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Research report

Role of the lateral hypothalamus in modulating responses of parabrachial gustatory neurons in the rat

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ABSTRACT

The lateral hypothalamus (LH) receives projections from the parabrachial nucleus (PBN) gustatory neurons and sends efferent projections to the PBN. To examine if the LH is involved in modulating activity of gustatory neurons in the PBN, we examined the effects of electrical stimulation and electrolytic lesions of the LH on the response of PBN gustatory neurons, using extracellular recording techniques. Among 45 PBN taste neurons recorded, 60% were affected by LH stimulation and 73% were affected by LH lesions. During LH stimulation, the responses of most affected PBN neurons were inhibited with the magnitude significantly lower than that obtained before stimulation ($P < 0.05$). In contrast, LH lesions facilitated the response. Based on the best-stimulus category, the responses of the NaCl-best neurons to NaCl and HCl and the QHCl-best neurons to HCl and QHCl were significantly suppressed during LH stimulation ($P < 0.05$). After lesions of the LH, however, the response to HCl in NaCl-best PBN neurons was significantly increased ($P < 0.05$). Analysis of across-unit patterns indicated that LH stimulation decreased the correlations between NaCl and other stimuli, and increased those between QHCl and other stimuli. After LH lesions, the correlations between NaCl and other tastants were higher than those before lesions. These findings suggest that the LH mediates feeding and taste via modulating the activity and chemical selectivity of PBN gustatory neurons.

1. Introduction

Taste information is conveyed from the taste receptor cells on the tongue to the first relay station, the rostral nucleus of the solitary tract (rNST), via branches of the facial (chorda tympani, CT), glossopharyngeal and vagal nerves. In rodents, the parabrachial nucleus (PBN) is the second relay of the central taste system. Gustatory responsive neurons in the PBN are located primarily below, but also within and above the brachium conjunctivum (BC) in the posterior aspect of the nucleus [18]. The PBN receives taste information from the rNST and sends ascending fibers through two parallel pathways, one to the ventral posteromedial nucleus of the thalamus and then to the insular cortex, which involves the transmission of taste information, and the other directly to the lateral hypothalamus (LH), central nucleus of the amygdala (CeA) and bed nucleus of the stria terminalis, which involve the modulation of taste and feeding [6,14,19,24]. In turn, the PBN receives gustatory information from these nuclei [6,19,32].

It is well known that the LH is the feeding center and plays a critical role in regulating food intake and body weight [24]. Feeding is a complex process that is influenced by vision and olfaction, as well as by the internal state of the animal, but is largely guided by the sense of taste [25]. This is supported by the fact that the gustatory-evoked neurons have been recorded in the LH [15]. Substantial evidence also indicates that the LH is a part of the neural system that is involved in the central evaluation of gustatory inputs [2]. The LH is not only a major recipient of the PBN gustatory efferent axons, but also the major source of the PBN inputs [3,7,17]. Moreover, there are reciprocal connections between the LH and the taste-related areas, such as NST, CeA and gustatory cortex (GC) [30]. Behavioral studies have suggested that stimulation of the LH leads to increased food intake [26] and consumption of taste solutions [31]. However, damage to this area of the hypothalamus results in decreasing food intake and diminished taste preferences [29]. Lesions of the GC alter the activity of gustatory responsive neurons in the PBN [5]. Our previous study has shown that electrical stimulation or electrolytic lesions of the CeA can modulate the activity of gustatory neurons in the PBN [8]. Furthermore, the pontine taste

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activity is altered by stimulation of the LH [15]. However, in the study of Lundy and Norgren [15], they only analyzed the effects of electrical stimulation of the LH on the taste activity of the PBN based on the best-stimulus category, and no quinine-best neuron was recorded.

The aim of the present study was to further examine the effects of electrical stimulation and electrolytic lesions of the LH on the evoked response of PBN gustatory neurons to the four basic tastants, using the single PBN neuron recording technique.

2. Materials and methods

2.1. Animals and surgery

Forty adult male Sprague–Dawley rats (220–290 g) were housed individually in stainless steel cages with laboratory chow and tap water freely available. Room lights were on a regular 12:12 h light–dark cycle and temperature was maintained at 24 ± 1 °C. Rats were initially anesthetized with ethyl carbamate (urethane, 1.4–1.5 g/kg, i.p.), and additional anesthetic was given as needed during the experiment. After tracheotomy and jugular vein cannulation were performed, the rat's head was positioned in a stereotaxic instrument by using non-puncture ear bars and a bite bar. The dermis and periosteum on the top of the skull were reflected through a midline incision and the skull was leveled between the bregma and lambda. The stainless steel electrodes for electrical stimulation and electrolytic lesion were penetrated so that the ventral tip was located in the LH (1.8 mm lateral to the midline, 2.5 mm posterior to bregma, 8.0 mm below the brain surface), as described by Paxinos and Watson [21]. For access to the taste-responsive area of the PBN, a small craniotomy was performed over the cerebellum. The transverse sinus overlying the PBN was ligated and retracted in order to insert a recording electrode to the PBN (9.0–10.1 mm posterior to the bregma, 1.5–2.1 mm lateral, 5.0–6.0 mm below the cortical surface). The electrocardiogram was continuously monitored and the rectal temperature was maintained at about 37 °C by using a heating pad throughout the experiment. All animal experiments were performed according to the "Principles of Laboratory Animal Care" (NIH publication No. 80-23).

2.2. Single-unit recording

Single-unit activity from PBN neurons was isolated using a glass microelectrode filled with 2% pontamine sky blue in 0.5 M sodium acetate (impedance 7–20 MΩ). Action potentials were recorded through conventional physiological equipment consisting of a preamplifier (MEZ-8201, Nihon Kohden, Japan), cathode-ray oscilloscope (VC-10, Nihon Kohden), and a recording system (PowerLab 4/SP, ADInstruments, Sydney, Australia). Once a PBN cell was identified as a taste-responsive neuron, the responses to four taste qualities were examined individually using the pre-stimulus water activity as a baseline. Uniform amplitude and waveform were used as the criteria for isolation of single units. The artifact signals resulting from the electrical stimulation of the LH were excluded from neural signals by using Chart 3.6.5 for Macintosh. For the LH stimulation experiment, the responses to four taste stimuli were recorded individually with or without concurrent LH stimulation in turn. For the LH lesion experiment, the spontaneous activity and gustatory responses before electrolytic lesion were recorded. Following the lesion, the gustatory responses were immediately tested repeatedly.

2.3. Gustatory stimulation

Gustatory stimuli were delivered to the tongue in the following order: NaCl (0.3 M), HCl (0.01 M), quinine HCl (QHCl) (0.003 M) and sucrose (0.5 M). All stimuli were made with reagent-grade chemicals dissolved in distilled water at room temperature. Fluid stimuli were delivered through a length of slender tubing, closed at the end and extensively perforated along its final 2 cm. The perforated end was placed in the animal's mouth and tastants were gravity-fed from an overhead reservoir and funnel. A pilot study by our group substituted a fast green dye for taste stimuli to ensure that this feeding method provided taste stimuli to almost all of the taste receptors on the tongue. Each stimulus trial consisted of a 10-s flow of distilled water, a 10-s taste stimulus, a 10-s wait, and a 20-s rinse with distilled water. The flow rate was 2 ml/s for all stimuli including the water. Ninety seconds were allowed to elapse between successive stimuli to avoid adaptation and possible effects of the preceding stimulus. Markers of the taste stimulus onset were stored concurrently in another channel to facilitate off-line analysis.

2.4. LH stimulation and lesions

Electrical stimulation in the LH was achieved by an electrical stimulator (SEN-7103, Nihon Konden), which was connected to the electrode via a stimulating isolator (SS-201J, Nihon Kihden). The stimulator was used to produce 20 Hz square wave monophasic pulses for 10 s. The duration and amplitude of an individual pulse were 0.2 ms and 0.4 mA, respectively. The parameters for the LH lesion were 0.4 mA constant current for 60 s.

2.5. Histology

At the end of the experiment, the last recording site of the day was marked by the electrophoretic deposition of the dye from the recording electrode with a cathode current of 10 μA for 20 min. When the LH was stimulated, the stimulation site was marked with an electrolytic lesion. Then, the rat was given a lethal dose of urethane and perfused intracardially with 0.9% saline followed by 10% formalin (containing 3% potassium hexacyanoferrate). The brain was removed, fixed, frozen-sectioned (50 μm) and stained with cresyl violet. The locations of recording sites, the stimulation sites, and the extent of the LH lesion were histologically examined.

2.6. Data analysis

Firing patterns of the parabrachial units were digitally analyzed on a computer using Chart 5 software (ADInstruments). A spike-amplitude discriminator was used to convert spike activity to digital form. Neuronal response to a taste stimulus was calculated by subtracting the 5-s discharge rate to each stimulus, beginning with the onset of a stimulus infusion, from its 5-s discharge rate to water. Excitatory responses were defined as an increase in the average firing rate over the first 5 s of stimulus presentation that exceeded the average base-line rate by ≥50%. Inhibitory responses were defined as a decrease in the average firing rate that was <50% of the base-line firing rate [4]. The neurons in the present study were classified as inhibited or augmented if these responses differed from their corresponding control rates by <20 or >20%, respectively.

Based on the response to a standard concentration of each of the four taste qualities, each neuron was categorized according to its best stimulus. To assess the across-unit differences among stimuli, the Pearson product–moment correlations across stimuli were determined for PBN units with and without LH stimulation or lesions. As an indication of the breadth of tuning, an uncertainty measure was calculated with the following formula: $H = -1.661 \sum_{i=1}^{4} \text{Pi} \log \text{Pi}$, where H is the breadth of responses, and Pi is the proportion of the response to each of the stimuli against the total response to four tastants [27].

One-way ANOVA were performed to detect significant differences between response rates. All averages were indicated as the mean ± S.E.M. Data were analyzed using SPSS, and $P < 0.05$ was considered to be statistically significant.

3. Results

3.1. Histology

Based on the stereotaxic coordinates of the recording loci, all recorded taste neurons were histologically identified within the medial PBN, lateral PBN and the BC, which have been described previously as the pontine taste areas (Fig. 1). Twenty of the 40 responsive sites were located in the medial PBN, 6 were in the BC, and 14 were in the lateral PBN. The experimental data were finally analyzed from animals in which the electrical stimulation or electrolytic lesions were found to be correctly located within the LH (Fig. 2). The distribution of affected PBN taste neurons did not appear to be distinctly different from that of the unaffected taste neurons.

3.2. Effects of LH stimulation on PBN gustatory responses

A total of 30 taste neurons in the PBN were recorded before and during LH stimulation in 25 rats. After confirming a taste neuron, electrical stimulation was delivered to the LH during taste stimulation trials. In 30 taste neurons recorded, 11 (37%) were inhibited and their taste-evoked responses were decreased by electrical stimulation of the LH. Spontaneous activity was suppressed significantly ($F(1, 20) = 4.99$, $P < 0.05$). The mean response of these PBN neurons to four taste stimuli before LH electrical stimulation was 9.96 ± 1.46 Hz. Application of stimulation to the LH while repeating the same taste trials significantly decreased the response to the taste stimuli (6.11 ± 0.90 Hz, $P < 0.05$). Seven (23%) taste neurons were significantly excited and their taste-evoked responses were increased during LH stimulation ($F(1, 12) = 4.92$, $P < 0.05$). The mean response to taste stimuli was significantly increased with the firing