Thermoreceptive neurons in the dorsal portion of the trigeminal principal nucleus in rats

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Abbreviations: PV, trigeminal principal nucleus; RF, receptive field; SpVc, caudal subnucleus of the spinal trigeminal nucleus

ABSTRACT

The dorsal margin of the trigeminal principal nucleus (PV) contains neurons responsive to innocuous thermal stimulation of the tongue and may be a thermal relay (Hayama and Hashimoto, 2011). The present electrophysiological study examined whether PV thermoreceptive neurons project to the thalamus and investigated response properties to cold (20°C) or warm (40°C) stimulation of the tongue. Twenty-three thermoreceptive neurons were identified in the dorsal portion of the PV. Twenty of the 23 neurons were examined but none projected to the thalamus. Impulse frequencies of 8 of the 11 thermoreceptive neurons examined rapidly increased with cold stimulation, then decreased and gradually increased to steady state level, and rapidly decreased with warm stimulation. Thermal receptive fields were examined for 6 PV thermoreceptive neurons; five had a large receptive field extending over the whole anterior tongue ipsilateral to the recording side. These findings suggest that the dorsal portion of the PV is not a thermal relay mediating thermal information from the tongue to the thalamus.

Keywords:
Trigeminal principal nucleus
Thermal response
Receptive field
Tongue
Rat

1. Introduction

The caudal subnucleus of the spinal trigeminal nucleus (SpVc), the primary thermal relay, receives thermal information conveyed by the trigeminal nerve from the trigeminal field and relays thermal information to the thalamic relay in many animal species [2-5,7,13-17,24]. Our recent electrophysiological study showed that the dorsal portion of the trigeminal principal nucleus (PV) contains neurons responsive to innocuous thermal stimulation of the tongue in rats [10]. Our previous neuroanatomical study demonstrated connections of the dorsal portion of the PV with the thalamic area and with the dorsal portion in the SpVc, which both receive thermal information from the tongue, and suggested that the dorsal portion of the PV is a possible new relay for thermal information from the tongue to the thalamus [10]. However, we did not examine whether thermoreceptive neurons in the PV project axons to the thalamus, which would demonstrate that the dorsal portion of the PV is a new relay.

The present electrophysiological study investigated the projection of PV thermoreceptive neurons to the thalamus and examined the response properties of such neurons. Our results were previously reported in abstract form [9].

2. Materials and methods

All surgical procedures were conducted following the Guidelines for Animal Treatment issued by our institution and by the Physiological Society of Japan. Ten albino Sprague-Dawley male rats weighing 200-350 g were anesthetized with intraperitoneal administration of urethane (1-1.5 g/kg) and maintained areflexia by additional intraperitoneal doses. The animals were mounted on a stereotaxic instrument. A portion of the parietal bone and/or a rostral portion of the interparietal bone were removed to allow electrical stimulation of the thalamic area, which receives thermal information from the tongue, and recording of neural activity in the dorsal margin of the PV, as described previously [10]. Rectal temperature was maintained at about 37˚C with a water heater. Electrocardiographical monitoring was continued throughout the experiment.

A glass micropipette (tip diameter 1-3 μm), filled with 0.5 M sodium acetate containing 2% pontamine sky blue or 2 M NaCl solution, was inserted into the dorsal portion of the PV through the occipital cortex rostrocaudally at 5-15˚ from the vertical to explore neuronal activities. An indifferent silver wire electrode was placed on the neck muscle. To examine projection of the PV thermoreceptive neurons to the thalamus, monopolar stimulating electrodes insulated down to the tip (tungsten, 0.2-0.5 MΩ) were inserted into the thalamus at 3.0-3.5 mm posterior to the bregma, 1.5-2.0 mm lateral to the midline, and to a depth of ca. 6.0 mm from the dorsal surface of the cerebral cortex [12]. The mouth of the subject animal was pulled open wide with a weight attached to the lower incisors with a piece of thread. The tongue was positioned ventrolaterally with its tip extending from the oral cavity.

Neuronal activities responsive to thermal stimulation of the tongue were explored by application of distilled water at 20˚C or 40˚C with a pipette to the tongue anterior to the intermolar eminence. Electrical pulses (duration 0.2 ms, intensity 300 μA) were then applied to the thalamic site receiving thermal information [12]. If electrical stimulation evoked impulses in PV thermoreceptive neurons, antidromic evocation of the impulse was examined with the collision test [8, 11]. The response properties of the neurons were investigated by passing a continuous flow of distilled water at 20˚C and 40˚C over the anterior tongue from an overhead funnel via gravity flow at a rate of about 3 ml/s for more than 2 minutes. A large thermal stimulation consisting of a 20 deg C change within the physiological range was used to ensure detection of even the slightest thermal responses [20]. The temperature of the surface of the anterior tongue was monitored with a thermistor (PTC-201; Unique Medical Co., Ltd., Komae, Tokyo, Japan). Single or multineuronal activity was passed to a preamplifier and displayed on a cathode ray oscilloscope, then to a data acquisition system (Unique Acquisition Ver.2.11.0.10; Unique Medical Co., Ltd.), digitized with a sampling frequency of 50 kHz, and stored in a hard disk for off-line analysis. Single neuronal activity was identified by examining the spike waveforms and impulse frequency histograms were prepared using a software program incorporated in the same data acquisition system.

Thermal receptive fields (RFs) on the anterior tongue were identified with a cold or warm metal probe (4 mm wide) connected with a glass tube containing water, which had been kept in a water bath at 20˚C or 40˚C. Responses to stimulation of other modalities, touch, mechanical nociceptive, and heat nociceptive, were also examined. Tactile stimulation was applied to the anterior tongue with a glass rod. Mechanical nociceptive stimulation was applied with pre-warmed non-serrated forceps. Heat nociceptive stimulation was applied using distilled water at 50˚C from a pipette soon after cessation of flow of distilled water at 40˚C. Stimulation sites in the thalamus and some recording sites of thermoreceptive neurons were marked by electrolytic lesions or electrophoretic deposition of dye from the recording electrodes. After the experiment, the animal was deeply anesthetized and perfused intracardially with 10% formalin solution in 0.1 M phosphate buffer (pH 7.4). The stimulation sites in the thalamus and recording sites of the thermoreceptive neurons were histologically identified on coronal brain sections (50 µm thick). The experiments were carried out at room temperature (25-28˚C).

3. Results

Twenty-three neurons responding to innocuous thermal stimulation of the tongue were identified in the dorsal portion of the PV at ca. 2.5 mm lateral to the midline, 0.8-2.5 mm posterior to the lambda, and at a depth of 6.2-7.2 mm from the dorsal surface of the cerebral cortex. Spontaneous activity of the neurons was suppressed by warm stimuli but potentiated by cold stimuli, indicating cold neurons. Twenty thermoreceptive neurons were examined to identify antidromic activation from the thalamus. Only 3 of these 20 neurons were activated with latencies of 0.9 ms, 1.0 ms, and 1.3 ms. Activated spikes did not collide with spontaneous spikes, thus excluding antidromic induction. Twenty-one neurons responding to tactile stimulation of the anterior tongue were also identified in the dorsal regions of the PV. Eight of these 21 neurons showed antidromic activation from the thalamus with a mean latency of 1.69±0.34 ms (mean±SD).

Impulse frequency histograms during cold and warm stimulation were prepared for 11 neurons. Impulse frequencies of 8 of the 11 neurons rapidly increased with cold stimulation, then significantly decreased and gradually increased to steady state level, and rapidly decreased with warm stimulation as shown in Fig. 1. Two of the remaining three neurons had both dynamic and static responses without an intervening suppression period. The other neuron showed only dynamic responses with cold stimulation and no spontaneous discharges. Mean impulse frequencies during the last 30 seconds of cold and warm water application were calculated as static frequencies of 15.7±5.9 imp/s at 20°C and 0.1± 0.3 imp/s at 40°C (mean ± SD, n=10), excluding the neuron with only dynamic responses. The suppression period (10 s) during cold stimulation was judged visually for the 8 neurons. The ratio of mean impulse frequencies in the suppression periods to the static state during cold stimulation was 0.22± 0.19 (n=8).

Thermal RFs were examined for 6 thermoreceptive neurons with cold and warm metal probes, which potentiated and suppressed the spontaneous activities of the neurons, respectively. RFs were detected on the anterior portion of the tongue ipsilateral to the recording side; five neurons had a large RF extending over the whole area of the anterior tongue (Fig. 2AB) and one neuron had an RF on the anterior quarter of the anterior tongue. These 6 neurons did not respond to tactile stimulation, and 3 of these 6 neurons were tested for other sensory modalities and responded to mechanical nociceptive (Fig. 2C) and heat nociceptive stimulation (not shown). RFs of the 21 neurons responding to tactile stimulation of the anterior tongue were located on the anterior tip area of the tongue. These RFs were much smaller than the thermal RFs of the thermoreceptive neurons, although the exact areas were not determined.

4. Discussion

Many thermoreceptive neurons in the SpVc, the first central relay for thermal information from the trigeminal fields, project to the thalamus, 25% of these neurons in the rat [14] and and 33% in the cat [3]. The present study showed that electrical stimulation of the thalamus failed to cause antidromic activation of 20 PV thermoreceptive neurons, whereas antidromic activation was observed for 8 of 21 PV neurons responding to tactile stimulation of the tongue. The thalamic area processing tactile information from the tongue is laterally adjacent to the area receiving thermal information from the tongue [12]. The present results indicate that there are no, or only a few PV thermoreceptive neurons projecting to the thalamus. This observation does not agree with previous findings on connections between the dorsal part of the PV and the thalamic area receiving thermal information from the tongue [10]. In the previous study, tracer molecules were injected into a thalamic site just after multineuronal activity responding to thermal stimulation of the tongue was observed with tracer-containing glass electrodes, and many retrogradely labeled neurons were found in the dorsal portion of the PV. Further, tracer injection into the dorsal PV responding to thermal stimulation of the tongue resulted in dense anterograde labeling at the thalamic thermal site. Possibly tracer molecules injected into the dorsal PV or the thalamic thermal area were incorporated by neurons situated close to the thermoreceptive neurons and were transported to the thalamic area or the dorsal PV.

Three of the 20 thermoreceptive PV neurons responded orthodromically to thalamic electrical stimulation with a short latency of ca. 1 ms, suggesting thalamic projection to the PV. However, the thalamic area responding to thermal stimulation of the tongue has not been shown to contain neurons projecting to the PV. Therefore, thalamic stimulation may have activated fibers of unknown origin passing through the thalamic thermal area or its vicinity resulting in activation of the thermoreceptive PV neurons.

In the present study, most PV thermoreceptive neurons showed dynamic and static responses with cold stimulation. These results are similar to previous findings for peripheral cold fibers and thermal relay nuclei [3,4,6,13,14,21–23]. The PV thermoreceptive neurons also showed a suppression period between the dynamic and static responses, which was previously described as a post-excitatory cessation of activity after cold stimulation in monkey peripheral fibers and the thalamus [22,23], but this was not seen in cats and rats [4,13,14,21]. These differences between the present and previous results, and the differences between species may be due to the stimulation methods; cold water at ca. 20°C was poured on the anterior tongue just after preadaptation at 35-40°C in the present study and studies on monkeys, whereas successive cooling or warming steps of about 5°C were used in other studies. The mean impulse frequency of PV thermoreceptive neurons at 20°C (15.7±5.9 imps/s) is similar to that of peripheral cold fibers in rats [5]. Differences in the impulse rates were reported between three types of cold neurons in the SpVc [14].

The PV thermoreceptive neurons had a large thermal RF extending over the whole ipsilateral anterior tongue, which is quite different from thermal RF size of thermoreceptive neurons in other nuclei. Peripheral cold fibers have spot-like thermal RFs [4] and cold neurons in the SpVc have RFs of 1-5 mm^2 [14]. Thalamic neurons responding to cold stimulation of the tongue have smaller thermal RFs than those of PV thermoreceptive neurons [12]. The present findings suggest that PV thermoreceptive neurons receive convergent input from peripheral cold fibers and/or cold neurons in the SpVc.

The PV thermoreceptive neurons were sensitive to nociceptive thermal and mechanical stimulation, although only a few neurons were examined. Innocuous thermal stimulation of the tongue has not been reported to influence salivary secretion or tongue movement, but nociceptive thermal and mechanical stimulation induces reflex salivation [18,19] and modifies the neural activities of hypoglossal neurons [25]. The dorsal part of the PV projects to the hypoglossal nucleus [1]. Consequently, PV thermoreceptive neurons might be involved in such reflex activities.

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LEGENDS

Fig. 1. Thermal responses of a single PV neuron (neuron no. 100827-1). (Aa) Thermal responses to cold water (20˚C). The arrow indicates onset of the stimulation. (Ab) Superimposed traces of field and/or action potentials activated with thalamic electrical stimulation (downward arrow) following action potentials of the thermoreceptive neuron (star mark). The thermoreceptive neuron was not considered to show antidromic activation because the shape of the action potential with latency of ca. 2.0 ms is similar but slightly different from that of the thermoreceptive neuron preceding thalamic stimulation. (B) Upper part, impulse frequencies during cold stimulation with bin width 2 s. The maximum discharge rate of the dynamic response was 41 imp/s. Lower part, temperature change of the tongue surface measured by a thermistor.

Fig. 2. Thermal RFs of a thermoreceptive PV neuron (neuron no. 100827-3) (A) and its responses to cold (B) and mechanical nociceptive stimulation (C). The maximum discharge rate of the dynamic response with cold water stimulation was 92 imp/s (not shown). The RF was located on the ipsilateral anterior tongue. Short lines (1-3) above the spike histogram in (B) indicate touch with a cold metal probe on 1-3 portions of the anterior tongue shown in (A). The maximum discharge rate with cold metal stimulation was 40-45 imp/s, much lower than that with cold water stimulation, probably because of the different stimulation methods. IME, intermolar eminence. White and black boxes above the histogram in (C) indicate touch and pinch stimulation of an anterior tip area of the tongue with pre-warmed forceps. Time scale is for both (B) and (C). Bin width was one second for both (B) and (C).