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Bed nucleus of the stria terminalis and the cardiovascular responses to chemoreflex activation

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ABSTRACT

Several studies from our group have indicated that the BNST play an important role in baroreflex modulation. However, the involvement of the BNST in the chemoreflex activity is unknown. Thus, in the present study, we investigated the effect of the local bed nucleus of stria terminalis (BNST) neurotransmission inhibition by bilateral microinjections of the non-selective synaptic blocker cobalt chloride ($CoCl_2$) on the cardiovascular responses to chemoreflex activation in rats. For this purpose, chemoreflex was activated with KCN (i.v.) before and after microinjections of $CoCl_2$ into the BNST. Reversible BNST inactivation produced no significant changes in the magnitude and durations of both pressor and bradycardic responses to intravenous KCN infusion. These findings suggesting that BNST neurotransmission have not influence on both sympathoexcitatory and parasympathoexcitatory components of the peripheral chemoreflex activation.

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1. Introduction

The peripheral chemoreflex plays an important role in regulation of ventilatory and circulatory responses to change in arterial oxygen partial pressure (Barros et al., 2002). This reflex is predominately stimulated by hypoxic stimulus producing increases in sympathetic activity, blood pressure, and minute ventilation (Reddy et al., 2005). There is considerable evidence suggesting an enhanced peripheral chemoreceptor reflex in disease states such as congestive heart failure (Narkiewicz et al., 1999; Sun et al., 1999) and hypertension (Zoccal et al., 2009). Given the importance of the peripheral chemoreceptors in the cardiovascular reflex regulation in normal and disease conditions, it is important to understand the neuroanatomical pathways and neurotransmitters involved in generation, processing, and modulation of complex cardiovascular responses to peripheral chemoreceptor activation.

Several studies have identified brain structures involved in the sympathoexcitatory component of chemoreflex (Haibara et al., 1995; Olivan et al., 2001; Braga et al., 2007; Costa-Silva et al., 2010; Granjeiro et al., 2011). However, most have focused on the pons and the medulla structures (Guyenet, 2000), which are the primary projection areas of chemosensitive afferents and the primary sites for respiratory and sympathetic integration (Mifflin, 1992). Apart from these brain areas, other studies suggest an involvement of the different limbic structures in neural pathways of peripheral chemoreceptors, including the bed nucleus of stria terminals (BNST) (Ma et al., 2008).

The BNST is a limbic forebrain structure situated ventrally to the lateral septal nucleus and dorsally to the preoptic area of the hypothalamus (Dong et al., 2001b; Forray and Gysling, 2004). It has extensive reciprocal connections with other limbic structures as well as with brainstem autonomic nuclei involved in reflexes modulation (Martin et al., 1991; Walker et al., 2003; Forray and Gysling, 2004; Shin et al., 2008), and it is an import relay for the integration of information from brain regions associated with the control of autonomic, endocrine and behavioral responses (Dong et al., 2001a; Alheid, 2003).

Functional studies have also indicated the important role of the BNST in the central nervous system cardiovascular modulation. Indeed, it was demonstrated that electrical and chemical BNST stimulation were able to evoke cardiovascular responses (Gelsema et al., 1993; Giancola et al., 1993; Hatam and Nasimi, 2007). This area also

Abbreviations: ACSF, Artificial Cerebrospinal Fluid; BNST, Bed nucleus of stria terminalis; $CoCl_2$, cobalt chloride; HR, heart rate; IL, infralimbic cortex; KCN, potassium cyanide; MAP, mean arterial pressure; NTS, nucleus of the tractus solitarius; PAP, pulsatile arterial pressure; PL, prelimbic cortex; RVLM, rostral ventrolateral medulla.

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plays an important neuromodulatory role on cardiovascular responses to baroreflex activation and dynamic exercise (Crestani et al., 2006, 2010). With respect to chemoreflex, it was observed an increase in c-fos protein expression, a marker of neuronal activity, in the BNST of rats submitted to 7 days of intermittent chromic hypoxia (Ma et al., 2008). This evidence could indicate a possible involvement of BNST on the neural pathway of peripheral chemoreceptors.

In spite of evidence in favor of participation of the BNST in several aspects of the autonomic control, there is no study investigating the role of this structure on acute cardiovascular changes in response to chemoreflex activation. Accordingly, in the present study we evaluated the effects of pharmacological inhibition of the BNST neurotransmission with cobalt chloride ($CoCl_2$), a non-selective synaptic blocker, on the pressor and bradycardiac responses to chemoreflex activation in unanesthetized rats.

2. Materials and methods

2.1. Animals

Male Wistar rats weighing 250–270 g were housed in groups of four per cage (41x33x17 cm) in a temperature-controlled room (24 ± 1 °C) under standard laboratory conditions, free access to food and water and a 12 h light/dark cycle (lights on at 06:30 A.M.). Experiments were performed during the morning period. Procedures were conducted in conformity with the Brazilian Society of Neuroscience and Behavior guidelines for the care and use of laboratory animals, which are in compliance with international laws and politics. The Institution's Animal Ethics Committee approved the housing conditions and experimental procedures (process number 167/2007).

2.2. Surgical procedures

Four days before the experiment the rats were anesthetized with 2,2,2-tribromoethanol (250 mg/kg ip., Sigma-Aldrich, USA) and fixed in a stereotaxic frame (Stoelting, USA). After scalp anesthesia with 2% lidocaine, the skull was surgically exposed and stainless steel guide cannulae (26 G) were implanted bilaterally into the BNST (antero-posterior = −8.4 mm from interaural; lateral = 4.0 mm from the medial suture, vertical = −5.5 mm from the skull with a lateral inclination of 23°, (Paxinos and Watson, 1997)). Cannulae were fixed to the skull with dental cement and one metal screw. An obturator inside the guide cannulae prevented obstruction. After surgery, the animals were treated with a polyantibiotic preparation of streptomycins and penicillins (i.m., Pentabiotico®, Fort Dodge, Brazil) to prevent infection and with the non-steroidal anti-inflammatory flunixine meglumine (s.c., Banamine®, Schering Plough, Brazil) for post-operative analgesia.

One day before the experiment, the rats were again anesthetized with 2,2,2-tribromoethanol (250 mg/kg, i.p.) and a catheter (a 4 cm segment of PE-10 that was heat-bound to a 13 cm segment of PE-50, Clay Adams, USA) was inserted into the abdominal aorta through the femoral artery, for cardiovascular recording. A second catheter was implanted into the femoral vein for infusion of KCN to evoke chemoreflex alterations (see below). Both catheters were tunneled under the skin and exteriorized on the animal's dorsum. In the end of the surgery the treatment with polyantibiotic and anti-inflammatory drug was repeated.

2.3. Measurement of cardiovascular responses

Pulsatile arterial pressure (PAP) of freely moving animals was recorded using an HP-7754A preamplifier (Hewlett Packard, USA) and an acquisition board (Biopac M-100, USA) connected to a computer. Mean arterial pressure (MAP) and heart rate (HR) values were derived from pulsatile recordings and processed on-line.

2.4. Drugs

The following drugs were used: KCN (Merck, Germany) was dissolved in saline (0.9%). $CoCl_2$ (Sigma-Aldrich, USA) were dissolved in artificial cerebrospinal fluid (ACSF composition: 100 mM NaCl; 2 mM Na3PO4; 2.5 mM KCl; 1 mM $MgCl_2$; 27 mM $NaHCO_3$; 2.5 mM $CaCl_2$; pH = 7.4).

2.5. Microinjections into the BNST in unanesthetized rats

All microinjections into the BNST were performed without any restraint or unnecessary handling of the rats. The needle (33-gauge, Small Parts, USA) used for microinjection into the BNST was 1.0-mm longer than the guide cannula and was connected to a 2 μL syringe (7002H; Hamilton, USA) through PE-10 tubing. The needle was carefully inserted into the guide cannula and drugs were injected in a final volume of 100 nL over a 30 s period. After a 45–60 s period, the needle was removed and inserted into the contralateral guide cannula for bilateral microinjections into the BNST.

2.6. Chemoreflex activation

The chemoreflex was activated by systemic intravenous injection of 0.1 mL of KCN (40 μg per rat; Merck, Germany) in accordance with our previously validated experimental conditions (Granjeiro and Machado, 2009; Oliva et al., 2010; Granjeiro et al., 2011).

The cardiovascular responses to chemoreflex activation were evaluated before (control), 10 and 60 min after bilateral microinjections of the unspecific synaptic blocker $CoCl_2$ (1 mM) into the BNST (n = 9). The control group received bilateral microinjections of ACSF into the BNST and were submitted to the same experimental protocol (n = 5). The magnitude of changes in MAP and HR in response to chemoreflex activation was quantified at the peak and the duration of the responses (Granjeiro et al., 2011).

2.7. Histological procedure

At the end of the experiments, rats were anesthetized with urethane (1.25 g/kg i.p., Sigma-Aldrich, USA) and 100 nL of 1% Evan's blue dye was bilaterally injected into the BNST as an injection site marker. The chest was surgically opened, the descending aorta occluded, the right atrium severed and the brain was perfused with 10% formalin through the left ventricle. Brains were postfixed for 24 h at 4 °C, and 40 μm sections were cut using a cryostat (CM-1900; Leica, Germany). Serial brain sections were stained with 1% neutral red. The injection sites were located using the rat brain atlas of Paxinos and Watson (1997). Rats receiving injections of the $CoCl_2$ outside the BNST were included in an additional group (OUT).

2.8. Statistical analysis

Data are expressed as means ± SEM. Baseline cardiovascular values before and after BNST treatment were compared using the Student's *t* test. MAP and HR changes produced by KCN were analyzed using two-way ANOVA with treatment (ACSF or $CoCl_2$) as independent factor and time (before, 10 and 60 min after bilateral microinjections) as the repeated measurement factor. When interaction between factors was observed, groups were compared by Dunnett´s post hoc test. Results of statistical tests with $P<0.05$ were considered significant.

3. Results

The diagrammatic representation indicating the injection sites of all drugs injected into the BNST and a representative photomicrograph are presented in Fig. 1.

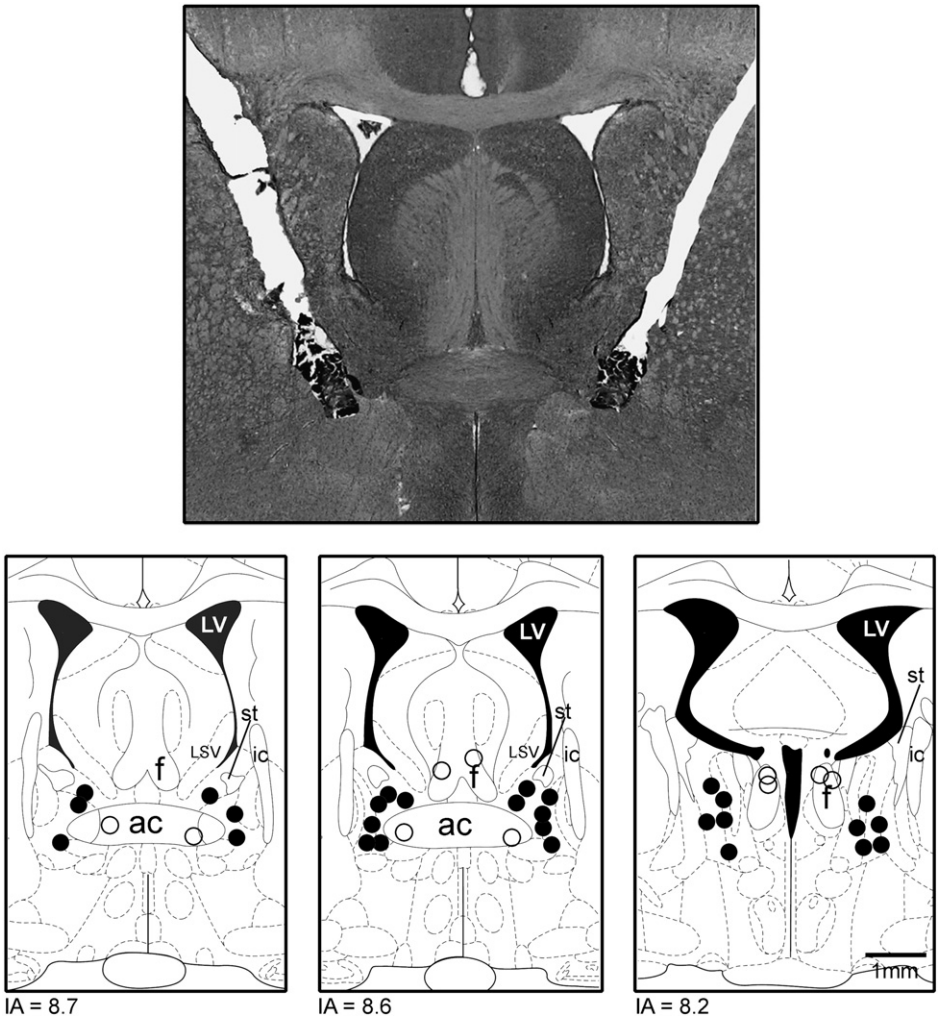

Fig. 1. Photomicrograph of a coronal brain section from one representative rat showing bilateral injection sites in the BNST. Diagrammatic representation is based on the rat brain atlas of Paxinos and Watson (1997), indicating injection sites of ACSF (n = 5) or $CoCl_2$ (n = 9) into the BNST of rats submitted to chemoreflex (black circles). $CoCl_2$ Microinjections into structures surrounding the BNST of rats submitted to baroreflex protocol are represented by white circles (n = 5). IA, interaural coordinate; ac, anterior commissure; ic, internal capsule; LV, lateral ventricle; LSV, lateral septal ventral, stria terminalis; and f, fornix.

3.1. Effect of bilateral microinjections of ACSF or $CoCl_2$ into the BNST on the cardiovascular responses to chemoreflex activation in unanesthetized rats

Artificial cerebrospinal fluid Bilateral microinjections of ACSF (n = 5) into the BNST did not affect baseline values of both MAP (before = 100 ± 3 and after = 106 ± 4 mmHg, t = 1.6, P > 0.05) and HR (before = 349 ± 11 and after = 368 ± 9 bpm, t = 2.03, P > 0.05) of animals submitted to chemoreflex activation protocol. ACSF did not affect chemoreflex responses.

$CoCl_2$ Bilateral microinjections of $CoCl_2$ (n = 9) into the BNST did not affect baseline values of both MAP (before = 99 ± 3 and after = 101 ± 4 mmHg, t = 0.5, P > 0.05) and HR (before = 344 ± 10 and after = 353 ± 11 bpm, t = 1.2, P > 0.05) of animals submitted to chemoreflex protocol.

Typical traces of PAP, MAP and HR of one representative animal that had the chemoreflex activated before and after bilateral microinjections of $CoCl_2$ into the BNST are shown in Fig. 2. Compared with baseline values, the systemic administration of KCN induced a marked increase of MAP (t = 13.35, P < 0.0001) and decrease in HR (t = 8.5, P < 0.0001). However, bilateral microinjections of $CoCl_2$ into the BNST produced no significant changes in the magnitude of both pressor ($F_{1,36}$ = 0.2, P > 0.05) and bradycardiac ($F_{1,36}$ = 3.2, P > 0.05) responses to chemoreflex activation (Fig. 3). Moreover, the durations of both pressor ($F_{1,36}$ = 1.2, P > 0.05) and bradicardiac ($F_{1,36}$ = 0.1, P > 0.05) responses induced by KCN also were similar to vehicle-treated animals (Fig. 4). In addition, no effects were observed when $CoCl_2$ was microinjected into structures surrounding the BNST (data not shown).

4. Discussion

The present work is the first study investigating the effects of the BNST neurotransmission inhibition on the pressor and bradycardiac responses to chemoreflex activation evoked by systemic administration of KCN. For this purpose, we used the local administration of $CoCl_2$, which produces an acute reversible synaptic inactivation by reducing Ca^{2+} pre-synapse influx (Kretz, 1984; Lomber, 1999). The study in unanesthetized rats was required because there is evidence that anesthesia may affect the neurochemical mechanisms involved in the cardiovascular adjustments at the brainstem level (Machado and Bonagamba, 1992), change the cardiovascular responses to chemoreflex activation (Franchini and Krieger, 1993) and also that anesthetics such as urethane may affect the electrophysiological properties of nucleus of the tractus solitarius (NTS) neurons (Accorsi-Mendonca et al., 2007).

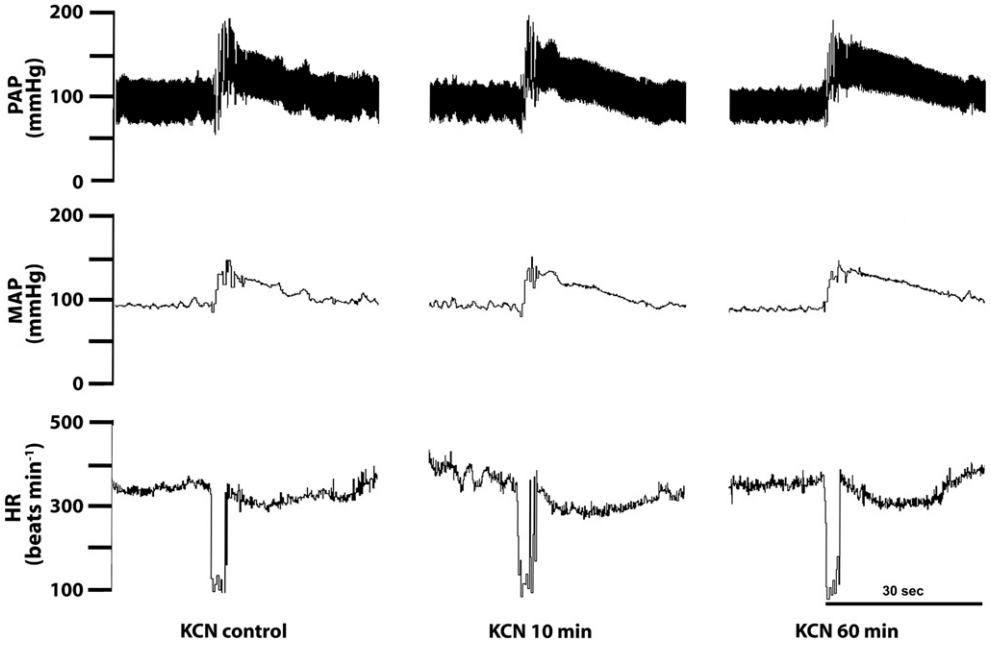

Fig. 2. Typical tracings of one rat representative of the group showing the records of pulsatile arterial pressure (PAP, mmHg), mean arterial pressure (MAP, mmHg) and heart rate (HR, bpm). This figure also shows the changes in these parameters produced by chemoreflex activation with KCN (40 μg/0.1 mL/rat; iv.) before (control), 10 and 60 min after bilateral microinjections of $CoCl_2$ (1 mM) into the BNST.

Our results showed that BNST neurotransmission inhibition did not affect any cardiovascular reflex elicited by selective stimulation of peripheral chemoreceptors. In contrast to chemoreflex, in a previous study from our group (Crestani et al., 2006), it was observed that bilateral microinjections of $CoCl_2$ in the BNST cause reversible significant increase in the magnitude of bradycardia to intravenous phenylephrine measured 10 min after $CoCl_2$ microinjections, without significantly effect on tachycardic responses to intravenous sodium nitruprusside.

The role of the BNST in the central regulation of the cardiovascular system has been extensively investigated by our laboratory. In this context, we previously demonstrated that blockade of the local BNST neurotransmission evokes a reversible bradycardia increases (Crestani et al., 2006), suggesting that BNST modulates the parasympathetic component of the baroreflex. This BNST tonic influence on baroreflex bradycardiac response seems involve activation of glutamate NMDA-receptors, α_1-adenoceptors, 5-HT_{1A} receptors, and nitric oxide (Crestani et al., 2008; Alves et al., 2009, 2010). In addition to

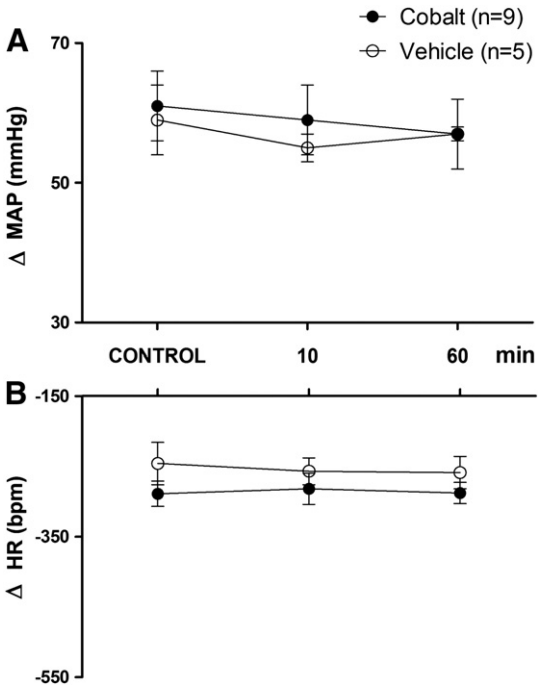

Fig. 3. Changes in mean arterial pressure (MAP, mmHg, panel A) and heart rate (HR, bpm, panel B) in response to chemoreflex activation with KCN (40 μg/0.1 mL/rat; iv.) before (control), 10 and 60 min after bilateral microinjections of $CoCl_2$ (1 mM, black circles, n = 9) or vehicle (open circles, n = 5) into the BNST. Data are means ± SEM.

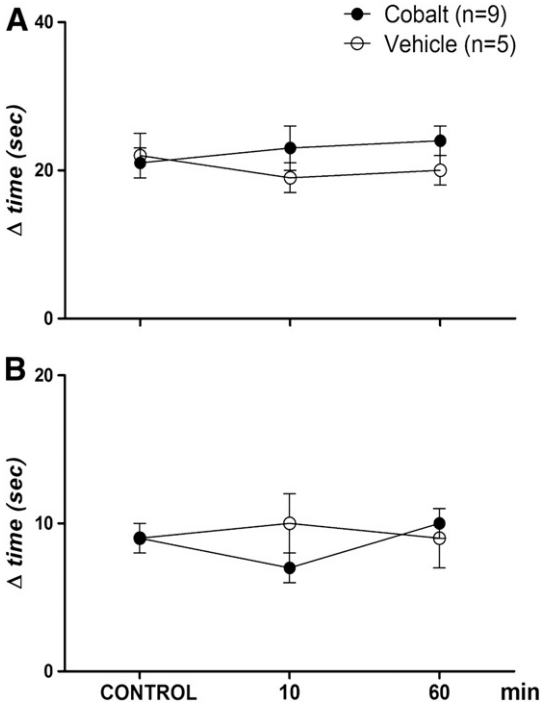

Fig. 4. Changes in the duration (Δ time, sec) of the pressor (panel A) and bradycardiac response (panel B) induced by chemoreflex activation before (control), 10 and 60 min after bilateral microinjection of $CoCl_2$ (1 mM, black circles, n = 9) or ACSF (open circles, n = 5) into the BNST. Data are means ± SEM.

baroreflex, BNST also plays an important role on cardiovascular adjustments during dynamic exercise, once that acute inhibition of this area reduced both pressor and tachycardic responses evoked by an acute bout of exercise on a rodent treadmill (Crestani et al., 2010). The pressor response to dynamic exercise seems involve activation of the BNST α_2-adrenoceptors (Alves et al., 2011). In spite of the fact, previous studies clearly demonstrated the important role of BNST on the cardiovascular reflex control, the possible influence of this area on the neural pathways of chemoreflex remain to be elucidated.

In the present study, KCN was used to briefly stimulate arterial chemoreceptors. Intravenous administration of KCN elicited pressor and bradycardiac responses, which were similar to that reported by previous studies (Granjeiro and Machado, 2009; Oliva et al., 2010; Granjeiro et al., 2011). The specificity of the cardiovascular components of the responses to KCN was established by complete elimination of the responses following bilateral ligature of the carotid body arteries (Franchini and Krieger, 1992, 1993; Haibara et al., 1995; Barros et al., 2002) or deafferentation of the carotid sinus nerve bifurcation (Franchini and Krieger, 1993) and supports the suggestion that the chemoreflex responses in the rat are primarily mediated through carotid chemoreceptor afferents (Kobayashi et al., 1999).

The afferents peripheral chemoreceptors in rats establish their first synapses in the caudal aspect of the commissural subnucleus of NTS bilaterally (Donoghue et al., 1984; Mifflin, 1992; Franchini and Krieger, 1993). There is evidence in favor of a direct projection from the NTS to the rostral ventrolateral medulla (RVLM), which could be part of the sympathoexcitatory component of the chemoreflex (Sequeira et al., 2000). Some studies in the literature indicate that peripheral chemoreflex neural pathways also may involve activation of mesencephalic and hypothalamic regions (Berquin et al., 2000; Olivan et al., 2001; Queiroz et al., 2011). Moreover, in a recent study from our laboratory, we observed that bilateral microinjections of $CoCl_2$ into the prelimbic (PL), but not infralimbic (IL) cortex, produced a significant reduction in the pressor response induced by KCN (Granjeiro et al., 2011), suggesting that PL plays an important neuromodulatory role in the processing of the sympathoexcitatory component of the chemoreflex. The neuromodulation of the pressor response to peripheral chemoreflex activation may involve postsynaptic neurons in the NTS projecting to the PL cortex in order to excite sympathoexcitatory projections from the PL cortex to the RVLM or from the PL cortex directly to the thoracic spinal cord (Owens and Verberne, 2000; Gabbott et al., 2005).

The BNST is proposed to be an important relay station linking important forebrain structures involved in autonomic, neuroendocrine and behavioral responses, such as the amygdala, hippocampus and medial prefrontal cortex, to the hypothalamus and autonomic regulatory brainstem areas (Herman et al., 1994; Herman and Cullinan, 1997; Dong et al., 2001a; Alheid, 2003; Vertes, 2006). Thus, it could be plausible to consider that the neuromodulation of the pressor response induced by chemoreflex activation could occur through a modulation of signals arising from PL cortex to the BNST. However, Massi et al. (2008), using retrograde tract-tracing methods, observed that IL strongly innervates the BNST through a direct projection, whereas PL only project sparingly to this site in rats (Massi et al., 2008). Accordingly, was demonstrated dense IL but minimal PL projections to the BNST (Vertes, 2004). Furthermore, our results did not show any functional evidence of involvement of the BNST in the modulation of cardiovascular responses elicited by acute chemoreflex activation.

These findings related to the lack of role of the BNST in the neurotransmission of the autonomic responses of acute chemoreflex does not rule out a possible involvement of this structure in the processing of the autonomic responses to the chronic activation of the peripheral chemoreceptors, as previously suggested (Ma et al., 2008). Additionally, considering that the BNST is also linked to different areas involved with respiratory control, including the NTS and the parabrachial nucleus (Dong and Swanson, 2004), future research is needed to elucidate the possible role of the BNST neurons on the modulation of the cardiovascular and respiratory responses to chronic chemoreflex activation.

In conclusion, the important finding of this study is that the pressor and bradycardic responses evoked by peripheral chemoreflex activation were not affected by blockade of BNST. These results suggest that the sympathoexcitatory and parasympathoexcitatory components of the chemoreflex do not involve reflex activation/modulation of neurons located in this brain region. However, considering that BNST is a cluster of 12 nuclei, which can be divided into anterior and posterior subdivisions and differ in their projection pattern and neurochemical identity (Dumont, 2009) further experiments using selective microinjections into different antero-posterior coordinates of BNST and specific antagonists are required to evaluate the possible neuromodulatory role of selective areas and/or BNST specific neurotransmitter on the cardiovascular responses elicited by acute or chronic chemoreflex activation.

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References

Accorsi-Mendonca, D., Leao, R.M., Aguiar, J.F., Varanda, W.A., Machado, B.H., 2007. Urethane inhibits the GABAergic neurotransmission in the nucleus of the solitary tract of rat brain stem slices. Am. J. Physiol. Regul. Integr. Comp. Physiol. 292, R396–R402.

Alheid, G.F., 2003. Extended amygdala and basal forebrain. Ann. N. Y. Acad. Sci. 985, 185–205.

Alves, F.H., Crestani, C.C., Resstel, L.B., Correa, F.M., 2009. Bed nucleus of the stria terminalis N-methyl-D-aspartate receptors and nitric oxide modulate the baroreflex cardiac component in unanesthetized rats. J. Neurosci. Res. 87, 1703–1711.

Alves, F.H., Crestani, C.C., Gomes, F.V., Guimaraes, F.S., Correa, F.M., Resstel, L.B., 2010. Cannabidiol injected into the bed nucleus of the stria terminalis modulates baroreflex activity through 5-HT1A receptors. Pharmacol. Res. 62, 228–236.

Alves, F.H., Resstel, L.B., Correa, F.M., Crestani, C.C., 2011. Bed nucleus of the stria terminalis alpha1- and alpha2-adrenoceptors differentially modulate the cardiovascular responses to exercise in rats. Neuroscience 177, 74–83.

Barros, R.C., Bonagamba, L.G., Okamoto-Canesin, R., de Oliveira, M., Branco, L.G., Machado, B.H., 2002. Cardiovascular responses to chemoreflex activation with potassium cyanide or hypoxic hypoxia in awake rats. Auton. Neurosci. 97, 110–115.

Berquin, P., Bodineau, L., Gros, F., Larnicol, N., 2000. Brainstem and hypothalamic areas involved in respiratory chemoreflexes: a Fos study in adult rats. Brain Res. 857, 30–40.

Braga, V.A., Soriano, R.N., Braccialli, A.L., de Paula, P.M., Bonagamba, L.G., Paton, J.F., Machado, B.H., 2007. Involvement of L-glutamate and ATP in the neurotransmission of the sympathoexcitatory component of the chemoreflex in the commissural nucleus tractus solitarii of awake rats and in the working heart-brainstem preparation. J. Physiol. 581, 1129–1145.

Costa-Silva, J.H., Zoccal, D.B., Machado, B.H., 2010. Glutamatergic antagonism in the NTS decreases post-inspiratory drive and changes phrenic and sympathetic coupling during chemoreflex activation. J. Neurophysiol. 103, 2095–2106.

Crestani, C.C., Alves, F.H., Resstel, L.B., Correa, F.M., 2006. The bed nucleus of the stria terminalis modulates baroreflex in rats. NeuroReport 17, 1531–1535.

Crestani, C.C., Alves, F.H., Resstel, L.B., Correa, F.M., 2008. Bed nucleus of the stria terminalis alpha(1)-adrenoceptor modulates baroreflex cardiac component in unanesthetized rats. Brain Res. 1245, 108–115.

Crestani, C.C., Alves, F.H., Resstel, L.B., Correa, F.M., 2010. The bed nucleus of the stria terminalis modulates exercise-evoked cardiovascular responses in rats. Exp. Physiol. 95, 69–79.

Dong, H.W., Swanson, L.W., 2004. Organization of axonal projections from the anterolateral area of the bed nuclei of the stria terminalis. J. Comp. Neurol. 468, 277–298.

Dong, H.W., Petrovich, G.D., Swanson, L.W., 2001a. Topography of projections from amygdala to bed nuclei of the stria terminalis. Brain Res Brain Res Rev. 38, 192–246.

Dong, H.W., Petrovich, G.D., Watts, A.G., Swanson, L.W., 2001b. Basic organization of projections from the oval and fusiform nuclei of the bed nuclei of the stria terminalis in adult rat brain. J. Comp. Neurol. 436, 430–455.

Donoghue, S., Felder, R.B., Jordan, D., Spyer, K.M., 1984. The central projections of carotid baroreceptors and chemoreceptors in the cat: a neurophysiological study. J. Physiol. 347, 397–409.

Dumont, E.C., 2009. What is the bed nucleus of the stria terminalis? Prog. Neuropsychopharmacol. Biol. Psychiatry. 33, 1289–1290.
Forray, M.I., Gysling, K., 2004. Role of noradrenergic projections to the bed nucleus of the stria terminalis in the regulation of the hypothalamic-pituitary-adrenal axis. Brain Res. Rev. 47, 145–160.
Franchini, K.G., Krieger, E.M., 1992. Carotid chemoreceptors influence arterial pressure in intact and aortic-denervated rats. Am. J. Physiol. 262, R677–R683.
Franchini, K.G., Krieger, E.M., 1993. Cardiovascular responses of conscious rats to carotid body chemoreceptor stimulation by intravenous KCN. J. Auton. Nerv. Syst. 42, 63–69.
Gabbott, P.L., Warner, T.A., Jays, P.R., Salway, P., Busby, S.J., 2005. Prefrontal cortex in the rat: projections to subcortical autonomic, motor, and limbic centers. J. Comp. Neurol. 492, 145–177.
Gelsema, A.J., Copeland, N.E., Drolet, G., Bachelard, H., 1993. Cardiovascular effects of neuronal activation of the extended amygdala in rats. Brain Res. 626, 156–166.
Giancola, S.B., Roder, S., Ciriello, J., 1993. Contribution of caudal ventrolateral medulla to the cardiovascular responses elicited by activation of bed nucleus of the stria terminalis. Brain Res. 606, 162–166.
Granjeiro, E.M., Machado, B.H., 2009. NO in the caudal NTS modulates the increase in respiratory frequency in response to chemoreflex activation in awake rats. Respir. Physiol. Neurobiol. 166, 32–40.
Granjeiro, E.M., Scopinho, A.A., Correa, F.M., Resstel, L.B., 2011. Prelimbic but not infralimbic cortex is involved in the pressor response to chemoreflex activation in awake rats. Exp. Physiol. 96, 518–527.
Guyenet, P.G., 2000. Neural structures that mediate sympathoexcitation during hypoxia. Respir. Physiol. 121, 147–162.
Haibara, A.S., Colombari, E., Chianca Jr., D.A., Bonagamba, L.G., Machado, B.H., 1995. NMDA receptors in NTS are involved in bradycardic but not in pressor response of chemoreflex. Am. J. Physiol. 269, H1421–H1427.
Hatam, M., Nasimi, A., 2007. Glutamatergic systems in the bed nucleus of the stria terminalis, effects on cardiovascular system. Exp. Brain Res. 178, 394–401.
Herman, J.P., Cullinan, W.E., 1997. Neurocircuitry of stress: central control of the hypothalamo-pituitary-adrenocortical axis. Trends Neurosci. 20, 78–84.
Herman, J.P., Cullinan, W.E., Watson, S.J., 1994. Involvement of the bed nucleus of the stria terminalis in tonic regulation of paraventricular hypothalamic CRH and AVP mRNA expression. J. Neuroendocrinol. 6, 433–442.
Kobayashi, M., Cheng, Z.B., Tanaka, K., Nosaka, S., 1999. Is the aortic depressor nerve involved in arterial chemoreflexes in rats? J. Auton. Nerv. Syst. 78, 38–48.
Kretz, R., 1984. Local cobalt injection: a method to discriminate presynaptic axonal from postsynaptic neuronal activity. J Neurosci. Meth. 11, 129–135.
Lomber, S.G., 1999. The advantages and limitations of permanent or reversible deactivation techniques in the assessment of neural function. J. Neurosci. Meth. 86, 109–117.
Ma, S., Mifflin, S.W., Cunningham, J.T., Morilak, D.A., 2008. Chronic intermittent hypoxia sensitizes acute hypothalamic-pituitary-adrenal stress reactivity and Fos induction in the rat locus coeruleus in response to subsequent immobilization stress. Neuroscience 154, 1639–1647.
Machado, B.H., Bonagamba, L.G., 1992. Microinjection of L-glutamate into the nucleus tractus solitarii increases arterial pressure in conscious rats. Brain Res. 576, 131–138.
Martin, L.J., Powers, R.E., Dellovade, T.L., Price, D.L., 1991. The bed nucleus-amygdala continuum in human and monkey. J. Comp. Neurol. 309, 445–485.
Massi, L., Elezgarai, I., Puente, N., Reguero, L., Grandes, P., Manzoni, O.J., Georges, F., 2008. Cannabinoid receptors in the bed nucleus of the stria terminalis control cortical excitation of midbrain dopamine cells in vivo. J. Neurosci. 28, 10496–10508.
Mifflin, S.W., 1992. Arterial chemoreceptor input to nucleus tractus solitarius. Am. J. Physiol. 263, R368–R375.
Narkiewicz, K., Pesek, C.A., van de Borne, P.J., Kato, M., Somers, V.K., 1999. Enhanced sympathetic and ventilatory responses to central chemoreflex activation in heart failure. Circulation 100, 262–267.
Oliva, W.M., Granjeiro, E.M., Bongamba, L.G., Mendes, R.A., Machado, B.H., 2010. Dopamine microinjected into brainstem of awake rats affects baseline arterial pressure but not chemoreflex responses. Auton. Neurosci. 155, 73–81.
Olivan, M.V., Bonagamba, L.G., Machado, B.H., 2001. Involvement of the paraventricular nucleus of the hypothalamus in the pressor response to chemoreflex activation in awake rats. Brain Res. 895, 167–172.
Owens, N.C., Verberne, A.J., 2000. Medial prefrontal depressor response: involvement of the rostral and caudal ventrolateral medulla in the rat. J. Auton. Nerv. Syst. 78, 86–93.
Paxinos, G., Watson, C., 1997. The rat brain in stereotaxic coordinates, 2.ed. Academic Press, Sydney.
Queiroz, E.A., Okada, M.N., Fumega, U., Fontes, M.A., Moraes, M.F., Haibara, A.S., 2011. Excitatory amino acid receptors in the dorsomedial hypothalamus are involved in the cardiovascular and behavioural chemoreflex responses. Exp. Physiol. 96, 73–84.
Reddy, M.K., Patel, K.P., Schultz, H.D., 2005. Differential role of the paraventricular nucleus of the hypothalamus in modulating the sympathoexcitatory component of peripheral and central chemoreflexes. Am. J. Physiol. Regul. Integr. Comp. Physiol. 289, R789–R797.
Sequeira, H., Viltart, O., Ba-M'Hamed, S., Poulain, P., 2000. Cortical control of somatocardiovascular integration: neuroanatomical studies. Brain Res. Bull. 53, 87–93.
Shin, J.W., Geerling, J.C., Loewy, A.D., 2008. Inputs to the ventrolateral bed nucleus of the stria terminalis. J. Comp. Neurol. 511, 628–657.
Sun, S.Y., Wang, W., Zucker, I.H., Schultz, H.D., 1999. Enhanced peripheral chemoreflex function in conscious rabbits with pacing-induced heart failure. J. Appl. Physiol. 86, 1264–1272.
Vertes, R.P., 2004. Differential projections of the infralimbic and prelimbic cortex in the rat. Synapse 51, 32–58.
Vertes, R.P., 2006. Interactions among the medial prefrontal cortex, hippocampus and midline thalamus in emotional and cognitive processing in the rat. Neuroscience 142, 1–20.
Walker, D.L., Toufexis, D.J., Davis, M., 2003. Role of the bed nucleus of the stria terminalis versus the amygdala in fear, stress, and anxiety. Eur. J. Pharmacol. 463, 199–216.
Zoccal, D.B., Bonagamba, L.G., Paton, J.F., Machado, B.H., 2009. Sympathetic-mediated hypertension of awake juvenile rats submitted to chronic intermittent hypoxia is not linked to baroreflex dysfunction. Exp. Physiol. 94, 972–983.