

Chemosensory control by commissural nucleus of the solitary tract in rats

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ABSTRACT

The commissural nucleus of the solitary tract (commNTS) is a main area that receives afferent signals involved in the cardiovascular and respiratory control like those related to chemoreceptor activation, however, the importance of the commNTS for the cardiorespiratory responses to chemoreceptor activation is still controversial. In the present study, we investigated the cardiorespiratory responses to hypoxia or hypercapnia in anesthetized and conscious rats treated with injections of the GABA-A agonist muscimol into the caudal portion of the commNTS. Male Holtzman rats (280–300 g) were used. In conscious rats that had a stainless steel cannula previously implanted into the commNTS, the injection of muscimol (2 mM) into the commNTS reduced the pressor response (16 ± 2 mmHg, vs. saline: 36 ± 3 mmHg) and the increase in ventilation (250 ± 17 ml/min/kg, vs. saline: 641 ± 28 ml/min/kg) produced by hypoxia (8–10% O₂). In urethane anesthetized rats, the injection of muscimol into the commNTS eliminated the pressor response (5 ± 2 mmHg, vs. saline: 26 ± 5 mmHg) and the increase in phrenic nerve discharge (PND) ($20 \pm 6\%$, vs. saline: $149 \pm 15\%$) and reduced the increase in splanchnic sympathetic nerve discharge (sSND) ($93 \pm 15\%$, vs. saline: $283 \pm 19\%$ of baseline) produced by hypoxia. However, muscimol injected into the commNTS did not change hypercapnia (8–10% CO₂) induced pressor response or the increase in the sSND or PND in urethane anesthetized rats or the increase in ventilation in conscious rats. The present results suggest that the cardiorespiratory responses to hypoxia are strongly dependent on the caudal portion of the commNTS, however, this area is not involved in the responses to hypercapnia.

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1. Introduction

The neural mechanisms involved in the control of breathing must be responsive to challenges affecting O₂, CO₂, and pH levels in the body, such as exercise, sleep, hypercapnia and hypoxia (Feldman et al., 2003; Nattie, 2006). The physiological process by which blood gases are detected, called chemoreception, depends on chemical sensors present in the aortic or carotid body (peripheral chemoreceptors) and within the central nervous system (CNS) (central chemoreceptors) (Ballantyne and Scheid, 2001; Feldman et al., 2003; Guyenet, 2008; Loeschcke, 1982).

The peripheral chemoreceptors, located mainly in the carotid body in the rat, detect changes in the partial O₂ pressure (P_{O_2}) or the CO₂ pressure (P_{CO_2}) in the arterial blood and send signals through the glossopharyngeal nerve to the commissural region of the nucleus of the solitary tract (commNTS) (Blessing, 1997; Campanucci and Nurse, 2007; Colombari et al., 1996; Finley and

Katz, 1992; Sapru, 1996; Smith et al., 2006). Similar to the hypoxia, the intravenous (iv) injection of low dose of potassium cyanide (KCN) activates the peripheral chemoreceptors producing tachypneic, pressor and bradycardic responses that are abolished by the bilateral ligation of the carotid body arteries (Braga et al., 2007; Franchini and Krieger, 1993; Haibara et al., 1999; Moreira et al., 2006). The pressor and bradycardic responses to i.v. KCN are also abolished by electrolytic lesions of the commNTS (Colombari et al., 1996). Under anesthesia, the activation of breathing and the rise in sympathetic nerve discharge (SND) caused by carotid body stimulation are blocked by the injection of glutamatergic antagonists into the commNTS, which suggests that the primary afferent neurons are likely glutamatergic (Sapru, 1996).

Detection of an increase in P_{CO_2} by central and peripheral chemoreception acts to maintain stable arterial P_{CO_2} (Feldman et al., 2003; Smith et al., 2006). It is still not clear whether the central chemoreception depends on a few specialized cell clusters located within the brainstem or on multiple types of acid-sensitive neurons (Chernov et al., 2008; Guyenet et al., 2010; Nattie and Li, 2009). The retrotrapezoid nucleus (RTN), locus coeruleus, medullary raphe, hypothalamic orexinergic neurons and the NTS neurons are the main sites suggested to be involved with the

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central chemoreception (Abbott et al., 2009; Biancardi et al., 2008; Dean et al., 1989; Deng et al., 2007; Johnson et al., 2008; Moreira et al., 2007; Mulkey et al., 2004; Nattie and Li, 2008; Richerson, 2004; Takakura et al., 2006; Williams et al., 2007).

The main focus of the present study is to reexamine the question whether the NTS, particularly the commissural division of the NTS caudal to the area postrema, is involved in chemoreception. Studies from the literature have suggested that acid-responsive neurons are located in the NTS and the acidification of the NTS region alters breathing (Dean et al., 1989; Nattie and Li, 2008). Additionally, previous studies have tested the effects of the lesions or the glutamatergic blockade in the commNTS, suggesting that this region is essential for the cardiorespiratory responses to the peripheral chemoreceptor activation (Colombari et al., 1996; Sapru, 1996; Braga et al., 2007). On the other hand, a more recent study evaluated the effects of muscimol microdialysis in a more rostral portion of the commNTS, suggesting that the rostral portion of the commNTS is involved mainly with respiratory responses to hypercapnia (Nattie and Li, 2008). Based on the assumptions described above, in the present study, also using muscimol injection to block the neuronal activity, we investigated the importance of the neurons located in a more caudal portion of the commNTS for the cardiorespiratory responses elicited by chemoreflex activation with hypoxia (8–10% O₂ in the breathing air) or hypercapnia (8–10% CO₂ in the breathing air) in conscious or anesthetized rats.

2. Methods

2.1. Animals

The experiments were performed on 36 male Holtzman rats weighing 300–330 g. The animals were housed individually in stainless steel cages in a room with controlled temperature (24 ± 2 °C) and humidity (55 ± 10%). Lights were on from 7:00 am to 7:00 pm. Standard Guabi rat chow (Paulinia, SP, Brazil) and tap water were available ad libitum. The experimental protocols were approved by the Animal Experimentation Ethics Committee of the Institute of Biomedical Science of University of São Paulo. All efforts were made to minimize animal discomfort and the number of animals used.

2.2. Surgical procedures

2.2.1. Conscious animals

Rats were anesthetized with intraperitoneal (i.p.) injection of ketamine (80 mg/kg of body wt) combined with xylazine (7 mg/kg of body wt) and placed in a stereotaxic frame (model 1760; David Kopf Instruments). A stainless steel cannula was implanted into the commNTS using the coordinates: 15.0 mm caudal to bregma, in the midline and 7.5 mm below dura mater. The cannulas were fixed to the cranium using dental acrylic resin and jeweler screws. Rats received a prophylactic dose of penicillin (30,000 IU) given intramuscularly and a subcutaneous injection of the analgesic Ketoflex (ketoprofen 1%, 0.03 ml/rat) post-surgically. After the surgery, the rats were maintained in individual box with free access of tap water and food pellets [Guabi rat chow (Paulinia, SP, Brazil)] for at least 7 days before the tests.

To record pulsatile arterial pressure (PAP), mean arterial pressure (MAP) and heart rate (HR) in unanesthetized freely moving rats, one day before the tests, rats were anesthetized again with i.p. injection of ketamine (80 mg/kg of body wt) combined with xylazine (7 mg/kg of body wt) to receive a polyethylene tubing (PE-10 connected to PE-50; Clay Adams, Parsippany, NJ, USA) inserted into the abdominal aorta through the femoral artery. Another polyethylene tubing was also inserted into the femoral vein for

drug administration. Both cannulas were tunneled subcutaneously to the back of the rats to allow access in unrestrained, freely moving rats. We have evidence that the animals recovery from the anesthesia and operative stress, because 1 day after the surgery the animals had normal drink and food intake and no impairment of motor activity. Although motor activity was not quantified, visual observation in their home cages and during handling revealed no apparent differences in reactivity or locomotion 1 day after the surgery.

2.2.2. Anesthetized animals

General anesthesia was induced with 5% halothane in 100% oxygen. The rats received a tracheostomy and surgery was done under artificial ventilation with 1.4–1.5% halothane in 100% oxygen. All rats were subjected to the following previously described surgical procedures: femoral artery cannulation for arterial pressure measurement, femoral vein cannulation for administration of fluids and drugs, removal of the occipital bone and retracting the underlying dura mater for insertion of a pipette for microinjection into the medulla oblongata via a dorsal trans cerebellar approach (Moreira et al., 2005, 2006). All animals were bilaterally vagotomized to prevent any influence of artificial ventilation on phrenic nerve discharge (PND). The phrenic nerve was accessed by a dorsolateral approach after retraction of the right shoulder blade. In a group of rats ($n=7$), used to test cardiorespiratory responses to hypercapnia, a complete baro- and peripheral chemoreceptor deafferentation was performed by sectioning the vagosympathetic trunks, the superior laryngeal nerves and the glossopharyngeal nerves (proximal to the junction with the carotid sinus nerves). Another rats ($n=6$), used to test the cardiorespiratory responses to hypoxia, was a group of baro- and chemo-receptor intact rats, that had the vagi nerves carefully separated from the vagosympathetic trunk and selectively transected bilaterally.

Splanchnic sympathetic nerve discharge (sSND) was recorded as previously described (Mandel and Schreihöfer, 2008; Moreira et al., 2006; Takakura et al., 2011). The right splanchnic nerve was isolated via a retroperitoneal approach, and the segment distal to the suprarenal ganglion was placed on a pair of teflon-coated silver wires that had been bared at the tip (250 μm bare diameter; A-M Systems, www.a-msystems.com). The nerves and wires were embedded in adhesive material (Kwik-Cast Sealant, WPI, USP), and the wound was closed around the exiting recording wires.

Upon completion of the surgical procedures, halothane was replaced by urethane (1.2 g/kg of body weight) administered slowly i.v. All rats were ventilated with 100% oxygen throughout the experiment. The rectal temperature was maintained at 37 °C and the end tidal-CO₂ (ETCO₂) were monitored throughout the experiment with a capnometer (CWE, Inc., Ardmore, PA, USA) that was calibrated twice per experiment against a calibrated CO₂/N₂ mix. The adequacy of the anesthesia was monitored during a 20 min stabilization period by testing for the absence of withdrawal response, the lack of arterial pressure change and lack of change in the PND rate or amplitude to firm toe pinch. After these criteria were satisfied, the muscle relaxant pancuronium was administered at the initial dose of 1 mg/kg i.v. and the adequacy of anesthesia was thereafter gauged solely by the lack of increase in arterial pressure and PND rate or amplitude to firm toe pinch. Approximately hourly supplements of one-third of the initial dose of urethane were needed to satisfy these criteria during the course of the recording period (4 h).

2.3. Intraparenchymal injections

In the anesthetized rats placed in a stereotaxic frame (model 1760; David Kopf Instruments), muscimol (Sigma Chemicals Co., St-Louis, MO, USA, 2 mM or 100 pmol/50 nl, in sterile saline pH

7.4) was pressure injected into the commNTS (50 nl in 5 s) through single-barrel glass pipettes (20 μm tip diameter). Injections into the commNTS were made 400 μm caudal to the calamus scriptorius, in the midline and 0.3–0.5 mm below the dorsal surface of the brainstem.

In conscious freely moving rats, the same dose of muscimol was injected into the commNTS using 1 μl Hamilton syringes connected by polyethylene tubing (PE-10) to the injection needle 1.5 mm longer than the guide cannulas implanted into the brain.

The solution of muscimol contained a 5% dilution of fluorescent latex microbeads (Lumafuor, New City, NY, USA) for later histological identification of the injection sites (Moreira et al., 2006).

2.4. Recordings of physiological variables

2.4.1. Conscious animals

Twenty-four hours after the artery and vein cannulation, when the rats were completely recovered from the surgery and adapted to the environment of the recording room, the arterial catheter was connected to a pressure transducer (MLT844, ADInstruments, Sydney, NSW, Australia) coupled to a preamplifier (Bridge Amp, ML221, ADInstruments, Sydney, NSW, Australia) that was connected to a Powerlab computer data acquisition system (PowerLab 16/30, ML880, ADInstruments).

The respiratory rate (fR, breaths/min) and the tidal volume (VT, ml/kg) in conscious, freely moving rats were measured by whole-body plethysmography as described in detail previously (Malan, 1973; Onodera et al., 1997). All experiments were performed at room temperature (24–26 °C). In brief, freely moving rats were kept in a plexiglass recording chamber (5 L) that was flushed continuously with a mixture of 79% nitrogen and 21% oxygen (unless otherwise required by the protocol) at a rate of 1 L/min. The concentrations of O₂ and CO₂ in the chamber were monitored on-line using a fast-response O₂/CO₂ monitor (ADInstruments, NSW, Australia). The pressure signal was amplified, filtered, recorded, and analyzed off-line using Powerlab software (Powerlab 16/30, ML880/P, ADInstruments, NSW, Australia). The values of fR and VT analyzed were those recorded for 2 min before the exposure to the stimulus and for 2 more min at the end of each stimulus, when breathing stabilized. Changes in the fR, VT, and minute ventilation (\dot{V}_E) (fR \times VT; ml/min/kg) were averaged and expressed as means \pm SEM.

2.4.2. Anesthetized animals

The mean arterial pressure, the discharge of the phrenic and splanchnic nerves and the tracheal O₂ and CO₂ were recorded as previously described (Moreira et al., 2006, 2007).

Before starting the experiments, the ventilation was adjusted to have the ET_{CO₂} at 3–4% at steady-state (60–80 cycles/s; tidal volume 1–1.2 ml/100 g). This condition was selected because 3–4% end-expiratory CO₂ was below the threshold of the PND. Variable amounts of pure CO₂ were added to the breathing mixture to adjust ET_{CO₂} to the desired level.

All analog data (ET_{CO₂}, sSND, PND and MAP) were stored on a computer via a micro1401 digitizer (Cambridge Electronic Design) and were processed off-line using version 6 of the Spike 2 software (Cambridge Electronic Design) as described previously (Takakura et al., 2006, 2011). The integrated phrenic nerve discharge (iPND) and the integrated splanchnic nerve discharge (iSND) were obtained after the rectification and smoothing ($\tau = 0.015$ and 2 s, respectively) of the original signal, which was acquired with a 30–300 Hz bandpass.

Neural minute \times volume (mvPND, a measure of the total phrenic nerve discharge per unit of time) was determined by averaging the iPND over 50 s and normalizing the result by assigning a value of 0 to the dependent variable recorded at the low levels of end-expiratory CO₂ (below threshold) and a value of 1 at the highest level of P_{CO₂}

investigated (between 9.5 and 10%). The iSND was normalized for each animal by assigning the value of 100 to the resting SNA and the value of 0 to the minimum value recorded either during the administration of a dose of phenylephrine that saturated the baroreflex (5 $\mu\text{g}/\text{kg}$, i.v.) or after the ganglionic blockade (hexamethonium; 10 mg/kg, i.v.).

2.5. Chemoreflex and baroreflex tests

2.5.1. Chemoreflex tests

In anesthetized rats, the hypoxia was done by switching the breathing mixture from 100% O₂ to 8–10% O₂ balanced with N₂ for 60 s, the same protocol used in a previous study (Takakura et al., 2006). The hypercapnia was done by increasing ET_{CO₂} from 3–3.5% to 8–10% in hyperoxia condition (100% O₂) for 5 min (Takakura et al., 2011).

Conscious rats were maintained for at least 30 min at normoxia/normocapnia (21% O₂, 79% N₂, and <0.5% CO₂) to adapt to the chamber environment before starting the measurements of the baseline arterial pressure and ventilation. Hypoxia was induced by lowering the O₂ concentration in the inspired air down to a level of 8–10% for 60 s. Hypercapnia was produced by adding CO₂ in the respiratory mixture up to 8–10% CO₂ for 5 min under hyperoxic condition (90–92% O₂), to minimize possible effects of peripheral chemoreflex activation (Trapp et al., 2008).

2.5.2. Baroreflex tests

In conscious or anesthetized rats, the arterial baroreflex was examined by raising the arterial pressure with phenylephrine (5 $\mu\text{g}/\text{kg}$ of body weight, i.v.) and lowering the arterial pressure with sodium nitroprusside (30 $\mu\text{g}/\text{kg}$ of body weight, i.v.). These doses of i.v. drugs were the same used in previous studies (Moreira et al., 2005, 2006; Takakura et al., 2009). For the i.v. injections, the drugs were prepared in sterile isotonic saline and the reflex tests were performed in the same order with drug injections separated by a 5 min interval.

2.6. Histology

At the end of the experiments, rats were deeply anesthetized with halothane and perfused transcardially with saline followed by 10% buffered formalin (pH 7.4). The brain was removed and stored in the fixative for 24 h at 4 °C. The medulla was cut in 40 μm coronal sections with a vibrating microtome (Vibratome 1000S Plus – Starter CE, 220 V/60 Hz, USA), and stored in a cryoprotectant solution at –20 °C (Nattie and Li, 2008). The injection site was verified with a conventional multifunction microscope (Olympus BX50F4, Japan). The section alignment between the brains was done relative to a reference section. To align the sections around NTS level, the mid-area postrema level was identified in each brain and assigned the level 13.8 mm (Bregma –13.8 mm) according to the atlas of Paxinos and Watson (1998). The coordinates of sections rostral and caudal of this reference section were calculated with respect to the reference section, using the number of intervening sections and the section thickness.

2.7. Statistics

The statistical analysis was done with Sigma Stat version 3.0 (Jandel Corporation, Point Richmond, CA). The data are reported as means \pm standard error of the mean (SEM). The *t*-test or one way parametric ANOVA followed by the Newman–Keuls multiple comparisons test were used as appropriate. The significance was set at $p < 0.05$.

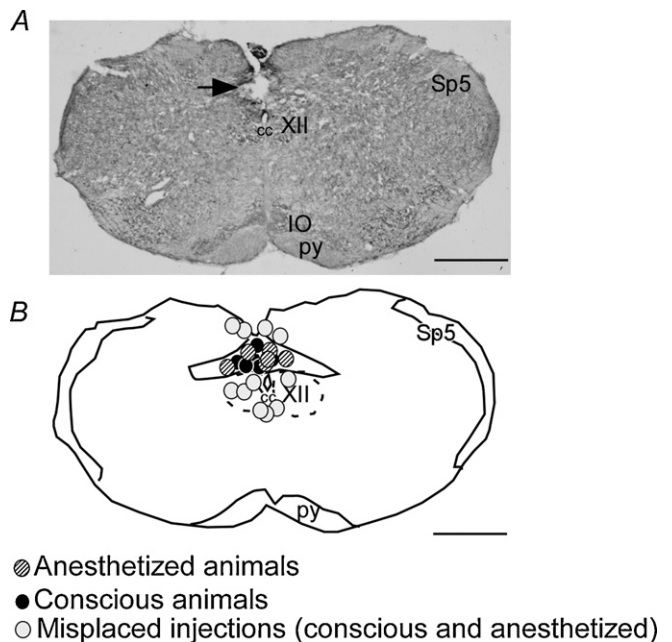


Fig. 1. (A) Photomicrograph showing the typical injection site into the commNTS in conscious rats (arrows). Scale = 1 mm. (B) The center of muscimol injections into the commNTS in the different rats tested represented on a single section (Bregma –14.3 mm, according to Paxinos and Watson, 1998). Scale = 1 mm. cc, central canal; IO, inferior olive; py, pyramide; Sp5, spinal trigeminal tract; XII, hypoglossal nucleus.

3. Results

3.1. Histological analysis

Muscimol injections into the commNTS were located about 400 μm caudal to the *calamus scriptorius* as illustrated in Fig. 1A and B. A single injection of muscimol was administered in or near the midline as represented in Fig. 1B. Based on the area of the distribution of the fluorescent microbeads, the injectate spread bilaterally (approximately 500 μm from the injection center) and a little less in the rostrocaudal direction (approximately 300 μm from the injection center).

3.2. Cardiorespiratory responses to hypoxia in anesthetized or conscious rats treated with muscimol into the commNTS

3.2.1. Anesthetized animals

In urethane-anesthetized rats, in control conditions (after saline injected into the commNTS), a brief period of hypoxia (8–10% O_2 in the breathing air for 60 s) produced an initial increase in MAP (26 ± 5 mmHg) in the first 5–10 s followed by a decrease in MAP (-47 ± 6 mmHg) that reach the maximum at the end of the period of hypoxia (Fig. 2A1 and B1). In these conditions, hypoxia also increased sSND ($283 \pm 19\%$ of the baseline) and mvPND (calculated as the product of phrenic nerve frequency and amplitude – $f \times a$ – a measure of the total phrenic neural output) ($149 \pm 25\%$ of the baseline) (Fig. 2A1, C and D).

Injection of muscimol (100 pmol/50 nl) into the commNTS did not change resting MAP (112 ± 3 mmHg, vs. saline: 110 ± 5 mmHg, $p > 0.05$), sSND and mvPND (Fig. 2A2). The PND amplitude ($98 \pm 6\%$ of control; $p > 0.05$) and duration (from 0.48 ± 0.02 to 0.47 ± 0.05 s, $p > 0.05$) also did not change. Muscimol injected within the commNTS blocked the pressor response (5 ± 2 mmHg, $p < 0.01$) and reduced sympathoexcitation ($93 \pm 15\%$ of the baseline, $p < 0.01$) and the increase in PND ($20 \pm 6\%$ of the baseline, $p < 0.01$) produced by hypoxia (Fig. 2A2, 2B–D). Muscimol into the commNTS also

increased the hypotension produced by 60 s of hypoxia in anesthetized rats (-63 ± 4 mmHg, $p < 0.05$) (Fig. 2A2 and B).

3.2.2. Conscious animals

In conscious rats, in control conditions (after saline injected into the commNTS), 60 s of hypoxia (8–10% O_2 in the inspired air) under normocapnia increased MAP (36 ± 3 mmHg), fR (60 ± 4 breaths/min), VT (4 ± 0.3 ml/kg) and \dot{V}_E (641 ± 28 ml/min/kg) and reduced HR (-96 ± 6 bpm) (Fig. 3A–E). Injection of muscimol (100 pmol/50 nl) into the commNTS, in conscious rats, did not change resting MAP (113 ± 6 mmHg, vs. saline: 117 ± 5 mmHg, $p > 0.05$) and HR (335 ± 21 bpm, vs. saline: 341 ± 18 bpm, $p > 0.05$). Muscimol injection within the commNTS reduced the increase on MAP (16 ± 2 mmHg, $p < 0.05$), fR (26 ± 3 breaths/min, $p < 0.05$), VT (1.8 ± 0.2 ml/kg, $p < 0.05$) and \dot{V}_E (250 ± 17 ml/kg/min, $p < 0.01$) and blocked the bradycardia (1 ± 2 bpm, $p < 0.01$) produced by hypoxia (Fig. 3A–F).

3.3. Cardiorespiratory responses to hypercapnia in anesthetized or conscious rats treated with muscimol injected into the commNTS

3.3.1. Anesthetized animals

In urethane-anesthetized rats, in control conditions (after saline injected into the commNTS), hypercapnia (from 5% to 10% CO_2 for 5 min) produced an immediate hypotension (-22 ± 4 mmHg) that was gradually reduced with MAP returning to or slightly above control levels at the end of hypercapnia. Immediately after stopping hypercapnia (returning to 5% CO_2), MAP increased (27 ± 5 mmHg) and returned to control values after around 5 min (Fig. 4A1 and B). In control condition, hypercapnia also increased sSND ($108 \pm 13\%$ of baseline at 5% CO_2) and mvPND ($111 \pm 8\%$ of the baseline at 5% CO_2) (Fig. 4A1, C and D). Injection of muscimol (100 pmol/50 nl) into the commNTS did not affect resting MAP, sSND or mvPND or hypercapnia-induced increase in MAP, sSND or mvPND in urethane anesthetized rats (Fig. 4A2, B–D).

3.3.2. Conscious animals

In conscious rats, in control conditions (after saline injected into the commNTS), hypercapnia (8–10% CO_2 in the inspired air) for 5 min under hyperoxic condition (92–98% O_2 , to minimize possible effects of peripheral chemoreflex activation) increased fR (55 ± 6 breaths/min), VT (3.7 ± 0.4 ml/kg) and \dot{V}_E (611 ± 19 ml/min/kg), however, produced no significant change in MAP (5 ± 2 mmHg) or HR (-4 ± 3 bpm) (Table 1). Injection of muscimol (100 pmol/50 nl) into the commNTS produced no change in resting MAP, HR and VE or on cardiorespiratory responses to hypercapnia in conscious rats (Table 1).

3.4. Cardiovascular responses to baroreflex activation in rats treated with muscimol injected into the commNTS

3.4.1. Anesthetized animals

Injections of muscimol (100 pmol/50 nl) within the commNTS in anesthetized rats did not affect the pressor response and sympathoinhibition to i.v. phenylephrine (PHE, 5 $\mu\text{g}/\text{kg}$ of body weight) or the hypotension and sympathoactivation to i.v. injection of sodium nitroprusside (SNP, 30 $\mu\text{g}/\text{kg}$ of body weight) (Table 2). PHE or SNP i.v. did not modify mvPND (Table 2).

3.4.2. Conscious animals

In conscious rats, injection of muscimol (100 pmol/50 nl) within the commNTS also did not affect the pressor and bradycardic responses to i.v. PHE or the hypotension and tachycardia to i.v. injection of SNP (Table 3). Activation or deactivation of baroreceptors by

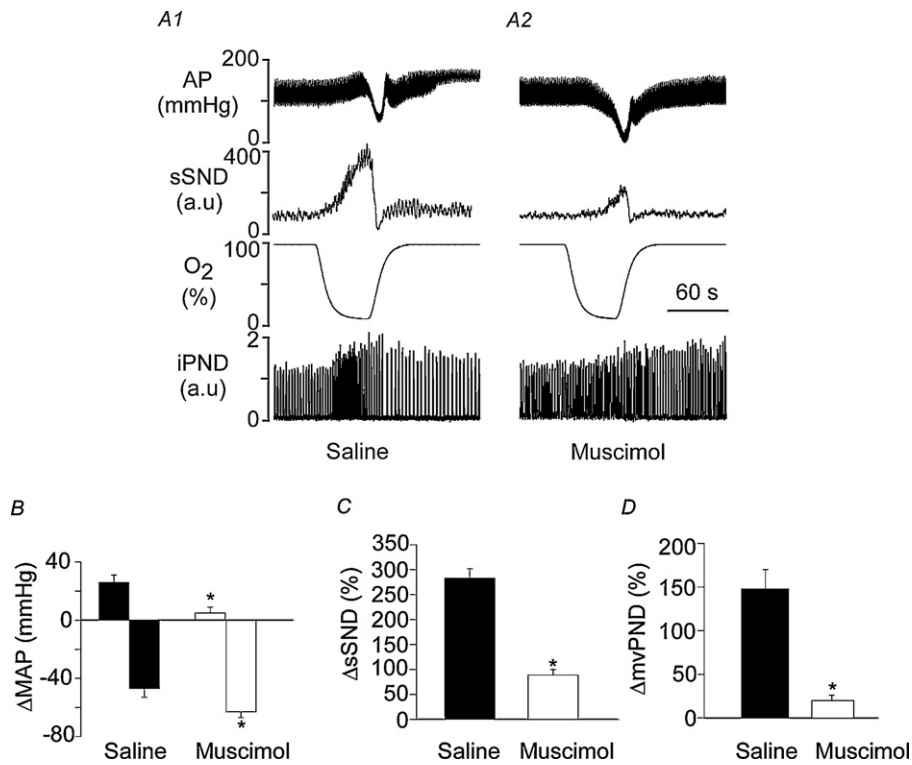


Fig. 2. (A) Typical recordings of arterial pressure (AP), splanchnic sympathetic nerve discharge (sSND) and phrenic nerve discharge (PND) in one rat representative of the group submitted to 60 s of hypoxia (8–10% O₂) after injection of (A1) saline or (A2) muscimol (100 pmol/50 nl) into the commNTS under anesthesia. (B, C and D) Changes in mean arterial pressure (ΔMAP), sSND (ΔsSND) and mvPND (ΔmvPND), respectively, produced by 60 s of hypoxia after injection of saline or muscimol into the commNTS in anesthetized rats. * Different from saline ($p < 0.05$); $n = 6$ rats/group.

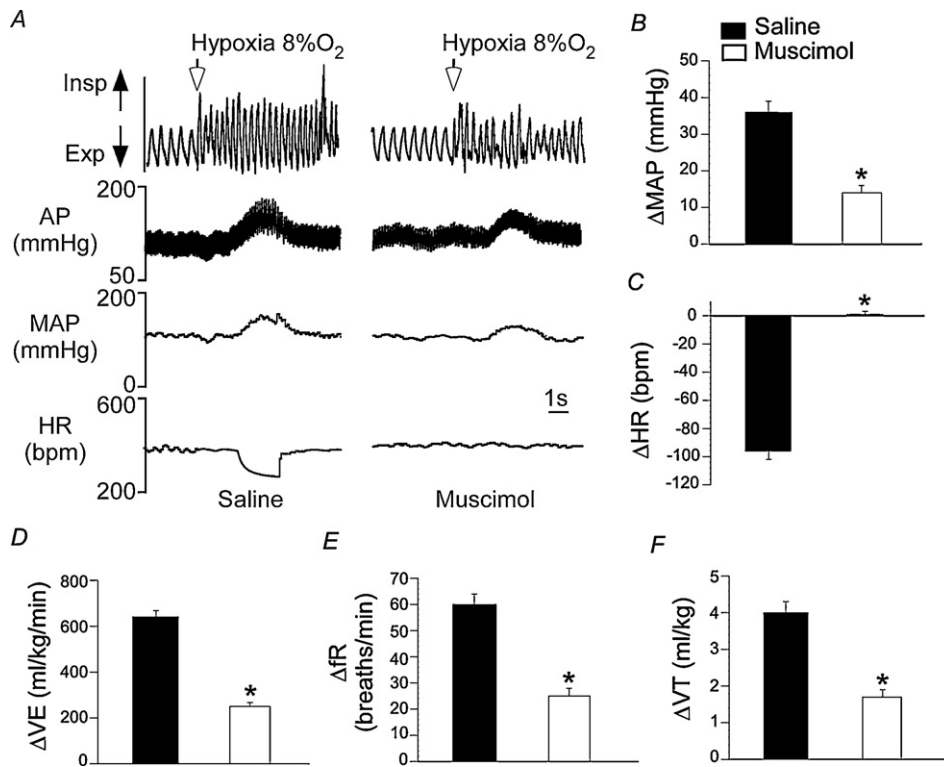


Fig. 3. (A) Typical recordings of breathing, arterial pressure (AP), mean arterial pressure (MAP) and heart rate (HR) in one rat representative of the group submitted to 60 s of hypoxia (8–10% O₂) after injection of saline or muscimol (100 pmol/50 nl) into the commNTS in conscious rats. (B–F) Changes in mean arterial pressure (MAP), heart rate (HR), ventilation (\dot{V}_E), respiratory frequency (fR) and tidal volume (VT), respectively, produced by hypoxia (8–10% O₂) before (saline injection) and after muscimol injection into the commNTS. * Different from saline ($p < 0.05$); $n = 6$ /group of rats.

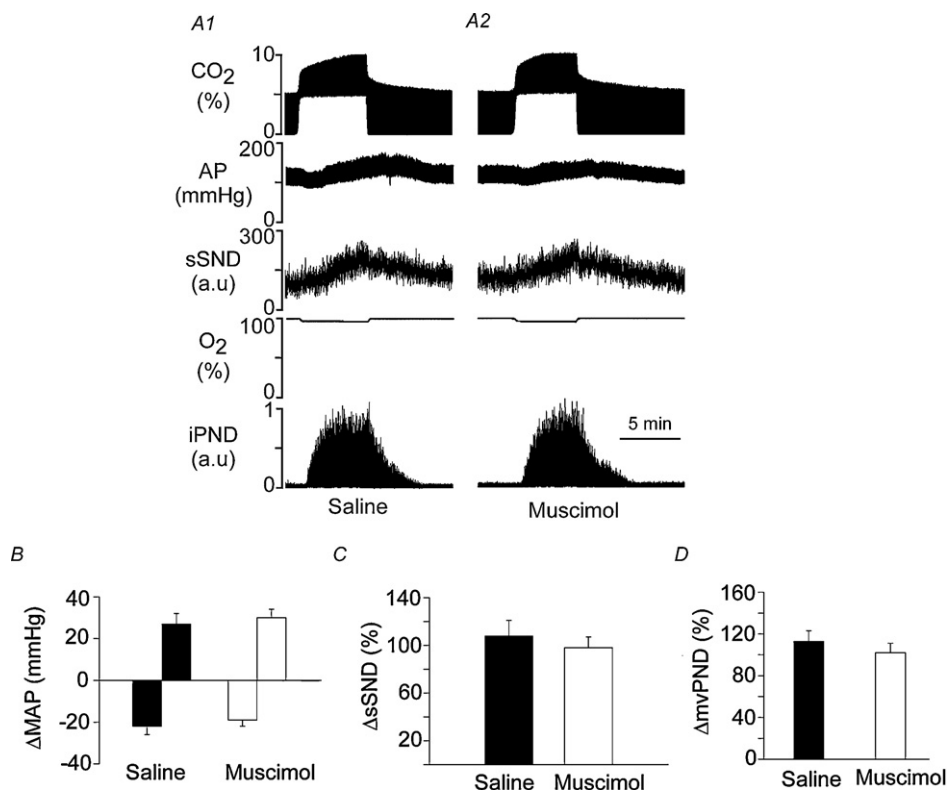


Fig. 4. (A) Typical recordings of arterial pressure (AP), splanchnic sympathetic nerve discharge (sSND) and phrenic nerve discharge (PND) in one rat representative of the group submitted to 5 min of hypercapnia (stepping ETCO_2 from 5 to 10%) after (A1) saline or (A2) injection of muscimol (100 pmol/50 nl) into the commNTS under anesthesia. (B–D) Changes in mean arterial pressure (ΔMAP), sSND (ΔsSND) and mvPND (ΔmvPND), respectively, produced by hypercapnia after injection of saline or muscimol into the commNTS in anesthetized rats. * Different from saline ($p < 0.05$); $n = 6$ rats/group.

Table 1
Cardiorespiratory responses to hypercapnia in conscious rats treated with muscimol injected into the commNTS.

Treatment	<i>n</i>	ΔMAP (mmHg)	ΔHR (bpm)	ΔfR (breaths/min)	ΔVT (ml/kg)	$\Delta\dot{V}_E$ (ml/min/kg)
Saline (control)	6	$+5 \pm 2$	-4 ± 3	$+55 \pm 6$	$+3.7 \pm 0.4$	$+611 \pm 19$
Muscimol	6	$+5 \pm 3$	-6 ± 2	$+53 \pm 5$	$+3.8 \pm 0.5$	$+668 \pm 28$

Values are means \pm SEM. n = number of rats. ΔMAP , changes in mean arterial pressure; ΔHR , changes in heart rate; ΔfR , changes in respiratory frequency; ΔVT , changes in tidal volume; $\Delta\dot{V}_E$, changes in ventilation. Muscimol (100 pmol/50 nl). Hypercapnia (8–10% CO_2 in the inspired air) for 5 min under hyperoxic condition (100% O_2).

Table 2
Cardiovascular responses to phenylephrine and sodium nitroprusside in anesthetized rats treated with muscimol injected into the commNTS.

Treatment	<i>n</i>	ΔMAP (mmHg)		ΔsSND (%)		ΔmvPND (%)	
		PHE	SNP	PHE	SNP	PHE	SNP
Saline (control)	5	$+32 \pm 3$	-34 ± 5	-98 ± 3	$+101 \pm 6$	$+9 \pm 9$	$+6 \pm 13$
Muscimol	6	$+34 \pm 7$	-38 ± 4	-94 ± 8	$+97 \pm 13$	$+10 \pm 4$	$+8 \pm 11$

Values are means \pm SEM. n = number of rats. PHE, phenylephrine (5 $\mu\text{g}/\text{kg}$ of body weight); SNP, sodium nitroprusside (30 $\mu\text{g}/\text{kg}$ of body weight); ΔMAP , changes in mean arterial pressure; ΔsSND , changes in splanchnic sympathetic nerve discharge; ΔmvPND , changes in phrenic nerve discharge.

PHE and SNP i.v., respectively, did not change \dot{V}_E in conscious rats (Table 3).

3.5. Specificity of commNTS as the site for the effects of muscimol

Injections of muscimol outside the commNTS ($n = 4$) did not change the pressor (25 ± 4 mmHg, $p > 0.05$), sympathetic ($270 \pm 15\%$ of baseline, $p > 0.05$) and phrenic ($136 \pm 9\%$ of baseline, $p > 0.05$) responses evoked by peripheral chemoreflex activation with brief period of hypoxia in anesthetized rats. In conscious rats, the injection of muscimol outside commNTS ($n = 7$) produced no change on pressor (33 ± 6 mmHg), fR (54 ± 9 breaths/min), VT (4.2 ± 0.4 ml/kg) and \dot{V}_E (631 ± 33 ml/min/kg) responses and on the bradycardia (-84 ± 11 bpm) produced by hypoxia.

4. Discussion

The present results provide functional evidence that the caudal portion of the commNTS is essential for the pressor response and the increase in the SND and breathing produced by hypoxia in conscious or anesthetized rats. However, the results show no evidence that this portion of the NTS is involved in mediating cardiorespiratory responses to hypercapnia. In addition, the inhibition of the caudal commNTS neurons did not modify the responses produced by baroreflex activation as previously demonstrated (Moreira et al., 2009).

The changes in arterial pressure produced by hypoxia or hypercapnia are the result of two opposite effects, a vasodilation due to the peripheral effect of the changes in O_2 or CO_2 and the

Table 3
Cardiovascular responses to phenylephrine and sodium nitroprusside in conscious rats treated with muscimol injected into the commNTS.

Treatment	n	Δ MAP (mmHg)		Δ HR (bpm)		$\Delta\dot{V}_E$ (ml/kg/min)	
		PHE	SNP	PHE	SNP	PHE	SNP
Saline (control)	6	+49 ± 5	–28 ± 6	–59 ± 8	+88 ± 4	+21 ± 8	+11 ± 5
Muscimol	6	+54 ± 6	–31 ± 5	–44 ± 11	+93 ± 8	+25 ± 7	+18 ± 8

Values are means ± SEM. n = number of rats. PHE, phenylephrine (5 µg/kg of body weight); SNP, sodium nitroprusside (30 µg/kg of body weight); Δ MAP, changes in mean arterial pressure; Δ HR, changes in heart rate; $\Delta\dot{V}_E$, changes in ventilation.

centrally mediated vasoconstriction that depends on chemoreceptor and sympathetic activation. Previous studies have suggested that anesthetics may affect neurotransmission on the brainstem and consequently reflex responses (Accorsi-Mendonça et al., 2007; Machado and Bonagamba, 1992). Therefore, in the present study, cardiorespiratory responses to hypoxia and hypercapnia were tested in anesthetized rats and also in conscious rats using whole-body plethysmography, which allows simultaneous measurements of respiratory and cardiovascular responses without restraining the animal (Biancardi et al., 2008; Braccialli et al., 2008; de Paula and Branco, 2005).

In urethane-anesthetized, vagotomized and artificially ventilated rats, in control conditions, hypoxia or hypercapnia produced a dual response on arterial pressure. The hypoxia produced an initial increase in MAP in the first 5–10 s that was followed by a decrease in MAP that reach the minimum value at the end of the period of hypoxia. The hypercapnia reduced arterial pressure in the first minute followed by an increase at the end of the 5-min hypercapnia. The hypoxia or hypercapnia rapidly increased PND and gradually increased sSND which reaches the maximum at the end of the test. In conscious rats, in control conditions, hypoxia or hypercapnia increased ventilation and hypoxia increased MAP, whereas hypercapnia produced no change in MAP.

The blockade of neuronal activity with muscimol injection into the commNTS almost abolished the pressor, sympathetic and phrenic responses to hypoxia in anesthetized rats and partially reduced the pressor and respiratory responses to hypoxia in conscious rats, whereas the same treatment in the commNTS produced no changes in the cardiorespiratory responses to hypercapnia in conscious or anesthetized rats. Therefore, in anesthetized or conscious rats, it seems that chemoreflex-mediated cardiovascular and respiratory responses to hypoxia are strongly dependent on caudal commNTS mechanisms. However, in conscious rats, neuronal blockade in the commNTS with muscimol only partially reduced cardiorespiratory responses to hypoxia. The effects of muscimol injected into the commNTS in conscious rats are similar to those previously demonstrated in the working heart-brainstem preparation after combining glutamatergic and purinergic receptor blockade in the commNTS (Braga et al., 2007), which suggest that in this case cardiorespiratory responses to hypoxia are also mediated by signals that arise from other central sites not related to commNTS.

A previous study showed that electrolytic lesions of the commNTS abolished the pressor and bradycardic responses to peripheral chemoreceptor activation with i.v. injection of KCN (Colombari et al., 1996). It is interesting to note that the results of the present study showed that muscimol into the commNTS only reduced the pressor responses to hypoxia in conscious rats, whereas in the previous study electrolytic lesions of the commNTS abolished the pressor response to i.v. KCN. These differences also suggest that, in conscious rats, besides the activation of peripheral chemoreceptors, additional mechanisms are activated by hypoxia, probably centrally, that do not depend on commNTS (Colombari et al., 1996).

Although the cardiorespiratory responses to hypoxia are strongly dependent on commNTS neuron activation, the present

results suggest that the same commNTS neurons involved in the responses to hypoxia are not involved in the cardiorespiratory responses to hypercapnia. Muscimol injections into the commNTS did not change the increase in arterial pressure, SND or breathing produced by hypercapnia. However, a previous study showed that it is possible to reduce the respiratory responses to hypercapnia by muscimol microdialysis in the commNTS, suggesting that commNTS may detect CO₂ (Nattie and Li, 2008). The same study also showed that muscimol microdialysis in the commNTS did not affect respiratory responses to hypoxia when rats were tested at room temperature of 24 °C, the same room temperature that rats were exposed in the present study. Therefore, the present and the previous study show different effects of the commNTS inhibition with muscimol in the control of the respiratory responses to hypoxia or hypercapnia. Possible reasons for the different results are the differences in the site of microdialysis/injections into the commNTS, the volume of microdialysis/injections and the concentration of muscimol released in the commNTS. In the previous study (Nattie and Li, 2008), microdialysis probes released muscimol into the commNTS bilaterally at the level of the area postrema, whereas in the present study just one injection was performed in the midline around 400 µm caudal to the area postrema, i.e., the previous study tested the effects of muscimol in a more rostral portion of the commNTS and the present study in a more caudal portion of the commNTS. Although, different sites of injections/microdialysis seem to be the main reason for the different results, in the previous study, the concentration of muscimol was 0.5 mM and the volume of microdialysis was 4 µl/min continuously throughout the entire experiment (Nattie and Li, 2008), whereas in the present study the concentration of muscimol was 2 mM and a volume of 50 nl was injected in a single injection. Although in both studies the nomenclature is the same (commissural NTS), they did not test the same area/neurons: the present study tested a more caudal portion of the commNTS and the previous study (Nattie and Li, 2008) tested a more rostral part of the commNTS. Therefore, based on the present and the previous study (Nattie and Li, 2008) it is possible to suggest that different parts of the commNTS are involved in the respiratory responses to hypoxia and hypercapnia. According to the present results, a more caudal portion of the commNTS is involved in cardiorespiratory responses to hypoxia, whereas a previous study suggests that a more rostral portion of the commNTS is the site of the pH-sensitive cells of the NTS important mainly for the respiratory responses to hypercapnia. These suggestions are coherent with the massive projections from the commNTS to the respiratory central pattern generator (CPG) (Aicher et al., 1996; Ezure and Tanaka, 2004; Koshiya and Guyenet, 1996; Kubin et al., 2006) that probably convey important signals from central and peripheral chemoreceptors.

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