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Hypoxic Ventilatory Response in Platelet-Derived Growth Factor Receptor-*β*–Knockout Mice

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Abstract. The present study investigated whether the platelet-derived growth factor receptor (PDGFR)-*β*–mediated mechanisms are involved in the hypoxic ventilatory response through modulating the *N*-methyl-D-aspartate (NMDA) function. The ventilatory changes during hypoxic challenge (10% O_2, 30 min) were measured plethysmographically in mice selectively lacking the PDGFR-*β* in neurons (KO mice) and in control wild-type mice (WT mice) before and after blockade of NMDA receptors. In baseline breathing at rest, respiratory rate, tidal volume, and minute ventilation were similar between WT and KO mice. Hypoxia caused an increase of ventilation during the early period of exposure (an initial excitation), followed by a progressive decrease along with the exposure period (a late decline). The initial excitation occurred similarly in KO and WT mice, while the late decline was markedly attenuated in KO mice. Administration of an antagonist of NMDA receptors, dizocilpine (0.3 mg/kg, i.p.) decreased the initial excitation and hastened the late decline of hypoxic ventilatory response. Furthermore, the hypoxic ventilatory response in KO mice was indistinguishable from that in WT mice after blockade of NMDA receptors. The present study suggests that the PDGF-BB/PDGFR-*β* signal axis contributes to the hypoxic ventilatory response by its inhibitory effect on the NMDA receptor–mediated function.

Keywords: hypoxic ventilatory response, platelet-derived growth factor receptor (PDGFR)-*β*, *N*-methyl-D-aspartate (NMDA) mechanism, dizocilpine

Introduction

In mammals, the function of the hypoxic ventilatory response is not only to adapt respiration during hypoxic conditions but also to ensure cell survival. The acute hypoxic response in adult mammals is characteristic and biphasic (1, 2); ventilation is temporarily activated in the early phase (an initial excitation) to increase oxygen inhalation, followed by a progressive reduction in the late phase (a late decline) to save energy consumption. The initial excitation is mediated by activation of peripheral chemoreceptors. It requires intact central relays within the nucleus tractus solitarius (NTS), and the neuronal transmission of hypoxic afferent signals is critically dependent on glutamatergic signaling (3 – 6). In contrast, the late decline appears to develop as a result of complex interactions between excitatory and inhibitory influences on peripheral chemoreceptors, central respiratory neurons, metabolic pathways, and signaling substrates including by-products of hypoxia, nitric oxide, and peptides (7 – 10).

Gozal and coworkers (8) have presented interesting results that the hypoxic ventilatory response in rats is inhibited by microinjection of platelet-derived growth factor (PDGF)-BB, but not PDGF-AA, into the dorsocaudal brainstem, which activates the PDGF receptor (PDGFR)-*β*. Moreover, marked reductions in the magnitude of late decline of hypoxic ventilatory response occurred in mice heterozygous for a mutation in the PDGFR-*β*. Administration of an antagonist of PDGFR-*β* to wild-type littermates elicited similar declines in hypoxic ventilation. These results indicate that the

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PDGF-BB/PDGFR-β signal axis modulates the hypoxic ventilatory response. On the other hand, several lines of evidence suggest that the *N*-methyl-D-aspartate (NMDA) receptor–mediated mechanisms are also involved in the hypoxic response of ventilation (5, 6, 11, 12). For instance, an increase in glutamate concentration occurs in the NTS during hypoxia, which coincides with an excitation of ventilation (3). Systemic injection of an NMDA-receptor antagonist, dizocilpine, depresses the hypoxic ventilatory response and the peripheral chemoreceptor response to NaCN in rats (5). Thus, the hypoxic ventilatory responses are critically dependent on activation of NMDA receptors.

Intriguingly, it has been demonstrated that PDGF-BB elicits a marked inhibition of NMDA receptor–dependent excitatory postsynaptic currents in the CA1 pyramidal neurons of rat hippocampal slices (13). Furthermore, the PDGFs exert the neuroprotective effect against the NMDA receptor–dependent Ca^{2+}-overload that induces neuronal death (14 – 17). These results lead us to postulate that modification of the hypoxic ventilation by the PDGF-BB/PDGFR-β signal axis is ascribed to its action on the NMDA receptor–mediated mechanism. To address the issue, the hypoxic ventilatory response was evaluated with or without blockade of NMDA receptors by systemic dizocilpine using mutant mice (knockout: KO mice) of which the PDGFR-β gene in neurons was conditionally deleted (18).

Materials and Methods

The present study was approved by the Animal Care Committee at the Aichi Gakuin University and performed in accordance with the Guiding Principles for the Care and Use of Laboratory Animals approved by The Japanese Pharmacological Society.

Preparation of mutant mice

The mutant mice lacking the PDGFR-β gene in neurons were generated as described previously (18, 19). Briefly, for the conditional deletion of PDGFR-β, two lines of mutant mice were crossed: one harboring the PDGFR-β floxed allele and the other expressing Cre recombinase under the control of the nestine promoter and enhancer (nestine-Cre^+ mouse). The functional expression of Cre recombinase in the mutant mouse was tested by crossbreeding the nestine-Cre^+ mouse and the CAG-CAT-LacZ reporter mouse, which expresses LacZ under the control of the CAG promoter. Induction of recombination was estimated by LacZ expression as detected by β-galactosidase staining (see refs. 19 and 20). Adult C57BL/6 wild-type mice (WT mice, 22.6 ± 0.57 g body weight) or mutant mice (KO mice, 20.6 ± 0.81 g body weight) were housed under standard conditions (constant temperature of 24°C – 25°C and a 12-12 h light-dark cycle) with the standard diet and water available ad libitum.

Plethysmographical measurements

Ventilatory activity was measured by the whole-body plethysmography technique (21). The freely behaving, unrestrained, and unanesthetized animal was placed in a plethysmograph chamber (300 ml) perfused with humidified air at a constant rate of 500 ml/min. Pressure changes derived from breath-to-breath ventilation were measured by using a differential pressure transducer (TP-602T; Nihon Kohden, Tokyo) at 23°C – 25°C. The pressure–volume relation was calibrated by injecting a standard volume of room air into the chamber at the end of each experiment with the animal kept inside. The concentrations of CO_2 and O_2 in the chamber were monitored continuously with CAPNOMAC (Datex, Helsinki, Finland). All signals were digitized, monitored on computer display, and stored on a hard disk (Macintosh-PowerLab; AD Instruments Pty Ltd., Castle Hill, Australia). After recording the stable spontaneous breathing at rest (for at least 60 min), the ventilatory response to hypoxia was measured. Hypoxia was introduced by infusing a gas mixture of 90% N_2 and 10% O_2 for 30 min. The speed of perfusion was set at 500 ml/min throughout the experiment. An antagonist of NMDA receptors, dizocilpine (dizocilpine maleate; Research Biochemical, Natick, MA, USA), was dissolved in the saline and administered intraperitoneally ca. 120 min before the hypoxic challenge. Referring to the previous studies (6, 22, 23), we determined the dose of dizocilpine in the preliminary experiment. A high dose (1.0 mg/kg) of dizocilpine caused abnormal behaviors such as abnormal gait, moving, and/or seizure; and a small dose (0.1 mg/kg) had no detectable effect on ventilation. Finally, a dose of 0.3 mg/kg was selected in this study, which produced a long-lasting, constant effect on ventilation without any effect on behaviors.

Data analyses

Respiratory rate (RR, breaths/min), tidal volume (V_T, μl/g), and minute ventilation (V_E, ml/min/g) were calculated by averaging the sequential 30 breaths. They were obtained before (normoxia); at 5, 10, 20, and 30 min after the onset of hypoxia; and at 10 min after the end of hypoxia (recovery). Changes in ventilatory parameters induced by hypoxia were presented as percents of the before values. The statistical significance was determined by using repeated measures ANOVA followed by multiple comparisons or *t*-test. All values are presented as means ± S.E.M. The significance

threshold was set at $P<0.05$.

Results

Ventilatory response to hypoxia in KO mice

In the normal condition (normoxia), no significant difference was detected between KO and WT mice for any of the three ventilatory parameters (RR, V_T, and V_E) analyzed, suggesting that the basic ventilation in KO mice was not impaired (Table 1). Figure 1A shows typical ventilatory responses to hypoxic challenge. The ventilation increased during the early phase of hypoxia (an initial excitation) and then gradually decreased along with the exposure to hypoxic gas (a late decline) in both mice. The magnitude and time course of the hypoxic ventilatory response in KO mice were different from those in WT mice. As shown in Fig. 2, V_E in KO mice increased to twice the baseline during the early phase of hypoxia, which was similar to the change in WT mice. During the late phase of hypoxia, V_E in KO mice was kept at increased levels when compared with those in WT mice, indicating that the late decline of V_E in KO mice was attenuated significantly. This difference in V_E responses between KO and WT mice was derived from RR changes, since V_T changes were similar in both mice.

Table 1. Baseline ventilatory measurements in WT and KO mice with or without blockade of NMDA receptors by dizocilpine (0.3 mg/kg, i.p.)

	Control	Dizocilpine
WT mice		
V_E (ml/min/g)	5.04 ± 0.28	6.54 ± 0.58**
V_T (μl/g)	31.18 ± 1.70	38.15 ± 1.46**
RR (breaths/min)	162.7 ± 8.0	170.5 ± 11.8
n	6	6
KO mice		
V_E (ml/min/g)	4.67 ± 0.44	8.06 ± 0.79**
V_T (μl/g)	27.01 ± 1.33	39.34 ± 2.91**
RR (breaths/min)	171.8 ± 12.4	203.1 ± 9.6*
n	6	6

Data are represented as the mean ± S.E.M., n = number of animals. V_E: minute ventilation, V_T: tidal volume, RR: respiratory rate. *$P<0.05$, **$P<0.01$: significantly different from the corresponding control value.

Effects of NMDA blockade on hypoxic responses

In normoxia, systemic administration of dizocilpine at a dose of 0.3 mg/kg increased the basal ventilation. This stimulating effect of dizocilpine on all three parameters (V_T, RR, and V_E) was observed in both KO and WT mice (Table 1). After treatment with dizocilpine, only a small excitation of ventilation occurred during the early phase of hypoxia (Fig. 1B). This response returned to the baseline level during the hypoxic challenge in both WT and KO mice. Consequently, the hypoxic ventilatory response in KO mice was indistinguishable from that in WT mice after blockade of NMDA receptors (Fig. 3).

Discussion

We plethysmographically measured the acute venti-

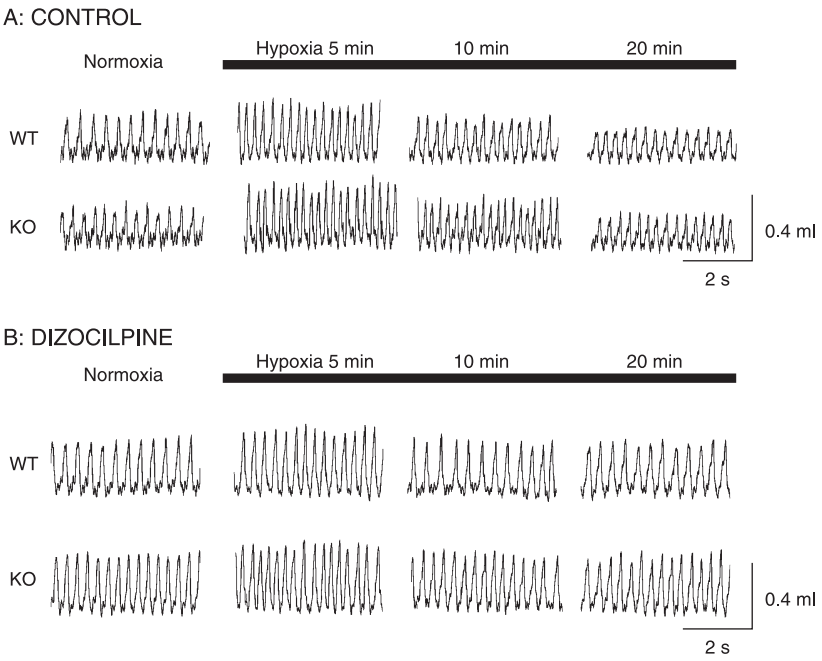

Fig. 1. Ventilatory responses to hypoxic challenge. A: Hypoxic ventilatory responses in a WT mouse (WT) and a KO mouse (KO) under the control condition. B: Hypoxic ventilation responses in a WT mouse and a KO mouse after treatment of dizocilpine (0.3 mg/kg, i.p.). Plethysmographical records were taken during normoxia (21% O_2) and at 5, 10, and 20 min after the onset of hypoxia (10% O_2).

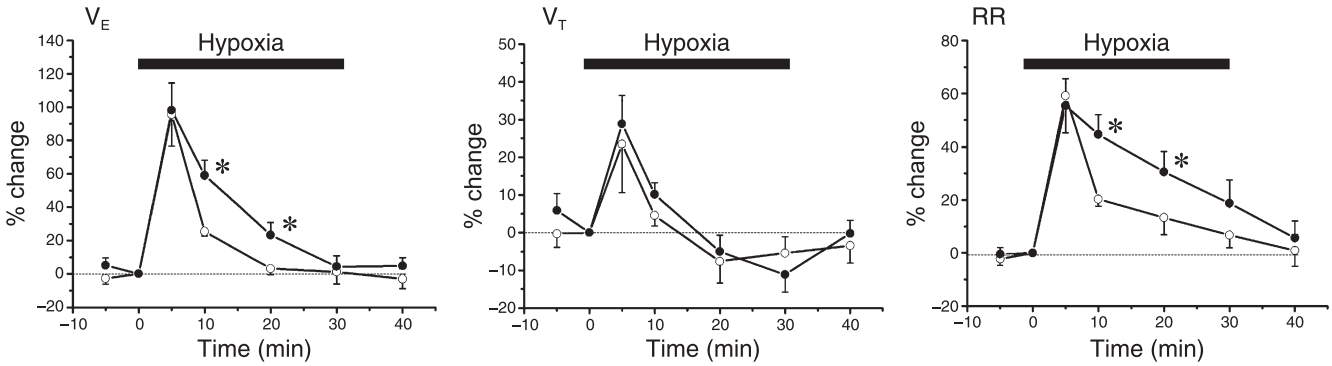

Fig. 2. Percent changes in ventilatory responses to a 30-min hypoxic challenge in WT (open circles) and KO mice (filled circles). V_E: minute ventilation, V_T: tidal volume, RR: respiratory rate. Data are presented as the mean ± S.E.M. (n = 6). **P*<0.05, significantly different from the corresponding value in WT mice.

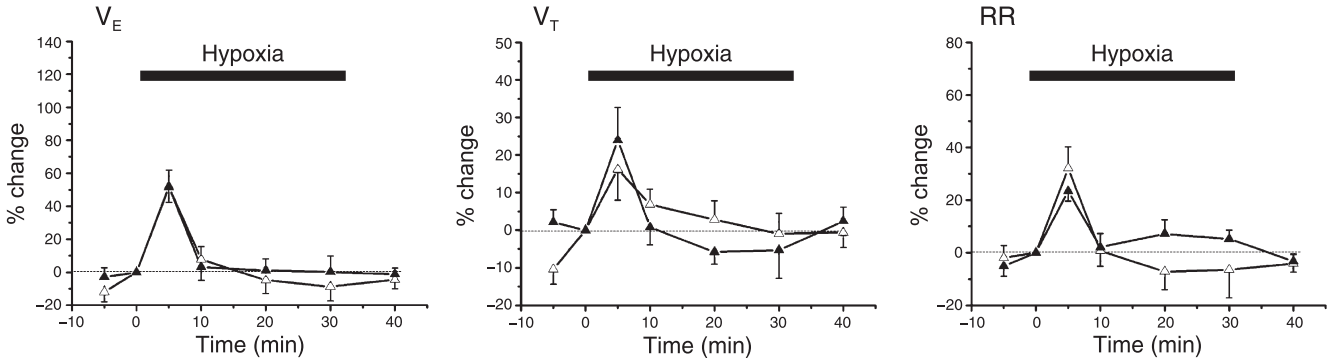

Fig. 3. Percent changes in ventilatory responses to a 30-min hypoxic challenge in WT (open triangles) and KO mice (filled triangles) following treatment with dizocilpine (0.3 mg/kg, i.p.). V_E: minute ventilation, V_T: tidal volume, RR: respiratory rate. Data are presented as the mean ± S.E.M. (n = 6). Note that there is no difference in hypoxic ventilatory response between KO and WT mice.

latory response to hypoxia in adult mice. The baseline values of ventilatory parameters during normoxia are comparable to those reported previously (22, 24). The present study demonstrated that the late decline of the hypoxic ventilatory response in PDGFR-β KO mice was significantly attenuated, while the initial excitation was unchanged when compared with responses in WT mice. This is consistent with the result demonstrated by Gozal et al. using mice heterozygous for a mutation in PDGFR-β (8) and agrees with their suggestion that PDGFR-β–mediated mechanisms modulate the late decline of hypoxic ventilation. That the response was confined to the late phase of hypoxia may be explained by the delayed onset of PDGF-BB action because administration of an antagonist of PBGF-BB, CGP 57148B, attenuated only the late hypoxic decline in mice (8). It is thought that PDGF-BB is released and accumulates along with the period of hypoxic challenge, and its concentration is sufficient for being effective during the late hypoxic phase but not enough during the initial phase. This is also parallel to the evidence that hypoxia increased the content of PDGF-B chain mRNA and PDGFR-β expression in the NTS in a time-dependent manner (8).

In the present study, systemic administration of dizocilpine increased the baseline ventilation in both WT and KO mice. Foutz and colleagues (22, 23) reported a similar result that dizocilpine, which blocks NMDA receptors involved in respiratory functions, had a stimulating effect in adult mice, while it did not have any or a slightly depressive effect in neonate mice. They have discussed that the increase in ventilation may be attributed to a stimulating action of dizocilpine on forebrain structures controlling respiration (25). In addition, this drug influenced the hypoxic ventilatory response; it greatly decreased the initial excitation and the ability to sustain V_E above its baseline value in KO and WT mice. This suggests that NMDA receptor–mediated mechanisms preferentially or mainly contribute to both the initial and late responses to hypoxia. There

have been a number of reports describing the important roles of NMDA receptor–mediated mechanisms in the NTS during hypoxia. Hypoxia induced an enhancement of glutamate release in the rat NTS that coincides with an increase in the ventilation (3) and NMDA antagonists attenuated the hypoxic ventilatory response (4 – 6, 26). Hoffman et al. (27) also demonstrated that hypoxia induced the modification of NMDA receptors in the piglet brain. The most important finding obtained in the present study is that blockade of NMDA receptors eliminated the attenuation of the late decline observed in KO mice and hence the hypoxic ventilatory response became similar in WT and KO mice. PDGF-BB is abundantly expressed in the NTS (8, 28), and sustained hypoxic stimulation induces the release of PDGF-BB and activates PDGFR-β in the NTS (8, 29, 30). Therefore, it is possible that during hypoxia the activation of PDGFR-β by endogenously released PDGF-BB effectively inhibits the NMDA receptors. The mechanism by which the PDGF-BB/PDGFR-β signal down-regulates the NMDA function is thought to modulate the open channel probability of NMDA receptors through the phospholipase Cγ–induced elevation of intracellular calcium levels and activation of the related signal transduction pathways (13, 15, 17). Additionally, Simakajornboon and Kuptanon (31) discussed that activation of PDGFR-β is an important contributor to the hypoxic ventilatory depression at all postnatal ages. Maturation of these NMDA- and PDGFR-β–mediated pathways occurs primarily during the early postnatal period. Perturbation of these processes may result in short-term or sustained alterations to the hypoxic ventilatory response and may also affect neuronal survival during hypoxia.

In conclusion, the present study revealed that the hypoxic ventilatory response is essentially mediated by NMDA receptor–mediated mechanisms that are modulated by the PDGF-BB/PDGFR-β signal axis. This result would provide useful information for understanding the hypoxic ventilatory response and, moreover, about the physiological role of the PDGFR-β in promoting the neuronal cell survival in mammals.

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