Brain stem NO modulates ventilatory acclimatization to hypoxia in mice

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Nitric oxide (NO) is assumed to be a possible mediator of the ventilatory response to hypoxia (9, 10, 11, 35). NO attenuates the chemosensory function of the carotid body (6, 28) and increases ventilation when a NO donor is injected into the nucleus tractus solitarii (NTS) (19). Recent evidence also supports the idea that NO could regulate ventilation by acting on the control of central breathing in the brain stem. Subsequent studies show that NO plays an important role in the signaling pathway leading to the HVR (6, 28). In acute hypoxia, NO production in the brain stem is associated with an activation of N-methyl-d-aspartate (NMDA) glutamate receptor (32). The activation of NMDA receptors induces a calcium elevation in intracellular stores by opening a voltage-dependent calcium channel, which seems to activate neuronal nitric oxide synthase (nNOS) (32). Reid and Powell (30) have recently shown that the systemic injection of the NMDA receptor blocker MK-801 (dizocilpine) eliminates HVR in normoxic control and chronically hypoxic rats. However, MK-801 caused a greater decrease in HVR after acclimatization, which is consistent with a role of NMDA receptor contributing to VAH. Therefore, the responsiveness of central nervous system to the afferent input received from the carotid body is increased. Thus the NO release in response to calcium-dependent activation of nNOS isoform may play a significant role in sustaining ventilation during hypoxia (6, 10). Furthermore, in chronic hypoxia, NO production is associated with increased nNOS activity and expression in the rat brain (5, 23), suggesting that NO pathway could contribute to VAH. Considering the effects of NMDA receptor blockade coupled to the increase in nNOS expression after chronic hypoxia, we hypothesize that NMDA receptors will be increased as well as nNOS in the medulla, therefore contributing to VAH in mice. The objective of our study was to determine if the exposure to chronic hypoxia would alter the NMDA receptor expression in the NMDA receptor expression in the medulla of acclimatized mice breathing 21% and 8% oxygen and used a nNOS inhibitor, and assessed the expression of N-methyl-d-aspartate (NMDA) glutamate receptor and nNOS (mRNA and protein). Two groups of Bl6/CBA mice (n = 60) were exposed during 2 wk either to hypoxia [barometric pressure (PB) = 420 mmHg] or normoxia (PB = 760 mmHg). At the end of exposure the medulla was removed to measure the concentration of nitric oxide (NO) metabolites, the expression of NMDA-NR1 receptor, and nNOS by real-time RT-PCR and Western blot. We also measured the ventilatory response [fraction of inspired O2 (FiO2) = 0.21 and 0.08] before and after S-methyl-l-thiocitrulline treatment (SMTC, nNOS inhibitor, 10 mg/kg ip). Chronic hypoxia caused an increase in ventilation that was reduced after SMTC treatment mainly through a decrease in tidal volume (Vt) in normoxia and in acute hypoxia. However, the difference observed in the magnitude of acute hypoxic ventilatory response [minute ventilation (Ve) 8% − Ve 21%] in acclimatized mice was not different. Acclimatization to hypoxia induced a rise in NMDA receptor as well as in nNOS and NO production. In conclusion, our study provides evidence that activation of nNOS is involved in the ventilatory acclimatization to hypoxia in mice but not in the hypoxic ventilatory response (HVR) while the increased expression of NMDA receptor expression in the medulla of chronically hypoxic mice plays a role in acute HVR. These results are therefore consistent with central nervous system plasticity, partially involved in ventilatory acclimatization to hypoxia through nNOS.

ventilatory acclimatization to hypoxia; nitric oxide synthase; control of breathing

EXPOSURE TO HYPOXIA elicits chemoreflexes increasing ventilation. In chronic hypoxia, the rise in ventilation is greater than during acute exposure and is called ventilatory acclimatization to hypoxia (VAH). VAH is characterized by an increase in resting ventilation and in the sensitivity of the respiratory control system (3, 4, 9, 22, 26, 27). Two mechanisms are described as contributing to VAH: an increase in the chemosensitivity to low levels of oxygen (3, 24), inducing an elevated afferent input to respiratory centers in the central nervous system (CNS) and an increase in the responsiveness of the CNS to the afferent input received from the carotid body (25).

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Mean body weight decreased after acclimatization to hypoxia (NX 26.4 ± 0.6 g, CHX 24.3 ± 0.2 g, P < 0.05). All procedures were performed in agreement with the Guide for the Care and Use of Laboratory Animals published by the National Institutes of Health (NIH Publication No. 85-23, Revised 1996) and with the approval of the French “Ministère de l’Agriculture” Guidelines (authorization number: A-93-008-01).

Mice undergoing chronic hypoxic exposure were housed for 14 days in a hypoxic chamber connected to a gas pump where air exposed at a pressure of 420 mmHg (~4,200 m). Hypobaric hypoxia was maintained by a vacuum source at flow rates sufficient to prevent CO2 buildup. The chamber was returned to sea level (760 mmHg) three times per week for 30 min to clean the cages and to feed and give water to the animals. The normoxic group was kept outside the hypobaric chamber at 760 mmHg. All animals had free access to food and water at all times.

At the end of hypoxic exposure, animals were killed by cervical dislocation. Mice were then decapitated, the brain was rapidly removed, and the medulla was quickly dissected and frozen in liquid nitrogen and stored at ~80°C until used either to quantify protein (n = 6 for each group and each protein), mRNA (n = 6 for each group) and NO, (n = 6 for each group). All procedures were done on ice to avoid protein degradation.

Other mice (n = 6 for each group) were used to study ventilation as described below.

**Basal Ventilation and Hypoxic Ventilatory Response**

Ventilation was measured in conscious animals via a whole body double-chamber plethysmograph. The system consists of a 250-ml Plexiglas experimental chamber. The chamber provides airflow at 0.85 l/min via a manual flowmeter system (Sho-Rate II 111355). Differential pressure between the experimental and reference chamber was measured with a differential pressure transducer (model DP45-18, Valdyine Engineering, Northridge, CA). The pressure signal was sent to a demodulator (model CD1, Valdyine Engineering, Northridge, CA), and data were recorded at a sampling rate of 200 Hz by a Biopac system (MP35, BIOPAC System, Santa Barbara, CA). The hypoxic gas mixture was obtained by mixing room air with N2 in a 250-ml mixing chamber connected to the plethysmographic chamber by using an oxygen regulator (PROOX model 110, BioSpherix, Redfield, New York). O2 and CO2 were monitored using a gas analyzer for O2 (ST105 Qubit Systems, Kingston, Ontario, Canada) and CO2 (S155 Qubit Systems).

The pressure signal was calibrated by injections of an adequate volume of air (0.2 ml) into the animal chamber and by recording the related changes in pressure. Barometric pressure was measured routinely before experiments, and temperature and humidity inside the chamber were continuously monitored with a digital thermometer and hygrometer (Thermo Frigo OTAX). Rectal temperature was assessed before and after ventilation measurement using an electronic thermometer. Ventilation (Ve, ml/min BTPS/kg), respiratory frequency (fR, breaths/min), and tidal volume (VT, ml BTPS) were computed breath-by-breath throughout all baseline and experimental periods and were stored for offline analysis. Ventilation was calculated following methods of Bartlett and Tenney (2).

Measurements were performed in normoxia [fraction of inspired O2 (FiO2): 0.21] and after acute hypoxic exposure (FiO2: 0.08). Mice were left in the plethysmograph for at least 30 min acclimation period during which they were exposed to 21% O2 and to 8% O2. Once the mice were quiet, the inlet and outlet tubes of the chamber were closed, and pressure fluctuations related to breathing were recorded several times as described above to get a robust normoxic ventilation. Then the inflowing gas composition (FiO2: 0.08) was changed, and the ventilation was immediately recorded during 5 min. Nevertheless, we analyzed the data during the first 3 min exposure to get a steady-state measurement and not the hypoxic “roll-off.” Ventilation was constant during these 3 min, and we considered these measures as the hypoxic ventilatory response.

After measurement of ventilation in normoxia and in hypoxia, animals received an intraperitoneal injection of 5-methyl-t-thiocitrulline (SMTc), a selective nNOS inhibitor (8) at a dose of 10 mg/kg as previously described (10). Thirty minutes after injection, mice were exposed to FiO2 = 0.21 and 0.08, and ventilation was measured.

**nNOS and NMDA-NR1 Western Blot Analysis**

Experiments were performed in the medulla of normoxic and hypoxic mice. Tissues were weighed and sonicated at low amplitude on ice-cold buffer (1 g in 4 ml of extraction buffer) containing 0.32 M sucrose, 10 mM Tris·HCl, 1 mM EDTA, 1 mM DTT, and a protease inhibitor cocktail, pH 7.5, for the nNOS study. Another extraction buffer containing 50 mM Tris·HCl, pH 9, to which NaCl and SDS were added to a final concentration of 200 mM and 1%, respectively, was used for the NMDA-NR1 study (17). Homogenates were then directly ultracentrifuged for 30 min at 100,000 g 4°C for nNOS and for 1 h at 100,000 g 4°C for NMDA-NR1 after the incubation of homogenates at 37°C for 30 min to solubilize and denature the NMDA receptor complexes. Then supernatants were directly stored at −20°C until used for nNOS, while supernatants of NMDA-NR1 were diluted in an equal volume of extraction buffer containing 0.1% Triton X-100. Supernatant protein content was determined by Bio-Rad protein assay using BSA as standard.

Proteins, 25 μg for nNOS and 100 μg for NMDA-NR1, were separated by electrophoresis on 7.5% SDS-polyacrylamide gel and transferred to PVDF membrane (Amersham, UK). Membranes were kept overnight in 3% BSA/Tris-buffered saline-0.5% Tween 20 (TBST-T) at 4°C to block nonspecific binding. Membranes were incubated overnight at 4°C with rabbit polyclonal antibody raised against a peptide mapping at the carboxy terminus of nNOS (Santa Cruz Biotechnology, Santa Cruz, CA) and with a rabbit polyclonal antibody raised against a synthetic peptide corresponding to the carboxy terminus of the NMDA-NR1 subunit (Chemicon, Temecula, CA). Antibodies were diluted 1:2,000 for nNOS and 1:500 for NMDA-NR1 in 1% BSA/TBS-T. Membranes were washed with TBST-T and incubated for 2 h at room temperature with anti-rabbit IgG antibody-horseradish peroxidase conjugate (Santa Cruz Biotechnology) diluted 1:2,000 in 1% BSA/TBS-T. Immunodetection was accomplished using ECL Western blotting kit detection (Santa Cruz Biotechnology). Membranes were then probed with anti-β-actin (Santa Cruz Biotechnology) as loading control, and mouse brain extract (Santa Cruz Biotechnology) was used as positive control for nNOS and NMDA-NR1 proteins.

To compare nNOS and NMDA-NR1 expression between both groups studied, equal amounts of protein from animals of the same group were loaded on to the same gel, and Western blotting was carried out as described above. Densitometric scanning was used to attain proteins quantification per sample. The density of each sample was referred to the density of the corresponding β-actin sample. Data were then expressed as the ratio of the control and experimental density to that of the β-actin density.

**Total RNA Isolation and cDNA Synthesis**

Total RNA was isolated from the medulla of mice from different groups using TRI-Reagent (Euromedex) and digested with RNase-free DNase I (Invitrogen) for 1 h 30 min at room temperature to remove any contaminating genomic DNA. cDNA synthesis was performed with the SuperScript III First-Strand Synthesis System for RT-PCR (Invitrogen) using 5 μg total RNA/20 μl cDNA synthesis reactions [50 μM oligo(dT)20, 10 mM DNTP mix]. The reactions were incubated for 5 min at 65°C and then placed on ice for at least 1 min followed by 50 min at 50°C after addition of 1× RT buffer, 25 mM
MgCl2, 0.1 M DTT, 40 U/μl of RNaseOUT, and 200 U/μl of SuperScript III RT. The reactions were finished at 85°C for 5 min, chilled on ice, and stored at −20°C. Before usage, the cDNA was diluted in water.

**Real-Time RT-PCR**

The LightCycler FastStart DNA Master SYBR Green I (Roche Biochemicals, Stockholm, Sweden) was used for quantitative analyses of the generated cDNA. The cDNA sequences for the genes of interest were obtained from GenBank, and the primer sequences were designed as described in Table 1. PCR amplifications were performed in a total volume of 20 μl, containing 5 μl cDNA sample, 4 μl LightCycler-FastStart DNA Master SYBR Green I, 0.5 μM of each primer, and 2.5 mM MgCl2. For each reaction, the polymerase was activated by a preincubation at 95°C for 10 min, and amplification was then performed at 45 cycles of switching between 95°C for 10 s, 62°C for 5 s for NR1 gene, 63°C for 5 s for nNOS gene, and 72°C for 8 s followed by melting point analysis from 65 to 95°C. The results were represented as threshold cycle (Ct) values. Control cDNA from cortex of normoxic wild-type mouse was diluted and run in the PCR amplification to create standard curves by plotting Ct values vs. the dilution of the cDNA templates. Relative amounts of mRNA normalized by β-actin were calculated from Ct numbers according to the manufacturer’s description (Roche Biochemicals).

**Calculation of Relative Quantification Values**

The relative quantification values were calculated according to the manufacturer’s description (Roche Biochemicals). Cp represents the PCR cycle at which an increase in fluorescence above a baseline signal can be detected. A Cp value was used to calculate the amount of PCR product compared with the internal control, β-actin. The Cp value for actin was subtracted from the NMDA-NR1 or nNOS Cp value to calculate the mean change in Cp in each experiment group.

**Nitrate and Nitrite Measurements**

Due to short NO half-life, quantification of NO production was determined by measuring NO oxidation end products, nitrate (NO3−) and nitrite (NO2−). Since the relative proportion of each metabolite may vary, the index of total NO production is best assessed by their sum. Hence the NOx (NO3− plus NO2−) method was used via the Griess reaction. Briefly, samples were homogenized in 4 vol (wt/vol) of PBS (pH 7.6) at 4°C. Homogenates were then sonicated and ultracentrifuged at 100,000 rpm for 60 min at 4°C. NOx determination was carried out in supernatants. The nitrate reductase reaction was first employed to convert nitrate to nitrite followed by Griess reaction to measure metabolites through photometric absorbance using an ELISA plate reader (nitrate/nitrite colorimetric assay kit, Cayman Chemical, MI).

**Statistical Analysis**

The effects of acclimatization, environment (21%, 8%), and SMTC were evaluated using analysis of variance with repeated measures (ANOVA-MANOVA). Newman-Keuls test was used for post hoc test. All statistical analysis were done using the Statistica software (StatSoft, Tulsa, OK). A Student’s t-test was used to assess statistical significance between normoxic (NX) and chronically hypoxic mice (CHX) for mRNA, protein, and NOx. A P value <0.05 was considered as a significant difference.

**RESULTS**

**Ventilatory Response to Normoxia (FiO2 = 0.21) and Acute Hypoxia (FiO2 = 0.08)**

**Effect of chronic hypoxia before SMTC treatment.** Acute exposure to hypoxia induced an increase in ventilation in control mice (Fig. 1A) through an increase in VT (Fig. 1B).

Chronic hypoxia caused a significant increase in ventilation in both normoxia and acute hypoxia (Fig. 1A, P < 0.001), mainly due to an increase in VT (Fig. 1B, P < 0.01). Respiratory frequency (fR) was significantly increased with acclimatization and decrease in FiO2 (Fig. 1C, P < 0.01).

Chronic hypoxia significantly increased the magnitude of the polikilocapnic acute HVR (Fig. 2A, P < 0.001) essentially through an increase in VT (Fig. 2B).

**Effect of chronic hypoxia after SMTC treatment.** The ventilation of the control nonacclimatized mice was not changed by SMTC in both normoxia and acute hypoxia (Fig. 1A).

The injection of SMTC induced a decrease in ventilation (Fig. 1A, P < 0.001) in mice acclimatized to chronic hypoxia in normoxia as well as in acute hypoxia, through a decrease in VT (P < 0.001) (Fig. 1B). The normoxic value in acclimatized mice was then not different compared with control mice in normoxia but without reaching the baseline normoxic ventilation or acute HVR observed in control mice.

The magnitude of the acute HVR was similar in both groups following SMTC injection (Fig. 2A). However, fR tended to decrease in control mice after SMTC injection (Fig. 2C, P = 0.06).

**nNOS and NMDA Western Blot**

Western blot analysis of denatured homogenates from medulla samples of all groups of mice revealed that both regions contain a 155- to 160-kDa and a 120-kDa protein band detected, respectively, by nNOS and NMDA antibodies (Fig. 3). nNOS and NMDA bands disappeared on incubation of membranes with the corresponding blocking peptide.

**Table 1. Primers sequences of genes studied**

<table>
<thead>
<tr>
<th>Name</th>
<th>Sequence (5’→3’)</th>
<th>Amplicon Length, bp</th>
<th>GenBank Accession No.</th>
<th>Position of Primer Sequence</th>
</tr>
</thead>
<tbody>
<tr>
<td>nNOS RV</td>
<td>TGAGGGTGAGCCCAAAAAAGAGG</td>
<td>90</td>
<td>NM_008712</td>
<td>4296</td>
</tr>
<tr>
<td>nNOS FW</td>
<td>GGGAAACTCTGGGAGGAGGA</td>
<td>125</td>
<td>NM_008712</td>
<td>4260</td>
</tr>
<tr>
<td>NMDA-NR1 RV</td>
<td>GTAGAGGCGCGATCATCTTCA</td>
<td>186</td>
<td>NM_008169</td>
<td>567</td>
</tr>
<tr>
<td>NMDA-NR1 FW</td>
<td>ACTCCCAACGGACACTTTCAC</td>
<td>186</td>
<td>NM_008712</td>
<td>382</td>
</tr>
<tr>
<td>β-Actin RV</td>
<td>CAATAGTGGATGACCTGGCGCT</td>
<td>138</td>
<td>NM_007393</td>
<td>831</td>
</tr>
<tr>
<td>β-Actin FW</td>
<td>AGAGGAAATATGGCGCTGAC</td>
<td>138</td>
<td>NM_007393</td>
<td>693</td>
</tr>
</tbody>
</table>

RV and FW, reverse and forward primers, respectively; nNOS, neuronal nitric oxide synthase; NMDA-NR1, N-methyl-d-aspartate receptor NR1.
Effect of Hypoxia on NO₃, nNOS, and NMDA Receptor Expression in Medulla

The injection of a nNOS inhibitor caused a decrease in ventilation secondary to a fall in tidal volume in acclimatized mice. The magnitude of the acute hypoxic ventilatory response was not affected by SMTC in both acclimatized and nonacclimatized mice. While VAH is characterized by an increase in resting normoxic ventilation and an enhancement of acute HVR, our study provides evidence that nNOS is only involved in normoxic ventilation, mainly by increasing VT. It seems then reasonable to think that NMDA and nNOS modulate differently the central nervous system plasticity involved in VAH.

Critique of the Methods

We injected the SMTC (nNOS inhibitor) intraperitoneally, which could affect both carotid body and medulla. It has been previously shown that NO could mediate chemoreceptor inhibition in the carotid body, leading to a decrease in ventilation...
However, the administration of the selective nNOS inhibitor to the cat carotid body did not increase the carotid body chemosensory response to hypoxia (34). Therefore, the decrease in ventilation observed after acclimatization to hypoxia in mice treated with a nNOS inhibitor would reflect a central effect of nNOS. nNOS inhibition led to a fall in ventilation in both normoxia and hypoxia, suggesting that nNOS contributed to VAH. The increase in arterial blood pressure following SMTC injection could reduce ventilation through a baroreceptor activation. However, arterial blood pressure has been shown to return to basal values 20 min after SMTC injection in rats (10). Since the whole medulla was removed in this study, we cannot localize which type of region is involved in VAH. Moreover, the intraperitoneal injection of SMTC could affect different regions in the brain, but these results bring evidence that nNOS is involved in VAH.

Oxygen consumption was not measured in this study since experiments have shown that NOS inhibition does not affect body metabolism (7, 14, 16).

**Role of NMDA Receptors on HVR After Chronic Hypoxia**

It is clearly demonstrated that the increase in the afferent input from the carotid body into the NTS results in an activation of NMDA receptors participating to the early ventilatory response to hypoxia (20, 21). Recently, Reid and Powell (30) demonstrated that systemic NMDA receptor blockade (MK-801) eliminates HVR in normoxic and chronically hypoxic rats. Othake et al. (21) have shown that NMDA receptor protein increased in the NTS in adult rat, which is in agreement with a role of NMDA receptor in HVR in normoxic rats. We did not perform experiments to confirm these data, but the activation of NMDA receptor, enhancing the early phase of HVR, seems not to be due to nNOS in our study, which is consistent with other studies (10). Our result using SMTC did not result in a decrease in ventilation in control mice exposed to acute hypoxia, suggesting that nNOS is not involved in the early phase of HVR in our model.

Reid and Powell (30) also observed a greater effect of MK-801 on ventilation primarily due to a decrease in VT after chronic hypoxia. The rise in NMDA receptor in chronically hypoxic mice could explain this larger response to NMDA receptor blockade. Taken together, these results imply that NMDA receptors are involved in HVR after chronic hypoxia and then support the idea of NMDA receptor plasticity with chronic hypoxia.

Reeves et al. (29) have studied the time course of NMDA receptor expression (subunit NR1) in the dorsocaudal brain stem in rats during 30 days of chronic exposure to hypoxia (10% O2). They showed a rapid increase of these receptors at the beginning of exposure to hypoxia which returned to the basal level after 3 days without modifications until day 30. These results disagree with an implication of these receptors in the dorsocaudal brain stem after chronic hypoxia. We used a

![Fig. 3. Representative Western blot of N-methyl-D-aspartate (NMDA), nNOS, and β-actin.](image)

![Fig. 4. Expression of mRNA (A) and protein (B) of NMDA receptors (NMDA-NR1) in the medulla in NX and CHX mice. Values are means ± SE. *(P < 0.001, CHX vs. NX.)*](image)
model of mice exposed for 14 days to hypoxia (12% O2), which may explain the difference in NMDA-NR1 expression. From our knowledge, no study has evaluated the expression of these receptors in mice; it is therefore probable that a different regulation occurs between species, rats and mice. Moreover, we measured the NMDA receptor expression in the whole medulla. Nevertheless, our results suggest that, in chronically hypoxic mice, the increase in NMDA receptor could participate in the increase in the responsiveness of the central nervous system to the afferent input from the carotid body (26).

Role of NO and nNOS in the Medulla After Chronic Hypoxia

It has been shown that NO enhances the excitability and spontaneous discharge rates of neurons within the NTS, leading to an increase in ventilation (10, 19, 33). In the carotid bodies, recent evidence suggests that NO production derived from inducible NOS blunts the hypoxic chemosensitivity during chronic hypoxia (37).

While Gozal et al. (10) have shown that nNOS was involved in hyperventilation following acute hypoxia, our results suggest that nNOS is implied in normoxic ventilation in chronically hypoxic mice. Some experiments have supported a central excitatory role of NO during hypoxia since significant ventilatory depression occurred in animals after NOS inhibition (10, 19, 27, 28). Kline et al. (16) have shown a decrease in HVR in mice lacking nNOS protein compared with wild-type control. This effect was partly attributed to an enhancement of peripheral chemosensory responsiveness. We have shown that, in acclimatized mice, SMTC decreased normoxic ventilation but did not affect the magnitude of the acute hypoxic response. Moreover, the increase in nNOS mRNA as well as protein in the whole medulla is consistent with a role of NO in VAH. The difference observed between our study and Kline et al. (16) could reflect a different role of nNOS on HVR with acclimatization to hypoxia.

NO has been shown to exert positive feedback on glutamate release in the NTS to augment HVR in rats (19). While we hypothesized that nNOS would be involved in the increase in HVR as well as NMDA NR1 receptor, our results do not support this idea. Indeed, MK-801 blocked HVR (30) while SMTC affected normoxic ventilation in acclimatized mice. These data suggested that two mechanisms take place after chronic hypoxia to contribute to VAH: one involving NMDA receptors to increase the responsiveness of afferent input from the carotid body and the other directly related to changes in the medulla, increasing normoxic ventilation. Nevertheless, both participate to the central nervous system plasticity to contribute to VAH.

When SMTC was injected in chronically hypoxic mice, normoxic ventilation decreased secondary to a fall in VT. These data support the idea that the increase in nNOS expression leading to a rise in NO modulates the plasticity of the central nervous system after chronic hypoxia, acting on the ventilatory pattern.

As previously described, VAH is due to several other factors which contribute in concert to the increase in ventilation after chronic hypoxia (1, 12, 13). Kline et al. (15) have shown, in mice partially deficient for hypoxia-inducible factor (HIF)-1α, HIF-1α was required to enhance VAH. Tyrosine hydroxylase activity was correlated with ventilation in the NTS (31), and more recently, it was shown that mice lacking the fos B gene did not develop VAH (18).

In conclusion, our results support the hypothesis that the increase in NMDA-NR1 receptors acts on VAH through the increase in the responsiveness of the central nervous system to the afferent input that it receives from carotid body.

The injection of a nNOS inhibitor caused a decrease in normoxic ventilation secondary to a fall in tidal volume in acclimatized mice while the magnitude of the acute hypoxic ventilatory response was not affected. Our study provides evidence that nNOS is only involved in normoxic ventilation, mainly by increasing VT, but the region of the respiratory centers involved remains to be determined. It seemed then reasonable to think that NMDA and nNOS modulate differently the central nervous system plasticity involved in VAH.
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