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BRAIN RESEARCH

Research Report

Comparison of somatostatin and corticotrophin-releasing hormone immunoreactivity in forebrain neurons projecting to taste-responsive and non-responsive regions of the parabrachial nucleus in rat

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ABSTRACT

Several forebrain areas have been shown to project to the parabrachial nucleus (PBN) and exert inhibitory and excitatory influences on taste processing. The neurochemicals by which descending forebrain inputs modulate neural taste-evoked responses remain to be established. This study investigated the existence of somatostatin (SS) and corticotrophin-releasing factor (CRF) in forebrain neurons that project to caudal regions of the PBN responsive to chemical stimulation of the anterior tongue as well as more rostral unresponsive regions. Retrograde tracer was iontophoretically or pressure ejected from glass micropipettes, and 7 days later the animals were euthanized for subsequent immunohistochemical processing for co-localization of tracer with SS and CRF in tissue sections containing the lateral hypothalamus (LH), central nucleus of the amygdala (CeA), bed nucleus of the stria terminalis (BNST), and insular cortex (IC). In each forebrain site, robust labeling of cells with distinguishable nuclei and short processes was observed for SS and CRF. The results indicate that CRF neurons in each forebrain site send projections throughout the rostral caudal extent of the PBN with a greater percentage terminating in regions rostral to the anterior tongue-responsive area. For SS, the percentage of double-labeled neurons was more forebrain site specific in that only BNST and CeA exhibited significant numbers of double-labeled neurons. Few retrogradely labeled cells in LH co-expressed SS, while no double-labeled cells were observed in IC. Again, tracer injections into

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Abbreviations: 3V, third ventricle; 7n, facial nerve; ac, anterior commissure; cCeA, caudal central nucleus of the amygdale; rCeA, ostral central nucleus of the amygdale; CnF, cuneiform nucleus; dBNST, dorsal bed nucleus of the stria terminalis; DMC, dorsal motor complex; fx, fornix; ic, internal capsule; IC, insular cortex; KF, Kolliker–Fuse nucleus; LH, lateral hypothalamus; LPBC, parabrachial nucleus central lateral; LPBCr, parabrachial nucleus lateral crescent; LPBD, parabrachial nucleus dorsal lateral; LPBE, parabrachial nucleus external lateral; LPBI, parabrachial nucleus internal lateral; LPBV, parabrachial nucleus ventral lateral; LC, locus coeruleus; Me5, mesencephalic trigeminal nucleus; MPB, medial parabrachial nucleus; MPBE, parabrachial nucleus external medial; Mo5, motor trigeminal nucleus; NST, nucleus of the solitary tract; ot, optic tract; PBN, parabrachial nucleus; PBW, parabrachial nucleus, waist part; Pr5, principle sensory trigeminal nucleus; PVN, paraventricular nucleus; scp, superior cerebellar peduncle; Su5, supratrigeminal nucleus; vBNST, ventral bed nucleus of the stria terminalis

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rostral PBN resulted in a greater percentage of double-labeled neurons in BNST and CeA compared to caudal injections. The present results suggest that some sources of descending forebrain input might utilize somatostatin and/or CRF to exert a broad influence on sensory information processing in the PBN.

1. Introduction

Learning and nutritional status are important for the development and maintenance of food preferences. For instance, a conditioned taste aversion (CTA) that develops following experience with negative gastrointestinal consequences of ingesting a taste stimulus (e.g., nausea, sickness, or vomiting) produces a switch from acceptance to avoidance of that and any like tasting stimulus. In contrast, the behavioral response to a negative body sodium balance is characterized by a switch from avoidance of concentrated sodium salt to avid ingestion. A common finding in conditions that alter taste preference is selective changes in the responses of taste neurons in the first and second central synapses of the ascending gustatory system, the nucleus of the solitary tact (NST) and parabrachial nucleus (PBN), respectively (Chang and Scott, 1984; Jacobs et al,. 1988; McCaughey et al., 1996, 1997; McCaughey and Scott, 2000; Nakamura and Norgren, 1995; Shimura et al., 1997a,b). In the case of CTA, neural responses to the conditioned taste stimulus are enhanced after acquisition, while expression of sodium appetite is accompanied by decreased sensitivity of NST and PBN taste cells to sodium salt, particularly at higher concentrations that are normally avoided. Whether these neural changes reflect a causal relationship is unsettled, although altered PBN taste responses induced by CTA acquisition are abolished following disruption of neural connections between the forebrain and the brainstem (Tokita et al., 2004).

Several forebrain regions that receive gustatory information like the insular cortex (IC), bed nucleus of the stria terminalis (BNST), central nucleus of the amygdala (CeA), and lateral hypothalamus (LH) send projections back to the NST and PBN to modulate taste-evoked neural activity (Cho et al., 2003; Li and Cho, 2006; Lundy and Norgren, 2001, 2004; van der Kooy et al., 1984; Veening et al., 1984). Despite the substantial data characterizing the influence of feedback from the LH, CeA, BNST, and IC on brainstem taste processing, the identification of the neurochemicals subserving centrifugal modulation remains in its infancy. Prior studies using stereotaxic coordinates alone to place retrograde tracer into the PBN have shown the presence of several neuropeptides like somatostatin, neurotensin, corticotrophin-releasing factor, cholecystokinin, enkephalin, substance P, and galanin in some LH, CeA, and/or BNST neurons (Moga et al., 1989, 1990a; Moga and Gray, 1985; Veening et al., 1984). However, these studies were limited in that a precise correspondence between the neurochemical content of forebrain neurons innervating different functional segments of the PBN could not be discerned with certainty.

The caudal classically defined pontine taste area corresponds to the locations where taste neurons responsive to chemical stimulation of the anterior oral cavity are concentrated (Nishijo and Norgren, 1997; Perrotto and Scott, 1976). Regions rostral to this gustatory-responsive area are primarily concerned with processing nociceptive, cardiovascular, respiratory, and gastric information (Baird et al. 2001a; Bester et al., 1995, 2000; Chamberlin and Saper, 1992, 1994; Cohen, 1971; Ezure and Tanaka, 2006; Hayward, 2007; Lara et al., 1994; Mraovitch et al., 1982). Nevertheless, neurons responsive to tactile stimulation of the oral cavity as well as some responsive to gastric distention are found in the caudal pontine taste area (Baird et al. 2001b; Karimnamazi et al. 2002). Moreover, a small number of neurons responsive to chemical stimulation of the posterior oral cavity reside in the external medial (MPBE) and external lateral (LPBE) subnuclei adjacent to the respiratory-responsive Kolliker–Fuse nucleus (Halsell and Travers, 1997). Whether any or all of the previously identified forebrain–PBN peptidergic pathways play a role in descending modulation of gustatory processing per se remains to be established.

To this end, the present study used electrophysiological techniques to compare the expression of somatostatin and corticotrophin-releasing factor immunoreactivity in LH, CeA, BNST, and IC neurons projecting to caudal regions of PBN responsive to anterior tongue application of NaCl and more rostral regions unresponsive to anterior tongue stimulation. In one group of animals we electrophysiologically located the caudal gustatory-responsive PBN and made small injections of retrograde tracer, while in a separate group we made similar injections in more rostral areas that were unresponsive to anterior tongue application of NaCl.

2. Results

2.1. *Injection sites*

Fig. 1A to D shows photomicrograph examples of tracer injected into the taste-responsive PBN and more rostral non-taste-responsive regions. Microscopic examination of each gustatory-responsive injection site revealed that predominately the medial, ventral lateral, and waist portions of the caudal PBN were targeted with minimal spread into the rostral regions. Injections placed rostral to this gustatory-responsive area primarily targeted the medial, external lateral (medial portion), central lateral, dorsal lateral, ventral lateral, and internal lateral subnuclei. A summary diagram of the tracer injections and their relative spread is shown in Fig. 2.

2.2. *Distribution of retrogradely labeled neurons*

Since the distributions of forebrain–PBN projecting neurons were similar to those described in earlier tracing studies, the present findings are only briefly summarized (Kang and Lundy, 2009; Moga et al., 1989, 1990b; Moga and Gray, 1985;