PDGF-β receptor expression and ventilatory acclimatization to hypoxia in the rat

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The acute ventilatory response to hypoxia in adult mammalian species is biphasic. After an initial ventilatory enhancement, there is a subsequent decrease in ventilation to levels that, although exceeding those during normoxia, are lower than the peak early ventilatory increase (47). However, sustained exposure to environmental hypoxia will lead to a time-dependent increase in ventilation, which has been termed ventilatory acclimatization to hypoxia (VAH) and occurs in humans, goats, ponies, and also in rats (10, 14, 17, 30, 32, 40, 47). Although increased sensitivity of arterial chemoreceptors has been shown to contribute to VAH (5, 6, 16, 41, 54, 59), it has become increasingly clear that central mechanisms are also operative in this process (13, 50, 60) and may contribute to the increased ventilatory output that is readily measurable when normoxic or isocapnic hypoxic mixtures are breathed (1, 9). Indeed, the process of adaptation to hypoxia that results in VAH appears to involve both ends of the primary synaptic pathways underlying the ventilatory response, such that development of increased sensitivity of the peripheral chemoreceptors occurs in parallel with integration of afferent input and amplification of centrally generated efferent output (13).

In a previous study, we found that the early phase of the acute hypoxic ventilatory response is attenuated by platelet-derived growth factor BB (PDGF-BB) but not by PDGF-AA in the rat dorsocaudal brain stem (19), such that this attenuation is mediated by activation of PDGF-β receptors within the nucleus of the solitary tract (nTS) (19). Furthermore, the second or late phase of the biphasic hypoxic ventilatory response was dependent on the presence and activation of PDGF-β receptors, such that pharmacological inhibition of PDGF-β receptors in the rat resulted in significant attenuation of the hypoxic ventilatory depression (19). Similarly, diminished expression of PDGF-β receptors in transgenic mice was associated with almost complete abolition of the typical ventilatory decline that characteristically occurs with ongoing hypoxia (19). Additional examination revealed that both PDGF-B chain and PDGF-β receptors, but not PDGF-α receptors, were abundantly expressed in a large proportion of nTS neurons undergoing functional recruitment (as evidenced by c-Fos induction) during hypoxia, and that
acute hypoxia induced PDGF-B chain mRNA enhancements (19). On the basis of these data, we hypothesized that VAH could be mediated at least in part by temporal changes in PDGF-β receptor function, such that reciprocal relationships would emerge between the magnitude of VAH and the expression of PDGF-β receptor within the nTS.

METHODS

Animals

The experimental protocols were approved by the Institutional Animal Use and Care Committee and are in close agreement with the National Research Council publication, Guide for the Care and Use of Laboratory Animals. All efforts were made to minimize animal suffering, to reduce the number of animals used, and to utilize alternatives to in vivo techniques. Survival experiments were performed under pentobarbital sodium anesthesia (50 mg/kg ip) on 38 male Sprague-Dawley young adult rats (200–225 g) as previously described (37). In one set of experiments (group I), a small diameter hole was drilled into the occipital skull, and a small cannula (22G; Plastics One, Roanoke, VA) was surgically implanted at, or in close proximity to, the nTS according to standard stereotaxic coordinates (−13.85 mm bregma, 0.2 mm off midline, 8.0 mm depth) (44). The cannula was then connected via PE-50 tubing to preloaded osmotic pumps (model 1002; Alza, Palo Alto, CA) preset to deliver the designated solution at 0.25 µl/h, consisting of either vehicle (Veh) or PDGF-BB (80 nM). After surgery, animals were allowed to recover for 24 h as demonstrated by return to spontaneous feeding and drinking patterns.

In a second set of experiments (group II), a small cannula was placed as in group I and occluded with a guide obturater. Rats were then allowed to recover for 24 h, after which 100 nl of L-glutamate solution (L-Glu; 1 M) were microinjected with the animal in a barometric chamber to verify that the anticipated ventilatory enhancements indeed occurred and thus confirm adequate position of the cannula. In addition, 100-nl nTS microinjections of Veh and PDGF-BB (80 nM) were carried out 2 h apart into the nTS after collection of baseline ventilatory monitoring and maintained at <1,500 ppm by appropriate adjustments in the overall chamber ventilation. Humidity was maintained at 40–50%, while ambient temperature was kept at 22–24°C. On days 0, 7, and 14 of hypoxia, 100-nl microinjections of Veh and PDGF-BB (80 nM) were performed 2 h apart into the nTS after collection of baseline ventilatory measures in room air for 15 min. Fifteen to twenty minutes after each microinjection, 20-min hypoxic challenges (10% O2 balanced in N2) were performed, after which animals were returned to the chamber or euthanized with a pentobarbital overdose for assessment of cannula location. In addition, on days 0, 1, 7, and 14 of chronic hypoxia, L-Glu microinjections were also performed, and ventilatory responses were measured as described in the next section.

Ventilatory and Cardiovascular Recordings

Cardiorespiratory measures were continuously acquired in the freely behaving animals by use of the barometric method (Buxco Electronics, Troy, NY) (2, 43). To minimize the long-term effect of signal drift due to temperature and pressure changes outside the chamber, a reference chamber of equal size in which temperature was measured using a T-type thermocouple was used. In addition, a correction factor was incorporated into the software routine to account for inspiratory and expiratory barometric asymmetries (15). Environmental temperature was maintained slightly below the thermoneutral range (24–26°C). At least 60 min before the start of each protocol, animals were allowed to acclimate to the chamber, in which humidified air (70–90% relative humidity) was passed through at a rate of 4 l/min as appropriate, using a precision flow pump-reservoir system. Pressure changes in the chamber due to the inspiratory and expiratory temperature changes were measured using a high gain differential pressure transducer (model MP45–1, Validyne) (11). Analog signals were continuously digitized and analyzed on-line by a microcomputer software program (Buxco Electronics). A rejection algorithm was included in the breath-by-breath analysis routine and allowed for accurate rejection of motion-induced artifacts. Tidal volume, respiratory frequency, and minute ventilation (V˙E) were computed and stored for subsequent off-line analysis.

Measurement of blood gas values. Arterial blood samples were obtained from the implanted arterial catheter in the rats subjected to experiment I. After withdrawal of 75–100 µl

Exposures to Hypoxia

In group I, ventilatory measures were recorded in room air conditions and during a 20-min exposure to 10% O2 in N2 by use of a preset gas mixture, 1 day before (−1), on the day of surgery (0), and on days 1, 2, 4, and 7 after implantation of the osmotic pump. To ascertain that no degeneration of PDGF-BB occurred within the osmotic pump over the 7-day duration of these experiments, samples of PDGF-BB (80 nM) were kept in an incubator at 37°C for 7 days and 100-nl microinjections were then carried out on day 7 in those animals receiving chronic administration of vehicle via the osmotic pump, followed by a 20-min hypoxic challenge 20 min after administration.

Group II animals underwent 14 days of chronic hypoxic exposures by sojourning in a custom-designed chamber (volume ~0.2 m3; Oxycycler, Reming Bioinstruments, Redfield, NY) that was operated under a 12:12-h light-dark cycle. O2 concentration was continuously measured by an O2 analyzer and maintained at 10% by a servo-controlled system, such that deviations from the desired concentration were met by addition of N2 or O2 through computer-driven solenoid valves. Ambient CO2 in the chamber was continuously monitored and maintained at 2.4% ppm by appropriate adjustments in the overall chamber ventilation. Humidity was maintained at 40–50%, while ambient temperature was kept at 22–24°C. On days 0, 7, and 14 of hypoxia, 100-nl microinjections of Veh and PDGF-BB (80 nM) were performed 2 h apart into the nTS after collection of baseline ventilatory measures in room air for 15 min. Fifteen to twenty minutes after each microinjection, 20-min hypoxic challenges (10% O2 balanced in N2) were performed, after which animals were returned to the chamber or euthanized with a pentobarbital overdose for assessment of cannula location. In addition, on days 0, 1, 7, and 14 of chronic hypoxia, L-Glu microinjections were also performed, and ventilatory responses were measured as described in the next section.

Measurement of blood gas values. Arterial blood samples were obtained from the implanted arterial catheter in the rats subjected to experiment I. After withdrawal of 75–100 µl
of blood in the dead space of the catheter, another 150 μl were sampled for immediate analysis of PO2, PCO2, and pH with a blood gas analyzer (model 178, Ciba Corning). Measurements were always performed before the hypoxic gas switch and during the last minute of each hypoxic challenge.

**Immunoblotting of PDGF-β receptor.** Pooled dorsocaudal brain stem tissues (n = 5–6 animals/sample) primarily corresponding to the nTS were harvested from normoxic or chronically hypoxic rats by punch sampling with a 17-gauge thin-walled hypodermic needle as previously described (21). Six separate experiments corresponding to a total of 30–36 rats/condition were conducted. Samples were homogenized in lysis buffer (PBS pH 7.6, 0.5% sodium deoxycholate, 1% Nonidet P-40, 0.1% SDS, 20 mM sodium orthovanadate, 10 μg/ml aprotinin, 10 μg/ml leupeptin, and 0.5 mM phenylmethylsulfonyl fluoride). Supernatants obtained from 30-min centrifugation at 15,000 g were assayed for protein content (Bio-Rad, Hercules, CA). Three hundred micrograms of protein were used for the immunoprecipitation of PDGF-β receptor protein and incubated overnight at 4°C with 12 μg of PDGF-β receptor antibody (UBI, Lake Placid, NY) in a total volume of 250 μl, after which 20 μl of protein A-Sepharose were added and incubated for 1 h at 4°C. The immunoprecipitates were washed three times with lysis buffer and resuspended in a mixture of 20 μl lysis buffer and 20 μl SDS-sample buffer (0.5 M Tris, pH 6.5, 20% glycerol, 4% SDS, and 100 mM dithiothreitol). Proteins were separated by electrophoresis on a 8% Tris-glycine gel (Novex, San Diego, CA) and transferred to a 0.2-μm nitrocellulose membrane. Nonspecific binding was blocked by 1 h of incubation with 5% BSA in TBS-Tween 20 (TBS-T). The membrane was incubated overnight at 4°C with PDGF-β receptor antibody (1:300; UBI). Membranes were washed with TBS-T and incubated with secondary antibodies for 1 h. After extensive washing, proteins were detected by enhanced chemiluminescence (Amersham, Arlington Heights, IL).

**Northern Blots**

Pooled dorsocaudal rat brain specimen (n = 2–3/samples) primarily corresponding to nTS regions were immediately frozen in liquid nitrogen then stored at −70°C until use. Three separate experiments, corresponding to a total of 6–9 rats/condition, were conducted. Total RNA was isolated from the samples using a guanadinium/cesium chloride isolation method, exactly as previously described (29). Fifteen micrograms of total rat brain or lung RNA (as control) in denaturation buffer were added per well of a 1.2% formaldehyde/agarose gel and separated overnight via electrophoresis. RNA was transferred from the gel to a nylon membrane (Immobilon-N) by capillary action overnight. Prehybridization took place for 3 h at 62°C, and hybridization took place overnight at 62°C. cDNA templates were random-primed with 32P-radiolabeled dCTP by the random primer method using the Ready-To-Go DNA labeling kit (Pharmacia). A plasmid designated b15a, which includes the sequence for the rat PDGF-β receptor cDNA, was kindly provided by Dr. Michael Pech, Basel, Switzerland. The EcoR U/Hind III restriction enzyme fragment from the b15a plasmid was used as a template to generate the radiolabeled PDGF-β receptor probe. A plasmid with the murine 18S cDNA construct was obtained from American Type Culture Collection (Rockville, MD) and used to generate labeled probes as a loading control. Labeled probes were separated from unincorporated nucleotides using the TE Midi SELECT-D, G-50 spin columns (5 Prime-3 Prime, Boulder, CO). Hybridized membranes were washed with 2× standard sodium citrate (SSC) with 0.5% SDS, then 0.2× SSC with 0.5% SDS at 37 and 62°C. Images were generated for the hybridized autoradiographic signal by exposing the membrane to Biomax Film (Kodak) for 2 days. The hybridized autoradiographic signal was quantified using a Fuji phosphomager plate (Fugix BAS 1000) and the Mc-BAS 2.5 software (Fuji USA, Stanford, CT). PDGF-β receptor results were adjusted using the 18S loading control.

**Data Analysis**

Values are reported as means ± SD. Baseline ventilation before each hypoxic run was defined as the average of ventilatory measures during the 3-min period immediately preceding the gas switch. For ventilatory challenges, mean V̇e values in 1-min bins were calculated, and the peak V̇e value of the hypoxic run was considered as representative of the hypoxic ventilatory response. To normalize across the various experiments, the overall peak V̇e increase during hypoxia was calculated using the normoxic baseline preceding each challenge and was therefore expressed as %baseline.

For Western and Northern blot procedures, semiquantitative analysis of the bands was performed by scanning densitometry or by using the count values derived from the phosphorimager plates. Comparisons across treatment groups of numerical data were performed using either Student’s t-tests or two-way ANOVA (treatment and time or hypoxia) followed by Newman-Keuls post hoc tests, as appropriate. Linear regression between PDGF-β receptor levels and corresponding normoxic ventilation was also examined in the rats exposed to chronic hypoxia, and the correlation coefficient was calculated from the regression analysis. A P value of <0.05 was considered to achieve statistical significance.

**RESULTS**

**Group I Experiments**

As previously shown with acute nTS microinjections of PDGF-BB (19), continuous administration of the growth factor within nTS locations (Fig. 1) resulted in significant attenuation of the acute ventilatory response to hypoxia (HVR) at days 1 and 2 compared with days −1 and 0 (Fig. 2; Table 1; n = 8; P < 0.001) and compared with Veh-treated rats (n = 8; P < 0.001). However, at day 4, the PDGF-BB effect on HVR was not as pronounced, and by day 7, the HVR reduction had virtually disappeared [Table 1; P < 0.0001 ANOVA for time; P was nonsignificant (NS) day 0 vs. day 7]. To ascertain that PDGF-BB was still active at the later time points, and that the attenuation of the PDGF-BB effect was not the result of diminished PDGF-BB activity over time, on day 7 we performed in Veh-treated rats 100-nl microinjections of PDGF-BB kept for 7 days at 37°C, and we then assessed their HVR. Significant attenuation of HVR occurred after PDGF-BB administration (Fig. 2). Arterial blood gases confirmed the dynamic ventilatory changes associated with prolonged PDGF-BB administration, such that PCO2 was higher in PDGF-BB-treated rats during acute hypoxic challenges than in Veh-treated animals at days 1 and 4 (Table 2). This is in contrast with day 7, at which time point PCO2 during the hypoxic challenge was similar in both treatment groups (Table 2). Of note, the microcannulas were not in place in four rats.
Table 1. Baseline and peak ventilation during a 20-min hypoxic challenge with 10% O2 in conscious freely behaving rats before and during chronic nTS administration of PDGF-BB or vehicle

<table>
<thead>
<tr>
<th></th>
<th>Baseline V̇E</th>
<th>Peak V̇E</th>
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</thead>
<tbody>
<tr>
<td>Day 0</td>
<td>97.2 ± 5.8</td>
<td>211.0 ± 12.9</td>
<td>96.8 ± 5.3</td>
<td>205.9 ± 11.4</td>
</tr>
<tr>
<td>Day 1</td>
<td>92.6 ± 5.2</td>
<td>143.1 ± 8.9</td>
<td>94.3 ± 5.5</td>
<td>194.3 ± 10.6*</td>
</tr>
<tr>
<td>Day 2</td>
<td>93.7 ± 5.2</td>
<td>149.0 ± 8.2*</td>
<td>101.2 ± 6.3</td>
<td>204.3 ± 12.1*</td>
</tr>
<tr>
<td>Day 4</td>
<td>99.4 ± 6.0</td>
<td>182.9 ± 10.1†</td>
<td>94.6 ± 5.7</td>
<td>192.0 ± 10.8†</td>
</tr>
<tr>
<td>Day 7</td>
<td>97.3 ± 6.1</td>
<td>201.2 ± 10.2</td>
<td>99.1 ± 5.7</td>
<td>214.1 ± 11.3</td>
</tr>
</tbody>
</table>

Values are means ± SD; n = 8 rats/group. Chronic administration of platelet-derived growth factor BB (PDGF-BB) was made to the nucleus of the solitary tract (nTS) of rats from day 1 to day 7. V̇E, minute ventilation expressed in ml/min. PDGF-BB vs. vehicle: *P < 0.0001, †P < 0.01, both by 2-way ANOVA.

The effect was reduced at day 4, and at 37°C for 7 days elicited significant attenuation of HVR (Veh-treated rats, a single 100-nl microinjection of PDGF-BB stored at 37°C for 7 days elicited significant attenuation of HVR (●, n = 8; P < 0.001 vs. Veh), which was comparable in magnitude to results seen in the PDGF-BB group (P = NS).

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Values are means ± SD; n = 8 rats/group. Chronic administration of platelet-derived growth factor BB (PDGF-BB) was made to the nucleus of the solitary tract (nTS) of rats from day 1 to day 7. V̇E, minute ventilation expressed in ml/min. PDGF-BB vs. vehicle: *P < 0.0001, †P < 0.01, both by 2-way ANOVA.

As previously shown, PDGF-BB at day 0 was associated with attenuation of HVR (19). In contrast, identical treatments at days 7 and 14 of chronic hypoxia failed to elicit any significant effect on HVR (Fig. 4; n = 10). In the other eight animals designated for these experiments, the cannula was either not in the right location (n = 2) or became dislodged during the chronic hypoxic exposure (n = 6), such that data were not available.

Chronic Exposure to Hypoxia

When rats not undergoing any surgery were exposed to chronic hypoxia, ventilatory measurements performed in normoxia revealed time-dependent V̇E increases indicative of VAH (Fig. 5; n = 34/time point). These animals were then euthanized for tissue harvesting for PDGF-β receptor immunoreactivity (see next section).

PDGF-β Immunoblots and Northern Blots

Exposure to chronic hypoxia was associated with decreases in PDGF-β receptor immunoreactivity that became statistically significant at 7 and 14 days (Fig. 6). Linear regression of normoxic ventilation (as a correlate measure of VAH) plotted against PDGF-β receptor densitometric readings revealed a significant relationship between these two measurements (r = −0.56; P < 0.005; Fig. 7).

In contrast, no significant changes occurred over time during prolonged hypoxia in PDGF-β receptor mRNA (Fig. 8).

DISCUSSION

This study shows that long-term administration of PDGF-BB is associated with blunting of its attenuating effect on the acute hypoxic ventilatory response and that such changes are concordant with the temporal characteristics of VAH emergence in the rat. This observation is further reinforced by the decreasing
effect of acute PDGF-BB administration on the attenuation of the acute ventilatory response to hypoxia in rats exposed to chronic hypoxia at time points in which VAH is either developing or fully established. Thus, the physiological data and the changes in PDGF-β receptor expression suggest that the latter may underlie components of VAH. Indeed, evidence for decreasing expression of the PDGF-β receptor, albeit without significant changes in receptor mRNA, occurred over time during exposure to chronic hypoxia within the dorsal-caudal brain stem, and such changes in PDGF-β receptor expression correlated with ventilatory evidence of VAH.

Ventilatory Measurements

The magnitude and time course of ventilatory changes associated with VAH vary across the various mammalian species, ranging from a few hours and ventilatory increases during normoxia by 30–60% above prehypoxic normoxic levels to up to several weeks and doubling of ventilatory output during hypoxia (6, 10, 32). The ventilatory measurements during normoxia in the chronically hypoxic rats (Fig. 3), as well as the overall increases in the acute ventilatory response to L-Glu (Fig. 4) and to acute hypoxia after microinjection of vehicle in group II rats (data not shown) are in close concordance with previous studies on VAH in rats (1, 40, 51) and display some degree of biological variability, particularly during the first day of chronic hypoxic exposure (see Fig. 7). Notwithstanding this interindividual variability in ventilatory adaptation, the abolition of the ventilatory effect of PDGF-BB on the acute ventilatory response, after either long-term administration of this growth factor or after chronic hypoxia, suggests that PDGF-related pathways have undergone functional modifications during the process of acclimatization in this species.

Peripheral and Central Mechanisms of VAH

In an interesting series of experiments using an extracorporeal oxygenator to allow for separate control of blood gas characteristics at the carotid body and brain tissue levels, application of sustained carotid body hypoxia in the absence of brain tissue hypoxia in goats was found to be a prerequisite for the development of VAH in the goat (4, 7). Furthermore, initiation of the VAH process appeared to be critically dependent on the increased afferent neural input originating from peripheral chemoreceptor activation during hypoxia, such that elimination of peripheral chemosensory input virtually abolished VAH (3, 41). Furthermore, chronic exposure of glomus type 1 cells to chronic hypoxia resulted in increased cell excitability and cal-

Table 2. Mean blood gases during the last minute of a 20-min hypoxic challenge with 10% O₂ in conscious freely behaving rats before and during chronic nTS administration of PDGF-BB or vehicle

<table>
<thead>
<tr>
<th></th>
<th>PDGF-BB</th>
<th>Vehicle</th>
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<tbody>
<tr>
<td>Po₂</td>
<td>38.4±1.7</td>
<td>38.2±1.5</td>
</tr>
<tr>
<td>PcO₂</td>
<td>26.9±1.3</td>
<td>27.0±1.3</td>
</tr>
<tr>
<td>pH</td>
<td>7.53±0.04</td>
<td>7.54±0.03</td>
</tr>
<tr>
<td>Po₂</td>
<td>38.6±1.5</td>
<td>37.8±1.9</td>
</tr>
<tr>
<td>PcO₂</td>
<td>27.3±1.2</td>
<td>27.9±1.3</td>
</tr>
<tr>
<td>pH</td>
<td>7.52±0.04</td>
<td>7.53±0.03</td>
</tr>
<tr>
<td>Po₂</td>
<td>32.2±1.7*</td>
<td>38.7±1.6*</td>
</tr>
<tr>
<td>PcO₂</td>
<td>33.9±1.3*</td>
<td>27.5±1.4*</td>
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<tr>
<td>pH</td>
<td>7.46±0.03*</td>
<td>7.54±0.03*</td>
</tr>
<tr>
<td>Po₂</td>
<td>34.8±1.8*</td>
<td>38.5±1.7*</td>
</tr>
<tr>
<td>PcO₂</td>
<td>31.5±1.4*</td>
<td>28.1±1.2*</td>
</tr>
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<td>pH</td>
<td>7.49±0.03*</td>
<td>7.53±0.04*</td>
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<td>Po₂</td>
<td>37.9±1.6</td>
<td>38.0±1.5</td>
</tr>
<tr>
<td>PcO₂</td>
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<tr>
<td>pH</td>
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Values are means ± SD; n = 8 rats/group. Chronic nTS administration of PDGF-BB or vehicle shown here for days 1, 4, and 7. *PDGF-BB vs. vehicle: P < 0.05.
cium mobilization, resulting in part from recruitment of cAMP-dependent pathways (56) as well as from changes in sodium and potassium channel conductances (22).

In contrast, application of isolated brain hypoxia to awake goats did not induce VAH, suggesting that central mechanisms may not be operative in the initial process of ventilatory acclimatization (61). However, administration of carbon monoxide to awake ponies, such as to induce a reduction of arterial oxygen content without stimulation of the carotid chemoreceptors, resulted in ventilatory increases over time that were compatible with VAH, thereby suggesting that both an early peripherally mediated component and a subsequent central component of VAH occur and are required for the complete expression of VAH (31). To further explore the nature of this central component to VAH, 31P nuclear magnetic resonance spectroscopy of brain was performed in humans to assess whether brain intracellular acidosis provided the supplemental stimulus to ventilatory acclimatization to high altitude (18). However, these experiments clearly ruled out the possibility that changes in intracellular pH contribute to VAH (18). In addition, no definitive role was found for endogenous opioids and serotonin centrally, or for dopamine peripherally, as potential modulators of VAH (23, 27, 28, 38, 39, 42, 45, 46, 60).

Fig. 5. Mean ventilatory changes (ΔVE) during normoxia in 34 conscious rats over the course of a 14-day exposure to chronic hypoxia (10% O2 balance N2). *P < 0.01 vs. normoxic baseline (established 1 day before beginning of hypoxic exposures).

Fig. 6. Top: representative Western blot of PDGF-β receptor immunoprecipitates probed with an antibody against the PDGF-β receptor from dorsocaudal brain stem lysates harvested from rats at 0, 1, 2, 7, and 14 days of hypoxia (10% O2 balance N2). Decreases in PDGF-β receptor immunoreactivity are apparent at day 2 and continue to decrease thereafter. Bottom: mean scanning densitometry values (expressed in arbitrary units) from 6 sets of dorsocaudal brain stem tissue lysates exposed to room air or hypoxia (10% O2 balance N2) for 1, 2, 7, or 14 days.

Fig. 7. Scattergram of PDGF-β receptor immunoreactivity expressed as a ratio with normoxic control in the denominator plotted against corresponding mean ventilatory measurements during normoxia (VE, expressed as a ratio with normoxic control in the denominator) from rats exposed to 1 day (■), 2 days (○), 7 days (▲), and 14 days (▼) of hypoxia (10% O2 balance N2). A significant linear relationship emerged as shown by the straight line (dotted lines indicate 95% confidence intervals) as follows: y = 1.28–0.23 × x (r: −0.56; *P < 0.005).

Fig. 8. Top and middle: representative Northern blots for PDGF-β receptor and S18 (as internal control) in dorsocaudal brain stem harvested from rats at 0, 1, 2, 7, and 14 days of hypoxia (10% O2 balance N2). A control blot from a normoxic rat lung is also shown. No consistent changes in PDGF-β receptor mRNA were apparent. Bottom: mean phosphoimager counts (adjusted for S18 internal control and expressed as degrees of change from day 0) from 3 sets of dorsocaudal brain stem tissue exposed to room air or hypoxia (10% O2 balance N2) for 1, 2, 7, or 14 days. No significant changes in PDGF-β receptor mRNA density occurred over time (*P = NS).
More recently, Schmitt et al. (51) reported that tyrosine hydroxylase content and norepinephrine turnover exclusively increased in the caudal part of the nTS in rats, were preceded by ventilatory evidence of VAH, and, more importantly, that the biochemical and physiological parameters were highly correlated. Thus VAH is associated with intrinsic modifications of gene expression within dorsocaudal brain stem neurons that primarily subserve components of the ventilatory reflex arc (12, 55). Such genomic modifications may in turn induce changes in the physiological properties of these neurons, as shown by Nolan and Waldrop (35), who found enhanced discharge frequencies in response to hypoxia in ventrolateral medullary neurons from rats after acclimatization to hypoxia compared with normoxic controls. In addition, Dwinnell and Powell (13) recently demonstrated centrally mediated increases in phrenic nerve output developing over the course of acclimatization to hypoxia in rats. We believe that the modest, albeit significant, increases in ventilation in response to microinjection of L-Glu in the acclimatized rats provide additional evidence for evolving changes in the responsivity of nTS neurons to excitatory stimuli such as increased peripheral chemoreceptor input. Therefore, we support the current conceptual framework for development of VAH to include early dependency on peripheral chemoreceptor integrity, which is followed by substantial alterations in the physiological properties of brain stem neurons. In this context, the present study further adds to this concept and proposes an important role for PDGF-β receptors in mediating components of such centrally dependent VAH in a rat model.

Role of PDGF-BB and PDGF-β Receptors in the Hypoxic Ventilatory Response

It is now quite well established that nuclei within the brain stem show high levels of expression for both the PDGF-B chain (49) and the PDGF-β receptors (19, 53). In contrast, PDGF-α receptors are preferentially expressed in glial cells and display different topographic abundance compared with PDGF-β receptors (19, 48, 58, 62–64).

The abundance of PDGF-related pathways within many of the nuclei underlying the neural substrate for the hypoxic ventilatory response was highly suggestive that PDGF isoforms and their receptors could play a functional role in both the immediate and the long-term mechanisms of adaptation to hypoxia. Several lines of evidence led to this assumption. 1) PDGF-B chain expression is greatly increased in neural regions after focal ischemia (24–26, 36), where it has been assigned a putative role in the prevention of neuronal cell death (8). 2) Activation of PDGF-β receptors by their specific ligand, PDGF-BB, will inhibit N-methyl-D-aspartate (NMDA) receptor-dependent excitatory postsynaptic currents in CA1 pyramidal neurons of rat hippocampal slices (57), possibly through a recently uncovered pathway that involves activation of GTPase-activating protein of Ras (RasGAP) and binding of RasGAP to the Src kinase to form a complex that prevents the phosphorylation of phospholipase C (50). Phospholipase C is prominently involved in mediating the ventilatory response to hypoxia within an NMDA glutamate receptor-protein kinase C pathway (18, 21, 52). 3) Pharmacological studies from our laboratory have demonstrated that PDGF-BB attenuates the acute phase of the hypoxic ventilatory response, whereas reduction of PDGF-β receptor function by use of either transgenic or pharmacological approaches prevents development of the hypoxic ventilatory depression that characteristically follows the acute response (19). Therefore, in the setting of acute hypoxia, activation of PDGF-β receptors within dorsocaudal brain stem nuclei reduces the magnitude of the acute hypoxic ventilatory response and thereby modulates the onset of the late phase of this response. We now show that development of VAH is closely linked to the reduction of PDGF-β receptor expression in the dorsocaudal brain stem. Because the decreased expression of the receptor as evidenced by the Western blots was not accompanied by a parallel decrease in PDGF-β receptor mRNA, it is possible that receptor downregulation may have occurred via sustained autophosphorylation, followed by internalization and degradation of the receptor through the ubiquitin proteasome proteolytic pathway (33, 34). However, we cannot exclude with certainty that the absence of PDGF-β receptor mRNA changes with chronic hypoxia may be due to alterations in posttranscriptional regulation.

In summary, we present novel evidence supporting a role for PDGF-β receptors within the dorsocaudal brain stem in mediating ventilatory components that characterize the phenomenon of ventilatory acclimatization to hypoxia. These findings further suggest that central mechanisms involving PDGF are indeed operative in VAH and that VAH may in fact represent a complex interplay between peripheral and central neurons. Each of these two intimately linked systems undergoes complex cellular and molecular changes that will ultimately result in optimal organismal adaptation and survival.

Perspectives

Differential activation of glutamate receptors, such as NMDA receptors (37), and growth factor receptors, such as the PDGF-β receptor (19) within the nTS during the early phases (<1 h) of the hypoxic ventilatory response, suggests that the unique downstream signaling intracellular pathways linked to these receptors are not only involved in moment-to-moment ventilatory output regulation but may also be critical for induction of protective and/or adaptive genes that permit ventilatory acclimatization, as shown in the present study. It is therefore possible that the time domains of complex receptor-receptor and protein-protein interactions induced by hypoxia will in turn induce transcriptional and posttranslational regulation of multiple genes aiming to secure functional adapta-
tion, cell survival, and/or programmed cell death and synaptic plasticity.

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REFERENCES


