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Interaction of central and peripheral chemoreflexes in neonatal mice: Evidence for hypo-addition

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ABSTRACT

The potential for interaction between the peripheral (PCR) and central (CCR) chemoreflexes has not been studied in the neonatal period, when breathing is inherently unstable. Based on recent work in adult rodents, this study addresses the hypothesis that in neonatal mice there is a hypoadditive interaction between the chemoreflexes. To test this, a mask-pneumotach system was used to expose postnatal day (P) 11–12 mouse pups to square-wave hyperoxia (100% O_2; $n=8$) or hypoxia (10% O_2; $n=11$), administered in normocapnic conditions (inspired CO_2 (F_ICO_2) = 0.001–0.005), or following an episode of re-breathing to increase F_ICO_2 by 0.015–0.02. The immediate (*i.e.* PCR-mediated) responses of frequency (f_B), tidal volume (V_T) and ventilation ($\dot{V}_E$) to square-wave hyperoxia and hypoxia were assessed. When given in a normocapnic background, hyperoxia induced an immediate (within the first 20 breaths, or ~6 s) but transient fall in f_B (-46 ± 9 breaths/min) and $\dot{V}_E$ ($-149\pm41\,\mu l\,min^{-1}\,g^{-1}$) ($P<0.001$ for both), with no effect on V_T. In contrast, hyperoxia had no influence on breathing when it was administered following re-breathing. Similarly, the hypoxia-induced increase in f_B was greater when applied under normocapnic conditions (50 ± 8 breaths/min) then when applied following re-breathing (21 ± 5 breaths/min) ($P=0.02$). These data demonstrate a hypo-additive interaction between the PCR and CCR with respect to the immediate frequency response to inhibition or excitation of the PCR. Hypoaddition of the chemoreflexes could cause or mitigate neonatal apnea, depending on the prevailing PCO_2.

1. Introduction

The peripheral and central chemoreceptors provide negative feedback control of respiratory motor output in response to fluctuating partial pressures of O_2 and CO_2. The interaction between CO_2 and O_2 at the level of the carotid body chemoreceptors is well-known (hypercapnia potentiates the hypoxic response (Lahiri and DeLaney, 1975)). How the peripheral (PCR) and central (CCR) chemoreflexes interact centrally to modify respiratory motor output is less clear, seemingly varying with species and/or experimental approach. The nature of PCR–CCR interaction has been of interest to respiratory physiologists since at least 1940, when Gesell et al. (1940) showed in dogs that cooling of the carotid sinus nerve (thus inhibiting the PCR) has a greater inhibitory effect on breathing when systemic CO_2 is first reduced via artificial ventilation, and has a smaller inhibitory effect when inspired CO_2 is elevated. At the time, the authors proposed "that the progressively increasing hypocapnia leads to a progressively increasing potentiation of the reflexogenic signals [they used "reflexogenic" to mean the PCR]". This may have been the first demonstration of PCR–CCR interaction, and it was hypo-additive (*i.e.* the influence of one chemoreflex on respiratory motor output was enhanced when the other chemoreflex was inhibited). There are two other possibilities: hyper-addition (or multiplicative interaction), where the influence of one chemoreflex is enhanced or reduced when the other chemoreflex is stimulated or inhibited, respectively, or no interaction whatsoever, where the change in respiratory motor output is the sum of the change in PCR and CCR activity.

Since the work of Gesell and colleagues, investigations too numerous to list have demonstrated all three types of interactions (Forster and Smith, 2010). The interpretation of some of these experiments is hampered by the use of anesthesia and its inhibitory effects on central chemosensitivity (Dripps and Severinghaus, 1955). Elegant studies have since been performed without the use of anesthesia, using innovative approaches to stimulate or inhibit the PCR and CCR independently of one another (Blain et al., 2010; Day and Wilson, 2009; Smith et al., 1984; Tin et al., 2012). Using goats with extra-corporeal perfusion of the carotid bodies

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(isolating them from systemic blood), Smith et al. (1984) showed that activation of the PCR has less influence on breathing when central CO_2/H^+ is high; *i.e.* an hypo-additive interaction. Similar results have been obtained from decerebrate and awake rats in which the PCR is stimulated or inhibited at varying central CO_2/H^+ (Day and Wilson, 2009; Tin et al., 2012). In contrast, Blain et al. (2010), who used dogs with extra-corporeal carotid body perfusion, showed that the hyperventilation in response to increasing central CO_2/H^+ was greater when the PCR was first stimulated (*i.e.* a hyper-additive interaction). Recent experiments in humans, exploiting the delay between the activation of the PCR and CCR, failed to identify a PCR–CCR interaction, at least in terms of minute ventilation (Cui et al., 2012).

The issue of PCR–CCR interaction has not been studied in early life when apneas are common due to, among other factors, an arterial PCO_2 that lies close to the apneic threshold, as well as immature chemoreflexes (Khan et al., 2005; Nock et al., 2004). Data from carotid body-denervated lambs suggests no interaction, in that the PCR dominates the hypercapnic ventilatory response over the initial 20 breaths, but has no influence on the later (central) response (Carroll et al., 1991). Similar conclusions come from experiments using neonatal rats challenged with square-wave hypercapnia, administered in a hyperoxic background to dampen the PCR (Cummings and Frappell, 2009). A direct test of PCR–CCR interaction in neonatal mammals, to my knowledge, has not been performed.

This study attempts to characterize the nature of PCR–CCR interaction using ~2 week-old mice. At this age, mice are arguably equivalent to human newborns (Darnall, 2010) and, importantly, demonstrate strong (yet still developing) respiratory responses to hypoxia and hyperoxia. Based on recent data from rodents, here it was hypothesized that in neonatal mice a hypo-additive interaction exists between the PCR and CCR. To address this hypothesis a mask-pneumotach system was used that allows re-breathing to elicit mild systemic hypercapnia, followed by fast (near square-wave) wash-in of hyperoxia or hypoxia. This approach temporally and chemically isolates the PCR, which can then be quantified at varying CCR activity.

2. Materials and methods

2.1. Animals

Wild-type, mixed-strain (C57Bl/6 and 129Sv) male mice were tested at postnatal days (P) 11–12. Average mass of animals used was 6.8 ± 0.3 g. Dams were provided food and water *ad libitum* and were housed with a 12 h light–dark cycle and a room temperature of 21–23 °C. Pups were selected from the litter, weighed and immediately tested. A total of 27 pups from 10 litters of 6 breeding pairs were used. All experimental protocols were approved by the Institutional Animal Care and Use Committee of the University of Missouri at Columbia, MO.

2.2. Rationale for methodology

The methodology was designed to take advantage of the experimental set-up insofar as it allows re-breathing and near-square wave gas challenges. Hypoxia and hyperoxia are used to stimulate and inhibit, respectively, the PCR. The magnitude of immediate ventilatory suppression in response to hyperoxia (*i.e.* Dejours test) is a classic test of PCR strength (Dejours, 1962). Given the temporal delay between the activation of the PCR and CCR, measuring the initial respiratory response to sudden changes in inspired O_2 allows for the strength of the PCR to be assessed at normal or elevated CCR activity. If the PCR and CCR interact in a simple additive fashion, the respiratory response to hypoxia and hyperoxia will be the same at each level of CO_2. If there is hyperaddition, both hypoxia and hyperoxia will elicit a larger respiratory response at the higher background CO_2 (*i.e.* the change carotid body afferent activity with hyperoxia or hypoxia will be amplified centrally by elevated CO_2). Finally, if there is hypoaddition, hypoxia and hyperoxia will elicit a larger respiratory response at the lower background CO_2 (*i.e.* the change carotid body afferent activity with hyperoxia or hypoxia will be dampened centrally by elevated CO_2).

2.3. Experimental setup

Measurements were made using a head-out system where respiratory flow is measured directly via a mask and pneumotach (Cummings and Frappell, 2009). Importantly, normal values for V_T, f_B and $\dot{V}_E$ have been obtained in several previous studies that utilize this system (Cummings et al., 2013, 2010).

The experimental setup is shown in supplemental Video 1. Animals rested in a jacketed glass chamber (~40 ml volume), perfused with warmed water from a programmable water bath (Fisher Scientific, Pittsburgh, PA) in order to hold ambient temperature constant, at 32–33 ± 0.5 °C, throughout the experiment. The snout of the animal was sealed into a small mask (~4 ml volume) and away from the body chamber using a polyether material (Impregum F, Polyether Impression material, 3M, St. Paul, MN). Gas was delivered from tanks into the surrounds of the pneumotach via the open end of a syringe tube (supplemental Video 1). A pump (AEI Technologies, Naperville, IL) pulled room air, or several different gas mixtures through the mask at a flow rate of ~120 ml min^{-1}. With this flow and given the mask volume, wash-in of gases was complete in ~2–3 s (~6–10 breaths). The mask was open to the atmosphere via the pneumotach, and pressure within the mask, measured with a water column, was negligible.

Inspiratory and expiratory airflows were detected by connecting both side-arms of the pneumotachograph to a differential pressure transducer (Validyne Engineering, Northridge, CA). Integration of the flow trace provided respiratory volume. The system was calibrated by injecting 25 and 50 μl of air into the mask with a micropipetter. All analogue signals were recorded and analyzed in LabChart 7 (ADInstruments, Colorado Springs, CO, USA) using Powerlab data acquisition system (ADInstruments).

2.4. Experimental protocol

Pups were individually removed from the litter and weighed. Pups were then placed within in the pre-heated chamber. The snout of the animal was sealed in the mask, and the mask placed via the surrounding rubber gasket into the anterior portion of chamber. Mice typically rested comfortably in the chamber, with periodic arousals. The experimental protocol used is shown in Fig. 1. All animals were given a 10 min settling period, and then baseline (room air) variables were recorded for 2 min. Animals were then exposed to either poikilocapnic hypoxia (10% O_2/balance N_2; $n = 11$) or poikilocapnic hyperoxia (100% O_2; $n = 9$) from tanks. Control experiments were performed using 21% O_2/balance N_2 ($n = 7$). Pups in each group were exposed to the gas twice – once in a normocapnic background (fractional inspired CO_2 (F_ICO_2) of 0–0.005), and once in a mildly hypercapnic background (F_ICO_2 of 0.015–0.02). F_ICO_2 was increased by stopping flow through the mask for ~30 s, thus allowing re-breathing of expired gases (supplemental Video 2). The exact time period of re-breathing depended on the size and of the animal and hence the rate of CO_2 production and its accumulation in the mask. To avoid the mask becoming hypoxic during the re-breathing, the gas flowing through the mask was switched from room air to 25% O_2 (balance N_2) ~30 s prior to the start of re-breathing. When F_ICO_2 reached 0.015–0.02, flow was resumed