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CAUDAL NUCLEI OF THE RAT NUCLEUS OF THE SOLITARY TRACT DIFFERENTIALLY INNERVATE RESPIRATORY COMPARTMENTS WITHIN THE VENTROLATERAL MEDULLA

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Abstract—A substantial array of respiratory, cardiovascular, visceral and somatic afferents are relayed via the nucleus of the solitary tract (NTS) to the brainstem (and forebrain). Despite some degree of overlap within the NTS, specificity is maintained in central respiratory reflexes driven by second order afferent relay neurons in the NTS. While the topographic arrangement of respiratory-related afferents targeting the NTS has been extensively investigated, their higher order brainstem targets beyond the NTS has only rarely been defined with any precision. Nonetheless, the various brainstem circuits serving blood gas homeostasis and airway protective reflexes must clearly receive a differential innervation from the NTS in order to evoke stimulus appropriate behavioral responses. Accordingly, we have examined the question of which specific NTS nuclei project to particular compartments within the ventral respiratory column (VRC) of the ventrolateral medulla. Our analyses of NTS labeling after retrograde tracer injections in the VRC and the nearby neuronal groups controlling autonomic function indicate a significant distinction between projections to the Bötzinger complex and preBötzinger complex compared to the remainder of the VRC. Specifically, the caudomedial NTS, including caudal portions of the medial solitary nucleus and the commissural division of NTS project relatively densely to the region of the retrotrapezoid nucleus and rostral ventrolateral medullary nucleus as well as to the rostral ventral respiratory group while avoiding the intervening Bötzinger and preBötzinger complexes. Area postrema appears to demonstrate a pattern of projections similar to that of caudal medial and commissural NTS nuclei. Other, less pronounced differential projections of lateral NTS nuclei to the various VRC compartments are additionally noted.

Key words: respiratory control, cardiovascular control, retrotrapezoid nucleus, ventral respiratory group, preBötzinger complex, rostral ventrolateral medulla.

The nucleus of the solitary tract (NTS) is a complex neuronal aggregate providing a critical interface between afferent information from the viscera, and the central circuits controlling a broad range of functions (Barraco, 1994; Saper, 2004; Bradley, 2007). Respiratory, autonomic, volitional, and emotional behaviors acting in conjunction with central, innate and/or learned sensory associations are all responsive to visceral feedback via the caudal NTS.

Breathing, in particular, is a voluntary and highly motivated somatomotor behavior supported by automatic rhythm and pattern generating circuits located in the rhombencephalon, and particularly within the ventrolateral medulla (Feldman and del Negro, 2006; McCrimmon et al., 2009; Smith et al., 2009). Medullary respiratory circuits include a column of cells in the ventrolateral medulla termed the ventral respiratory column (VRC) that is comprised of at least five serial compartments. Each of these appears to be functionally distinct with respect to the firing pattern evinced by their complement of respiratory neurons, and with respect to their ultimate influence on the control of breathing (Alheid and McCrimmon, 2008; Smith et al., 2009). Similarly, neurons controlling the sympathetic outflow also occupy a series of compartments that are organized ventrally adjacent to the respiratory network and more-or-less parallel to the VRC (Goodchild and Moon, 2009; Pilowsky et al., 2009).

Caudal NTS efferents strongly and differentially influence the activity of the neurons in ventral medullary cardiorespiratory compartments. Understanding the particular targets of various NTS subdivisions in the brainstem is an important key to understanding the functional organization of both the NTS and ventral medulla. However, the hodology of NTS projections to specific cardiorespiratory brainstem targets remain incompletely characterized. This is particularly true for the targeting of medullary respiratory compartments by various NTS nuclei.

Several studies have addressed the question of NTS efferents to the medulla in the context of efferents to individual respiratory compartments within the VRC (e.g. Ellenberger and Feldman, 1990a,b, 1994; Rosin et al., 2006), as well as with respect to projections from individual

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Abbreviations: AmbC, nucleus ambiguus—compact part; AP, area postrema; arVRG, anterior part of the rostral ventral respiratory group; BötC, Bötzinger complex; cc, central canal; CVL, caudal ventrolateral nucleus of the medulla; cVRG, caudal division of the ventral respiratory group; C1, adrenergic neurons in rostral ventrolateral medulla; DAB, diaminobenzidine; DAPI, 4′6-diamidino-2-phenylindole-2HCl; DLH, DL-homocysteic acid; FB, Fast Blue; FG, FluoroGold; FITC, fluorescein isothiocyanate; Gr, gracile nucleus; Li, linear nucleus of the medulla; LRt, lateral reticular nucleus; NeuN, neuronal specific nuclear protein; NTS, nucleus of the solitary tract; pFRG, parafacial respiratory group; preBötC, preBötzinger complex; prVRG, posterior rostral ventral respiratory group, RTN, retrotrapezoid nucleus; RVL, rostral ventrolateral nucleus of the medulla; rVRG, rostral division of the ventral respiratory group; SO, superior olive; sol, solitary tract; SolC, commissural nucleus; SolCe, central nucleus; SolDL, dorsolateral nucleus; SolG, gelatinous nucleus; SolIcp, interstitial nucleus compact part; SolIdf, interstitial nucleus diffuse part; SolIM, intermediate nucleus; SolM, medial nucleus; SolV, ventral nucleus; SolVL, ventrolateral nucleus; SubP, subpostrema nucleus; VRC, ventral respiratory column; VRG, ventral respiratory group; 7n, facial nerve; 7N, facial nucleus; 10N, dorsal motor nucleus of the vagus; 12N, hypoglossal nucleus.

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subregions of the NTS to the VRC and to the medulla (Otake et al., 1992; Geerling and Loewy, 2006; Rinaman, 2010). Other studies have addressed the overall distinctions between projections to multiple compartments within the VRC, both in the cat (Smith et al., 1989), and in the rat (Núñez-Abades et al., 1993). In the intervening years, however, considerable refinement has occurred both in defining the detailed structure of the NTS (e.g. Herbert et al., 1990; Ciriello et al., 1994; Ruggiero et al., 1996; Paxinos et al., 1999) and in the identification of the compartmental organization of the VRC (Alheid et al., 2002, 2008; Alheid and McCrimmon, 2008; Feldman and del Negro, 2006; McCrimmon et al., 2009).

Accordingly, we have reexamined the question of which specific NTS nuclei project to particular VRC compartments. Our analyses of NTS labeling after retrograde tracer injections in the VRC and the nearby autonomic column indicate a significant distinction between projections to the Bötzinger complex (BötC) and preBötzinger complex (preBötC) compared to the remainder of the VRC. Specifically, the caudomedial NTS, including caudal portions of the medial solitary nucleus (SolM; alongside and caudal to area postrema) and the commissural division of NTS (SolC) projects relatively densely to the region of the retrotrapezoid nucleus (RTN) and rostral ventrolateral medullary nucleus (RVL) as well as to the rostral ventral respiratory group (rVRG) while avoiding the intervening BötC and preBötC. Area postrema appears to demonstrate a pattern of projections similar to that of caudal SolM and SolC. Other, less pronounced differential projections of lateral NTS nuclei to the various VRC compartments are additionally noted.

These data have previously been presented in abstract form (Alheid et al., 2010).

EXPERIMENTAL PROCEDURES

Animals

Experiments were performed on 14 male Sprague–Dawley rats (Charles River, Wilmington, MA, USA) weighing 200–400 g. All surgeries were performed using sterile procedures adapted for small rodents, in accordance with guidelines recommended by the NIH and by the Society for Neuroscience. All procedures were approved by the Northwestern University Animal Care and Use Committee. One normal rat was prepared for examination of NTS nuclei by injecting FluoroGold (FG) subcutaneously (25 mg/kg). This directly labels the area postrema, which lies outside of the blood–brain barrier, and retrogradely labels motoneurons in the dorsal motor nucleus of the vagus (10N) and hypoglossal nucleus (12N; Leong and Ling, 1990) whose axons project outside of the blood–brain barrier. The same brain was subsequently immunolabeled for neuronal specific nuclear protein (NeuN) in order to examine neuronal clusters within the NTS and their disposition relative to the area postrema, 10N and 12N motoneurons. Four animals used in the present analysis also had received a prior injection of the transganglionic tracer DiI (1,1-dioctadecyl-3,3,3,3-tetramethyl-indocarbocyanine perchlorate; Invitrogen, Carlsbad, CA, USA, #D-282) in the carotid body in order to examine carotid afferents terminating on retrogradely labeled neurons. The results of this experiment will be described elsewhere.

Surgery

Anesthesia was induced with 5% isoflurane in an induction chamber. Animals were then rapidly switched from the induction chamber to a nose cone attached to a stereotaxic frame where the animal was allowed to freely breathe isoflurane (2.5–3%) for maintenance. The depth of anesthesia was frequently assessed (10–15 min intervals) and judged by the absence of overt retraction responses to a strong noxious paw pinch, and by the absence of changes in heart rate or breathing in response to the noxious stimulation.

Rectal temperature was monitored and maintained at 37.5±1 °C by means of a thermistor-controlled heating pad and heat lamp. EMG and ECG activities were continuously monitored using transcutaneous needle electrodes placed on the caudal thorax with a ground wire placed laterally on the abdomen. Oxygen saturation as well as heart rate and respiratory rate were monitored via a pulse oximeter (Mouse Ox, Starr Life Sciences, Oakmont, PA, USA). In a subset of rats a femoral artery was cannulated (PE-50) for monitoring arterial pressure.

For extracellular recording and retrograde tracer injections a dorsal midline incision was made to expose the skull and/or neck. For tracer injections into rostral portions of the VRC (RTN, BötC or preBötC) a ~2×2 mm^2 opening was made in the skull and a trans-cerebellar approach was used. Stereotaxic coordinates were obtained from the atlas of Paxinos et al. (2009). Nominally, rostral tracer injections (in the BötC or preBötC complex) were targeted at approximately 1.8 mm lateral to the midline and approximately 3.5 to 4.5 caudal to lambda suture (using the average of this suture as described in the atlas by Paxinos et al., 2009).

Injections into more caudal VRC regions (rVRG) were made after separating the dorsal neck muscles and exposing the dorsal surface of the medulla by opening the cisterna magna. In the latter instance, obex was used as the initial reference point, the skull was mounted with the bregma suture located ~1.5 mm lower than the lambda suture, and the electrode was angled at 16° (dorsocaudal to rostroventral). For caudal portions of the VRC (i.e. the rVRG) nominal targets were at 0.0–1.5 rostral to the most caudal point of the area postrema (obex).

An analgesic, meloxicam (1 mg/kg, s.c.), was administered 30 min prior to the end of the experiment. Following the completion of surgery and the recovery of the righting response, the opiate analgesic buprenorphine hydrochloride was administered (0.03 mg/kg, s.c.). Buprenorphine was repeated 12 h post-operatively and meloxicam at 24 h. Following survival intervals sufficient for transport of retrograde tracers (≥7 days), animals were deeply anesthetized (ketamine-xylazine; 100–20 mg/kg) and perfused transcardially with fixatives. Subsets of animals were subjected to multiple surgeries either for injection of anterograde tracers in peripheral nerves or for single cell recordings in the NTS. These data and the accompanying procedures will be reported separately.

Retrograde tracer injections

FG (Fluorochrome Inc., Denver, CO, USA) and/or Fast Blue (FB, #F-5756 Sigma-Aldrich, St. Louis, MO, USA) were injected in the VRC of the medulla or in ventrally adjacent regions related to control of the sympathetic nervous system at rostral (RVL) or mid-levels of the medulla (caudal ventrolateral medullary nucleus; CVL).

In all instances, retrograde tracer injections were targeted by extracellular recording of unit activity in the VRC using the same micropipette used for the tracer injections. Three different recording and injection combinations were used: (1) FG was iontophoresed from a single barrel pipette that was also used for extracellular unit recording; (2) Since the solution containing FB was inappropriate for extracellular recording, FB was injected from a double barrel pipette where one barrel was used for extracellular unit