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Most neurons in the nucleus tractus solitarii do not send collateral projections to multiple autonomic targets in the rat brain

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Abstract

The nucleus tractus solitarii (NTS) receives primary visceral afferents and sends projections to other autonomic nuclei at all levels of the neuroaxis. However, it is unknown if distinct populations of NTS neurons project to individual autonomic targets or if individual neurons in the NTS project to multiple autonomic targets. Understanding the basic circuitry of visceral reflex pathways is essential for the analyses of functional central autonomic networks. We examined projections from the NTS to autonomic targets within the hypothalamus (paraventricular nucleus, PVN), pons (parabrachial nucleus, PB), and medulla (caudal ventrolateral medulla, CVL) using retrograde tracing and immunohistochemistry. Dual retrograde tracer microinjections were made into pairs of targets (PVN + CVL; PVN + PB; PB + CVL), and the pattern of retrograde labeling was examined within NTS. The extent of collateralization, seen as dual retrogradely labeled neurons, was negligible for combined PVN and CVL injections and increased for injections combining PB with either PVN or CVL, but the majority of NTS neurons project to only one autonomic target. Immunohistochemistry for tyrosine hydroxylase (TH) was used to examine the pattern of TH-immunoreactivity (TH-ir) within retrogradely labeled NTS neurons. TH-ir was seen predominantly in projections to PVN, to a lesser degree in projections to PB, and was largely absent from projections to CVL. The percentage of dual retrogradely labeled neurons displaying TH-ir corresponded to the target displaying the most TH-ir, and TH-ir was not predictive of collateralization. Together, these results indicate that NTS neurons project to individual autonomic targets in the brain.

Keywords: Caudal ventrolateral medulla; Parabrachial; Paraventricular; Hypothalamus

Introduction

The nucleus tractus solitarii (NTS) is the primary central target of visceral afferents (Ciriello, 1983; Hamilton and Norgren, 1984; Kalia and Richter, 1988; Kalia and Sullivan, 1982; Loewy, 1990; Norgren, 1978; Panneton and Loewy, 1980) and in turn sends projections to other autonomic nuclei at all levels of the neuroaxis, including major targets in the hypothalamus (paraventricular nucleus, PVN), pons (parabrachial nuclei, PB), and medulla (caudal ventrolateral medulla, CVL) (Aicher et al., 1995; Loewy, 1990; Loewy and Burton, 1978; Ricardo and Koh, 1978). Projections from the NTS to the PVN are involved in the regulation of hormonal and neural control of the cardiovascular system (Kannan and Yamashita, 1985; Sawchenko and Swanson, 1982; Stern, 2001). Projections from the NTS to the PB are important for the integration of autonomic information with higher brain centers (Chamberlin and Saper, 1992; Jhamandas and Harris, 1992; Jia et al., 1994; Otake et al., 1992; Saleh and Cechetto, 1995). Projections from the NTS to the CVL are involved in the minimal necessary pathway of the baroreflex response (Agarwal et al., 1990; Aicher et al., 1995; Jeske et al., 1993). We chose to examine efferent projections from NTS to targets at different levels of the neuroaxis that are involved in distinct autonomic effector responses but are potentially regulated by similar afferent information to NTS. We also examined three different rostrocaudal levels of NTS that receive primary visceral afferents and are generally considered to be functionally distinct, with cardiopulmonary afferents projecting to caudal regions of NTS and subdiaphragmatic afferents projecting to more rostral regions.

Many studies suggest a role for the NTS as an intermediate relay in pathways from peripheral afferents to diverse targets within the brain (Loewy, 1990), however, the potential role of

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the NTS as a substrate for preliminary integration of afferent information is not fully understood. For example, it is unknown if single neurons in the NTS project to multiple autonomic targets or if the projections to these various targets arise from distinct populations of neurons. The organization of these projections has important implications for the integration of autonomic information throughout the central nervous system. We sought to address this important question by examining projections from NTS to targets at distinct levels of the neuroaxis that are involved in autonomic responses, including cardiovascular control. In the present study, we used retrograde tracing methods to determine if NTS neurons send collateral projections to specific targets by examining two targets at a time, with combinations of injections into PVN, PB and CVL. The degree and pattern of retrograde labeling at three distinct rostrocaudal levels of NTS, as well as the prevalence of two retrograde tracers within single neurons, were examined. A high occurrence of dual retrogradely labeled neurons would indicate that many NTS neurons send collaterals to both targets, while a paucity of dual retrogradely labeled neurons would suggest that distinct populations of NTS neurons project to each target region. We hypothesize that the extent of dual retrogradely labeled neurons may reflect the degree of integration of afferent information within the NTS prior to transmission to other autonomic targets in the brain.

Additional studies sought to characterize the neurochemical phenotype of some of these projection neurons. Prior research has demonstrated distinct catecholaminergic projections from NTS to PVN (Petrov et al., 1993; Sawchenko and Swanson, 1982) and PB (Mantyh and Hunt, 1984; Milner et al., 1984). We combined immunohistochemistry for tyrosine hydroxylase (TH), the rate-limiting enzyme for catecholamine synthesis, with dual retrograde injections. The pattern of TH-immunoreactivity (TH-ir) within retrogradely labeled NTS neurons projecting to PVN, PB, and CVL was evaluated at three rostrocaudal levels of NTS to determine if TH is preferentially localized to subpopulations of neurons that collateralize to these targets.

Materials and methods

Animals and retrograde tracing methods

Male Sprague–Dawley rats (250–375 g; Taconic Farms, Germantown, NY) were utilized for these experiments, and all protocols were in accordance with the Institutional Animal Care and Use Committee at Oregon Health & Science University. For tract tracer injections, rats were anesthetized with isoflurane (4% for induction, 2% for maintenance) and placed into a stereotaxic apparatus. The brain region of interest was accessed by removal of a small portion of the occipital bone with ronguers (brainstem) or by drilling through the skull at appropriate coordinates (pons or hypothalamus) and opening the dura. The retrograde tracers FluoroGold (FG, 2% in 0.9% saline; Fluorochrome Inc.) and red RetroBeads™ (Rhod, undiluted Rhodamine-labeled latex microspheres; Lumafluor) were individually pressure-injected (Picospritzer II, General Valve Inc.) through single-barrel glass micropipettes into pairs of three distinct nuclei: PVN (150 nl), PB (50 nl), or CVL (50–80 nl). Injections were made into the left side of the brain. Injectate volume was determined by observing the displacement of the meniscus across a calibrated reticule in a dissecting microscope. All three dual labeling permutations, PVN + CVL (n = 9), PVN + PB (n = 9), and PB + CVL (n = 6), were performed with the location of injections into the appropriate nuclei determined by stereotaxic coordinates as follows: PVN injections were 1.9 mm caudal, 0.6 mm lateral, and 7.6 mm ventral from Bregma; PB injections were 9.7 mm caudal, 2.1 mm lateral, and 5.7 mm ventral from Bregma; and CVL injections were 1.0 mm rostral, 2.0 mm lateral, and 2.0 mm ventral from calamus scriptorius. Following each injection, the micropipette was left in place for 5 min and slowly removed. Surgical wounds were closed with wound clips, and after recovering from anesthesia, rats were returned to the colony and housed individually. No postoperative complications were observed in injected animals.

Perfusion and tissue processing

Five to seven days after tracer injections (Halsell et al., 1996; Schmued and Fallon, 1986; Van Bockstaele et al., 1994), each rat was overdosed with sodium pentobarbital (150 mg/kg, i.p.) and perfused transcardially through the ascending aorta with 20 ml of heparinized saline followed by 600 ml of 4% paraformaldehyde (in 0.1 M phosphate buffer, pH 7.4). Blocks of tissue containing the NTS and the injection sites were removed and placed in 4% paraformaldehyde for 30 min at room temperature. The tissue was then placed in 0.1 M phosphate buffer, sectioned (40 μm for NTS, 50 μm for injection sites) on a vibrating microtome (Leica, Malvern, PA), and consecutive NTS sections were mounted on gelatin-coated slides in 0.05 M phosphate buffer, with every third section placed into storage solution (30% sucrose, 30% ethylene glycol in 0.1 M phosphate buffer) and stored at −20°C, to be used later for immunohistochemistry. Slides with NTS sections were immediately coverslipped with Prolong™ antifade media (Molecular Probes, Eugene, OR).

Slides containing sections of FG injection sites were placed in a vacuum chamber overnight, and alternating sections were processed through either an alcohol and xylene dehydration series, or Nissl-stained with thionin, and coverslipped with DPX. Slides containing Rhod injection sites were immediately coverslipped with antifade media. Due to the light sensitivity of the Rhodamine-labeled latex microspheres, sections were processed under as little ambient light as was feasible, and once coverslipped, slides were wrapped in aluminum foil and stored at 4°C, until analyses were completed.

Confirmation of injection sites and specificity of labeling

Images of injection sites and NTS sections were captured with a Spot 2 camera (Spot 2, version 3.5.2, 1.4 × 10^6 pixels, Diagnostics Instruments) attached to a Zeiss Axiophot