Respiratory Physiology & Neurobiology 205 (2015) 66–76

Contents lists available at ScienceDirect

Respiratory Physiology & Neurobiology

journal homepage: www.elsevier.com/locate/resphysiol

Role of posterior hypothalamus in hypobaric hypoxia induced pulmonary edema

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ARTICLE INFO

Article history:
Accepted 20 October 2014
Available online 24 October 2014

Keywords:
GABA
High altitude
Hypothalamus
Nitric oxide
Pulmonary edema

ABSTRACT

To investigate the role of posterior hypothalamus and central neurotransmitters in the pulmonary edema due to hypobaric hypoxia, rats were placed in a high altitude simulation chamber (barometric pressure—294.4 mmHg) for 24 h. Exposure to hypobaric hypoxia resulted in increases in mean arterial blood pressure, renal sympathetic nerve activity, right ventricular systolic pressure, lung wet to dry weight ratio and Evans blue dye leakage. There was a significant attenuation in these responses to hypobaric hypoxia (a) after lesioning posterior hypothalamus and (b) after chronic infusion of $GABA_A$ receptor agonist muscimol into posterior hypothalamus. No such attenuation was evident with the chronic infusion of the nitric oxide donor SNAP into the posterior hypothalamus. It is concluded that in hypobaric hypoxia, there is over-activity of posterior hypothalamic neurons probably due to a local decrease in GABA-ergic inhibition which increases the sympathetic drive causing pulmonary hypertension and edema.

1. Introduction

High altitude pulmonary edema (HAPE) is a life-threatening form of non-cardiogenic pulmonary edema which is characterized by augmented hypoxia-induced sympathetic drive, pulmonary hypertension and fluid accumulation in the lungs that impairs gas exchange (Malhotra et al., 1976; Duplain et al., 1999; Scherrer et al., 1999; Bärtsch and Gibbs, 2007). HAPE occurs in otherwise healthy and un-acclimatized mountaineers ascending up at altitudes rapidly. Even though ventilating these victims with oxygen mixed with nitric oxide (NO), administering acetazolamide and moving them to low altitudes has reduced the mortality rate (Forwand et al., 1968; Evans et al., 1976; Grissom et al., 1992; Anand et al., 1998), the pathogenesis of HAPE is still under intense investigation. Hypoxic pulmonary vasoconstriction is considered to be a prime factor in HAPE (Hultgren, 1997; Eldridge et al., 2006). Hypoxia *per se* causes constriction of small resistance pulmonary arteries and raises pressure in the pulmonary vasculature (Jensen et al., 1992; Sartori et al., 2007). Hypobaric hypoxia induced sympathetic excitation and endothelial dysfunction also contribute to vasoconstriction and fluid extravasation (Bernardi et al., 1998; Berger et al., 2005). However, there is very little information in the literature on how the higher nervous control systems regulate the sympathetic outflow and influence the lung fluid accumulation on exposure to hypobaric hypoxia.

Among the various centers, the posterior hypothalamus may have an important role to play in hypobaric hypoxia as it is involved in regulating sympathetic outflow (Scherrer, 1962; Wible et al., 1988; Waldrop and Bauer, 1989; Dillon and Waldrop, 1993). In normoxic rats, electrical stimulation of posterior hypothalamus resulted in an increase in heart rate, dilation of pupils, fall in skin temperature (Selvamurthy et al., 1978), pulmonary hypertension and increase in sympathetic nerve activity (Cross and Silver, 1963; Ninomiya et al., 1970; Takeda and Buñag, 1978; Scherrer et al., 1999). Interestingly, it resulted in an increase in the lung wet weight/dry weight ratio also (Selvamurthy et al., 1978) suggesting thereby that the posterior hypothalamus may function as the edema center and the increase in lung water may be due to a generalized increase in sympathetic nerve activity (Selvamurthy et al., 1978). It is possible that in hypobaric hypoxia, there would be an increase in neuronal activity at the posterior hypothalamus which would result in an increase in sympathetic discharge, pulmonary hypertension and edema. Indeed, ventilation with hypoxia for 3 h has been reported to increase Fos expression in posterior hypothalamus of rats suggesting that these neurons are excited by hypoxia (Horn et al., 2000). Similarly, there are studies in man which show that there is a highly elevated sympathetic outflow 18–24 h after arrival at an altitude of 4559 m (Duplain et al., 1999) or

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http://dx.doi.org/10.1016/j.resp.2014.10.010
1569-9048/

during acclimatization following exposure to hypobaric hypoxia for 4 weeks at an altitude of 5260 m (Hansen and Sander, 2003). All these studies would suggest that changing the concentration of excitatory or inhibitory neurotransmitters in the posterior hypothalamus would attenuate pulmonary hypertension and edema by suitably modifying sympathetic outflow.

Even though there is a consensus that hypoxia/hypobaric hypoxia augments sympathetic outflow and the indication that posterior hypothalamus may be activated by hypoxia, there is no comprehensive study which has examined all the aspects, namely acute hypobaric hypoxia resulting in pulmonary edema with an increase in sympathetic outflow and their attenuation either by extirpation or by decreasing the activity of neurons in posterior hypothalamus. For the present study, we hypothesized that the increased sympathetic discharge, pulmonary hypertension and pulmonary edema occurring in rats exposed to hypobaric hypoxia for 24 h would be attenuated by bilateral electrical lesion of posterior hypothalamus and by prior infusion of γ-aminobutyric acid ($GABA_A$) receptor agonist muscimol or nitric oxide (NO) donor *S*-nitroso-*N*-acetylpenicillamine (SNAP) into the posterior hypothalamus. To observe the cumulative effects, muscimol and SNAP were given as chronic infusions. The renal sympathetic nerve activity (RSNA) was used for quantifying sympathetic outflow as it has been reported that exposure to hypoxia produces changes in RSNA which are qualitatively similar to those in pulmonary sympathetic nerves (Shirai et al., 1995). Pulmonary hypertension was assessed by recording right ventricular systolic pressure (RVSP) (Rashid et al., 2013). For quantification of lung water, along with the wet weight dry weight ratio, the vascular permeability using Evans blue was determined also. For investigating the role of neurotransmitters, first we administered muscimol into the posterior hypothalamus as it has been reported that muscimol in posterior hypothalamus reduces sympathetic nerve activity (SNA), heart rate and blood pressure (Waldrop and Bauer, 1989; Wible et al., 1988). Then, in a separate set of rats, we administered the NO donor SNAP into the posterior hypothalamus with the purpose of increasing local NO as a decrease in NO by L-NAME in this region increased blood pressure (Gerová et al., 1995) possibly by increasing SNA.

2. Methods

Healthy male Wistar rats (body weight, 280 ± 20 g) were used for this study. The experiments were performed in accordance with the regulations specified by the Institute's Animal Ethical Committee and conform to the national guidelines on the care and use of laboratory animals, India. All measures were taken to minimize any suffering during the experimentation.

2.1. Study design

The animals were divided into three main groups: Group-I, Group-II and Group-III. Each group was divided into four sub-groups, **a**–**d**. Common animals were used in sub-groups **a** and **c** of Groups II and III. With seven animals in each sub-group, the various protocols were carried out on a total of 70 rats.

In Group-I, sub-group I**a** served as sham-operated rats ventilated with room air; I**b** consisted of rats with bilateral lesion of posterior hypothalamus and ventilated with room air; I**c** served as sham operated rats exposed to hypobaric hypoxia and I**d** consisted of rats with bilateral lesion of posterior hypothalamus and exposed to hypobaric hypoxia.

In Group-II, in sub-group II**a**, artificial cerebrospinal fluid (aCSF) was infused into posterior hypothalamus and the rats ventilated with room air (sham); in II**b**, muscimol prepared in aCSF was infused chronically into posterior hypothalamus and the rats were ventilated with room air; II**c** rats were infused with aCSF into posterior hypothalamus (sham) and exposed to hypobaric hypoxia. II**d** rats were chronically infused with muscimol in aCSF into posterior hypothalamus and exposed to hypobaric hypoxia.

In Group-III, all preparations were similar as Group-II excepting that instead of muscimol, SNAP in aCSF was infused chronically in sub-groups III**b** and III**d**. In this group, the data from the sham animals in Group-II**a** and II**c** were used for comparison within the group.

2.2. Stereotaxic surgery preparation

2.2.1. For lesion of the posterior hypothalamus (Group-I)

All rats from Group-I were anesthetized with ketamine HCl (Neon Pharmaceutical, India, 50 mg/kg, i.m) and xylazine (Indian Immunologicals Ltd., India, 5 mg/kg i.m). Each rat was fixed on a stereotaxic apparatus (Stoelting, USA). The cranium was exposed and posterior hypothalamus was reached as per coordinates: 3.6 to 4.2 mm caudal to bregma, 0.5 mm lateral to midline, and 8.2 to 8.9 mm ventral to the skull surface (Paxinos and Watson, 2009, 6th edition). Bipolar electrodes were implanted bilaterally in the posterior hypothalamus. The electrodes used were fabricated locally, insulated within 0.5 mm of the tip and a current strength of 3 mA DC for 15 s was delivered through these electrodes to the posterior hypothalamus to produce electrolytic lesion in rats of Group-I**b** and I**d**. In rats of Group-I**a** and I**c**, all preparations including electrode implantations were similar as mentioned above excepting that no current was passed through the electrodes (sham). After lesion or sham preparation, the animals were kept in room with temperature controlled (25° C) to prevent from hypothermia (Bell et al., 1981).

2.2.2. For chronic infusion of muscimol into the posterior hypothalamus (Group-II)

After induction of anesthesia and fixing the animals on the stereotaxic apparatus, stainless steel cannulae (OD-0.55 mm, ID-0.31 mm) were implanted bilaterally in posterior hypothalamus using the co-ordinates as described above. The cannulae were fixed to the skull with stainless steel screws and dental cement. Their outer ends were connected to osmotic pumps (Model 2001; Alzet, CA) one on each side. These pumps were placed in the neck subcutaneously. Using the pumps and the cannulae, aCSF was infused into the posterior hypothalamus of rats of Group-II**a** and II**c** for 7 days; in the rats of Group-II**b** and Group II-**d**, muscimol (Sigma, USA) prepared in aCSF (5 μg/day/kg b.w.) was infused for 7 days. The flow rate was kept at 1 μl/h. The doses of muscimol used in this study were based on pilot studies performed in the laboratory. With these doses, the animals were stable with no apparent adverse drug reactions such as sedation and impaired motor actions (Chandra et al., 2010).

2.2.3. For chronic infusion of SNAP into the posterior hypothalamus (Group-III)

Whereas rats from Group-III**a** and III**c** were infused with aCSF into posterior hypothalamus (same animals from Group-II), rats from Group-III**b** and III**d** were infused with SNAP (34 μg/day/kg; flow rate, 1 μl/h) prepared in aCSF for 7 days using mini osmotic pumps as described above. The doses of SNAP used in this study were based on pilot studies performed in our laboratory. With these doses, there was no significant decrease in mean arterial blood pressure from the control group and there was no adverse NO mediated effect such as diarrhea (Uchida et al., 2000; Mascolo et al., 1994).

After stereotaxic surgery, each rat was kept under observation for six days to recover from surgical trauma with proper post-operative care. Post operative care included intramuscular injections of Gentamycin and Diclofenac (2 mg/kg b.w.) for 3 days