Please cite this article in press as: Winter A et al. Differential behavioral sensitivity to carbon dioxide (CO_2) inhalation in rats. Neuroscience (2017), http://dx.doi.org/10.1016/j.neuroscience.2017.01.003

Neuroscience xxx (2017) xxx–xxx

DIFFERENTIAL BEHAVIORAL SENSITIVITY TO CARBON DIOXIDE (CO_2) INHALATION IN RATS

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Abstract—Inhalation of carbon dioxide (CO_2) is frequently employed as a biological challenge to evoke intense fear and anxiety. In individuals with panic disorder, CO_2 reliably evokes panic attacks. Sensitivity to CO_2 is highly heterogeneous among individuals, and although a genetic component is implicated, underlying mechanisms are not clear. Preclinical models that can simulate differential responsivity to CO_2 are therefore relevant. In the current study we investigated CO_2-evoked behavioral responses in four different rat strains: Sprague–Dawley (SD), Wistar (W), Long Evans (LE) and Wistar-Kyoto, (WK) rats. We also assessed tryptophan hydroxylase 2 (TPH-2)-positive serotonergic neurons in anxiety/panic regulatory subdivisions of the dorsal raphe nucleus (DR), as well as dopamine β hydroxylase (DβH)-positive noradrenergic neurons in the locus coeruleus, implicated in central CO_2-chemosensitivity. Behavioral responsivity to CO_2 inhalation varied between strains. CO_2-evoked immobility was significantly higher in LE and WK rats as compared with W and SD cohorts. Differences were also observed in CO_2-evoked rearing and grooming behaviors. Exposure to CO_2 did not produce conditioned behavioral responses upon re-exposure to CO_2 context in any strain. Reduced TPH-2-positive cell counts were observed specifically in the panic-regulatory dorsal raphe ventrolateral (DRVL)-ventrolateral periaqueductal gray (VLPAG) subdivision in CO_2-sensitive strains. Conversely, DβH-positive cell counts within the LC were significantly higher in CO_2-sensitive strains. Collectively, our data provide evidence for strain dependent, differential CO_2-sensitivity and potential differences in monoaminergic systems regulating panic and anxiety. Comparative studies between CO_2-vulnerable and resistant strains may facilitate the mechanistic understanding of differential CO_2-sensitivity in the development of panic and anxiety disorders. © 2017 Published by Elsevier Ltd on behalf of IBRO.

Key words: panic, CO_2 sensitivity, serotonergic, noradrenergic, dorsal raphe, locus coeruleus.

INTRODUCTION

Inhalation of CO_2-enriched air produces psychological and physiological responses that can promote anxiety and fear-like behavior. In humans CO_2 sensitivity lies on a continuum (Colasanti et al., 2008), as the composition, frequency, and severity of CO_2-evoked phenotypes have been found to be quite heterogeneous within the population. First described in 1951 by Cohen and White (Cohen and White, 1951), CO_2 inhalation is an established biological challenge, as individuals with a heightened risk for panic disorder (PD) elicit CO_2-hypersensitivity indexed by exaggerated emotional and respiratory responses (Papp et al., 1993; Rassovsky and Kushner, 2003; Leibold et al., 2013). In the extracellular fluid, CO_2 is hydrolyzed to carbonic acid (H_2CO_3) by carbonic anhydrase which readily dissociates into bicarbonate (HCO_3^-) and H^+ (Huckstepp and Dale, 2011). The resulting acidosis is thought to be the trigger for the panic and fear symptoms, and neuroimaging studies on PD patients support a role of homeostatic pH disturbances in panic physiology (Maddock et al., 2008).

Evidence from genetically informed studies support risk factors that influence liability to heightened sensitivity to CO_2, an endophenotype to PD (Battaglia et al., 2014). Heightened sensitivity to CO_2 has been associated with both childhood separation anxiety and adult panic disorder (PD) with predispositions to either disorder founded largely on genetic factors (Battaglia et al., 2009). Additionally, twin studies have shown significant association with shared genetic components to CO_2-sensitivity (Bellodi et al., 1998; Battaglia et al., 2007), and the degree of familial relationships to panic disorder patients has been shown to be associated with CO_2 sensitivity (Perna et al., 1996; Coryell et al., 2001). Collectively, these observations strongly support CO_2-hypersensitivity as a valid biological risk and trait marker for panic and anxiety disorders. Currently, biological underpinnings of individual variance in CO_2 sensitivity

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Abbreviations: BSA, bovine serum albumin; DR, dorsal raphe; DRD, dorsal region; DRVL, dorsal raphe ventrolateral; DβH, dopamine β hydroxylase; LC, locus coeruleus; NA, noradrenaline; PD, panic disorder; SD, Sprague–Dawley; TPH, tryptophan hydroxylase; TPH-2, tryptophan hydroxylase 2; VLPAG, ventrolateral periaqueductal grey.

http://dx.doi.org/10.1016/j.neuroscience.2017.01.003
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are not well understood. Rodent and human studies provide evidence for a role of gene × environment interactions toward heightened CO_2 reactivity (D'Amato et al., 2011; Spatola et al., 2011). Although a strong genetic predisposition and gene × environment contributions to CO_2 hypersensitivity has been proposed, contributory mechanisms are not clear. Development of preclinical models that can simulate differential responsivity to CO_2 inhalation is relevant and can facilitate mechanistic understanding of this phenomenon.

Rodent models of CO_2 inhalation-evoked behavior and physiological responses have been studied previously (see Battaglia et al., 2014; Johnson et al., 2014; Vollmer et al., 2015 and references therein), however, studies on variation in CO_2 responsivity have been limited. Previously, strain-dependent variation in CO_2-evoked ventilation was reported in mice (Tankersley et al., 1994). The current study assessed differential behavioral sensitivity to CO_2 inhalation in four rat strains with distinct genetic backgrounds. Our primary objective was to determine whether CO_2 inhalation evoked distinct strain-dependent responses in rats with the purpose to develop a rodent model of susceptibility/resistance to CO_2 inhalation. We selected three commonly used outbred strains (Sprague–Dawley (SD), Wistar (W) and Long Evans (LE) rats) and one inbred (Wistar-Kyoto, WK) strain. In this initial study, our objective was to capture the wide genetic diversity and phenotypic variation between these commonly used rat strains as there are no studies available comparing CO_2-inhalation responses between these strains. We anticipated greater CO_2 response variation within outbred strains compared with the inbred WK animals where genetic homogeneity and lower variability may be useful for mechanistic studies. In addition to behavioral measurements, we also investigated tryptophan hydroxylase 2 (TPH-2)-positive serotonergic neurons in anxiety and panic regulatory subdivisions of the dorsal raphe nucleus (DR), as well as dopamine β hydroxylase (DβH)-positive noradrenergic neurons in the locus coeruleus (LC). The DR houses topographically organized subsets of serotonergic neurons. These include subpopulations in the dorsal region (DRD) that project to forebrain circuits modulating anxiety-related behaviors, while neurons within the dorsal raphe ventrolateral (DRVL)-ventrolateral periaqueductal grey (VLPAG) division provide inhibitory input to the dorsal PAG to attenuate panic-relevant responses (Hale and Lowry, 2011). TPH-2-positive serotonergic neurons in the dorsal raphe are CO_2-chemosensitive and therefore have the potential to impact CO_2-evoked behavior and physiology (Severson et al., 2003). Serotonergic neurons within the DRVL are significantly activated by CO_2 inhalation (Johnson et al., 2011) and may represent a 'sympathomotor control system' that normally limits autonomic/behavioral responses to interoceptive threats.

The LC is an established central CO_2-chemosensitive site (Gargaglioni et al., 2010) and contains the major group of noradrenaline (NA) synthesizing neurons, the A6 cell group. NA cell bodies are well connected to brain regions regulating arousal, anxiety, autonomic responses, and memory. LC activation may regulate CO_2-sensitivity, as lesioning of rat LC noradrenergic neurons has been associated with attenuated physiological responses to CO_2 inhalation (Biancardi et al., 2008). Neuroimaging studies also reveal increased blood oxygen level dependent (BOLD) signal in brain stem areas including the LC following CO_2 inhalation in humans (Pattinson et al., 2009). In addition to their well characterized regulatory role in panic and anxiety, the LC and the DR have reciprocal interactions, with the DR exerting an inhibitory effect on LC while the locus is reported to exert excitatory action on the DR (Vandermaelen and Aghajanian, 1983; Szabo and Blier, 2001).

We hypothesized a strain-dependent variance in CO_2 responsivity, accompanied by reduced TPH-2 and enhanced DβH immunoreactivity in the DR and LC respectively in CO_2-sensitive strains.

EXPERIMENTAL PROCEDURES

Animals

All experiments reported here were performed on adult rats (300–350 g) purchased from Harlan. All rat strains (WI, WK, LE and SD) were housed under constant temperature (23–28 °C) with a 12-h light, 12-h dark cycle (lights on at 06:00 h). Food and water were provided ad libitum. All behavioral experiments were performed between 8 am and 1 pm during the 12-h light cycle. Study protocols were approved by the Institutional Animal Care and Use Committee of University of Cincinnati in a vivarium accredited by the Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC). A total of 48 animals were used for the study (n = 6 rats/group).

CO_2 inhalation

Animals were exposed to a three day paradigm (see Fig. 1) consisting of habituation (day 1), air or CO_2 exposure (day 2) and CO_2-context exposure (day 3) in the absence of CO_2 as described in previous studies from our group (Vollmer et al., 2016), with modifications. This enabled an assessment of unconditioned (day 2) and conditioned (day 3) behavioral responses to CO_2 inhalation. Briefly, rats were habituated to a Plexiglas CO_2 chamber (8″ × 8″ × 6.5″) for 10 min one day prior to the CO_2 challenge. On the following day, animals were

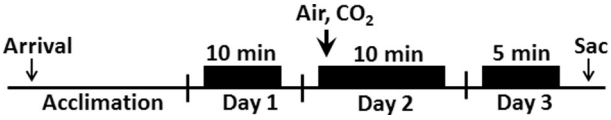

Fig. 1. Schematic showing the experimental layout for measuring CO_2-evoked behavior and conditioned responses to context exposure. Following arrival animals were acclimated to the facility for a week prior to behavioral measurement. The CO_2 inhalation paradigm (Day 1–3) consisted of habituation (Day 1) where animals were exposed to the CO_2 chamber for 10 min. On Day 2, animals were exposed to either air or CO_2 (10%) for 10 min while being videotaped. 24 h post inhalation animals were returned to the CO_2 context for 5 min in the absence of gas inhalation and were videotaped. Following behavior, animals were sacrificed and brain tissue collected for further analyses.

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