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Bilateral carotid sinus nerve transection exacerbates morphine-induced respiratory depression

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ABSTRACT

Opioid-induced respiratory depression (OIRD) involves decreased sensitivity of ventilatory control systems to decreased blood levels of oxygen (hypoxia) and elevated levels of carbon dioxide (hypercapnia). Understanding the sites and mechanisms by which opioids elicit respiratory depression is pivotal for finding novel therapeutics to prevent and/or reverse OIRD. To examine the contribution of carotid body chemoreceptors OIRD, we used whole-body plethysmography to evaluate hypoxic (HVR) and hypercapnic (HCVR) ventilatory responses including changes in frequency of breathing, tidal volume, minute ventilation and inspiratory drive, after intravenous injection of morphine (10 mg/kg) in sham-operated (SHAM) and in bilateral carotid sinus nerve transected (CSNX) Sprague-Dawley rats. In SHAM rats, morphine produced sustained respiratory depression (e.g., decreases in tidal volume, minute ventilation and inspiratory drive) and reduced the HVR and HCVR responses. Unexpectedly, morphine-induced suppression of HVR and HCVR were substantially greater in CSNX rats than in SHAM rats. This suggests that morphine did not compromise the function of the carotid body-chemoafferent complex and indeed, that the carotid body acts to defend against morphine-induced respiratory depression. These data are the first in vivo evidence that carotid body chemoreceptor afferents defend against rather than participate in OIRD in conscious rats. As such, drugs that stimulate ventilation by targeting primary glomus cells and/or chemoafferent terminals in the carotid bodies may help to alleviate OIRD.

1. Introduction

The use of opioids to manage acute and chronic pain is limited by opioid-induced respiratory depression (OIRD) in operative and perioperative settings (Dahan et al., 2010). Morphine binds with relatively high affinity to μ-opioid receptors (μ-ORs) and with lesser affinity to δ-ORs and κ-ORs (Chen et al., 1991; Christensen and Reiff, 1991; Frances et al., 1990, 1992). Analgesic doses of morphine depress ventilation in humans by central and peripheral actions (Cepeda et al., 2003; Cashman and Dolin, 2004; Taylor et al., 2005) via activation of μ-ORs although co-activation of δ- or κ-ORs modulates μ-OR responses (Dahan et al., 2010; Trescot et al., 2008). In animals, opioids depress ventilation via central and peripheral mechanisms including (a) centrally-mediated depression of ventilatory drive, (b) chest-wall muscle rigidity, (c) increases in airways resistance, and (d) an increase pulmonary vascular resistance, which decreases gas-exchange (see Henderson et al., 2013, 2014; Shook et al., 1990).

Systemic morphine blunts hypoxic (HVR), hypercapnic (HCVR) and hypoxic-hypercapnic ventilatory responses in conscious rats (Emery et al., 2016; May et al., 2013a, 2013b; Murphy et al., 1995) probably by actions in the brain since μ-ORs are expressed in numerous nuclei involved in respiratory control such as the nucleus tractus solitarius (NTS) (see Zhang et al., 2007). Moreover, microinjections of the μ-OR agonist, DAMGO, into medullary raphe regions of anesthetized rats blunt HVR whereas microinjections of DAMGO into the commissural NTS suppress HVR and HCVR (Zhang et al., 2007, 2009, 2011). The ability of morphine to depress HVR (Berkenbosch et al., 1997; Dahan et al., 1998; Sarton et al., 1999) and HCVR (Sarton et al., 1999) in humans, suggests that opioids and/or metabolites of opioids (Peat et al., 1991) depress carotid body (CB) function and central mechanisms responsive to these challenges (Dahan et al., 1998; Sarton et al., 1999). The possibility that opioids suppress HVR and HCVR via actions in CBs has been examined

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by intra-carotid administration of the δ-OR agonist methionine-enkephalin (ME), and morphine on chemoreceptor activity in the carotid sinus nerve (CSN) of anaesthetized cats (McQueen and Ribeiro, 1980). ME profoundly inhibited chemoreceptor discharge whereas morphine was minimally active with lower doses *increasing* discharge (McQueen and Ribeiro, 1980). Chemoexcitation evoked by intracarotid injections of CO_2-saturated solutions were reduced by ME but were *augmented* by morphine. Kirby and McQueen (1986) confirmed that the rank order of opioid-induced depression of chemosensory discharge in cats was compatible with involvement of δ-ORs rather than μ-ORs. In contrast, Zimpfer et al. (1983) found that morphine blunted hemodynamic responses elicited by chemoafferent stimulation in conscious dogs although whether this involved CBs or brain were not determined. The effects of opioids on resting CB and/or chemoafferent discharge or responses to hypoxic and/or hypercapnic challenges have not been examined in rats.

Despite the recent progress in understanding the mechanisms of OIRD (see Dahan et al., 2010; Boom et al., 2013), the role of CB chemoreceptors in these deleterious effects of morphine in vivo are unknown. To address this issue, we examined the effects of morphine (10 mg/kg, i.v.) on ventilatory responses elicited by a hypoxic (HX) or hypercapnic (HC) gas challenges in conscious Sprague-Dawley rats with prior sham-operation (SHAM) or bilateral CSN transection (CSNX) to disrupt chemoafferent input to the NTS. Our novel findings that morphine-induced suppression of ventilatory responses to HX and HC is exacerbated in CSNX rats provide the first in vivo evidence that CB chemoreceptors defend against, rather than participate in, the ventilatory depressant effects of morphine.

2. Materials and methods

2.1. Animals and surgeries

All studies were carried out in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals (NIH Publication No. 80-23) revised in 1996. The protocols were approved by the Institutional Animal Care and Use Committee at Galleon Pharmaceuticals, Inc. (Philadelphia, PA). Thirty adult male Sprague Dawley rats (275–300 g) obtained from Harlan Laboratories (Indianapolis, IN) were used for this study. All rats were anesthetized with isoflurane (2.5%) and placed on a surgical station allowing body temperature to be maintained at 37 °C via a thermal blanket (Harvard Apparatus, Holliston, MA). The adequacy of the anesthesia was regularly checked by nociceptive stimulus (toe pinch). The left jugular vein of all animals was catheterized (PE-50; Instech Solomon, Plymouth Meeting, PA) for delivery of morphine and exteriorized to the back of the neck. Bilateral CSNX was then performed in 15 rats while another 15 rats were sham-operated (SHAM). In CSNX rats, both CSNs were denervated at the point where they entered the glossopharyngeal nerve (Palmer et al., 2013; Gaston et al., 2014). In SHAM rats, the nerves were identified but not cut. The rats were allowed 7 days to recover from surgery. On the day of study, the catheters were flushed with normal saline (Hospira, Inc., Lake Forest, Illinois) at least 4 h before starting the study. All studies were done in a quiet laboratory with relative humidity of 50 ± 2% and room temperature of 21.2 ± 0.2 °C.

2.2. Ventilatory parameters

Ventilatory parameters were continuously recorded in unrestrained freely-moving rats via a whole-body 12-chamber plethysmography system (PLY 3223; BUXCO Inc., Wilmington, NC, USA), as detailed previously (Henderson et al., 2013, 2014; May et al., 2013a, 2013b) and as diagrammed in Fig. 1. Parameters were frequency of breathing (f_R), tidal volume (V_T), minute ventilation (V_E), inspiratory time (T_I), and V_T/T_I, an index of inspiratory drive (Young et al., 2013; Laferriere et al., 2005). The software constantly corrected digitized values for changes in chamber temperature and humidity and a rejection algorithm excluded motion-induced artifacts (Getsy et al., 2014).

2.3. Protocols

Rats were placed into the plethysmograph chambers and allowed to acclimatize for at least 60 min. Upon development of stable baseline recordings, the rats received a bolus injection of saline or morphine (10 mg/kg, i.v.). After 15 min, all rats were then subjected to a HX (10% O_2, 90% N_2) or to a HC (5% CO_2, 21% O_2, 74% N_2) gas challenge of 20 min in duration. At this time, room-air was reintroduced to the chambers and the ventilatory parameters recorded for a further 15 min. The dose of morphine was chosen because it elicits a sustained decrease in V_E in conscious rats (May et al., 2013b; Young et al., 2013).

2.4. Data analysis

Data was continuously recorded and ventilatory parameters were averaged into 1 min time periods for statistical analyses and graphing. Morphine-induced respiratory depression in SHAM and CSNX rats were calculated as percentage change from pre-morphine baseline values. The post-morphine period was used to calculate percentage changes in ventilatory parameters elicited by the HX and HC challenges and upon return to room-air. All values are expressed as mean ± SEM. The data was analyzed by one-way or two-way repeated measures analyses of variance followed by Bonferroni corrections for multiple comparisons between means (May et al., 2013a, 2013b).

3. Results

3.1. Ventilatory responses during the hypoxic and hypercapnic challenges - morphine studies

Prior to injection of morphine, all recorded ventilatory parameters in CSNX rats were similar to those of SHAM rats in both the hypoxia and hypercapnia studies (Table 1).

3.1.1. Frequency of breathing

As shown in Fig. 2, the injection of morphine (10 mg/kg, i.v.) elicited an initial increase in f_R in the HX (lower panel) and HC (middle panel) studies which was followed by a decrease in f_R in the HX but not the HC study. As shown in Table 1, the maximum responses (column designated "%Max") and total cumulative responses (column denoted %Total) elicited by morphine were similar in the SHAM and CSNX rats. As seen in the bottom left panel of Fig. 2, neither the HX nor HC challenge elicited significant increases in f_R at any time-point in morphine-treated SHAM or CSNX rats. However, as seen in the bottom right panel of Fig. 2, there was a small cumulative (total) fall in f_R during the HX challenge that was of equal magnitude in SHAM and CSNX rats. Moreover, there was a cumulative increase in f_R during the HC challenge in morphine-treated SHAM rats that was absent in morphine-treated CSNX rats.

3.1.2. Inspiratory and expiratory times

Morphine produced an increase in T_I that was similar in the SHAM and CSNX rats in the HX (top panel) and HC (middle panel) studies (Fig. 3). Neither the HX nor the HC challenges elicited significant changes in T_I (bottom panels). As shown in Fig. 4, morphine produced a decrease in T_E (expiratory duration was shorter) that was similar in the SHAM and CSNX rats in both the HX (top panel) and HC (middle panel) studies. Although neither HX nor HC elicited significant changes in T_E at any time point, the HX challenge did elicit a cumulative increase in T_E (lengthening of expiratory duration) of equal magnitude in the SHAM and CSNX rats. The HC challenge did not elicit a cumulative increase in T_E in SHAM or CSNC rats.