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Short communication

Respiratory chemoreflex response inhibition by dorsomedian hypothalamic nucleus activation in rats

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ABSTRACT

Recent observations from our group seem to indicate that repeated stress-evoked dorsomedian hypothalamic nucleus (DMH) activation in rats can lead to persistent bradypnea. One possibility was that respiratory responses to peripheral chemoreceptor activation were reduced by DMH stimulation. In the present study, we therefore investigated the effect of minimal supra-threshold DMH stimulation on respiratory carotid chemoreflex responses. For this purpose, the chemoreflex was activated by potassium cyanide (KCN, 40 μg/rat, i.v.) during electrical and chemical stimulation of the DMH. In both situations, changes in breathing frequency but not tidal volume responses to KCN administration were reduced. These findings suggest that low DMH neurotransmission negatively affects respiratory chemoreflex responses and may be involved in stress-induced bradypnea.

1. Introduction

Several nuclei in the hypothalamus constitute a brain aversive system that coordinates aggressive or defense-type behavioral patterns (for review see Depaulis et al., 1994). In particular, activation of dorsomedial hypothalamic nucleus (DMH) neurons by microinjection of either excitatory amino acids or the γ-aminobutyric acid (GABA) receptor antagonist bicuculline methiodide results in dynamic vascular changes such as blood flow redistribution among organs and increases in arterial blood pressure and heart rate (Bandler and Carrive, 1988). In addition, rapid and deep breathing, characteristic of the response to pain and excitement, has been known for a long time to be a component of the overall response to electrical stimulation of the defense areas in the hypothalamus and mid-brain. This response could produce an anticipatory reduction of blood pCO_2. However, we have recently observed that repeated exposure to stress in a social defeat model results in long-term bradypnea in the rat (unpublished observations). As this model is associated with DMH activation (Sévoz-Couche et al., 2013), it could be postulated that DMH stimulation depresses the respiratory component of the peripheral chemoreflex, as chemodenervation reduces respiratory rate (Roux et al., 2000).

The aim of this study was to determine the effect of low electrical and chemical activation of DMH neurons on the ventilatory response to carotid chemoreflex activation induced by intravenous administration of potassium cyanide (KCN).

2. Material and methods

2.1. General procedures

Experiments were performed using male Sprague-Dawley rats (330–350 g). Procedures involving animals and their care were all conducted in accordance with institutional guidelines, which are in compliance with European Directives (2010/63/EU). Animals were anesthetized with urethane (1.5 g/kg, i.p.). The depth of anesthesia was regularly assessed by pinching a hind paw and monitoring the stability of arterial blood pressure and heart rate recordings. Rectal temperature was maintained at 37 °C with a thermostatically controlled heating blanket. The methods used for cardiovascular recordings were derived from those previously described (Sévoz-Couche et al., 2003; Netzer et al., 2011). A cannula was inserted into the femoral vein for administration of KCN or additional doses of urethane.

2.2. Respiratory measurements

The trachea was cannulated to monitor respiration (Brouillard et al., 2014). The cannula was connected to a pneumotachograph

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(Fleisch 0000), linked to a volume transducer (Digitimer Neurolog, NL 905). Respiratory signals were relayed to a 1401 interface (1401 Plus, CED, UK) and were processed by Spike 2 Software (6.14, CED, UK). Breathing frequency (f_B) and tidal volume (V_T) were derived from the flow signal.

2.3. Neuronal stimulation in the DMH

We first determined the level of electrical and chemical stimulation of the DMH that would be sufficient to induce small arousal cardiovascular responses but no breathing alteration, in order to avoid addition of ventilatory responses following chemoreflex activation during DMH activation. Such "minimal" suprathreshold DMH activation was found to be elicited by electrical stimulation (bipolar electrode, 15 s, 50 Hz, 1 ms pulse duration, $n = 6$) with low intensity (30 μA), or local microinjection of bicuculline methiodide at a dose of 5 pmol in 100 nl ($n = 6$). In both cases, DMH activation produced first signs of arousal (mydriasis, vibrissae and body movements, piloerection) associated to minimal cardiovascular responses (increases in heart rate up to 10 bpm and mean blood pressure up to 5 mmHg) that were not associated to significant modifications of f_B and V_T. Sham procedures were performed with zero electrical current or saline (Sévoz-Couche et al., 2003) microinjection, respectively. Stereotaxic coordinates were those used previously: P 3.0–3.6, L 0.5, V 8–9 (Sévoz-Couche et al., 2003).

2.4. Carotid chemoreceptor reflex activation

This reflex was induced by injections of KCN (40 μg/rat, 0.1 ml) into the femoral vein at least 30 min after induction of anesthesia, resulting in a decrease in heart rate and an increase in blood pressure associated with hyperventilation assessed by an increase in f_B and V_T. The time interval between two tests for repetition of respiratory reflex responses was >15 min (Sévoz et al., 1997). As previously performed (Sévoz et al., 1997), we verified that chemodenervation abolished both respiratory responses. KCN was first administered in addition to sham DMH activation procedures (i.e. when zero current was applied or after saline microinjection, respectively), and 15 min later during DMH activation (i.e. 3 s after the beginning of electrical stimulation or 5 min after microinjection of bicuculline into the DMH, respectively).

2.5. Statistics

Paired Student *t*-test was used to compare respiratory parameters after sham and experimental treatments in the same animals. Analyses were done with Prism 5.04 (Graph Pad Software).

3. Results

3.1. Effects of electrical DMH stimulation on KCN-induced ventilatory responses

Activation of peripheral chemoreceptors by intravenous administration of KCN (40 μg/rat) without passing current into the DMH induced increases in f_B (+35 ± 3 from 105 ± 2 cpm, $p < 0.001$) and V_T (+1.3 ± 0.1 from 1.9 ± 0.1 ml, $p < 0.001$) (Fig. 1A and C). When minimal suprathreshold activation of the DMH was induced (30 μA, Fig. 1B), minor but non-significant changes in baseline ventilatory parameters were observed (f_B: +5 ± 3 from 111 ± 2 bpm, $p = 0.85$; V_T: +0.3 ± 0.1 from 1.9 ± 0.3 ml, $p = 0.91$). KCN administration during DMH activation failed to induce any modification of f_B ($p = 0.2$), but V_T responses were unaffected ($p < 0.01$) (Fig. 1B and C). As a control, we verified that both responses occurred again when KCN was administered 15 min later with no application of current to the DMH ($p < 0.001$ each, Fig. 1C).

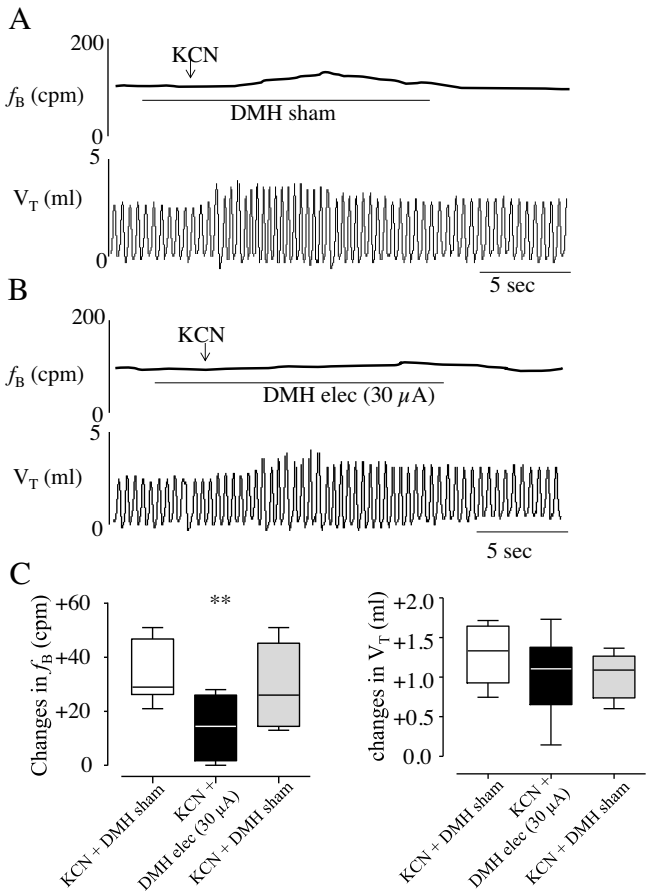

Fig. 1. (A and B) Representative breathing frequency (f_B) and tidal volume (V_T) responses to KCN (40 μg/rat) administration (arrows) as obtained by pneumotachograph measurements connected to the tracheal cannula, without (A, sham) or during electrical (elec, 30 μA, B) DMH activation (solid line). (C) Box-and-Whisker plots with minimum and maximum values, lines represent the medians, showing KCN-induced changes in tracheal f_B and V_T without or during DMH electrical stimulation. $^{**}p < 0.01$ versus sham.

3.2. Effects of chemical DMH stimulation on KCN-induced ventilatory responses

In a second group of animals, administration of KCN after saline into the DMH induced increases in f_B (+33 ± 7 from 110 ± 2 cpm, $p < 0.001$) and V_T (+1.2 ± 0.1 from 1.9 ± 0.3 ml, $p < 0.001$) (Fig. 2A). After bicuculline, non-significant changes in baseline ventilatory parameters were observed (f_B: +8 ± 4 from 110 ± 4 bpm, $p = 0.85$; V_T: +0.4 ± 0.2, $p = 0.90$). Five minutes later, only V_T was increased after KCN administration ($p < 0.001$, Fig. 2B and C). As a control, we verified that both responses occurred when KCN was administered 1 h later ($p < 0.001$ each, Fig. 2C).

4. Discussion

This study shows, seemingly for the first time, that minimal stimulation of the DMH reduces the tachypneic response to peripheral chemoreflex activation, without affecting tidal volume.

The DMH is classically considered to be part of the "hypothalamic defense area" based on the finding that electrical and chemical stimulation of this region induced a defense reaction, a pattern of adjustments characterized by behavioral and autonomic (sympathetic) changes typically seen when the organism is confronted

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