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Research Report

Interaction between delta opioid receptors and benzodiazepines in CO_2-induced respiratory responses in mice

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ABSTRACT

The false-suffocation hypothesis of panic disorder (Klein, 1993) suggested δ-opioid receptors as a possible source of the respiratory dysfunction manifested in panic attacks occurring in panic disorder (Preter and Klein, 2008). This study sought to determine if a lack of δ-opioid receptors in a mouse model affects respiratory response to elevated CO_2, and whether the response is modulated by benzodiazepines, which are widely used to treat panic disorder. In a whole-body plethysmograph, respiratory responses to 5% CO_2 were compared between δ-opioid receptor knockout mice and wild-type mice after saline, diazepam (1 mg/kg), and alprazolam (0.3 mg/kg) injections. The results show that lack of δ-opioid receptors does not affect normal response to elevated CO_2, but does prevent benzodiazepines from modulating that response. Thus, in the presence of benzodiazepine agonists, respiratory responses to elevated CO_2 were enhanced in δ-opioid receptor knockout mice compared to wild-type mice. This suggests an interplay between benzodiazepine receptors and δ-opioid receptors in regulating the respiratory effects of elevated CO_2, which might be related to CO_2 induced panic.

1. Introduction

A panic attack is a brief episode characterized by marked fear or discomfort accompanied by an array of symptoms that may include: dyspnea, pounding heart, sweating, shaking, chest pain, nausea/abdominal distress, dizziness, paresthesias, chills, and hot flushes (APA, 2000). Panic disorder is defined by repeated panic attacks that often seem causeless, spontaneous, "out of the blue" and involving flight to help, resulting in chronic anxiety anticipating future attacks, worry over what the attacks mean, and avoidance of circumstances that prevent access to help (APA, 2000). Acute dyspnea (air hunger) during a panic attack differentiates such attacks from attacks limited to palpitations, sweating and trembling. These last are typical of danger induced fear (Klein, 1993).

It has been hypothesized that an evolved suffocation alarm system may become episodically hypersensitive due to endogenous opioidergic dysfunction, predisposing to false alarms. These are experienced as dyspneic panics, inciting rapid escape (Klein, 1993). Panic attacks can be reliably induced in about 70% of those with panic disorder by lactate infusions and, in a subset of these patients, by inhalation of 5%

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CO_2 (Gorman et al., 1988; Klein, 1993; Papp et al., 1997). Such elicitation occurs very rarely in normal subjects or other anxiety disorders, and does not occur during infusions of such stressors as physostigmine, insulin, 5HTP, etc. (Brambilla et al., 1995; den Boer and Westenberg, 1990; Di Lorenzo et al., 1987; Rapaport et al., 1991; Strawn et al., 2008). Hypersensitivity to elevated CO_2 may also help identify childhood groups at familial risk for subsequent development of panic disorder (Roberson-Nay et al., 2010; Spatola et al., 2011).

Having hypothesized this opioidergic dysfunction underlying panic disorder, it was of interest to note that reductions in delta opioid receptor (DOR) efficacy induce substantial anxiety in mice. DOR knockout mice have been reported to display enhanced anxiety on elevated plus mazes and other standardized tests compared to wild-type controls (Filliol et al., 2000). Furthermore, DOR-specific agonists produce anxiolytic effects that are blocked by DOR-specific antagonists. The same antagonists administered alone and at a higher dose produce anxiogenic effects (e.g., based on performance in an elevated-plus maze) (Perrine et al., 2006; Saitoh et al., 2004). DORs have further been implicated in normative anxiety regulation, most likely through the action of endogenous opioids (Jutkiewicz, 2007; Nieto et al., 2005). DORs also modulate the anxiolytic effects of the benzodiazepine diazepam. For example, the anxiolytic effects of diazepam in an elevated-plus maze can be reduced by the DOR antagonist naltrindole (Primeaux et al., 2006). These results seem consonant with experimental evidence in normal human subjects that show 2 mg/kg naloxone given before lactate infusions regularly elicited a tidal volume accentuation resembling those in clinical panic attacks. Less doses ranging from 0.5 to 1.5 mg/kg were less effective (Preter and Klein, 2008; Sinha et al., 2007). In humans it requires about 2 mg/kg to block DOR while as little as 0.15 mg/kg blocks mu-opioid receptors (Preter and Klein, 2008; Sinha et al., 2007).

Given these results, we hypothesized here that DOR knockout mice should be particularly vulnerable to hypercarbia. Two basic questions stemming from earlier hypotheses (Preter and Klein, 2008) were examined: 1) does the absence of DORs exacerbate the respiratory response to elevated CO_2, and 2) do benzodiazepines decrease this response? The results suggest that while DORs are not required for a normal respiratory response to elevated CO_2, they are required for benzodiazepine modulation of this response.

2. Results

To record baseline differences in respiratory response under normal breathing air and 5% CO_2, WT (n=12) and DOR-KO (n=14) mice were injected with saline and placed in the whole body plethysmograph. Representative examples of the raw data traces are seen in Fig. 1B. As noted in Experimental procedures, tidal volume, breathing rate, and

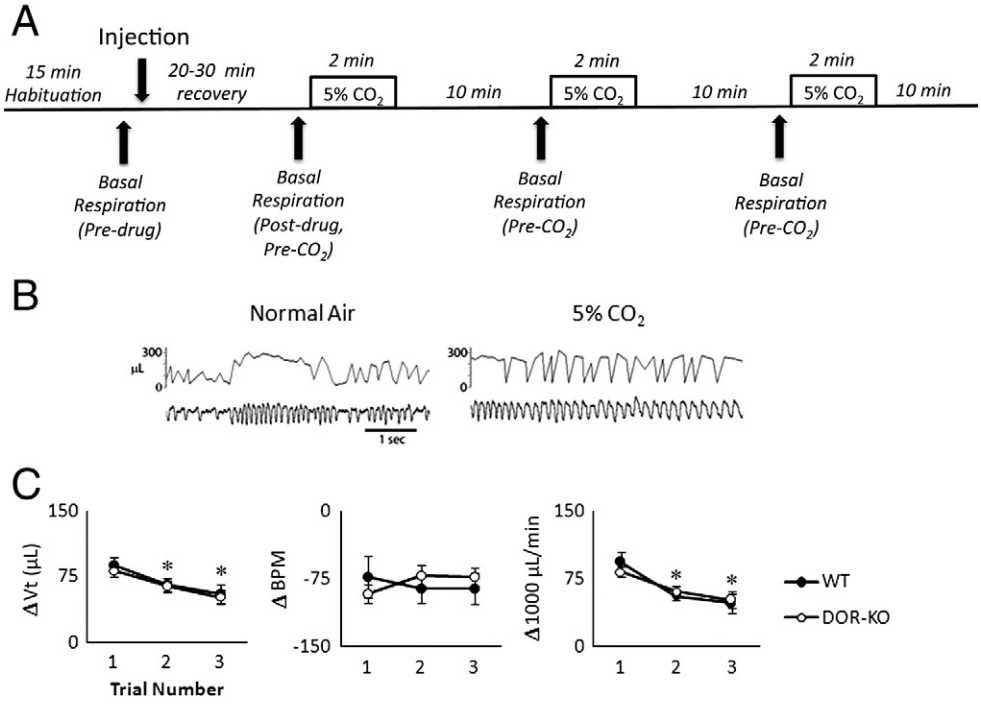

Fig. 1 – A) Experimental design of individual drug trials. Each mouse was tested in this protocol following saline, alprazolam and diazepam injections. B) Typical respiratory response to CO_2. Raw data trace (bottom trace) and calibrated tidal volume (top trace) in a saline-injected wild-type (WT) mouse exposed to normal breathing air and 5% CO_2 respectively. There is a marked change in breathing pattern between the two conditions. DOR-KO mice followed the same response pattern. C) The respiratory response to CO_2 habituated across trials. This profile is for saline and shows that both genotypes habituate and showed no difference in their CO_2 response pattern for change in breath volume (ΔVt) and change in minute volume (ΔμL/min) in the absence of benzodiazepine administration. Note that for breathing rate (ΔBPM) no clear habituation was observed. Asterisks signify significant difference ($p<0.05$) from trial 1.