Respiratory Physiology & Neurobiology 189 (2013) 624–631

Contents lists available at ScienceDirect

Respiratory Physiology & Neurobiology

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

Petrosal ganglion responses to acetylcholine and ATP are enhanced by chronic normobaric hypoxia in the rabbit

Gabriel Icekson, Claudia V. Dominguez, Valentina P. Dedios, Jorge Arroyo, Julio Alcayaga*

Laboratorio de Fisiología Celular, Facultad de Ciencias, Universidad de Chile, Santiago, Chile

ARTICLE INFO

Article history:
Accepted 22 July 2013

Keywords:
Chemoreflex
Afferent neuron
Ventilatory control

ABSTRACT

In mammals, adaptation to chronic hypoxia requires the integrity of the arterial chemoreceptors, specially the carotid body (CB). Chronic hypoxia increases the sensibility of the CB by acting on the receptor cells, but there is limited information on the effects of chronic hypoxia on the sensory neurons that innervate the CB. Therefore, we studied the responses evoked by ACh and ATP, the main transmitters that generate the chemoafferent activity, on the petrosal ganglion (PG) of rabbits exposed to chronic normobaric hypoxia (CNH) during fourteen days. ATP and ACh increased the activity of PG neurons in a dose-dependent manner, in a similar way than in rabbits not exposed to hypoxia (naïve). However, the duration of the responses were significantly increased by CNH, with the mean maximal responses to ACh and ATP increased by a factor of two and four, respectively. Our results suggest that CNH increases duration of the responses by modifying the expression and/or content of ACh and ATP receptors.

© 2013 Elsevier B.V. All rights reserved.

1. Introduction

In mammals, the tissue oxygen supply is controlled by reflex cardiovascular and ventilatory mechanisms that match oxygen supply with oxygen consumption. Acute hypoxia produces a brisk increase in tidal volume and/or ventilation frequency, response termed hypoxic ventilatory response (HVR), which is peripherally driven by the arterial chemoreceptors (Gonzalez et al., 1994; Teppema and Dahan, 2010). On the other hand, long lasting hypoxia increases basal ventilation progressively until the ventilation stabilizes at a higher level (Powell et al., 1998), phenomenon known as hypoxic ventilatory acclimatization (HVA). Moreover, during the HVA the HVR increases, indicating an increase in sensibility and/or reactivity of the ventilatory control. Initially the HVA was postulated to be originated within the central nervous system (Severinghaus et al., 1963) without the participation of the peripheral chemoreceptors (Sørensen, 1970), but it has been shown that peripheral chemoreceptors are necessary for the development of HVA (Smith et al., 1986). Denervation of the carotid bodies reduces basal ventilation and precludes the development of the HVA (Forster et al., 1981; Smith et al., 1986). In animals exposed to chronic hypobaric hypoxia the HVR is significantly larger than in control animals (Aaron and Powell, 1993), and the carotid sinus nerve single fiber basal activity (Vizek et al., 1987) as well as the responses to acute hypoxia are increased (Barnard et al., 1987; Vizek et al., 1987), without changes in the responses induced by anoxia or hypercapnia, suggesting an increased gain of the chemoreceptor to hypoxia.

Recordings from rat carotid bodies *in vitro*, exposed to hypobaric hypoxia for 3–20 days, show an increased frequency discharge under basal conditions and an increased response to acute hypoxia (Chen et al., 2002a; He et al., 2005, 2006). Carotid body cells of rats gestated and reared hypoxic for 5–10 days, present K^+ currents that are depressed by hypoxia as in control animals (Hempleman, 1995, 1996), although the current density could be reduced because of hypertrophy of glomus cells (McGregor et al., 1984; Stea et al., 1992). In glomus cells from adult rabbits cultured in hypoxic media for 24–48 h, acute hypoxia inhibits the O_2-sensitive K^+ current to a larger extent than in cells obtained from control animals, producing a larger depolarization (Kääb et al., 2005). Similarly, TTX-sensitive sodium (Caceres et al., 2007; Stea et al., 1992, 1995) and nifedipine-sensitive (L-type) calcium currents (Hempleman, 1995, 1996) are increased in magnitude and density by chronic hypoxia with respect to control cells, although no modification of the calcium current density has also been reported (Peers et al., 1996; Stea et al., 1992, 1995). These data suggests that chronic hypoxia increases glomus cells excitability and exocytotic release of transmitters, increasing in that way the afferent discharge.

It is generally accepted that depolarization of glomus cells induce the release of one or several transmitters that, acting both in pre- and postsynaptic receptors generate and/or maintain the afferent chemosensory activity (Gonzalez et al., 1994; Iturriaga and Alcayaga, 2004; Nurse and Piskuric, 2013). Acetylcholine (ACh)

* Corresponding author at: Laboratorio de Fisiología Celular, Facultad de Ciencias, Universidad de Chile, Casilla 653, Santiago, Chile. Tel.: +56 2 2978 7366; fax: +56 2 2271 2983.
E-mail address: jalcayag@uchile.cl (J. Alcayaga).

1569-9048/$ – see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.resp.2013.07.023

and ATP play a major role in the generation of the afferent discharge (Conde et al., 2012; Iturriaga and Alcayaga, 2004; Nurse and Piskuric, 2013). Afferent discharge of identified cat petrosal chemosensory neurons increases by stop-flow or acidification of the carotid body, responses partially blocked by either a nicotinic receptor antagonist (mecamylamine) or a nucleotide receptor antagonist (suramin), and completely blocked by the join application of the antagonists (Varas et al., 2003). Similar responses have been recorded in co-cultures of rat petrosal ganglion neurons and carotid body tissue (Nurse and Zhang, 1999; Prasad et al., 2001; Zhang et al., 2000). These responses are mediated by receptors located on the petrosal ganglion neurons (Alcayaga et al., 1998, 2000, 2007; Prasad et al., 2001; Shirahata et al., 1998; Soto et al., 2010; Zhang et al., 2000).

Both the basal discharge and the responses to acute hypoxia are increased in the peripheral chemoreceptors after HVA, indicating an increased sensibility, but the mechanisms underlying this increased sensibility are still not fully understood. Because a change in synaptic efficacy in the carotid body may be partly responsible for the increased gain, we hypothesized that chronic hypoxia could increase the responses of petrosal ganglion neurons to the carotid body putative transmitters, thus increasing the responses of the afferent arm of the reflex pathway. To test our hypothesis, we studied the responses evoked by ACh and ATP in petrosal ganglia obtained from male adult rabbits exposed to normobaric hypoxia ($PO_2 \sim 75$ mm Hg) for approximately 15 days.

2. Methods

2.1. Animals

Experiments were performed in 7 male New Zealand white rabbits. Experiments were conducted in accordance to the guidelines of the Fondo Nacional de Desarrollo Científico y Tecnológico (FONDECYT, Chile) and the Bio-Ethics Committee from the Facultad de Ciencias of the Universidad de Chile approved the experimental protocols.

2.2. Chronic normobaric hypoxia exposure

On each experiment, two individually caged (D × H × W; 0.55 × 0.42 × 0.38 m) animals were exposed to normobaric hypoxia for 14.4 ± 0.4 days ($n = 7$) in a 300 L (0.6 × 0.5 × 1.0 m) sealed plastic chamber. The O_2 content was continuously measured with an oxygen sensor (AX300, Teledyne Analytical Instruments, USA) which output was connected to an automatic programmable controller (Zelio SR2 B121BD, Schneider Electric, France), which maintained the oxygen content (F_{IO_2}) around 9.1%. The controller opened solenoid valves (2026BV172, Jefferson Solenoid Valves, USA), admitting compressed air or N_2 to the chamber if F_{IO_2} values were below ~8.2% or over ~10.4%, respectively, and simultaneously a relief valve that remained opened for 40 s after the closure of the admitting ones. Additionally, two mechanic relief valves opened whenever the gauge pressure exceeded 17 mm Hg inside the chamber. Four internal fans homogenized the atmosphere in the chamber. Ventilatory CO_2 was trapped using $CaCO_3$ (250 g) and the urinary ammonia with H_3BO_3 (60 g). Every other day, the chamber was opened for about 5 min to clean the cages and chamber walls and replenish the food and water containers; $CaCO_3$ and H_3BO_3 were changed when necessary. The chamber internal pressure was measured continuously with a gauge transducer and along with the F_{IO_2} signal was recorded (WinDaq, DATAQ Instruments, Inc., USA) at 1 Hz with an analog to digital acquisition system (DI-158U, DATAQ Instruments, Inc., USA). Throughout the hypoxic period the temperature and the relative humidity within the chamber were recorded at 5 min intervals with a data logger (EL-USB-2, Lascar Electronics Inc., USA).

2.3. Physiological recordings

Male New Zealand white rabbits were anesthetized with a ketamine/xylazine mixture (75/7.5 mg/kg, i.m.), with additional intramuscular doses (1/3 of the initial dose) applied when necessary to maintain the surgical anesthetic level. The animals were placed in supine position and the glossopharyngeal nerve dissected through an incision in the midline of the neck, exposing the carotid bifurcation and the carotid nerve was severed close to the CB. The PG was exposed by eroding the tympanic bulla and the petrosal bone and the glossopharyngeal nerve was cut cephalically to the central apparent limit of the ganglion. The ganglion with its attached nerves was placed in ice-chilled Hanks' balanced salt solution (HBSS), and the surrounding connective tissue removed from over the ganglion and along the full extension of the nerves. The ganglion was placed over a pair of stimulating electrodes, secured to the bottom of a 0.2 mL chamber, and superfused with air-equilibrated HBSS supplemented with 5 mM HEPES buffer, pH 7.4 at 38 ± 0.5 °C, flowing at 1.2–1.5 mL/min.

The carotid nerve was placed on paired platinum–iridium electrodes, and covered with mineral oil in an upper compartment of the superfusion chamber. The electrodes were connected in turn to an AC-preamplifier (Model 1800, A-M Systems, USA) and the recorded electroneurogram was amplified, displayed in oscilloscope, and stored in videocassette tape after digital encoding. The electroneurogram was also fed to a spike amplitude discriminator which standardized output pulses were digitally counted to assess the carotid nerve frequency of discharge (f_{CN}), in Hz. The temperature of the chamber was monitored with a thermistor (YSI, USA) and with the f_{CN} were digitized (DI-145, Dataq Instruments Inc., USA), displayed and recorded (WinDaq, Dataq Instruments Inc., USA) at 1 Hz. Bethanechol, ACh and ATP, in doses of 1–1000 μg in 10 μL boluses, were applied over the ganglion.

2.4. Data evaluation

The change in frequency of discharge (Δf_{CN}) was calculated as the difference between the maximal frequency achieved during a single response and the mean basal frequency (bas f_{CN}), computed in a 30 s interval prior to every evoked response. The relation between Δf_{CN} and the doses of any of the used drugs was assessed by fitting the evoked responses to a sigmoid curve ($\Delta f = \Delta f_{max}/[1 + (ED_{50}/D)^S]$), where D, applied dose; ED_{50}, the dose that evoked half-maximal response, and S, Hill slope factor determining the steepness of each curve. Duration of the responses (Δf_D) was assessed measuring the time f_{CN} remained increased over the 99% confidence limit of the bas f_{CN}, and were related to the dose with a sigmoid curve ($\Delta f_D = \max \Delta f_D/[1 + (ED_{50}/D)^S]$), where max Δf_D, corresponds to the maximal duration of the response. Curve fitting was performed using CurveExpert Professional© (version 1.6.3, Daniel G. Hyams). Comparisons between responses of animals exposed CNH and animals not exposed to hypoxia (naïve) were assessed using our previously published data (Soto et al., 2010).

2.5. Atmospheric variables

Yearly barometric pressure values for Santiago (717.2 ± 0.3 mm Hg; altitude, 567 m) and relative humidity (63.3 ± 2.5%) were obtained from the web database (http://164.77.222.61/climatologia/) of the Dirección Meteorológica de Chile of the Dirección General de Aeronáutica Civil.

Download English Version:

https://daneshyari.com/en/article/5926065

Download Persian Version:

https://daneshyari.com/article/5926065

Daneshyari.com