Respiratory Physiology & Neurobiology 224 (2016) 100–103

Contents lists available at ScienceDirect

Respiratory Physiology & Neurobiology

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

Sex-specific response to hypoxia in a reduced brainstem preparation from *Xenopus laevis*

Jean-Philippe Rousseau*, Stéphanie Fournier, Richard Kinkead

Département de Pédiatrie, Université Laval, Centre de Recherche du CHU de Québec, Hôpital St-François d'Assise, Québec, QC G1L 3L5, Canada

ARTICLE INFO

Article history:
Received 29 July 2015
Received in revised form 21 October 2015
Accepted 23 October 2015
Available online 31 October 2015

Keywords:
Amphibian
Xenopus laevis
Control of breathing
Brainstem
Hypoxic chemoreflex
Sexual dimorphism

ABSTRACT

Respiratory reflexes and tolerance to hypoxia show significant sexual dimorphism. However, the data supporting this notion originates exclusively from mammals. To determine whether this concept is limited to this group of vertebrates, we examined the sex-specific response to acute hypoxia in an adult reduced brainstem preparation from *Xenopus laevis*. Within the first 5 min of exposure to hypoxic aCSF (98% N_2/2% CO_2), recordings of respiratory-related activity show a stronger increase in fictive breathing frequency in males than females. This initial response was followed by a decrease in respiratory-related activity; this depression occurred 6 min sooner in males than females. These results represent new evidences of sexual dimorphism in respiratory control in amphibians and provide potential insight in understanding the homology with other groups of vertebrates, including mammals.

1. Introduction

There is growing awareness that physiological processes regulating behavior and homeostasis exhibit significant sexual dimorphism (McCarthy et al., 2012). This basic principle applies to the respiratory system (Behan and Kinkead, 2011) and the higher risk of cardio-respiratory disease in males and the superior hypoxia tolerance of female rodents illustrate this notion (Garcia et al., 2013). The mechanisms underlying sexual dimorphism in respiratory control are not well understood. We know, however, that perinatal release of testosterone plays a crucial role in brain masculinisation and recent results from our laboratory indicate that stressful conditions interfering with this process have deleterious (and sex-specific) consequences on respiratory control during early life and beyond (Fournier et al., 2013, 2015). Furthermore, the recent work of Garcia et al. (2013) using an *in vitro* slice preparation from newborn mice revealed sex-based differences in its response to bath hypoxia, thereby indicating that the function of the core network generating respiratory rhythm differs between males and females. However, this fascinating aspect of respiratory control is yet to be investigated in another group of vertebrates or in a sexually mature organism.

In amphibians, many studies have evaluated the chemicals respiratory drive and their receptors involved. The response to hypoxia in adult frogs is mainly elicited by peripheral arterial chemoreceptors situated in the aortic arch and carotid labyrinth (Van Vliet and West, 1992). However, electrophysiological experiments on reduced brainstem preparations from bullfrogs indicate that O_2-sensitive structures located within the pons modulate the hypoxic chemoreflex (Winmill et al., 2005; Fournier and Kinkead, 2008). The brainstem response to hypoxia undergoes significant developmental changes as the same stimulus elicits fictive hyperventilation in tadpoles and respiratory depression in adults; regardless of the developmental stage, the pons is necessary to complete expression of the response (Fournier and Kinkead, 2008). Even though the amphibian hypoxic response is well documented, little is known about the sex-based differences in respiratory regulation ectotherms. To address this shortcoming, we tested the hypothesis that the fictive breathing response to hypoxia from adult *Xenopus laevis* preparations shows sexual dimorphism.

2. Methods

Animals: Experiments were performed on 11 (6 males and 5 females) brainstem preparations from *X. laevis*. Animals were obtained from a commercial supplier (Nasco, Fort Atkinson, WI, USA) and were housed in aquaria supplied in flowing, filtered and

* Corresponding author at. Centre de Recherche du CHU de Québec, Hôpital St-Françoisd'Assise (D0-700), 10 rue de l'Espinay, Québec, QC G1L 3L5, Canada. Fax: +1 4185254195.
E-mail address: jean-philippe.rousseau.3@ulaval.ca (J.-P. Rousseau).

http://dx.doi.org/10.1016/j.resp.2015.10.014
1569-9048/

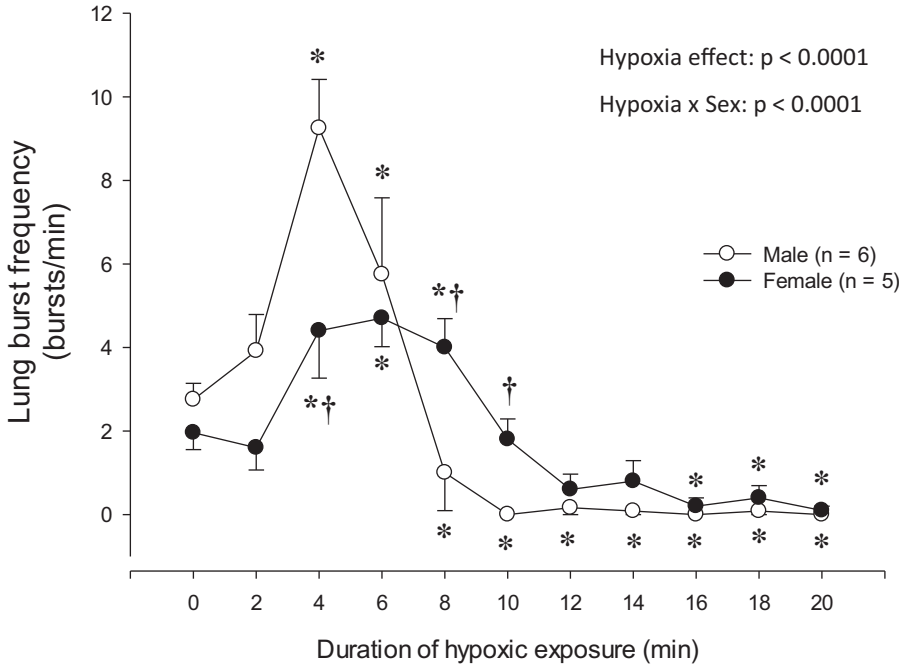

Fig. 1. Lung burst frequency in adult male and female *Xenopus laevis* over 20 min of hypoxia. Data are reported as mean ± s.e.m. *Significantly different from the corresponding baseline value at $P < 0.05$. † Significantly different from the opposite sex at $P < 0.05$.

dechlorinated Québec city water maintained between 19° and 22 °C (photoperiod: 12 h light/dark). Animal care met local ethics committee standards.

2.1. In vitro *brainstem preparations*

Male and female frogs were distinguished according to size (females larger than males) and the presence of the cloaca (visible on females). Animals were anesthetized by immersion in ice cold tricaine methane sulfonate (1 g/L) buffered to pH 7.0 with $NaHCO_3$ (Sigma–Aldrich Canada Cie. Oakville, Ontario). Once unresponsive to body pinch, they were decerebrated by a transection just rostral to the eyes and the cranium was opened to expose the brainstem and rostral spinal cord to allow dissection of the cranial nerves. During dissection, the brainstem was superfused with oxygenated artificial cerebrospinal fluid (aCSF) kept at cold temperature (0–5 °C) to avoid a sudden change in temperature and reduce axonal conductance throughout the dissection procedure (Fournier et al., 2007). The composition of the adult aCSF consisted of (in mM): NaCl (75.0); KCl (2.0); $MgCl_2$ (0.5); $NaHCO_3$ (25.0); $CaCl_2$ (2.0); D–glucose (11.0). The superfusate was equilibrated with a 98% O_2/2% CO_2 gas mixture and had a pH 7.90 ± 0.10. The brainstem was finally transected between the optic tectum and the forebrain and then caudal to the hypoglossal nerve before being transferred to the recording chamber coated with Syglard (Dow Corning; Midland, MI, USA) where it was immobilised with insect pins ventral side up. The arachnoid and pia membranes were then carefully removed.

2.2. Electrophysiological recordings

Using suction electrodes, bursts of respiratory-related motor activity were recorded simultaneously from the rootlets of the trigeminal (V) nerve and the vagal (X) nerve. Vagal nerve activity was used as a sensitive marker of fictive lung activity to distinguish between lung- and buccal-related signals. The pipettes were constructed from borosilicate glass (1.5 mm) pulled to a fine tip with a vertical microelectrode puller (Stoelting Co., Wood Dale, IL, USA). The tip was broken and bevelled to achieve an appropriate tip diameter. Neural activity signal recorded from the suction electrodes were amplified (10k) and filtered (low cut-off 10 Hz, high cut-off 1 kHz) using a differential AC amplifier (model 1700; A-M Systems, Everett, WA, USA). Signals were then full-wave rectified and integrated (time constant 100 ms) using a moving average (model MA-821; CWE, Ardmore, PA, USA). The raw and integrated nerve signals were digitised for recording with a data acquisition system (model DI-720; Dataq Instrument, Akron, OH, USA). The sampling rate of the analog to digital conversion for the raw signal was 1250 Hz.

2.3. Experimental protocols

Once the recording electrodes were in place, the brainstem preparation was superfused with aCSF at room temperature (19–21 °C) continuously delivered at 7 ml min^{-1}. The aCSF was not recycled. The preparation was allowed to return to ambient temperature and recover from the isolation procedure until stable rhythmic neural activity was recorded from both nerves (~60 min). Once recovery from dissection was complete, baseline lung breathing was recorded for 20 min. Brainstem preparations were then superfused with aCSF bubbled with 98% N_2/2% CO_2. In preliminary experiments, PO_2 measurements of the aCSF with a blood gas analyser showed that after 5 min of superfusion, the PO_2 of the solution in the chamber was ~20 mm/Hg. Fictive breathing was recorded for a 20 min period of hypoxia.

2.4. Data analysis

Baseline frequency and amplitude values for respiratory burst activity were obtained by analysing the 20 min recording prior to the onset of hypoxia. During hypoxia, data were analysed every minute of the hypoxic period (20 min); the results were then averaged for a 2 min period. Fictive breathing in *in-vitro* adult brainstem preparations consisted solely of low frequency, high amplitude bursts reflecting fictive lung ventilation. High frequency, low amplitude bursts, characteristic of non-ventilatory fictive buccal activity in adults frog, were only present in one preparation and were not