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Hydrogen sulfide ameliorates prenatal cigarette smoke exposure-induced impairment of respiratory responses to hypercapnia in neonatal rats

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ABSTRACT

The present study was designed to investigate whether H_2S could improve the respiratory responses to hypercapnia blunted by prenatal CSE in neonatal rats in vivo. Respiratory activities were recorded with head-out body plethysmography. The results showed that during baseline, respiratory frequency (F_R), tidal volume (V_T) and minute ventilation (V_E) were similar among tested groups; frequency of spontaneous apnea (F_{SA}), not post-sigh apnea (F_{PA}), was significantly elevated by prenatal CSE. During hypercapnia, the increases in F_R and V_E were significantly reduced, but V_T was not markedly different, in CSE group; both F_{SA} and F_{PA} were decreased, although F_{SA} remained higher in CSE group. All the aforementioned effects induced by CSE on respiratory activities were relieved by NaHS (donor of H_2S, 56 μmol/kg by intraperitoneal injection). These data indicate that H_2S could ameliorate the disruption of respiratory responses to hypercapnia induced by prenatal CSE in neonatal rats.

1. Introduction

Prenatal cigarette smoke exposure (CSE) adversely affects fetal development and is notably linked to spontaneous abortion, placental abruption, preterm birth, low birth weight, and fetal growth retardation (Ananth et al., 1996; Cnattingius, 2004). Prenatal CSE also impairs the development of the brainstem which is involved in control of some vital activities of the body, including respiration (Lavezzi et al., 2005; Slotkin et al., 2011). It has been reported that prenatal CSE is a primary independent risk factor for the occurrence of sudden infant death syndrome (SIDS), which indicates the sudden and unexpected death of infants under 1 year of age (Fleming and Blair, 2007; Friedmann et al., 2016). Victims of SIDS showed abnormal brainstem function (Machaalani and Waters, 2014; Paine et al., 2014), reduced chemoreflexes (Kinney et al., 2009), and central apnea (Kato et al., 2001). Studies have confirmed that prenatal CSE blunts respiratory responses to hypercapnia in neonatal rats (Pendlebury et al., 2012) and that maternal smoking during pregnancy increased apnea in infants (Gunnerbeck et al., 2011; Toubas et al., 1986). We have previously reported that prenatal CSE results in apoptotic cell death in brainstem regions, leading to dysfunctional respiratory regulations (Nie et al., 2013) and that prenatal CSE weakens central chemoreception in neonatal rats (Lei et al., 2015).

Hydrogen sulfide (H_2S) has been considered as the third biological gaseous signaling molecule in addition to nitric oxide and carbon monoxide (Wang, 2002). It participates in a wide range of physiological and pathological processes in mammals, such as stimulating angiogenic properties of endothelial cells (Papapetropoulos et al., 2009), relaxing smooth muscles (Dunn et al., 2016), protecting organs from chronic degeneration (Jin et al., 2015). Most importantly, as a neuromodulator and neuroprotectant (Panthi et al., 2016), H_2S facilitates the induction of hippocampal long-term potentiation by enhancing the activity of N-methyl D,L-aspartate receptors (Abe and Kimura, 1996), exerts a beneficial effect as a treatment for Alzheimer's disease by preventing amyloid-β peptide-induced neurotoxicity (Li et al., 2016b), protects neurons from oxidative stress (Kimura and Kimura, 2004), and plays a protective action against cerebral hypoxia-induced neuronal death (Tay et al., 2010).

Furthermore, our previous studies have shown that H_2S participates in central control of respiration (Hu et al., 2008; Li et al., 2014) and protects medullary respiratory centers from injury induced by chronic intermittent hypoxia in adult rats (Li et al., 2013) and from injury induced by acute hypoxia in medullary slices of neonatal rats (Pan et al., 2010). H_2S also protects rat medullary respiratory centers from prenatal CSE-induced injury via anti-apoptotic effect (Nie et al., 2013). Thus, we propose that H_2S would relieve prenatal CSE-induced impairment of ventilatory responses to hypercapnia.

The aim of the present study was to elucidate our hypothesis that

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H_2S can relieve prenatal CSE-induced impairment of respiratory responses to hypercapnia in neonatal rats in vivo.

2. Methods

2.1. Animal grouping

Adult Sprague-Dawley rats were obtained from the Sichuan University Experimental Animal Center, with females weighing 240–260 g and males weighing 320–360 g. All experimental procedures were complied with the Sichuan University Committee Guidelines on the Use of Live Animals in Research, in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals (NIH Publications No. 80–23), revised in 1978. The rats were kept in a room with a light/dark cycle of 12 h/12 h at 22 ± 1 °C and were provided with access to food and water ad libitum.

Pregnancies were established by overnight mating of one mature male with two nulliparous female rats. The presence of spermatozoa on the vaginal smear was considered as the evidence of successful mating and the day was used to indicate gestational day (gd) 0. Pregnant rats were randomly divided into four groups: NaCl (9 rats), NaHS (donor of H_2S, 7), CSE (8) and CSE + NaHS (7) groups. One or two rat pups (post-natal 2 days) were randomly chosen from each litter to be used in the subsequent experiments of plethysmography. Post-natal day 2 was chosen because this age corresponds to approximately 43.7 days in human infants (Quinn, 2005), a postnatal age within the SIDS risk group (Fleming and Blair, 2007; Friedmann et al., 2016).

2.2. Prenatal cigarette smoke exposure

CSE was performed during gd 7–20 with a modified method extracted from that of Drummond et al. (2017). In brief, animals were placed in a restraining exposure box (80 cm × 60 cm × 50 cm) with cigarette smoke delivered cyclically (2 cigarette/12 min, 10 min with the box closed and the remaining 2 min with the box open, repeated five times, to mimic typical smoking behavior) by lighting cigarettes (Tianxiaxiu, 11 mg of tar and 1 mg of nicotine per cigarette, China Tobacco Chuanyu Industrial Co., China). CSE was conducted twice a day (starting at 9:00 a.m. and 16:00 p.m., respectively).

In addition to the CSE treatment described above, the pregnant rats in CSE and CSE + NaHS group received an equivalent volume of physiological saline and NaHS (56 μmol/kg), respectively, intraperitoneally administered at 2.5 ml/kg body weight 30 min before the first smoke exposure each day. Previous studies in our laboratory have determined the serum cotinine level by means of ELISA. Using this regimen, the serum cotinine concentration (92.3 ± 15.7 ng/ml) (Nie et al., 2013) achieves a level of smoking exposure that simulates active smoking during pregnancy (Klebanoff et al., 1998; Vasankari et al., 2011). The pregnant rats in NaCl and NaHS groups were exposed to air under a similar condition and were intraperitoneally injected with an equivalent volume of physiological saline and NaHS (56 μmol/kg), respectively, from gd 7–20.

None of the pregnant rats in the four groups became severely ill or moribund during the experiment. Thus, all animals survived until the experimental endpoint.

2.3. Respiratory measurements in vivo

Measurements of respiratory frequency (F_R), tidal volume (V_T), minute ventilation (V_E), frequency of spontaneous apnea (F_{SA}) and post-sigh apnea (F_{PA}) in awake neonates were performed using head-out body plethysmography. The head and body chambers of the plethysmograph were self-made from plastic tubes with a similar design to that described by Saetta and Mortola (1987).

The plethysmograph consisted of separate head (50 ml) and body (40 ml) chambers, separated by a flexible latex seal. The neonatal rat was placed in the body chamber with its head protruding through an adjustable hole in the latex seal into the head chamber. Inside the body chamber, the movements of neonates were restrained by fixations of their hindlimbs with scotch tape. The temperature around the neonatal body was maintained at 29 ± 0.5 °C by the application of an intelligent temperature control instrument (Taimeng Biotech. Co., Chengdu, China). The body chamber was connected to one of the two ports of a respiratory flow transducer (Model HX200, Xinhang Xingye Trade Co., Beijing, China), while the other was connected to a biological signal collecting and processing system (MedLab-U/4C501H, Medease Sci. Tech. Co., Nanjing, China). Data were collected with a sampling rate of 1 kHz and analyzed using MedLab6 software. Calibration was done by injecting air with volumes of 50, 100, 150, 200, 250, 300, 350, 400, 450 and 500 μl into the recording chamber, and a standard curve and the corresponding equation were obtained according to the fluctuation of the recorded signals.

Respiratory responses to hypercapnia (8% CO_2, 21% O_2, balance nitrogen) of the neonatal rats from different groups were recorded. The head of the neonate was placed inside the head chamber. The air flowed into the head chamber through one port with the tested gas occupying the chamber completely and out of the chamber through the other port. The air flow rate was controlled at 100 ml/min. Evaluation of respiratory responses to 10-min hypercapnia began once the neonate lay quietly in the plethysmographic chamber and regularly breathed fresh air for at least 30 min; subsequently fresh air was given for another 10 min as the washout. Values of F_R, V_T and V_E were calculated every 2 min. In the present study, we defined apnea in a manner similar to that used previously “an interruption of airflow for at least two breathing cycles, including both spontaneous and post-sigh apneas” (Fournier et al., 2013; Han et al., 2002; Montandon et al., 2006; Zanella et al., 2008), and was calculated every 10 min of recording.

2.4. Statistical analysis

The changes of F_R, V_T, and V_E induced by hypercapnia over time within one group were analyzed with repeated-measures ANOVA, and the changes among groups were analyzed with two-way mixed factorial ANOVA. The body weights, F_{SA} and F_{PA} were analyzed using one-way ANOVA. All data were presented as mean ± SEM and analyzed using SPSS software (version 17.0), and p values less than 0.05 were considered statistically significant.

3. Results

The body weight of the pups in the CSE group (6.59 ± 0.14 g) was significantly lowered as compared with that of NaCl, NaHS and CSE + NaHS groups (7.52 ± 0.28 g, 7.46 ± 0.29 g and 7.45 ± 0.28 g, respectively, $p < 0.05$). Nevertheless, we have excluded the effect of body weight on the ventilatory volumes because we have processed the data as ml/kg body weight. Hence the decreased ventilatory responses of the CSE pups to hypercapnia, as presented in the following parts, were not due to the decrease in body weight.

3.1. Respiratory frequency response to hypercapnia

No significant differences in F_R were found among the NaCl, NaHS, CSE and CSE + NaHS groups during the baseline period under room air condition (107.81 ± 5.09, 106.30 ± 8.85, 102.68 ± 5.85 and 99.92 ± 5.36 breaths/min, respectively, $p > 0.05$, Figs. 1 and 2A).

However, statistically significant differences were observed during exposure to hypercapnia. In the neonates of NaCl, NaHS and CSE + NaHS groups, F_R was significantly increased during hypercapnia (25.24 ± 5.28%, 23.19 ± 2.85%, 23.09 ± 4.59%, respectively, $p < 0.05$) and gradually restored after the exposure ($p > 0.05$, Figs. 1 and 2B). F_R in the neonates of CSE group, however, showed a biphasic change: an initial increase (11.42 ± 2.11%, $p < 0.05$)