Available online at www.sciencedirect.com
ScienceDirect
Respiratory Physiology & Neurobiology 160 (2008) 181–186
RESPIRATORY PHYSIOLOGY & NEUROBIOLOGY
www.elsevier.com/locate/resphysiol

Age specific effect of MK-801 on hypoxic body temperature regulation in rats

Mirza Shafiulla Baig, Vincent Joseph *

Department of Pediatrics, Laval University, Centre de Recherche CHUQ, Hôpital St-François d'Assise, Local D0-711, 10 Rue de l'Espinay, Quebec, QC G1L 3L5, Canada

Accepted 17 September 2007

Abstract

Hypoxic exposure produces a consistent decrease of rectal temperature (Tb), which is recognized as a potent protective response. While some of the neural mechanisms underlying this response have recently been described, it remains poorly known how these mechanisms evolve during post-natal development. We recently reported that in rat pups NMDA glutamate receptor limits Tb drop upon hypoxic exposure, an effect that has not been reported by others in adult rats. Accordingly, we tested the hypothesis that the implication of NMDA receptors on temperature control during hypoxic exposure evolves during development. To this aim, we evaluated the hypoxic (30 min – 12% O_2) responses of Tb, metabolic rate, and ventilation in rats after injection of vehicle, or the NMDA receptor antagonist MK-801, at different ages (post-natal days 4, 10, 20 and 2–3 month-old – P4, P10, P20 and P60). MK-801 amplified the magnitude of the hypoxic-induced Tb drop in P4, P10 and P20 rats, but this effect was not apparent in adults. In P20 rats MK-801 tripled the hypoxic induced Tb drop, which was 0.5 °C in control and 1.4 °C in treated rats ($p < 0.0001$). This effect was specific to temperature regulation, and was not accompanied by similar changes of other recorded parameters. MK-801 induced a significant decrease of the hypoxic ventilatory response in adults only. We conclude that NMDA glutamate receptor acts as a counter-regulatory factor that limits the hypoxic-induced drop of rectal temperature during post-natal development in rats.

Keywords: Development; Hypoxia; Rectal temperature; NMDA glutamate receptor

1. Introduction

The pattern of systemic responses elicited by acute hypoxic exposure in newborn mammals includes a decrease of metabolic rate, rectal temperature, and minute ventilation (Cross and Oppe, 1952; Mortola, 1999; Mortola, 2004). This response is highly effective to increase the O_2 related convection ratio (i.e. the ratio of $\dot{V}_E/\dot{V}_{O_2}$), hence resulting in effective hyperventilation relative to metabolic needs, contributing to maintain alveolar and arterial oxygen pressure within physiological boundaries. During development, this pattern of response evolves gradually, with ventilatory response becoming prominent, while the drop of metabolic rate becomes less important. Experimental studies have been directed to understand the factors underlying the development of respiratory responses, both at the level of peripheral chemoreceptors (Wasicko et al., 1999; Bairam and Carroll, 2005; Carroll and Kim, 2005), and at their central projection sites (Ohtake et al., 2000). On the other hand, we are not aware of any studies specifically addressing age-dependent changes of the neural responses underlying the decrease of metabolic rate and/or rectal temperature during hypoxic exposure. Nonetheless, it has been clearly established that these responses are not passive mechanisms, but are tightly regulated. For example, in adult rats, the decrease of body temperature during hypoxia involves a reduction of the thermoregulatory set point of hypothalamic neurons of the pre-optic area, regulated by an interplay of neurochemical factors either negatively or positively regulating rectal temperature (Steiner and Branco, 2002; Bicego et al., 2006; Branco et al., 2006). Accordingly, as most homeostatic functions, this response should be submitted to a developmental regulation.

Glutamate, which is released in the central nervous system during hypoxic exposure, is also involved in thermoregulation. In anesthetized rats, hypothalamic glutamate injection increases heat production (Amir, 1990), an effect that is mimicked by *N*-methyl-D-aspartate (NMDA) (Hara et al., 1997b) and abolished by MK-801, the specific NMDA glutamate receptor antagonist (Hara et al., 1997a). Accordingly, glutamate has been suspected

* Corresponding author. Tel.: +1 418 525 4444x52371; fax: +1 418 525 4195.
E-mail address: joseph.vincent@crsfa.ulaval.ca (V. Joseph).

1569-9048/$ – see front matter © 2007 Elsevier B.V. All rights reserved.
doi:10.1016/j.resp.2007.09.008

to participate in regulation of body temperature under hypoxic exposure. Nonetheless, in adult rats, this hypothesis has not been confirmed (de Paula and Branco, 2004), but in younger animals (10-day-old) blockade of NMDA receptors resulted in a marked reduction of rectal temperature during acute hypoxic exposure (Baig and Joseph, 2006). Hence we hypothesized that glutamate control on body temperature response to hypoxia should decrease during development. Accordingly, the main objective of the present study was to test this hypothesis. To this aim, we used rats of either sex, at the age of 4, 10, 20 and 60–90 days (P4, P10, P20, and P60), receiving either a saline control injection, or MK-801, the specific NMDA-glutamate receptor antagonist. Ventilation, metabolic rate, and rectal temperature were then measured in normoxia and after exposure to moderate hypoxia (12% O_2) for 30 min. Our results are consistent with the hypothesis of a transient implication of NMDA glutamate receptor as a counter-regulatory mechanism that specifically avoids excessive decrease of rectal temperature during hypoxic exposure in rats, at least until the third post-natal week. This response is no longer present in adult rats.

2. Materials and methods

All the experimental protocols have been approved by the local (Laval University) committee on animal care and use. Based on daily vaginal smears, female Sprague–Dawley rats (purchased from Charles River, St. Constant, Canada) were put overnight with mating males on the day of estrus. Pregnancy was confirmed after observation of spermatozoids in vaginal smear the next day (considered as day 0 of gestation; G0) and by monitoring weight gain during the subsequent days. Pregnant female rats were housed separately and left undisturbed. At birth, all litters were normalized to 12 pups, and the animals (both males and females) were studied at the age of 4, 10, 20 and 60–90 post-natal days.

2.1. Experimental design

On the day of recording the animals were randomly divided into two groups receiving either an i.p. injection of saline vehicle or the selective NMDA receptor antagonist MK-801 (0.5, 1.0, 1.5 and 2.0 mg/kg for P4, P10, P20 and P60 rats, respectively). The doses used are in the range of previous studies in newborn (1 mg/kg) and adult (3 mg/kg) rats (Ohtake et al., 1998, 2000). In P4 and P10 rats, higher doses induced undesired side effects with convulsions. Immediately following the injection, the animal was placed in a flow-through plethysmographic chamber. For P4 and P10 rats, the temperature inside the chamber was maintained at a mean value of $32.2 \pm 0.1\,^{\circ}C$ via a temperature control loop (Physitemp, Clifton, NJ, USA). The animals were allowed 15–20 min of habituation, then body temperature (Tb) was measured with a rectal thermocouple probe. The animal was replaced in the chamber for another 10 min before normoxic recordings for 15 min. Then, the inflow line gas was connected to a nitrogen tank and the animals were exposed for 30 min to 12–12.5% O_2. At the end of the hypoxic exposure, the chamber was open and rectal temperature immediately measured.

One week before the recordings, adult rats underwent a small surgery under halothane anaesthesia for i.p. implantation of a thermoprobe (PDT-4000 E-Mitter) allowing telemetric temperature measurements before and during hypoxic recordings. Body weight was measured before the surgery, then daily to ensure post-surgery recovery (i.e. gain weight). Analgesics (Anafin – 0.5 mg/100 g) and antibiotics (Baytril – 0.5 mg/100 g) were given immediately after the surgery and for 3 consecutive days.

2.2. Respiratory and metabolic recordings

Respiratory recordings were performed in normoxia and hypoxia in freely behaving, unrestrained rats by whole body flow-through plethysmography as previously described (Baig and Joseph, 2006). Briefly, a pneumotachograph connected to a differential pressure transducer was inserted into the walls of the recording chamber (Emka Technologies), allowing recordings of flow related to respiratory gas exchange within the chamber. Calibration was performed by a rapid injection (i.e. within 2–3 s) of a known volume of air, and recording the related flow change. For each respiratory cycle, the flow is integrated to volume (inspiratory and expiratory—IOX software, Emka Technologies), inspiratory volume was used to calculate tidal volume with the standard equation proposed by Bartlett and Tenney for whole body plethysmography, all necessary parameters (atmospheric pressure, chamber temperature and relative humidity, rectal temperature) being recorded during measurements (Bartlett and Tenney, 1970).

During recordings, sub-samplings of inlet and outlet gas were dried and directed towards a dual channel oxygen analyzer (S-3A/II, AEI technologies, Pittsburgh, PA, USA), the flow through the chamber was continuously recorded by a mass flowmeter (TSI series 4140 mass flowmeter—TSI, Shoreview, MN, USA). All signals (from the plethysmograph, oxygen analyzer and flowmeter) were directed towards a computer for storage and online calculation of respiratory parameters and oxygen consumption ($[\%O_2\text{in} - \%O_2\text{out}] \times \text{flow}$) during recording using the IOX software (Emka Technologies). The reported respiratory variables were, respiratory frequency (breaths/min), tidal volume (mL/100 g) and minute ventilation (mL/min/100 g).

2.3. Statistical analysis

All analyses were performed using the statview 5.0 software. To test the age specific effects of MK-801 on the selected parameters, we performed a two-way ANOVA using treatment (saline versus MK-801) and age as independent variables. When significant effects of MK-801, or interactions between age and MK-801 appeared, we performed a simple ANOVA using treatment as the independent variable for each age. Changes of a particular variable during hypoxic exposure were tested using an ANOVA for repeated measures for each age and treatment group. At all ages, we also performed a two-way ANOVA (for all tested parameters under normoxia and hypoxia) using treatment and gender as independent variables, to test the hypothesis that MK-801 injection had different