml pulses from a gas-tight syringe attached to a port on top of the chamber were injected into the plethysmograph at a rate similar to \( f \). The chamber-ambient pressure differential was measured with a water manometer and ranged from 6 to 18 cm\( \text{H}_2\text{O} \).

**Metabolic measurements.** We also measured \( V_{\text{O}_2} \) by using dried and \( \text{CO}_2 \)-scrubbed gas (Drierite and soda lime, respectively) to provide a determination of \( V_{\text{O}_2} \) independent of the respiratory quotient (\( V_{\text{O}_2} \text{/} V_{\text{I}} \)). A differential \( \text{O}_2 \) analyzer (models S-A3II and N-37M sensor, Applied Electrochemistry) determined the change in \( \text{O}_2 \) fraction by comparing the excurrent metabolic chamber \( \text{O}_2 \) fraction with that measured in the reference chamber. Metabolic chamber flow (600–650 ml/min) was measured with a Fleisch pneumotachograph calibrated against a glass float flowmeter (Cole-Parmer Instrument, ±2% accuracy). The signal output from the \( \text{O}_2 \) analyzer was acquired simultaneously with the ventilation signal onto a computer. After analog low-pass filtration to eliminate noise spikes, the signal was sampled at 250 Hz.

**Protocols.** Ventilatory responses and \( V_{\text{O}_2} \) were measured before (day 0) and after 2 and 8 days of exposure to chronic hypoxia. At the start of an experiment, a mouse was removed from its cage, weighed, and then placed into the plethysmograph. The chamber setup was then immersed in a water bath controlled at 30°C. If a mouse became noticeably agitated in the chamber, it was removed and the experiment was ended.

The mouse was allowed to acclimatize to the chamber at its chronic \( \text{Po}_2 \) for 1 h before data collection. The poikilocapnic HVR was determined using three to four different \( \text{Pt}_2 \) levels (57–214 Torr). We used 214 Torr \( \text{Pt}_2 \) (sea level fraction of \( \text{O}_2 \) = 0.30) as our normoxic level to minimize afferent input from arterial chemoreceptors. Pilot measurements of \( V_t \) in three mice showed that \( V_t \) decreased from 1,758 ± 85 to 1,472 ± 33 ml·min⁻¹·kg⁻¹ when \( \text{Po}_2 \) increased from 150 to 214 Torr. This is similar to results reported for rats (1) and suggests that 30% \( \text{O}_2 \) breathing at sea level can minimize ventilatory drive from \( \text{O}_2 \)-sensitive arterial chemoreceptors. \( V_t \) was measured 2 min after a change in \( \text{Pt}_2 \) and again 10–15 min after a change, before the animal was returned to its chronic \( \text{Pt}_2 \) level for 15 min between tests. \( V_{\text{O}_2} \) was measured continuously during the protocol, but the values reported here correspond to the ventilatory periods selected for analysis.

**Data analysis.** Data were analyzed for \( V_t \), \( V_{\text{r}} \), and \( f \) with Labview software. \( V_{\text{O}_2} \) values were measured during the ventilatory periods selected for analysis. A two-way ANOVA was done at each \( \text{Pt}_2 \) to determine significant ventilatory responses and interactions between the different durations of acclimatization to hypoxia (0, 2, and 8 days) and genotype (\( \text{D}_2(+/+) \) and \( \text{D}_2(−/−) \)). \( F \) tests for simple effects were used when the ANOVA yielded a significant interaction of day and genotype. Paired \( t \)-tests were used to compare \( V_t \), \( V_{\text{r}} \), \( f \), and \( V_{\text{O}_2} \) after 2 and 15 min of hypoxia to test for hypoxic “roll-off.”
RESULTS

Ventilatory Responses to Acute Hypoxia and Hypercapnia

The average weight for the male D\textsubscript{2}(\(+\)/\(+\)) mice was significantly lower than that for male D\textsubscript{2}(\(+\)/\(-\)) mice (29.5 ± 0.7 and 35.5 ± 0.8 g, respectively). In contrast, the average weight was not significantly different between female D\textsubscript{2}(\(+\)/\(+\)) and D\textsubscript{2}(\(+\)/\(-\)) mice (22.3 ± 1.4 and 24.0 ± 1.1 g, respectively).

Effects of gender. The effects of gender, independent of genotype, were studied by comparing ventilatory responses in male and female D\textsubscript{2}(\(+\)/\(+\)) mice. There was no significant effect of gender on weight-normalized V\textsubscript{I} in the mice breathing room air. V\textsubscript{I} in room air for D\textsubscript{2}(\(+\)/\(+\)) mice was 2,629 ± 135 and 2,549 ± 59 ml\textperiodcentered min\textperiodcentered kg\textperiodcentered 2 for male and female mice, respectively. Similarly, ventilatory responses to acute hypoxia were not affected by gender (Fig. 1). In addition, the time-dependent decline in V\textsubscript{I} during sustained hypoxia was similar between genders in D\textsubscript{2}(\(+\)/\(+\)) mice.

In contrast to room air breathing and hypoxia, HCVR were significantly affected by gender in the D\textsubscript{2}(\(+\)/\(+\)) mice (Fig. 2). HCVR were significantly greater in female than in male D\textsubscript{2}(\(+\)/\(+\)) mice; V\textsubscript{I} after 5 min of 10\% CO\textsubscript{2} inhalation was 4,843 ± 374 and 3,583 ± 225 ml\textperiodcentered min\textperiodcentered kg\textperiodcentered 1, respectively. This greater HCVR was primarily due to a larger V\textsubscript{T} in the female D\textsubscript{2}(\(+\)/\(+\)) mice (15.4 ± 1.4 vs. 11.6 ± 0.9 ml/kg at 5 min for females and males, respectively), inasmuch as ƒ was similar (322 ± 10 and 313 ± 10 min\textsuperscript{-1} at 5 min for females and males, respectively). The significant gender effect on HCVR was not evident when the D\textsubscript{2}(\(+\)/\(+\)) mice were exposed to hypoxia and hypercapnia (Fig. 3).

Effects of D\textsubscript{2} receptor genotype and gender. There were no significant effects of genotype on V\textsubscript{I} in mice breathing room air. V\textsubscript{I} in the male and female D\textsubscript{2}(\(+\)/\(+\)) mice were similar to V\textsubscript{I} reported above for the D\textsubscript{2}(\(+\)/\(+\)) mice [2,896 ± 83 and 2,674 ± 59 ml\textperiodcentered min\textperiodcentered kg\textperiodcentered 2 for male and female D\textsubscript{2}(\(+\)/\(+\)) mice, respectively]. In male and female mice there was a significant HVR (8\% O\textsubscript{2}) that was independent of gender but significantly affected by genotype (Fig. 1). The significant V\textsubscript{I} response to hypoxia was the result of significant VT and ƒ responses; however, only ƒ was significantly affected by gender and genotype. Although the acute HVR was not significantly affected by gender alone, there was a significant interaction of gender, genotype, and the time-dependent ventilatory response to hypoxia. For example, in the female mice, peak V\textsubscript{I} in hypoxia was different between genotypes, occurring after 30 and 60 s for the D\textsubscript{2}(\(+\)/\(+\)) and D\textsubscript{2}(\(+\)/\(-\)) mice, respectively (3,344 ± 118 and 3,603 ± 203 ml\textperiodcentered min\textperiodcentered kg\textperiodcentered 1, respectively). In contrast, in the male mice, peak V\textsubscript{I} in hypoxia occurred after 30 s for both genotypes (3,144 ± 259 and 3,532 ± 224 ml\textperiodcentered min\textperiodcentered kg\textperiodcentered 1 for D\textsubscript{2}(\(+\)/\(+\)) and

Fig. 1. Time course of ventilation (V\textsubscript{I}), frequency (ƒ), and tidal volume (V\textsubscript{T}) responses to 5 min of hypoxia (8\% O\textsubscript{2}) in male and female D\textsubscript{2}(\(+\)/\(+\)) (●) and D\textsubscript{2}(\(+\)/\(-\)) (○) mice. Hypoxic responses were not affected by gender alone but were significantly greater in female D\textsubscript{2}(\(+\)/\(-\)) than in female D\textsubscript{2}(\(+\)/\(+\)) mice. All groups exhibited ventilatory decline with sustained hypoxia. *Significant difference between genotypes at 5 min.
D2(-/-) mice, respectively]. After peak responses to hypoxia, all groups of mice exhibited ventilatory decline during sustained hypoxia or roll-off (Fig. 1). However, at the end of the 5-min exposure to hypoxia, V̇I was significantly different between genotypes in the female mice only.

Exposure to 5 min of hypercapnia significantly increased V̇I in male and female mice, and the responses were significantly affected by genotype and gender (Fig. 2). The significant V̇I responses to hypercapnia were the result of significant increases in f and VT. Gender and genotype significantly affected VT responses, whereas f was significantly affected by genotype only. There were also significant interactions between the time-dependent V̇I responses to hypercapnia and both genotype and gender. The time-dependent change in V̇I with hypercapnia was a slow “on” response, making ventilation stable after 60 s, in contrast to roll-off with hypoxia. Steady-state V̇I at the end of the 5-min hypercapnic exposure was significantly greater in both groups of female mice than in the male mice. However, genotype significantly affected hypercapnic V̇I in male mice only, inasmuch as it was significantly greater in D2(-/-) than in D2(+/+) mice. This was the result of a significantly greater VT in male D2(-/-) mice coupled with a nonsignificantly higher f.

The significant V̇I response to hypoxic hypercapnia (10% CO₂ and 8% O₂) in male and female mice was also dependent on gender and genotype (Fig. 3). Hypoxic hypercapnia significantly increased VT and f. Similar to hypercapnia alone, VT responses were significantly affected by gender and genotype, whereas f responses were significantly affected by genotype only. In addition, there were significant interactions between time-dependent changes in V̇I during exposure to hypoxic hypercapnia and both gender and genotype. The time-dependent changes with hypoxic hypercapnia were similar to the changes with hypercapnia alone, i.e., “roll-on,” not roll-off. Steady-state V̇I responses after 5 min of hypoxic hypercapnia were greater in D2(-/-) male and female mice than in D2(+/+) mice; however, the difference was only significant in female mice.

**Ventilatory Responses to Chronic Hypoxia**

Similar to the younger male mice used to study acute ventilatory responses, the average weight was significantly lower for male D2(-/-) mice than for D2(+/+) mice (31.2 ± 1.1 and 38.1 ± 1.4 g, respectively, n = 10/group). However, VO₂ normalized to body weight was not significantly different between the groups in normoxia or hypoxia (Table 1). Acute hypoxia produced a significant hypometabolism, independent of genotype, on all days.

Similar to the restrained mice studied during acute hypoxia, ventilatory decline was observed during sustained acute hypoxia in these unrestrained male mice. Between 2 and 15 min of exposure to hypoxia, there
was a significant decrease in V\textsuperscript{˙}I from 3,120 ± 280 to 2,741 ± 203 ml \cdot min\textsuperscript{−1} \cdot kg\textsuperscript{−1} in the D\textsubscript{2}(+/+) mice. This ventilatory decline was due to a significant decrease in V\text{r} and was accompanied by a significant decrease in V\textsuperscript{˙}O\textsubscript{2}. There was also a nonsignificant decrease in hypoxic V\textsuperscript{˙}I in the D\textsubscript{2}(-/-) mice, from 3,427 ± 163 to 3,121 ± 195 ml \cdot min\textsuperscript{−1} \cdot kg\textsuperscript{−1} (P = 0.06). In contrast to the D\textsubscript{2}(+/+) mice, ventilatory decline was due to a significant decrease in f between 2 and 15 min in the D\textsubscript{2}(-/-) mice. Similar to the D\textsubscript{2}(+/+) mice, the D\textsubscript{2}(-/-) mice also significantly decreased their V\textsuperscript{˙}O\textsubscript{2} between 2 and 15 min of hypoxia. All data reported below are measured at 10–15 min of acute hypoxia, i.e., after the decline has occurred.

Table 1. Effects of acute hypoxia on V\textsuperscript{˙}O\textsubscript{2} in D\textsubscript{2}(+/+) and D\textsubscript{2}(-/-) mice after 0, 2, and 8 days of continuous hypoxia

<table>
<thead>
<tr>
<th>Day</th>
<th>30% O\textsubscript{2}</th>
<th>10% O\textsubscript{2}</th>
<th>30% O\textsubscript{2}</th>
<th>10% O\textsubscript{2}</th>
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<tbody>
<tr>
<td>0</td>
<td>41.7 ± 5.0</td>
<td>31.1 ± 2.0*</td>
<td>35.3 ± 2.2</td>
<td>31.8 ± 1.8*</td>
</tr>
<tr>
<td>2</td>
<td>41.9 ± 4.3*</td>
<td>32.0 ± 1.7*</td>
<td>42.3 ± 1.9</td>
<td>29.1 ± 2.1*</td>
</tr>
<tr>
<td>8</td>
<td>42.1 ± 3.4*</td>
<td>32.8 ± 1.4*</td>
<td>39.2 ± 4.0</td>
<td>30.5 ± 2.5*</td>
</tr>
</tbody>
</table>

Values are means ± SE in ml \cdot min\textsuperscript{−1} \cdot kg\textsuperscript{−1}; n = 10 on days 0 and 2, n = 7 on day 8. V\text{O}\textsubscript{2}, O\textsubscript{2} consumption; D\textsubscript{2}(-/-) and D\textsubscript{2}(+/+), dopamine D\textsubscript{2} receptor-deficient and wild-type mice, respectively. *Significantly less than corresponding normoxic value within genotype (P < 0.05).

On all days, V\textsuperscript{˙}I significantly increased with decreasing P\textsubscript{O\textsubscript{2}} in both groups (Fig. 4). However, the HVR were significantly different between D\textsubscript{2}(+/+) and D\textsubscript{2}(-/-) mice. Under control conditions (day 0), V\textsuperscript{˙}I was similar in both genotypes, except at the lowest level of hypoxia, where V\textsuperscript{˙}I was significantly greater in the D\textsubscript{2}(+/+) mice. With longer hypoxic exposures, V\textsuperscript{˙}I became more different between D\textsubscript{2}(+/+) and D\textsubscript{2}(-/-) mice, as V\textsuperscript{˙}I increased more in the D\textsubscript{2}(+/+) mice. The differences were most pronounced on day 8, such that V\textsuperscript{˙}I was significantly lower in the D\textsubscript{2}(-/-) mice at all P\textsubscript{O\textsubscript{2}} levels. Differences in V\textsuperscript{˙}I between D\textsubscript{2}(+/+) and D\textsubscript{2}(-/-) mice on days 0 and 2 were due to significant differences in f, whereas the differences on day 8 were due to significant differences in f and V\text{t}.

With chronic hypoxia, D\textsubscript{2}(+/+) mice exhibited a progressive and significant increase in V\textsuperscript{˙}I at high P\textsubscript{O\textsubscript{2}} (P\textsubscript{O\textsubscript{2}} = 214 Torr) from days 0 to 2 and from days 2 to 8, whereas it was unchanged in D\textsubscript{2}(-/-) mice (Fig. 4). This indicates that “baseline” ventilatory drive (i.e., without significant hypoxic ventilatory drive) is changing differently between genotypes. The time-dependent change in baseline V\textsuperscript{˙}I from 0 to 8 days of hypoxia was significantly different between genotypes. The differences in baseline V\textsuperscript{˙}I from days 0 to 2 between genotypes were due to significant differences in f, whereas differences from days 2 to 8 were the result of significant differences in V\text{t}.
There were also significant differences in hypoxic (P_{O_2} = 70 Torr) ventilatory drive during acclimatization between D_{2}(-/-) and D_{2}(+/-) mice (Fig. 4). Hypoxic V\_\text{I} decreased in both groups from 0 to 2 days; however, the decrease was significant only in the D_{2}(-/-) group because of a significant decrease in f. Both genotypes showed a similar increase in hypoxic V\_\text{I} between days 2 and 8. In the D_{2}(+/-) mice, the increase in hypoxic V\_\text{I} from 2 to 8 days resulted in a significantly greater hypoxic V\_\text{I} after 8 days than in control. However, hypoxic V\_\text{I} after 8 days in D_{2}(-/-) mice remained below control levels, because hypoxic V\_\text{I} after 2 days was so low compared with control. The higher hypoxic V\_\text{I} on day 8 in the D_{2}(+/-) mice was due to a greater V\_\text{T} than in D_{2}(-/-) mice with a similar f.

**DISCUSSION**

These findings suggest that D\_\text{2} receptors are involved in acute ventilatory responses to hypoxia and/or hypercapnia and ventilatory acclimatization to hypoxia. The contribution of D\_\text{2} receptors to acute hypoxic, hypercapnic, and hypoxic-hypercapnic ventilatory responses is dependent on gender. Furthermore, data from male and female mice strongly support a net inhibitory effect of D\_\text{2} receptors on acute HVR and HCVR. Although not always significant, ventilation was higher with all acute ventilatory challenges in the D_{2}(-/-) mice. However, chronic hypoxia removed this net inhibitory effect of D\_\text{2} receptors on ventilation, and the HVR were lower in D_{2}(-/-) mice after 2 and 8 days of exposure to hypoxia.

**Acute HVR and HCVR in Mice**

Effector of experimental methods to measure V\_\text{I}. Differences in the methods used to measure ventilation may explain differences in the magnitude of the HVR between the two groups of transgenic mice we studied. Restraint has been shown to significantly increase absolute values of f and V\_\text{I}, but not V\_\text{T}, in adult female outbred mice, although increases in V\_\text{I} with hypoxia or hypercapnia were not affected (3). The first set of transgenic mice we used to measure acute ventilatory responses were restrained in a “head-out” plethysmograph, and this potential stress may explain why f and V\_\text{I} in normoxia were greater in this group than in the chronically hypoxic group studied with whole body plethysmography. The maximum levels of ventilation we observed were similar in both groups of animals we studied, so the change in ventilation with chemoreceptor stimulation (i.e., the HVR and HCVR) was larger in our unrestrained mice. This contrasts with results showing no significant difference between the HVR and HCVR in restrained and unrestrained mice (3). We speculate that the effect of restraint may have been larger on our first group of mice in baseline conditions, thereby decreasing the absolute HVR.

Male and female restrained mice exhibited ventilatory decline during the 5-min exposure to 8% O\_\text{2} independent of genotype (Fig. 1). The experimental system used to measure ventilation in unrestrained male mice was not suitable to measure the time course of ventilatory changes with the same resolution obtained for the restrained mice because of the time necessary to change gas mixtures in the whole body chamber. However, in the unrestrained male mice, we observed a significant decrease in V\_\text{I} and VO_{2} between 2 and 15 min of exposure to 10% O\_\text{2}. Because VO_{2} was not measured in the restrained mice, it is unknown whether the ventilatory decline observed in only 5 min of hypoxia was also accompanied by a fall in metabolic rate. Also, because isocapnia was not maintained in the restrained or unrestrained mice, the ventilatory decline may have resulted from decreased CO_{2} stimulation as Pa_{CO_2} was decreased during hypoxic hyperventilation. Thus, we cannot use these data to determine whether mice exhibit hypoxic ventilatory decline comparable to that described in humans and other mammals (29). Hypoxic ventilatory decline has been defined as a decline in ventilation during the first several
minutes of hypoxia that can occur independent of decreases in metabolism or $P_{a\text{CO}_2}$.

Effects of gender. In this study, gender did not significantly affect ventilation in our transgenic mice breathing room air or the peak acute HVR (Fig. 1). In contrast, acute HCVR were significantly greater in female than in male $D_2$-$(+/+)$ mice (Fig. 2). These data agree, in part, with a previous study measuring ventilation in aged (32- to 44-wk-old) male and female 129/Sv mice (27). In this study, ventilation in room air and hypoxia was similar in male and female mice. In the 129/Sv mice, $V_t$ in hypoxia was not greater than $V_t$ in room air. However, this may be due to the fact that hypoxic $V_t$ was reported at 5 min of hypoxia, after roll-off may have occurred. In this previous study, HCVR were greater in female mice; however, the difference was not statistically significant. Our hypocapnic results, taken together with the previous results showing a trend for greater responses to hypocapnia in female mice, agree with studies in humans and rats showing that female hormones (progestin and estrogen) increase ventilatory responses to $CO_2$ (21, 35).

Effects of $D_2$ receptor genotype. $D_2$ receptor genotype significantly affected acute hypoxic, hypocapnic, and hypocapnic responses, as well as ventilatory acclimatization to hypoxia (Figs. 1–4). The acute ventilatory responses strongly suggest a net inhibitory effect of $D_2$ receptors on acute HVR or HCVR, inasmuch as ventilation was consistently higher in $D_2$-$(−−)$ than in $D_2$-$(+/+)$ mice under these conditions. Furthermore, the effects of $D_2$ receptor genotype on acute ventilatory responses were gender dependent. Genotype significantly affected HVR in female mice only. In contrast, HCVR were significantly affected by genotype in male mice only. This suggests that the lower HCVR may be due to a $D_2$ receptor-dependent effect in males instead of (or in addition to) a lack of facilitation by female hormones. The significant interaction between genotype and ventilation during exposure to hypocapnic hypoxia in male and female mice may reflect the independent effects of $D_2$ receptor involvement in HVR and HCVR in female and male mice, respectively. The differences in HVR and HCVR seen between male and female mice may be due to hormonal differences (34).

Despite differences and age and experimental techniques, the effects of $D_2$ receptor genotype on normoxic and hypoxic ventilation are similar in the two groups of male mice studied. The acute responses to 8% $O_2$ in restrained male mice and the acute responses to 10% $O_2$ ($day 0$) in unrestrained male mice before acclimatization to hypoxia are in agreement, in that $D_2$ receptors do not have large effects on acute HVR in male mice. However, in both groups studied, $V_t$ was higher in $D_2$-$(−−)$ than in $D_2$-$(+/+)$ mice at all $O_2$ levels before exposure to chronic hypoxia (Figs. 1 and 4).

Ventilation in 10% inspired $CO_2$ was higher in male and female $D_2$-$(−−)$ mice than in the corresponding $D_2$-$(+/+)$ mice (Fig. 2). The significantly greater HCVR in the male $D_2$-$(−−)$ mice contrasts with a previous study in which $D_2$ receptor antagonists were used (26).

In male QS mice, total body $D_2$ receptor blockade with droperidol significantly decreased ventilation in 7.5% inspired $CO_2$. In addition, stimulation of CNS dopamine receptors by simultaneous administration of L-dopa and carbidopa significantly increased ventilation in 7.5% inspired $CO_2$ compared with administration of carbidopa alone (25). The differences between these previous studies and the present results may be due to side effects of droperidol, which could depress HCVR, independent of $D_2$ receptors. Additional possibilities are differences in HCVR among different strains of mice that have been previously documented (33); similar responses to hypocapnia (8% $CO_2$ and 21% $O_2$) and hypoxic hypocapnia (8% $CO_2$ and 10% $O_2$) were reported in C57 and 129 mouse strains; however, QS mice were not studied.

Ventilatory Acclimatization to Chronic Hypoxia in Mice

This is the first study to quantify the time course of changes in the HVR during acclimatization to hypoxia in mice. We measured poikilocapnic HVR in $D_2$-$(+/+)$ and $D_2$-$(−−)$ mice after 0, 2, and 8 days of exposure to 70 Torr $P_{a\text{O}_2}$ (Fig. 4). Olson and Saunders (26) investigated acclimatization to hypoxia in male QS mice, but normoxic $V_t$, $f$, and $V_t$ were not reported. Because hyperventilation in normoxia, in addition to increased ventilation during hypoxic stimulation, is considered an important component of ventilatory acclimatization to hypoxia (9), changes in normoxic ventilation are integral in characterizing the time course of acclimatization in mice. Our results demonstrate that the time course of ventilatory acclimatization to hypoxia in transgenic wild-type mice is similar to that previously reported in rats (23). In rats exposed to 14 days of hypobaric hypoxia, significant ventilatory acclimatization occurred within 4 days but was not complete until 1 wk (23). In another study on rats, normoxic $V_t$ increased up to 2 days of hypoxia and remained at that level through 8 days of hypoxia (28). In contrast, other animal species acclimatize to hypoxia more rapidly than rats or mice. Specifically, goats acclimatize to 4,300 m in 4–6 h (7, 32), whereas significant acclimatization to hypoxia is apparent in cats after 2 days (38).

Effects of $D_2$ receptors on ventilatory acclimatization to hypoxia. Our results in the $D_2$-$(−−)$ mice are consistent with a previous study investigating the acute effects of whole body $D_2$ receptor blockade with droperidol during acclimatization to hypoxia in QS mice (26). Systemic $D_2$ receptor blockade demonstrates the net effects of $D_2$ receptors on the HVR. The ventilatory responses of $D_2$-$(−−)$ mice compared with those of $D_2$-$(+/+)$ mice are similar to the effects of acute droperidol administration during chronic hypoxia. Before exposure to chronic hypoxia, droperidol significantly increased $V_t$ in mice acutely breathing 70 Torr $P_{a\text{O}_2}$, suggesting that the carotid body $D_2$ receptor effect predominates in control mice because $D_2$ receptors in the carotid body depress $O_2$ sensitivity and the HVR (13, 16, 18, 28, 36). Similarly, $V_t$ was higher in unac-
climatized $D_2(-/-)$ mice than in unacclimatized $D_2(+/+)$ mice at all $P_{O_2}$ levels, with the largest difference at 70 Torr $P_{O_2}$ (Fig. 4). After 2, 4, and 8 days of hypoxia, acute droperidol significantly decreased $V_t$ in the QS mice (26). Similarly, $V_t$ was greater in the $D_2(-/+)$ than in the $D_2(-/-)$ mice at all $P_{O_2}$ levels after 2 and 8 days of hypoxia (Fig. 4). This suggests a predominantly CNS effect in mice after exposure to chronic hypoxia, because $D_2$ receptors facilitate the HVR in rats and cats (14, 16, 25, 28, 31). The lack of significant increase in baseline ventilation ($P_{O_2} = 214$ Torr) in $D_2(-/-)$ mice over 8 days of hypoxia suggests that $D_2$ receptors are critical for components of ventilatory acclimatization other than changes in the HVR. The difference in baseline ventilation after chronic hypoxia between genotypes may involve the effects of $D_2$ receptors on ventilatory sensitivity to $CO_2$, as suggested by differences in the acute HCVR (Fig. 2).

Critique of Experimental Design and Methods

Arterial chemoreceptor stimuli. One limitation of the present study is that we were unable to obtain arterial blood gases and, therefore, measure the true arterial chemoreceptor stimuli. However, after 2 and 8 days of hypoxia, $V_t$ was lower in the $D_2(-/-)$ than in the $D_2(-/+)$ mice, and this would decrease arterial $P_{O_2}$ ($P_{O_2}$) at a given $P_{O_2}$, so the HVR would be even lower in the $D_2(-/-)$ mice if calculated as a function of $P_{O_2}$. Furthermore, $V_t$ was lower at 8 days in the $D_2(-/-)$ mice, so dead space would be greater, which would increase $P_{CO_2}$ with the assumption of similar $CO_2$ production. $V_o$ was similar (Table 1), and we would not expect respiratory exchange ratios to be different between groups. Consequently, the predicted changes in $P_{O_2}$ and $P_{CO_2}$ in $D_2(-/-)$ mice would stimulate $V_t$ and cannot account for the lower $V_t$ at a given $P_{O_2}$ with acclimatization compared with the $D_2(-/+)$ mice.

Transgenic model of $D_2$ receptor deficiency. Although genetically manipulated mice provide the opportunity to study physiological adaptations in the absence of the gene of interest, several limitations in this model must be considered. One limitation of the knockout methodology is that the genetic mutation is generally on a hybrid background. The genetic and behavioral differences between inbred mouse strains present some problems in the interpretation of mutant phenotypes as well as in the choice of appropriate control mice. The mice in this study are of a mixed 129 and C57BL/6 background (20). Further studies are necessary to determine whether the wild-type mice must be used for control studies or whether the phenotype is the same as one of the original strains.

In addition, results may be complicated by compensatory mechanisms during development, for example, in other dopamine receptor subtypes. However, the results show that even if these secondary effects occur, they are insufficient to completely overcome the direct effects of gene ablation. $D_2(-/-)$ mice lacked a normal ventilatory response to chronic hypoxia, despite a normal control HVR. It is also possible that the difference in ventilatory acclimatization in $D_2(-/-)$ mice does not involve $D_2$ receptors during exposure to chronic hypoxia but, rather, results from another $D_2$ receptor-dependent mechanism during development. However, our results are consistent with previous results with acute administration of a dopamine antagonist during acclimatization (26).

Conclusions

These results demonstrate gender-specific involvement of $D_2$ receptors in acute HVR and/or HCVR. Specifically, these results suggest a net inhibitory effect of $D_2$ receptors on the acute HVR and HCVR. In addition, the absence of the $D_2$ receptor gene prevented normal ventilatory acclimatization to hypoxia in male mice, despite a normal control HVR and metabolic rate compared with $D_2(-/+)$ mice. Results with knockouts are most clearly interpreted in experimental paradigms that take place over days to weeks, since the results can be interpreted within the context of complete and continuous absence of receptor function (e.g., acclimatization, addiction, and sensitization). Consequently, $D_2(-/-)$ mice provide a good model to study ventilatory acclimatization to hypoxia, which occurs over days to weeks, when continuous (rather than acute) $D_2$ receptor inactivation may be advantageous. Future studies with inducible or region-specific knockouts will further our understanding of the role of $D_2$ receptors in ventilatory acclimatization to hypoxia.

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