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Multi-source inputs converge on the superior salivatory nucleus neurons in anaesthetized rats

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ABSTRACT

Activation of parasympathetic nerves innervating salivary glands evokes not only salivation but also vascular responses. These parasympathetic nerves may have cardiac and/or respiratory-related activity as well as the cardiovascular sympathetic nerves that control vascular bed of salivary glands. Therefore, we investigated whether preganglionic superior salivatory nucleus (SSN) neurons projecting to the submandibular and intra-lingual ganglia exhibit pulse-related and/or respiratory-related activity, and whether they can be excited by electrical stimulation of the lingual nerve.

25% of SSN neurons were found to have pulse-related and tracheal pressure-related activities, implying that they receive cardiac and respiratory inputs. 44% of neurons exhibited only pulse-related activity, whereas 31% of the neurons had neither pulse-related nor tracheal pressure-related activity. Neurons with pulse and tracheal pressure-related activities, and those only with pulse-related activity, had B and C fibre range axons. 53% of SSN neurons received both cardiac and lingual nerve inputs. 16% of neurons recorded were found to receive only cardiac inputs, and 26% only lingual nerve inputs; whereas 5% received neither cardiac nor lingual nerve inputs.

We conclude that the inputs from diverse sources converge on the SSN neurons, and they can cooperate to modulate SSN neuronal activity.

1. Introduction

Salivation can be evoked by the application of various stimuli to the orofacial (Kawamura and Yamamoto, 1978; Kawamura et al., 1982; Matsuo and Kusano, 1984; Matsuo et al., 1989; Furukawa and Okada, 1994; Furukawa et al., 1998a,b) and visceral (Kay and Phillipson, 1959; Furukawa et al., 1998a,b) regions, and conversely it can be depressed during retching (Furukawa and Okada, 1994; Furukawa et al., 1998a,b). In support of this, electrical stimulation of afferent fibres in the trigeminal (Murakami et al., 1982, 1983; Oskutyte et al., 2004; Ishizuka et al., 2008), chorda tympani (Murakami et al., 1989), glossopharyngeal (Murakami et al., 1989) and vagus (Murakami et al., 1989) nerves produced excitatory responses in preganglionic superior salivatory nucleus (SSN) neurons projecting to the submandibular and intra-lingual ganglia. Further, these SSN neurons have been demonstrated to be excited by the inputs from the hypothalamus (Ishizuka and Murakami, 1995) and cerebral cortex (Ishizuka and Murakami, 1989). Thus, these suggest that preganglionic SSN neurons receive multi-modal inputs from various regions including CNS. A neuroanatomical study (Agassandian et al., 2002) has found a direct monosynaptic pathway from the neurons of the cardiovascular part of nucleus tractus solitarii to the SSN neurons projecting pterygopalatine ganglion, implying they receive visceral, including baroreceptor and pulmonary receptor, afferent inputs. The excitatory and inhibitory modulations of the SSN postganglionic nerve activity by inputs from the respiratory centre and/or receptor afferents has been proposed previously (Furukawa and Okada, 1994). These physiological and morphological studies inspired the hypothesis that preganglionic SSN neurons receive cardiac and/or respiratory inputs. To test this hypothesis, the pulse, tracheal pressure and lingual nerve modulations of SSN neurons projecting submandibular and intra-lingual ganglia were tested.

2. Materials and methods

The experiments were carried out using 28 Sprague–Dawley rats (300–450 g) under urethane–chloralose anaesthesia (500 mg/kg and 50 mg/kg, i.p.). Under this anaesthetic regime, no flexion withdrawal reflex was evoked upon pinching of the paw. The trachea was intubated, and the femoral artery and vein were cannulated to monitor arterial blood pressure (ABP) and to administer drugs. Arterial blood pressure and tracheal pressure (TP) by artificial ventilation were monitored by pressure-sensitive transducers and recorded. A lead II electrocardiogram (ECG) was recorded using leads attached to the limbs of the rats. Rectal temperature was monitored

Abbreviations: Os, superior olive nucleus; F, facial nucleus; V, vestibular nucleus; Sp5, spinal trigeminal nucleus; 7, facial nerve root.

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and maintained at 37 °C using a regulated heating pad. All animal procedures were performed in accordance with the Guiding Principles for Care and Use of Animals in the Field of Physiological Sciences provided by the Physiological Society of Japan, and were approved by the Laboratory Animal Committee of The Nippon Dental University School of Life Dentistry at Niigata.

A ventral sagittal skin opening was made at the neck, and the anterior and posterior bellies of the right digastric muscle were separated by incising the muscle tendon. Reflection of the anterior belly of the digastric muscle was performed. The right chorda–lingual nerve (CLN) or chorda tympani branches (CTBs) and the lingual nerve (LN) were carefully exposed, and were separated from the surrounding connective tissues using a binocular microscope. Electrodes were introduced for stimulation and were insulated from the surrounding tissues by silicone elastomer (Kwick-Cast, World Precision Instruments, USA). CTBs include preganglionic fibres of SSN neurons projecting to the submandibular ganglion. LN includes preganglionic fibres of SSN neurons projecting to the submandibular ganglion as well as those projecting to the intra-lingual ganglion. CLN includes all these preganglionic fibres of SSN neurons including both CTBs and LN (Oskutyte et al., 2004).

The special fixing metal plate with bolt developed in our laboratory was fixed on the head by the screws. The animals were fixed in a stereotaxic apparatus (SN-3, Narishige) by attaching a fixing metal plate to the SM 15 (Narishige), so as to prevent damage to the preganglionic fibres in the chorda tympani as this passes through the middle ear.

Craniotomy was performed and the cerebellum was removed by suction to expose the dorsal surface of the caudal brainstem. The brainstem was covered with mineral oil.

Following neuromuscular blockade with pancronium bromide (1 mg/kg), the animals were ventilated artificially with room air that included 20–35% O_2 (monitored by an Ohmeda 5120 oxygen monitor), and positive end expiratory pressure (1 cmH_2O) was maintained using a positive pressure ventilator (Harvard rodent ventilator, model 683). End-tidal CO_2 was continuously monitored using a CO_2 analyzer (Capstar 100, Carbon Dioxide Analyzer) and was maintained between 4% and 6%. The depth of anaesthesia in the paralysed animals was assessed by monitoring the stability of the arterial pressure and heart rate in response to pinching of the hind paw at regular intervals.

During surgical preparation and recording, anaesthesia was supplemented by injections of urethane–chloralose (50 and 5 mg/kg; i.v.) at regular intervals.

Arterial blood samples were taken regularly, and blood gases and pH were monitored with a pH-blood gas analyzer (model i-Stat, Hewlett Packard). Blood parameters were maintained in the ranges: PO_2, 90–130 mm Hg; PCO_2, 40–50 mm Hg; pH 7.3–7.4 by adjusting the supplemental O_2 supply and/or the ventilatory pump or slowly infusing sodium bicarbonate (1.0 M) intravenously.

Searching stimuli (1 pulse with 0.2 ms duration, 1 Hz) for antidromic activity of SSN neurons were delivered to the CLN or CTBs and LN. The antidromic activity evoked by the CLN, CTBs or LN was identified by standard criteria (Barman and Gebber, 1987, 1997; Lipski, 1981; Oskutyte et al., 2004).

Extracellular recordings of single unit activity were made using a single microelectrode glued to multi-barreled ionophoresis microelectrodes. Recording microelectrodes and multi-barreled ionophoresis microelectrodes were made from borosilicate glasses (respectively GC 150TF-10 and GC 150F-10, Harvard Apparatus, UK). The single recording microelectrode and one barrel of the multi-barrelled electrode for automatic current balancing were filled with 0.5 M sodium acetate and 2% Pontamine Sky Blue. The remaining barrels were filled with excitatory amino acid receptor agonist; (±)-a-Amino-3-Hydroxy-5-methilisoxazole-4-proprionic acid hydrate (AMPA, 20 mM, pH8.5, Sigma), and/or DL-homocysteic acid (DLH, 100 mM, pH8.5).

The calamus scriptorius was used as a reference point, and the tip of the microelectrode was lowered into the SSN at an angle of 30° (coordinates: 3.4–4.0 mm rostral from calamus scriptorius, 1.5–2.2 mm lateral from the midline and 2.0–2.7 mm ventral from the surface of the brainstem).

Data and stimulus control signals were recorded on magnetic tape (Teac, RD-130 TE DAT) for further analysis. The onset latency of each anridromic spike could be determined in 10–20 trials using commercially available software (Signal for Windows, Cambridge Electronic Design). Ratemeter histogram (bin resolution 1 s), ECG/ABP-triggered correlation histogram (bin width 4 ms, 150 bins, pretriggered time 0.2 s), TP-triggered correlation histogram (bin width 20 ms, 150 bins, pretriggered time 1.0 s) and pre-stimulus time histogram (bin width 1 ms, 400 bins, pretriggered time 0.02 s) were constructed and displayed using commercially available software (Spike 2 for windows, Cambridge Electronic Design). In the ECG-triggered correlation histogram, the maximum and minimum numbers of phase-locked counts averaged over three cardiac cycles were taken as the peak and background counts, respectively. A neuron was classified as having a pulse-related activity if cardiac coefficient (peak counts per background counts) was more than 2.0 (Barman and Gebber, 1998; Barman et al., 2002; Oskutyte et al., 2006).

We constructed an ECG-triggered correlation histogram for neurons with mean firing rate below 11.2 spikes/s as pulse-related activity was clearly discernible in an ECG-triggered correlation histogram constructed from lower rather than higher firing rate activity (McAllen and Spyer, 1978b).

In the TP-triggered correlation histogram, the maximum and minimum numbers of phase-locked counts averaged over two respiratory cycles were taken as the peak and background counts, respectively. A neuron was classified as having a TP-related activity if TP coefficient (peak counts per background counts) was more than 2.0.

In individual SSN neurons the mean firing rate (spikes/s) was calculated from the ratemeter histogram during 20–30 s of induced firing. All data are presented as mean ± standard deviation (SD) unless otherwise stated. Mean firing rates were compared using Student's t test, and fibre type responses and histological locations were compared using the Chi-square test. Differences were taken as significant when $P<0.05$.

In each experiment, the last recording site was marked by electrophoretic injection of the dye. The animals were given by additional anaesthetic of urethane–chloralose (50 and 5 mg/kg; i.v.) and were perfused through the left cardiac ventricle with 0.9% of saline, followed by 10% of buffered formalin solution (pH 7.4). The brains were removed and fixed in 10% formalin. Serial coronal/sagittal sections of the brain (50 µm thick) were cut and were stained with Cresyl Violet. The recording site was visualized and mapped onto standard sections of the brain (Paxinos and Watson, 1998). In a case where more than two neurons were recorded in the same animal, unmarked recording site was determined by the relative locations from marked site on the atlas.

3. Results

A total of 32 SSN neurons were recorded which produced antidromic spikes in response to stimulation of the chorda–lingual nerve (CLN, $n=3$) or the chorda tympani branches (CTBs, $n=17$) or the lingual nerve (LN, $n=12$); see Fig. 1A. The pulse, tracheal pressure and lingual nerve modulations were tested with neuronal activity induced by excitatory amino acids (EAAs), since none of these SSN neurons showed spontaneous activity (in agreement with our previous reports: Oskutyte et al., 2004, Ishizuka et al., 2008). The cardiac and/or respiratory modulations were determined whether excitatory amino acid induced SSN neuronal activity exhibited pulse- and/or respiratory-related activity. Neurons that were not activated