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ORIGINAL ARTICLE

Effect of Interaction between Adenosine and Nitric Oxide on Central Nervous System Oxygen Toxicity

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Abstract

The metabolism of adenosine (ADO) and nitric oxide (NO) in brain tissues is closely associated with the change of oxygen content. They have contrary effects in the onset of hyperbaric oxygen (HBO)-induced central nervous system oxygen toxicity (CNS OT): ADO can suppress the onset, while NO promotes it. We adopted the ADO-augmenting measure and NO-inhibiting measure in this study and found the combined use had a far superior preventive and therapeutic effect in protecting against CNS OT compared with the use of either measure alone. So we hypothesized that there is an interaction between ADO and NO which has an important impact on the onset of CNS OT. On this basis, we administered ADO-augmenting or ADO-inhibiting drugs to rats. After exposure to HBO, the onset of CNS OT was evaluated, followed by the measurement of NO content in brain tissues. In another experiment, rats were administered NO-augmenting or NO-inhibiting drugs. After exposure to HBO, the onset of CNS OT was evaluated, followed by measurement of the activities of ADO metabolism-related enzymes in brain tissues. The results showed that, following ADO augmentation, the content of NO and its metabolite was significantly reduced, and the onset of CNS OT significantly improved. After ADO inhibition, just the opposite was observed. NO promotion resulted in a decrease in the activity of ADO-producing enzyme, an increase in the activity of ADO-decomposing enzyme, and an aggravation in CNS OT. The above results were all reversed after an inhibition in NO content. Studies have shown that exposure to HBO has a significant impact on the content of ADO and NO in brain tissues as well as their biological effects, and ADO and NO might have an intense interaction, which might generate an important effect on the onset of CNS OT. The prophylaxis and treatment effects of CNS OT can be greatly enhanced by augmenting ADO and inhibiting NO.

Keywords Hyperbaric oxygen · Seizure · Convulsion · Superoxide · Peroxynitrite

Introduction

Hyperbaric oxygen (HBO) is widely applied in underwater missions, hyperbaric facility operation, and disease treatment. However, a person, if breathing in excessively high partial pressure oxygen for more than a certain period of time, will experience a toxic reaction, namely oxygen toxicity (OT). OT is mainly characterized by the central nervous system (CNS) functional disorder called CNS OT when the oxygen partial pressure is greater than 3 atm absolute (ATA); the most typical and most intense manifestation is grand mal seizure, i.e., oxygen convulsion, which was initially identified by Paul Bert in 1878 (Wingelaar et al. 2017; Zhu et al. 2016). At present, the exact pathogenesis of CNS OT remains unrevealed and the control measures available, which are mainly the control of oxygen partial pressure and the shortening of oxygen inhalation duration, are also very limited, which, to a large extent, limits HBO application and improvement in operation efficiency.

Studies have shown that nitric oxide (NO) plays an important role in the pathogenesis of CNS OT in the following manner. On one hand, it can substantially increase the amount of oxygen supplied to the brain tissues through reversing the vasoconstrictive effect of HBO, thereby promoting the

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occurrence of OT (Bitterman and Bitterman 1998; Demchenko et al. 2001); on the other hand, it can rapidly bind to the superoxide anion ($O_2\cdot^-$) massively produced by exposure to HBO, generating peroxynitrite ($ONOO^-$) to further oxidize and nitrosylate substances such as protein and, in this way, the normal functions of receptors, ion channels, etc., are impaired, eventually causing neurotoxicity and resulting in convulsion seizure (Chavko et al. 2003; Demchenko et al. 2003). NO has also been shown by recent studies to play an important role in acute lung injury (ALI) caused by oxygen convulsion, further aggravating the consequences of CNS OT (Demchenko et al. 2012; Demchenko et al. 2011).

Adenosine (ADO) is an inhibitory substance with confirmed efficacy in the CNS. Through binding to the A1 receptor on the presynaptic and postsynaptic membrane, it reduces the level of excitatory transmitters released by neurons and hyperpolarizes cells, thereby inhibiting the transmission of neuronal excitability (Boison 2013a; Boison et al. 2013). In a variety of animal epileptic models induced by different predisposing causes, seizures can be effectively suppressed by ADO augmenting (Boison 2016a). HBO exposure has been shown by our previous studies to have a significant impact on the metabolism of ADO in brain tissues; in addition, ADO also has an important effect on the occurrence of oxygen convulsion. The onset of oxygen convulsion can be significantly delayed, and the severity can be relieved, by increasing ADO content in the brain tissue or activating ADO A1 receptor (Chen et al. 2016).

It is apparent that NO and ADO are two substances with opposite effects for CNS OT; NO has a promoting effect, and ADO shows an inhibitory effect. We propose a hypothesis that inhibiting NO and augmenting ADO during the prophylaxis and treatment of CNS OT may achieve better efficacy. In this study, we investigated the above hypothesis. We examined the efficacy of the combined use of the aforementioned measures for the prophylaxis and treatment of CNS OT, and further explored the possibility of an interaction between NO and ADO during the disease occurrence as well as the effect of this interaction on the onset of CNS OT.

Materials and Methods

Animals

All procedures were performed in accordance with the Second Military Medical University (SMMU) Guide for the care and use of laboratory animals, and approved by the ethics committee for Animal Experiments of SMMU.

Adult male SD rats weighing 250 g ± 10 were used. The breeding environment was maintained at a temperature of 22 ± 1 °C and a humidity of 40–50%, and daylight hours were set to 12 per day. Animals were allowed free access to food and drinking water.

Drugs

ADO, 2-chloro-N6-cyclopentyla-denosine (CCPA), 1, 3-dipropyl-8-cyclopentylxanthine (DPCPX), 5-ITU, 3-morpholinosydnonimine (SIN-1), and 7-nitroindazole (7-NI) were purchased from Tocris Bioscience; sodium nitroprusside (SNP) was purchased from Sigma-Aldrich. ADO, CCPA, and SNP were soluble in normal saline; DPCPX and 5'-ITU were soluble in 2% DMSO; 7-NI was soluble in peanut oil; SIN-1 was soluble in PBS.

HBO Exposure and CNS OT Severity Evaluation

The experiment was composed of three parts:

I. Effect of ADO combined with 7-NI on the latency to the onset of seizure: Rats were divided into 6 groups, with 6 rats in each: (1) saline control group, (2) ADO (40 μg) group, (3) 7-NI (10 μg) group, (4) ADO (40 μg) + 7-NI (10 μg) group, (5) ADO (160 μg) group, and (6) 7-NI (50 μg) group. After anesthesia, the rats were fixed on a stereotaxic instrument for the brain, on which the skull was exposed. An injection cannula was inserted and sat 5 mm within the lateral ventricle after the perforation at a site 2.0 mm posterior to the bregma and 1.5 mm to the right. The cannula was fixed with adhesive onto the skull. The scalp was sutured, followed by the wound disinfection and processing, and they were fed for 3 days for subsequent tests. At 20 min before HBO exposure, the rats were anesthetized with ether, and the above drugs were injected through a preset lateral ventricle cannula at an injection volume of 40 μl. After the rats were awakened from anesthesia, they were placed in a hyperbaric chamber for animal experiments at one rat per time, followed by flushing the internal air with pure oxygen. When the oxygen concentration in the chamber reached 99% or higher, it was pressurized at a speed of 1 ATA/min to 6 ATA, and then the pressure was maintained stable. The rats' behavioral changes were closely observed till the occurrence of generalized epileptic seizures of tonic-clonic type (grand mal seizures). At this moment, the duration from the point of reaching 6 ATA to the grand mal seizure was recorded as the latency to onset of oxygen convulsion.
II. Effect of drugs on the severity of CNS OT: Rats were divided into 9 groups, with 20 rats in each: (1) saline control group; (2) ADO (160 μg) group; (3) DPCPX (60 μg) group; (4) CCPA (20 μg) group; (5) 5-ITU (40 μg) group; (6) ADO (40 μg) + 7-NI (10 μg) group, in which the drugs were injected into the lateral ventricle with the method the same as before; (7) SNP (10 mg/kg) group; (8) SIN-1 (5 mg/kg) group; and (9) 7-NI (25 mg/kg) group, in which the drugs were administered via intraperitoneal injection. The drugs were administered

at 20 min before HBO exposure. Rats were exposed to HBO at 6 ATA for 60 min. Then, the rats were removed from the chambers and changes in their behavior were observed, such as convulsion, oral and nasal bleeding, coma, and death, followed by the recording of the number of deaths in each group and the calculation of mortality.

III. Effect of ADO combined with 7-NI on ALI caused by oxygen convulsion: Rats were divided into 5 groups, with 8 in each: (1) normal group (without exposure to HBO), (2) saline control group, (3) ADO (160 μg) group, (4) 7-NI (50 μg) group, and (5) ADO (40 μg) + 7-NI (10 μg) group, in which the drugs were administered by injection into the lateral ventricle. Following drug administration, they were continuously exposed to HBO at 6 ATA for 30 min. After being removed from the chambers, the rats were anesthetized with pentobarbital and immediately sacrificed by cervical dislocation. The lungs were exposed to observe any pulmonary hemorrhage, followed by photography for recording. One lung was sampled, and the fluid left by alveolar lavage was taken, followed by the determination of protein content (mg/ml). The other lung was sampled and divided into two parts, one for tissue water content detection and the other for histopathology examination. The histopathology result was evaluated by a histopathology expert who was "blinded" to the experimental grouping.

Assay of NO and Nitrotyrosine in Brain Tissues

Rats were divided into 6 groups: solvent control (1) normal saline, (2) 2% DMSO) group, (3) ADO (160 μg) group, (4) CCPA (20 μg) group, (5) DPCPX (60 μg) group, and (6) 5-ITU (40 μg) group. Before the exposure to HBO, the rats were injected with various control solvents or drugs via a preset cannula inserted into the lateral ventricle, and then exposed to HBO at 6 ATA for 30 min. In the end, the rats were anesthetized with pentobarbital and immediately sacrificed by decapitation. The entire cerebral cortex was harvested. For NO assay, the cortex was homogenized in iced lysis buffer in the ratio of 1 to 10 (wt/vol). Then, the homogenate was centrifuged at 12,000*g* for 5 min at 4 °C. For nitrotyrosine assay, the cortex was homogenized in iced PBS in the ratio of 1 to 9 (wt/vol). Then, the homogenate was further processed with two freezing and thawing cycles (being frozen at − 80 °C for 15 min and then thawed at 37 °C for 5 min). The homogenate was then centrifuged at 3000*g* for 20 min at 4 °C. The contents of NO and nitrotyrosine in the supernatant were assayed according to the operating instructions of the NO assay kit (Beyotime Biotechnology, China) and nitrotyrosine assay kit (Shanghai Enzyme-linked Biotechnology Co, China).

The NO assay was performed using the Griess Reagent colorimetric method, and the final result was expressed as the amount of NO contained per unit weight of tissue (μM/g). The nitrotyrosine assay was performed by enzyme-linked immunosorbent assay (ELISA), and the final result was expressed as the amount of nitrotyrosine per unit weight of tissue (ng/mg).

Detection of Enzyme Activity Related with ADO Metabolism in Brain Tissues

Rats were divided into 6 groups: solvent control (1) salt saline, (2) PBS, (3) peanut oil) group, (4) SNP (10 mg/kg) group, (5) SIN-1 (5 mg/kg) group, and (6) 7-NI (25 mg/kg) group. Before HBO exposure, the rats were administered intraperitoneally various control solvents or drugs. Following exposure to HBO at 6 ATA for 30 min, the rats were anesthetized with pentobarbital and immediately sacrificed by decapitation. The entire cerebral cortex was harvested. The sample processing method was the same as that of nitrotyrosine assay. The activity of adenosine kinase (ADK), 5′-nucleotidase (5'-NT), and S-adenosylhomocysteine hydrolase (SAHH) was determinate according to the operation instructions of the enzyme activity assay kit (Shanghai Enzyme-linked Biotechnology Co., Ltd., China).

Enzyme activity was measured by ELISA. A standard curve was prepared using an enzyme standard with known activity, and the results of the determined sample were compared with a standard curve to obtain the corresponding enzyme activity. Enzyme activity was expressed as the unit of activity of the enzyme contained per unit tissue weight (U/g).

Statistical Analysis

The data were expressed as mean ± standard deviation ($\bar{x} \pm s$) and statistically analyzed by the SPSS 17.0 software package; the ANOVA was used for the intergroup comparison, and the SNK q test was used for the analysis of variance. A P value of less than 0.05 indicated the presence of a statistically significant difference.

Results

Effect of ADO Combined with 7-NI on Convulsion Latent Period

The results showed that the injection of 40 μg ADO into the lateral ventricle had no significant effect on the convulsion latent period, but 160 μg ADO significantly prolonged the latent period; the injection of 10 and 50 μg 7-NI significantly prolonged the latent period, and the effect of 50 μg was more pronounced. The combination of 40 μg ADO and 10 μg 7-NI could significantly extend the latent period, and the effect was

even significantly superior to that using 160 μg ADO or 50 μg 7-NI alone (Fig. 1).

The above experimental results suggested that NO could inhibit the anti-oxygen-convulsion effect of ADO to a large extent; the anti-convulsion ability of ADO could be fully released through the moderate reduction of NO production, indicating that NO impacted the efficacy of ADO in the CNS. In addition, the inherent toxicity of NO was also significantly involved in the occurrence of oxygen convulsion, and the convulsion latent period could be significantly prolonged by inhibiting its production (using 10 μg or 50 μg 7-NI). Furthermore, ADO had a significant effect on NO toxicity; increasing ADO content in brain tissues could significantly enhance the effect of NO inhibition and extend the convulsion latent period.

Effect of ADO Combined with 7-NI on Mortality Caused by CNS OT in Rats

The CNS OT caused by HBO exposure at 6 ATA for 60 min had a certain lethal effect on rats. In the study, we compared the effects of the following drugs on the lethal effect, including ADO, ADO A1 receptor agonist CCPA, antagonist DPCPX, ADK antagonist 5-ITU, NO donor SNP, $ONOO^-$ donor SIN-1, nNOS inhibitor 7-NI, and ADO combined with 7-NI. The results showed that ADO combined with 7-NI significantly reduced the OT-caused mortality compared with that in the solvent control group (5 vs. 55%). It was also significantly superior to other drugs with similar anti-oxygen-toxicity effects in terms of the mortality (35% for ADO, 20% for CCPA, 25% for 5-ITU, and 25% for 7-NI). Some drugs with OT-promoting effects significantly increased mortality (75% for DPCPX, 85% for SNP, and 75% for SIN-1) (Fig. 2).

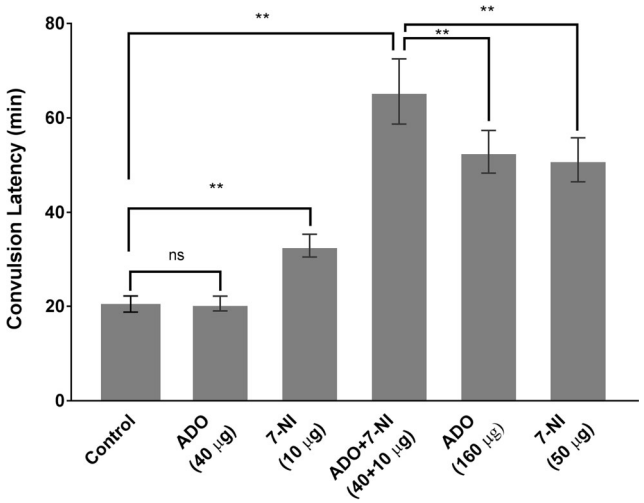

Fig. 1 Effect of ADO and nNOS inhibitor on convulsion latent period in rats. $^{**}P < 0.01$, $^{*}P < 0.05$. *ns* no statistical significance. $n = 10$

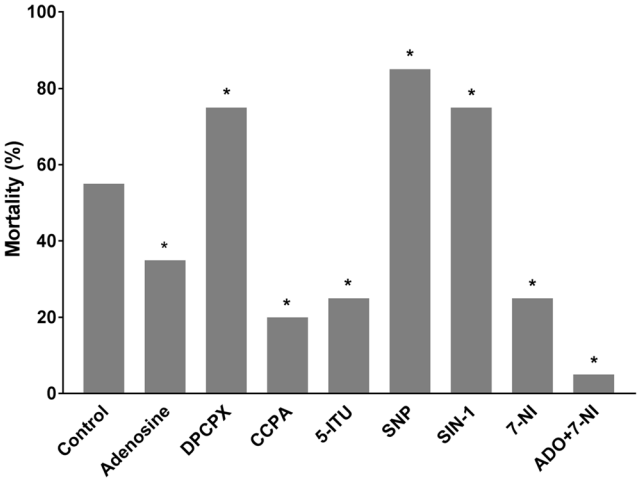

Fig. 2 Effect of different drugs on mortality caused by CNS OT in rats. A chi-square test was used for comparing two independent proportions. $^{*}P < 0.05$ vs. control group. $n = 20$

Effect of ADO Combined with 7-NI on ALI Induced by OT Onset in Rats

CNS OT caused ALI when it occurred. The macroscopical findings of the lungs showed the presence of severe blood spots on the pulmonary surface. Following the administration of 160 μg ADO, 50 μg 7-NI, and 40 μg ADO combined with 10 μg 7-NI, the surface bleeding of the lungs significantly decreased, and the improvement effect of ADO combined with 7-NI was more pronounced (Fig. 3a).

Histopathological results showed that following the occurrence of ALI caused by CNS OT, the alveolar wall became severely thickened; some alveolar walls were fused or ruptured, with massive hemorrhage, edema, and protein exudation in the alveolar space; and relatively apparent inflammatory cell infiltration was observed. Following the administration of 160 μg ADO, 50 μg 7-NI, and 40 μg ADO combined with 10 μg 7-NI, significant improvement was achieved as follows: the alveolar wall was only slightly thickened; the bleeding volume, edema, and inflammatory cell infiltration in the alveolar cavity significantly decreased; and the improvement effect of ADO combined with 7-NI was more significant (Fig. 3b).

The results of the protein assay in alveolar lavage fluid (ALF) showed that the control group had a significantly higher protein content than the normal group; the combined administration of 160 μg ADO, 50 μg 7-NI, and 40 μg ADO with 10 μg 7-NI could significantly reduce the protein content in the ALF; and the combined administration had an effect significantly superior to that of the single administration (Fig. 4a).

The results of water content detection in pulmonary tissues showed that the control group had more water in lung tissues compared with the normal group, indicating that edema occurred. The administration of 160 μg ADO, 50 μg 7-NI, and

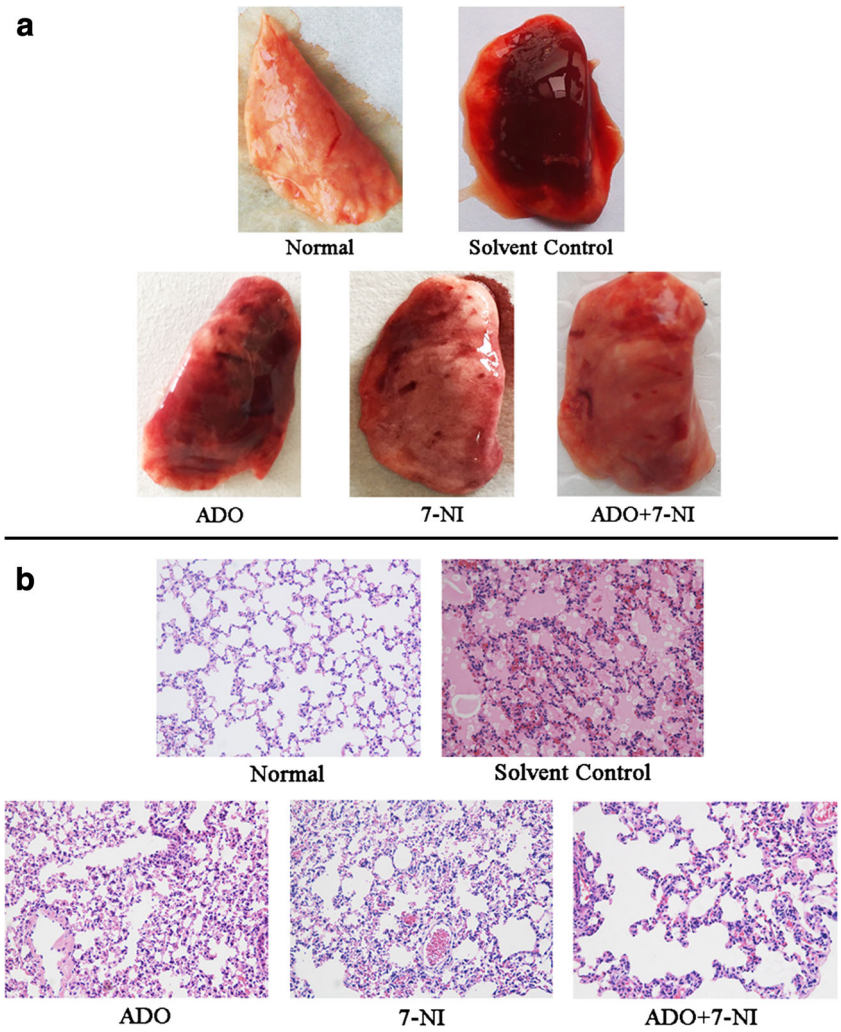

Fig. 3 Effects of ADO combined with 7-NI on surface bleeding (**a**) and histopathology (**b**) of rat's lung induced by OT onset. The quantity of lung used for histopathologic examination was six in each group

40 μg ADO combined with 10 μg 7-NI could significantly reduce the water content in lung tissues, and the effect of combined administration was more pronounced, so pulmonary edema could be relieved more effectively (Fig. 4b).

After taking the above research results into consideration, we concluded that ADO combined with 7-NI could more effectively ameliorate ALI caused by CNS OT onset, which proved in a more comprehensive manner that ADO combined with 7-NI substantially ameliorated the effects of CNS OT.

Effect of ADO Metabolism on NO Content in Brain Tissues

HBO exposure at 6 ATA for 30 min significantly increased NO content in the rats' cerebral cortex (Fig. 5a). ADO, ADK inhibitor 5-ITU, and ADO A1 receptor selective agonist CCPA significantly inhibited an increase in NO content (Fig. 5b, c, d). On the contrary, ADO A1 receptor selective inhibitor DPCPX promoted an elevation in NO content (Fig. 5e).

The aforementioned results indicated that increasing ADO content in brain tissues or activating the A1 receptor can inhibit excessive NO production, while the inhibition of the ADO A1 receptor promoted NO production.

Effect of ADO Metabolism on Nitrotyrosine Content in Brain Tissues

The effect of ADO metabolism on nitrotyrosine content in brain tissues was consistent with that of NO content. The HBO exposure at 6 ATA for 30 min resulted in a significant increase in nitrotyrosine content (Fig. 6a). ADO, 5-ITU, and CCPA both significantly inhibited their elevated levels (Fig. 6b, c, d), while DPCPX promoted elevated level (Fig. 6e).

As a metabolite of $ONOO^-$, nitrotyrosine is indicative of the $ONOO^-$ content in tissues. $ONOO^-$ is a product of the rapid binding between $O_2\cdot^-$ and NO; due to an association with NO content, its content is indirectly

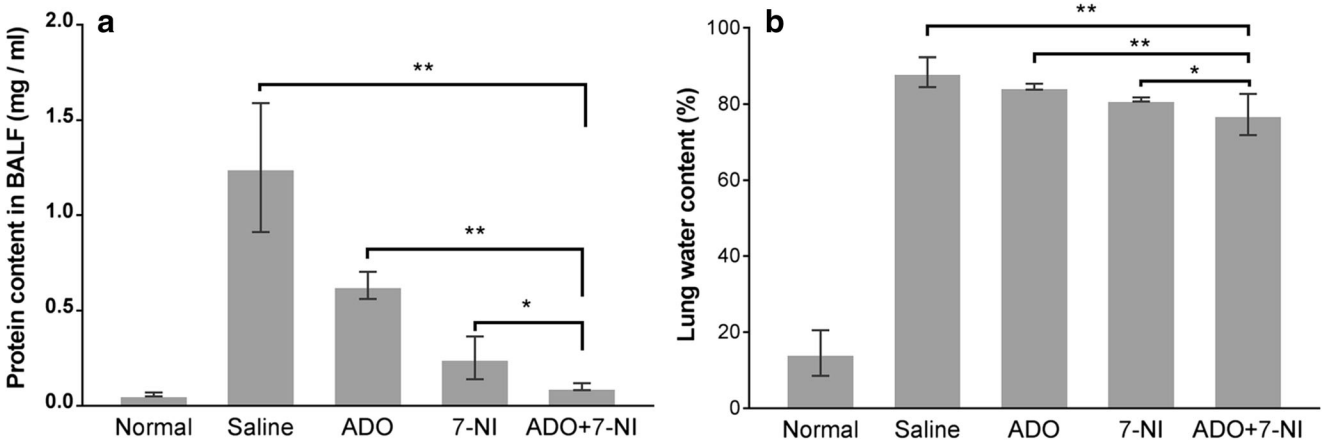

Fig. 4 Effects of ADO combined with 7-NI on protein effusion (**a**) and edema (**b**) of rat's lung induced by OT onset. $^{**}P < 0.01$, $^{*}P < 0.05$. $n = 6$

indicative of the changes in NO content. The aforementioned experimental results indicated that increasing ADO content in brain tissues or exciting the A1 receptor inhibited the increase of nitrotyrosine content caused by HBO exposure, while the inhibition of ADO A1 receptor promoted its production. The results provide further evidence that ADO metabolism in brain tissues has a significant effect on NO production.

Effects of SNP, SIN-1, and 7-NI on ADK, 5′-NT, and SAHH Activity

The experimental results showed that no matter whether the solvent was saline, PBS, or peanut oil, exposure to HBO at 6 ATA for 30 min could significantly reduce the activity of ADK, compared with the normal control group. Following the administration of NO donor SNP and $ONOO^-$ donor SIN-1 prior to exposure, ADK activity was significantly enhanced; the nNOS inhibitor 7-NI significantly inhibited the increase in ADK activity (Fig. 7a).

For 5'-NT, no matter whether the solvent was saline, PBS, or peanut oil, exposure to HBO at 6 ATA for 30 min could significantly elevate its activity, compared with the normal control group. However, the administration of NO donor SNP and $ONOO^-$ donor SIN-1 before exposure significantly reduced 5'-NT activity; the administration of the nNOS inhibitor 7-NI further augmented 5'-NT activity (Fig. 7b).

For SAHH, compared with the normal control group, no matter whether the solvent was saline, PBS, or peanut oil, exposure to HBO at 6 ATA for 30 min did not have a significant effect on its activity. However, the administration of NO donor SNP and $ONOO^-$ donor SIN-1 before exposure significantly reduced the SAHH activity; the administration of the nNOS inhibitor 7-NI significantly increased the SAHH activity (Fig. 7c).

Discussion

In this study, we confirmed two noteworthy findings: (1) In the course of oxygen convulsion generating, ADO and NO, two substances both have distinct impact on seizure, had intense interaction. ADO augmentation treatment could significantly reduce NO content and its metabolite. Increase of NO could influence the activity of ADO metabolism-related enzymes, and may further decrease ADO content. (2) Based on their interaction, we found combined use of ADO augment and NO inhibition measure could prevent against CNS OT more effectively, being superior to the use of either of the measures alone.

HBO exposure resulted in a significant increase in the content of NO and its metabolite nitrotyrosine in brain tissues. It could exert toxic effects on various kinds of intracellular and extracellular proteins, ion channels, and enzymes through intense oxidation and nitrification, eventually promoting and aggravating the onset of CNS OT (Allen et al. 2009; Chavko et al. 2003; Demchenko et al. 2003; Gasier et al. 2017). As a specific inhibitor of neuronal nitric oxide synthase (nNOS), 7-NI can effectively inhibit NO production in nerve tissues, and is a frequently used mechanism in studies on the regulation of the NO content in brain tissues. Studies have shown that 7-NI can effectively delay the onset of CNS OT due to reducing the NO content in brain tissues, inhibiting the cerebrovascular over vasodilation caused by continuous HBO exposure, and suppressing the NO-caused excessive increase of reactive nitrogen (Hagioka et al. 2005; Moskvin et al. 2003). As a specific inhibitor of adenosine kinase (ADK), 5-ITU can effectively inhibit ADK activity in brain astrocytes in converting ADO into adenine nucleotides, thereby inhibiting the transmission of ADO from extracellular to intracellular regions down the concentration gradient and further effectively increasing extracellular ADO content (Boison 2013b, 2016b). Our previous research has shown that 5-ITU can effectively delay the onset of oxygen convulsions (Chen et al. 2016). CCPA is an agonist of the ADO A1 receptor and DPCPX is

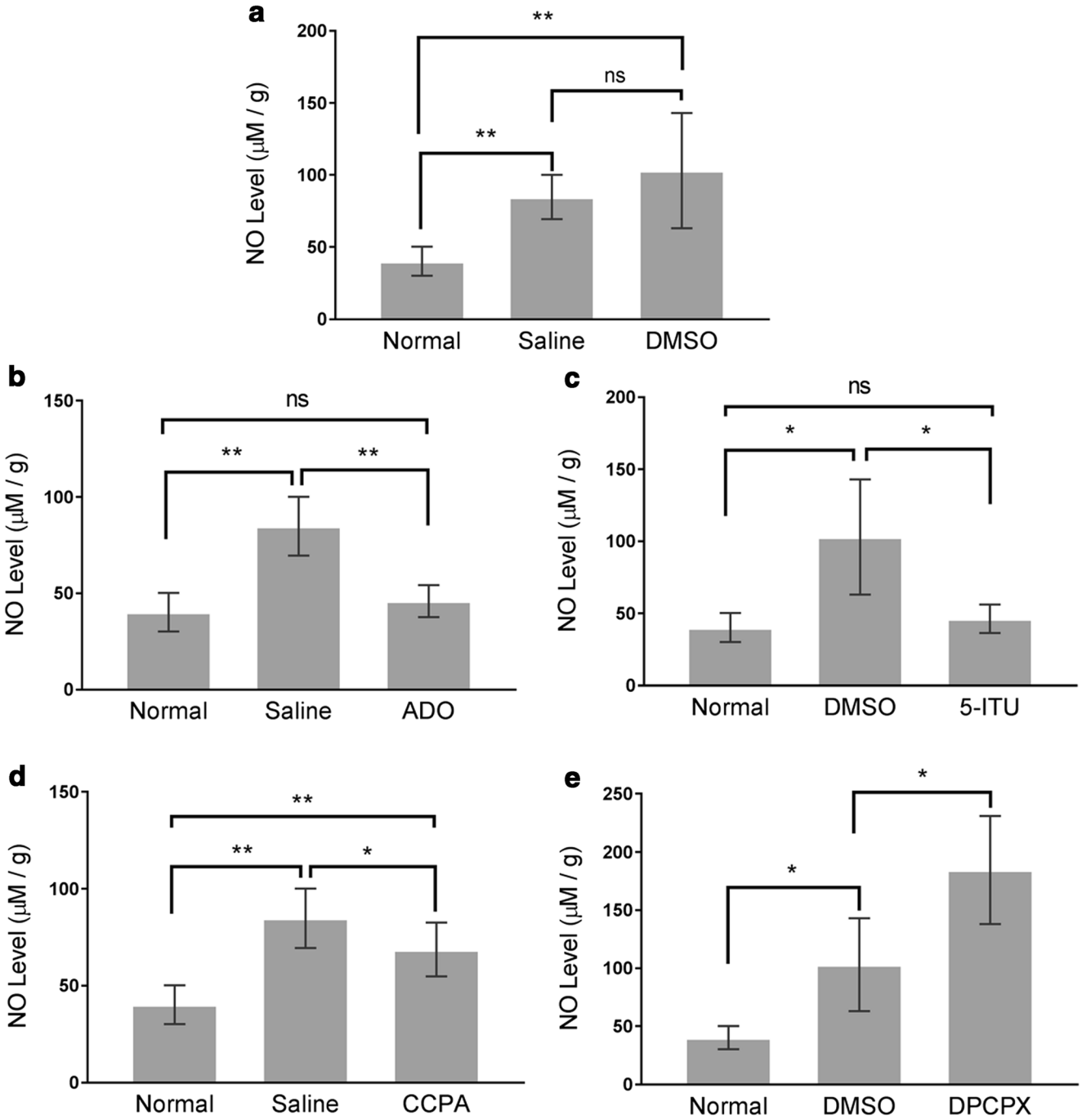

Fig. 5 Effects of ADO-augmenting measures and function inhibition on NO content in the rats' cerebral cortex during HBO exposure. **a** Solvent control, **b** ADO, **c** ADK inhibitor 5-ITU, **d** A1 receptor agonist CCPA, **e** A1 receptor inhibitor DPCPX. $^{**}P < 0.01$, $^{*}P < 0.05$, *ns* no significant difference. $n = 6$

its antagonist. Our previous studies have shown that CCPA can effectively delay the onset of oxygen convulsion by activating the A1 receptor, while DPCPX can promote the onset of oxygen convulsion by antagonizing the A1 receptor (Chen et al. 2016).

The onset of CNS OT can cause ALI through routes such as activating sympathetic nerves and leading to pulmonary vascular hypertension; ALI is mainly manifested as pulmonary hemorrhage and protein exudation etc., which can cause dyspnea, hypoxia, and even death within a short period of time (Demchenko et al. 2008, 2012, 2011). As a very important concomitant effect in the onset of CNS OT, ALI has become an important reference index for the investigation of the severity of CNS OT onset and the study of its pathogenesis and effectiveness of prophylaxis and treatment measures.

Based on our findings on protection against CNS OT, we can conclude that ADO combined with 7-NI can significantly delay the onset of oxygen convulsion, reduce mortality caused by CNS OT, and effectively reduce ALI manifestations such

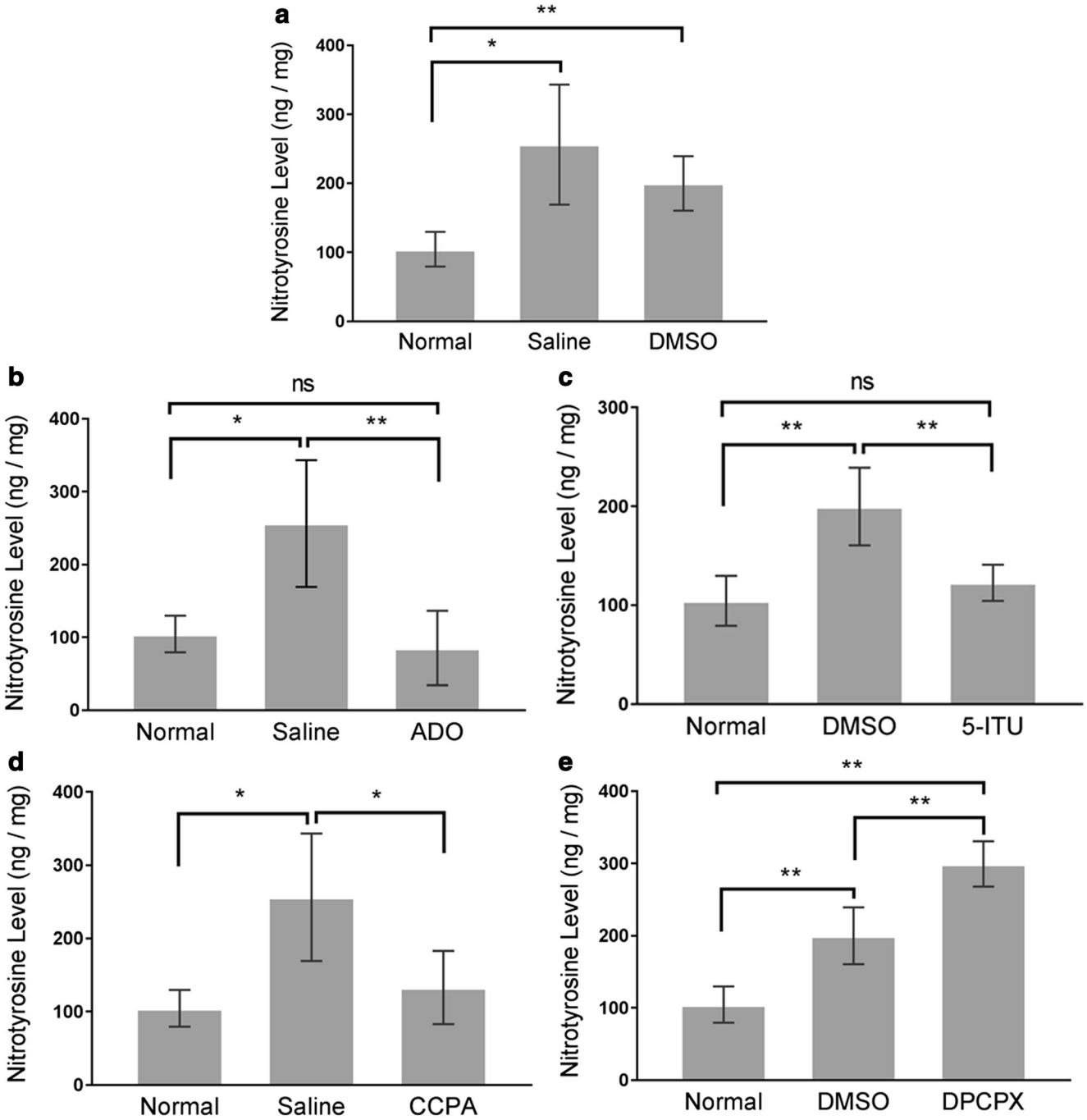

Fig. 6 Effects of ADO-augmenting measures and function inhibition on nitrotyrosine content in the rats' cerebral cortex during HBO exposure. **a** Solvent control, **b** ADO, **c** ADK inhibitor 5-ITU, **d** A1 receptor agonist CCPA, **e** A1 receptor inhibitor DPCPX. $^{**}P < 0.01$, $^{*}P < 0.05$, *ns* no significant difference. $n = 6$

as concomitant pulmonary hemorrhage, edema, protein exudation, and tissue injuries, indicating that the combined use of ADO and nNOS inhibitors has a confirmed and comprehensive protective effect on CNS OT.

Of note, the combined use of the above two measures is far more effective in preventing CNS OT than the use of either of the measures alone. Detailed explanations are as follows: (1) In case of simultaneously and substantially reducing the intensity (embodied as the dose) of the two interventional measures, the effect exerted by the combined use on the delay of oxygen convulsion onset was still superior to that exerted at the maximum interventional intensity (embodied as the highest dose) of either of the measures; when ADO had been reduced to the dose (40 μg) which turned out ineffective when used alone, the combined use with 7-NI could still achieve good prophylactic effects. (2) Likewise, in case of drastically reducing the doses of ADO and 7-NI, the combined use of the two measures could considerably reduce mortality caused by CNS OT, with an effect

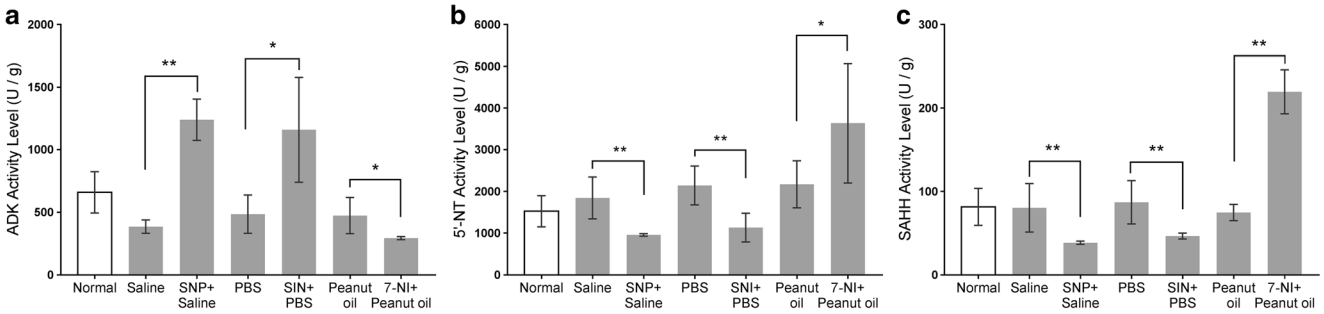

Fig. 7 Effects of SNP, SIN-1 and 7-NI on ADK (**a**), 5'-NT (**b**), and SAHH (**c**) activities in the rat's cerebral cortex during HBO exposure. $^{**}P < 0.01$, $^{*}P < 0.05$. $n = 6$

superior to various kinds of ADO-augmenting measures such as increasing the extracellular ADO content and activating the ADO A1 receptor, as well as NO-inhibiting measures. (3) In protection against ALI caused by CNS OT onset, the combined use also showed obvious superiority.

Therefore, a better prophylactic effect on CNS OT can be achieved by the combined use of ADO and nNOS inhibitors. In addition, combined use could greatly reduce the doses of the two drugs which, to a certain extent, should likely reduce the side effects caused by the use of either drug alone at an excessive dose. Indeed, ADO and 7-NI were all reported to have some side effects to the body. In CNS, ADO can function as an inhibitory neurotransmitter and may be used as a potential treatment for epilepsy. However, it cannot be given systemically because of its severe side effects such as decreased heart rate, blood pressure, and body temperature (Van Dycke et al. 2011). In addition, systemic application of the A1 receptor agonist can also lead to central sedative side effects (Guttinger et al. 2005). In our study, we also found that when ADO was injected intracerebroventricularly at large dosage (160 μg), rats manifested obvious sedation. 7-NI was reported to impair learning and memory functions, including disturbing spatial memory, emotional memory, visual memory, and olfactory memory (Akar et al. 2014; Mutlu et al. 2015). It was also reported that in conscious rats, 7-NI induced a rise in arterial blood pressure and significantly influenced levels of pCO2 in arterial blood, indicating its systemic effect. Behavioral tests showed that 7-NI affected locomotion and exploratory activity and induced walking incoordination. Electrophysiological recordings demonstrated 7-NI increased cortical excitability (Brozickova et al. 2014). Intracerebroventricular microinjection of 7-NI induced catalepsy in mice (Echeverry et al. 2007).

The aforementioned combination of ADO and 7-NI could significantly reduce the dose of either drug, suggesting that there may be an intense interaction between ADO and NO during the occurrence of CNS OT, manifested as follows: NO will restrain the CNS inhibition of ADO and vice verse; ADO also inhibits the NO-caused toxicity. The action between them can ultimately have an important impact on the onset of CNS OT. The presumed mechanism of interaction between ADO and NO and its effect on CNS OT is diagramed in Fig. 8.

Recent studies have also shown that there is a certain interaction between ADO and NO, which mainly occurs in vascular endothelial cells and involves the regulation of blood flow (El-Gowelli et al. 2013; Lamb and Murrant 2015; Persson et al. 2013; Singh et al. 2018). Few studies have aimed to investigate their interactions in neural tissue. ADO has been shown by some studies to need NO-mediated inhibition of epilepsy caused by various inducers (Akula et al. 2008; Yildirim et al. 2011); NO can also promote ADO production and release and enhance the function of the ADO A1 receptor, which may be linked to the protection against the NO-induced neurotoxicity (Fragata et al. 2006). Additional studies have shown that $ONOO^-$ produced by NO and $O_2{\cdot}^-$ can inhibit

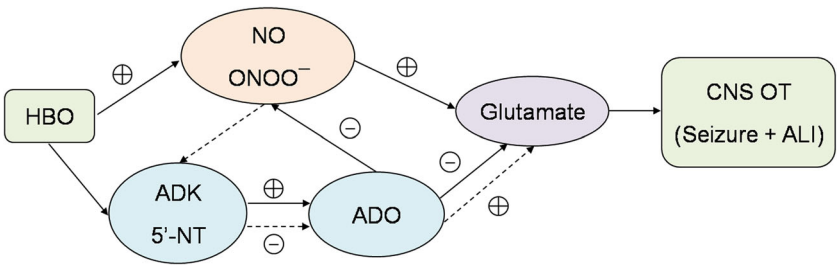

Fig. 8 Presumed mechanism diagram of interaction between ADO and NO and its effect on CNS OT. HBO exposure can increase NO content in the brain tissue, further increase the release of glutamate from neurocyte and induce CNS OT. HBO can also increase ADO content in brain tissue through modulating its metabolism-related enzymes, further enhancing its function to inhibit glutamate releasing and to prevent CNS OT. Increased ADO can limit NO generation and alleviate its neurotoxicity. Increased NO can reduce ADO through modulating its metabolism-related enzymes, further reversing its effect on glutamate releasing and CNS OT. Dotted arrow indicates the impact of NO on ADO metabolism and subsequent effect. *Circled plus* means promotion; *circled minus* means inhibition

the binding of the A1 receptor agonist and receptor and attenuate the CNS inhibition of ADO (Giuntini et al. 2004). In addition, other studies have shown that ADO can cause the release of NO from astrocytes (Janigro et al. 1996). These studies have shown that there is a complex interaction between ADO and NO in the CNS, and the action is presented in diverse forms, such as the synergism and inhibition, which is mainly linked to different physiological and pathological states.

The interaction between ADO and NO during the onset of CNS OT, including the effect of one drug on the content and function of the other, has not been previously reported. Our results suggest that changes in ADO or NO content in brain tissues can expressly have important effects on the content, metabolism, and function of NO or ADO, including: (1) ADO-augmenting measures such as supplementing ADO, inhibiting ADK activity, and activating the ADO A1 receptor can significantly inhibit the production of NO and its metabolite nitrotyrosine. Conversely, inhibition of ADO A1 receptor activity can significantly promote the production of NO and nitrotyrosine. (2) During HBO exposure, neurocytes can increase the production of ADO with inhibitory functions in brain tissues through a variety of regulatory pathways to antagonize enhanced CNS excitability. However, increasing the content of NO and $ONOO^-$ in brain tissues can lead to marked changes of the activity of some important ADO metabolism-related enzymes, mainly including ADK, 5'-NT, and SAHH. Present in astrocytes, ADK is a key substance in the regulation of extracellular ADO content in brain tissue (Aronica et al. 2013; Boison 2013b). The ADO in brain tissues is mainly derived from the rapid degradation of ATP from various types of neurocytes by extracellular nucleotidases such as 5'-NT, which ultimately produces ADO (Boison 2011). In addition to the ATP-derived ADO, the production of ADO via the transmethylation pathway also represents an important source of ADO in vivo, during which SAHH acts as a key enzyme (Boison 2013b; Williams-Karnesky et al. 2013). Our results show that the HBO exposure can inhibit ADK activity and increase 5'-NT activity. Such changes in activity of both enzymes are beneficial to inducing an increase of ADO content in brain tissues to consolidate protection against CNS OT. 7-NI, a nNOS inhibitor, can further amplify the changing trend of these two enzymes and the result will be more conducive to the increase of ADO content in brain tissues. On the contrary, the NO donor SNP and the $ONOO^-$ donor SIN-1 can reverse the changing trend of the above two enzymes, as a result may reduce ADO content in brain tissues correspondingly, and eventually aggravate the onset of CNS OT. HBO exposure alone had no significant effect on SAHH activity. However, the administration of 7-NI before HBO exposure increases SAHH activity while the administration of SNP and SIN-1 decreases SAHH activity, indicating a dramatic change in NO content also compromises SAHH activity during HBO exposure, which may further affect ADO content. The experimental results regarding the activity of the above three enzymes indicate that NO in brain tissues has an important effect on the activity of multiple ADO metabolism-related enzymes during HBO exposure, thereby may affect ADO content in brain tissues.

In summary, after completing our study, we can draw the following conclusions: ADO and NO can produce strong interactions in the pathogenesis of CNS OT; NO can limit the CNS inhibitory effect of ADO while ADO can attenuate NO toxicity. Combining the ADO-augmenting measures and neuronal NO-inhibiting therapy can greatly ameliorate the onset of CNS OT. These findings provide new insights for studies on the pathogenesis, prophylaxis, and treatment of this disease.

Our results also show that ADO can prevent the onset of CNS OT; in addition to inhibiting excessive enhancement of CNS excitability by directly activating the synaptic A1 receptor, it can also inhibit excessive production of NO and the toxicity induced by it. Therefore, we assume that the protection of ADO against CNS OT may be a result of the comprehensive effects of different mechanisms and different routes. This lays a foundation for a comprehensive understanding and further exploration of the role of ADO in preventing CNS OT, and further enriches the perception of ADO that functions as an important metabolic substrate and neuromodulator in the CNS.

The results also confirm there is an interaction between ADO and NO in the CNS, which can exert intense impacts on each other's production, metabolism, and function. Further exploration of the manifestation pattern and the mechanism of this interaction in other nervous system diseases could be of great significance for better understanding the mechanism of various seizure diseases and finding effective preventive measures.

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Compliance with Ethical Standards

All procedures were performed in accordance with the Second Military Medical University (SMMU) Guide for the care and use of laboratory animals, and approved by the ethics committee for Animal Experiments of SMMU.

Conflict of Interest The authors declare that they have no conflict of interest.

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