Respiratory Physiology & Neurobiology 200 (2014) 72–79

Contents lists available at ScienceDirect

Respiratory Physiology & Neurobiology

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

Consequences of gestational stress on GABAergic modulation of respiratory activity in developing newborn pups

Flavien Delhaes[b], Stéphanie Fournier[a], Jean-Francois Tolsa[b], Anne-Christine Peyter[b], Aida Bairam[a], Richard Kinkead[a,*]

[a] Département de Pédiatrie, Université Laval, Centre de Recherche du Centre Hospitalier Universitaire de Québec, Québec, QC, Canada
[b] Département médico-chirurgical de Pédiatrie, Centre Hospitalier Universitaire Vaudois, Lausanne, Switzerland

ARTICLE INFO

Article history:
Accepted 23 May 2014
Available online 11 June 2014

Keywords:
Control of breathing
Development
Prenatal stress

ABSTRACT

The GABAergic system modulates respiratory activity and undergoes substantial changes during early life. Because this maturation process is sensitive to stress, we tested the hypothesis that gestational stress (GS) alters development of GABAergic modulation of respiratory control in rat pups. The respiratory responses to the selective $GABA_A$ receptor agonist muscimol were compared between pups born to dams subjected to GS (bright light and predator odor; 20 min/day from G9 to G19) or maintained under standard (control) conditions. Respiratory activity was measured on 1 and 4 days old pups of both sexes using *in vivo* (whole body plethysmography) and *in vitro* (isolated brainstem-spinal cord preparation) approaches. In intact pups, muscimol injection (0.75 mg/kg; i.p.) depressed minute ventilation; this response was less in GS pups, and at P4, muscimol augmented minute ventilation in GS females. Bath application of muscimol (0.01–0.5 μM) onto brainstem preparations decreased inspiratory (C4) burst frequency and amplitude in a dose-dependent manner; the responsiveness decreased with age. However, GS had limited effects on these results. We conclude that the results obtained *in vivo* are consistent with our hypothesis and show that GS delays maturation of GABAergic modulation of respiratory activity. The differences in the results observed between experimental approaches (*in vivo versus in vitro*) indicate that the effect of prenatal stress on maturation of GABAergic modulation of respiratory control mainly affects the peripheral/metabolic components of the respiratory control system.

1. Introduction

Exposure to stress during early life alters the developmental trajectory of the central nervous system. Research performed on humans, primates, and rodents consistently show that exposure to stress during gestation (gestational stress, GS) increases the incidence of numerous neurological disorders in the offspring such as behavioral dysfunction, attention deficit, depression, schizophrenia, and drug abuse (Buitelaar et al., 2003; Hind and Audrey, 2008). In humans, stressful life events, depression, and anxiety during pregnancy have been associated with poor outcome at birth including a low APGAR score and increased need for resuscitation measures in infants (Markus and Miller, 2009; Ponirakis et al., 1998). These observations suggest that GS disrupts development of the respiratory control system prior to birth; however, confounding factors such as maternal age and life style complicate data interpretation. In light of these limitations, the use of rat as a model allows better control over environmental conditions. It has recently been shown that GS is sufficient to interfere with respiratory development in newborn pups and that these effects are sex-specific (Fournier et al., 2013). By comparison with controls, apneic events are more frequent in rats born to stressed dams and this effect is proportional to the intensity of the stress response measured in the dams. While a deficit in serotonin was revealed as an important mechanism in the manifestation of GS-related respiratory abnormalities in pups (Fournier et al., 2013), the broad effects of GS on brain maturation raises the possibility that other key transmitters contribute to this abnormal development.

The influence that the GABAergic system exerts on neuronal excitability undergoes substantial changes during the perinatal period. At first, activation of $GABA_A$ receptors depolarizes neurons, but as chloride gradients are established, GABA progressively becomes the predominant inhibitory neurotransmitter of the CNS. This maturation process is sensitive to various stressors and

* Corresponding author at: Centre de Recherche du CHU de Québec, Hôpital St-François d'Assise, 10 rue de l'Espinay, Québec, QC G1L 3L5, Canada.
Tel.: +1 418 525 4444x52577; fax: +1 418 525 4195.
E-mail address: Richard.Kinkead@crsfa.ulaval.ca (R. Kinkead).

http://dx.doi.org/10.1016/j.resp.2014.05.015
1569-9048/

consequently, disruption of GABAergic neurotransmission has been evoked in the pathophysiology of diverse respiratory disorders in the newborn (Abu-Shaweesh and Martin, 2008; Darnall et al., 2006; Luo et al., 2004; Zhao et al., 2011). To complete our understanding of the impact of GS on respiratory control development, the present study tested the hypothesis that GS disrupts GABAergic modulation of respiratory activity in newborn rats. To address this issue, we compared the effect of the selective $GABA_A$ agonist muscimol on respiratory activity of pups born to control (undisturbed) dams *versus* to those from mothers subjected to GS between day 9 and 19 of gestation. We first used plethysmography to measure the ventilatory and O_2 consumption responses of intact pups. The second series of experiments used a reduced "*en bloc*" brainstem-spinal cord preparation to quantify the impact of GS on the core elements of the respiratory control system. All experiments were performed on 1 or 4 days old males and females to assess sex- and age-specific effects.

2. Methods

2.1. Animals

Experiments were performed on male and female Sprague-Dawley rats of 1 and 4 days of age (post-natal days 1 and 4 (P1 and P4), respectively). These pups were born from 46 virgin females mated in our animal care facility. Dams were supplied with food and water *ad libitum* and maintained in standard animal care conditions (21 °C, 12:12-h dark–light cycle: lights on at 07:00 h and off at 19:00 h) and randomly assigned to one of two experimental groups: control or gestational stress (GS; see below). Potential litter effects are a valid concern when using multiparous species in developmental studies (Wainwright et al., 2007; Zorrilla, 1997). Accordingly, this confounding factor was considered in the experimental design and data analysis. Male and female pups from each litter were equally distributed between the two experimental approaches used to record ventilatory activity (*in vivo* or *in vitro*). For each series of experiments, pups were then assigned to one of the age (P1 or P4) or drug treatment group. A total of 375 newborns were used in this study. The number of pups and litters used in each group appear in the tables. All experiments complied with the guidelines of the Canadian Council on Animal Care. The institutional animal care committee approved the specific protocols used in this study.

2.2. Gestational stress (GS) protocol

Much like immobilization (Dumont et al., 2000), novelty, bright light, and predator odor activate the neuroendocrine response to stress in rodents (Fendt and Endres, 2008). On day 9 of gestation (G9), dams were arbitrarily selected to complete their pregnancy either under control condition (standard care; $n = 28$) or to be subjected to a GS protocol (GS; $n = 18$) adapted from our previous study (Fournier et al., 2013). Briefly, dams assigned to GS protocol were transported outside the main housing area and placed in a contention holder. The restrained rat was then placed in a clean cage containing a piece of filter paper wetted with 35 μl of fox anal gland extract (2,5,-dihydro-2,4,5,-trimethylethiazoline; TMT, Pherotech, Delta, BC, Canada). A bright light was placed near the cage. The protocol was performed at 9:00 AM daily from G9 to G19 and lasted 20 min. Because the predator odor was still perceptible after removal of the paper impregnated with TMT, the dam remained in the experimental cage (with restraint) for an additional hour prior to being housed with other rats. This procedure was necessary to avoid propagating the fox odor to the main housing area. To assess the efficacy of our GS protocol, corticosterone was also measured on the fourth day of exposure (*i.e.* at G12) by collecting a tail blood sample in control (undisturbed) and stressed rats.

2.3. Corticosterone measurement

Blood collection and plasma corticosterone measurements were performed according to standard laboratory procedures (Fournier et al., 2013). Blood was transferred from the syringe into a tube containing EDTA (microvette 500; Sarstedt). Plasma was separated by centrifugation, quickly frozen at −80 °C until assayed. Corticosterone levels were determined by an enzyme immunoassay (Assay Design). Corticosterone detection was done with a microplate spectrophotometer (μ-Quant; Bio-Tek Instruments). The corticosterone concentration was calculated from the four-parameter logistic standard curve using Prism 6 (Graph pad software Inc., La Jolla, CA).

2.4. In vivo plethysmography recordings

Respiratory activity was recorded using whole body, flow-through plethysmography (Emka Technologies, Paris, France) as previously described for newborn pups (Bairam et al., 2013; Fournier et al., 2013; Niane and Bairam, 2012). The gas flow through the experimental chamber was set at about 100 ml/min and measured with a mass flowmeter (TSI model 4140, Shoreview, MN), the temperature was maintained at 34 °C (P1) or 32 °C (P4) using a temperature control system (Physitemp, Clifton, NJ). Calibration of the system was performed by rapidly injecting 0.5 ml of air into the chamber with a syringe. Tidal volume (V_T) was calculated by integrating the pressure signal using specialized software (IOX, Version 1.8.9 EMKA Technology, Paris, France). Respiratory frequency (f_R) and V_T were recorded from the plethysmograph signal. Barometric pressure, body temperature, chamber temperature, and humidity were measured to correct and standardize V_T and values were expressed in ml BTPS (Bartlett and Tenney, 1970; Drorbaugh and Fenn, 1955). These values were subsequently used to calculate minute ventilation ($\dot{V}_E = f_R \times V_T$). Composition of the gas mixtures flowing in and out of the chamber was analyzed with an oxygen analyzer (model S-3A, Ametek, Pittsburgh, PA) for subsequent calculation of $\dot{V}_{O_2}$ as an index of metabolic rate (Mortola and Dotta, 1992).

2.4.1. Experimental protocol

Each rat received an intraperitoneal injection (2 μl/g) of saline (vehicle) or muscimol (specific $GABA_A$ receptor agonist; 0.75 mg/kg dissolved in saline). Preliminary experiments showed that this dose results in a robust inhibition of respiratory activity (∼50% drop in breathing frequency) without compromising survival of the animal over the entire duration of the protocol. After the injection, the pup was placed in a small cage under a heating lamp for 30 min. Preliminary "time-course" experiments showed that this period is sufficient for the drug to become active and respiratory depression to reach a steady state. The rat was then placed in the plethysmography chamber breathing room air (normoxia; 21% O_2). Breathing was recorded for 20 min once the pup appeared calm and breathing signal became regular (∼10 min). At the end of the measurement, the chamber was opened and body temperature was measured by gently placing a small thermocouple for rodents inside the mouth of the pup (Harvard, Holiston, MA, USA). Note that performing a single measurement (rather than "before and after" injection) reduces pup handling and the duration of the protocol by at least 20 min. This ensures full efficacy of the drug and minimizes experimental stress to the animal.