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Difference between male and female rats in cholinergic activity of parasympathetic vasodilatation in the masseter muscle

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ABSTRACT

We compared the changes in blood flow of the masseter muscle (MBF), lower lip (LBF) and common carotid artery (CCABF) evoked by electrical stimulation of the lingual nerve (LN) in order to examine whether high cholinergic activity of parasympathetic vasodilatation in females is specific for the masseter muscle, and whether sex-associated differences in cholinergic parasympathetic vasodilatation affect the regulation of blood flow to the orofacial area from the CCABF in urethane-anaesthetized, vago-sympathectomized male and female rats. Increases in the MBF, LBF and CCABF evoked by LN stimulation appear to be mediated via an activation of parasympathetic reflex vasodilatation since these increases were profoundly reduced by pretreatment with the autonomic cholinergic ganglion blocker hexamethonium (10 mg/kg). Although α- and β-adrenoceptor antagonists (phentolamine and propranolol, 100 μg/kg) had no effect on the LN stimulation-induced blood flow increases in either sex, a marked difference was found between males and females in the effects of the antimuscarinic agent atropine (1–100 μg/kg) on these blood flow increases. Pretreatment with atropine slightly attenuated the increase in the MBF in males, but in females it markedly reduced the increases in all three sites measured, especially in the MBF. Our results suggest that (1) cholinergic activity of the parasympathetic vasodilatation in females is higher than that in males in most orofacial tissues, but particularly in the masseter muscle and (2) cholinergic parasympathetic vasodilatations are more involved in the regulation of blood flow to the orofacial area from the CCABF in females than in males.

1. Introduction

Chronic pain and fatigue in jaw muscles are well known to be the most common symptoms in craniomandibular disorders (headache, bruxism, temporomandibular disorders).[1,2] Interestingly, these disorders in jaw muscles, especially in the masseter muscle, are accepted as being more prevalent in females than in males.[1,3] The precise reasons for the prevalence of jaw muscle disorders in females are not fully understood. However, haemodynamic properties of jaw muscles may be important for determining the functional or etiological differences in muscles between the sexes. Thus, there are several reports of sex-associated differences in the haemodynamic mechanisms that maintain homeostasis in limb or jaw muscles with respect to arteriovenous O_2 and CO_2 levels,[4,5] reactive hyperaemia[6,7] or vascular conductance.[6–8] This hypothesis would be supported by other earlier studies showing that muscle disorders are closely related to reduc-

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tions in intramuscular blood flow[9–11] and muscle tissue oxygen pressure.[12–14]

We recently reported the presence of parasympathetic vasodilator fibres originating from cell bodies in the otic ganglion in the rat masseter muscle,[15] as well as in other orofacial tissues such as lower lip[15–19] and submandibular gland.[20] These novel parasympathetic vasodilator fibres may play an important role in the regulation of haemodynamics in jaw muscles because (1) an increase in blood flow to the masseter muscle evoked by activation of these fibres occurred via the trigeminal mediated reflex,[15] and (2) it has been suggested that the inhibitory effect of excess sympathetic activity on the parasympathetic vasodilatation could be related to the development of disorders in the masseter muscle blood flow.[21] During the course of present studies on neural mechanisms underlying parasympathetic vasodilatation in jaw muscles, we found that parasympathetic vasodilatation in the masseter muscle was much more reduced in female than male rats by intravenous administration of the antimuscarinic agent atropine. This suggested that the cholinergic system would be more involved in the parasympathetic vasodilatation in the masseter muscle in females than in males. However, it is still questionable whether high cholinergic activity of parasympathetic vasodilatation in females is specific for the masseter muscle, and whether sex-associated difference in cholinergic parasympathetic vasodilatation affects the regulation of blood flow to the orofacial area from the common carotid artery (CCA).

We explored these questions in the present study by comparing changes in blood flow in the masseter muscle (MBF), lower lip (LBF) and common carotid artery (CCABF) evoked by the trigeminal mediated reflex in deeply urethane-anaesthetized, artificially ventilated, cervically vagotomized and sympathectomized male and female rats.

2. Materials and methods

2.1. Preparation of animals

The present study was designed to investigate sex differences rather than the effects of the estrous cycle on vasomotor response. For this reason, experiments were performed on adult male and female Wistar rats between 15 and 25 weeks of age and weighing 200–450 g since the previous report[22] showed that the plasma concentrations of 17β-estradiol levels in female rats aged 3–21 months are stable over time and remain elevated when compared with male rats. After induction with inhalation anaesthesia (ether), urethane (1 g/kg) in a volume of 1 ml/(100 g body weight) was injected subcutaneously into the backs of the animals. One femoral vein was cannulated to allow drug injection, and one femoral artery was cannulated and connected to a Statham pressure transducer to monitor the systemic arterial blood pressure (SABP) and heart rate. The anaesthetized animals were intubated, paralysed by intravenous (i.v.) injection of pancuronium bromide (Mioblock; Organon, Teknika, Arnhem, The Netherlands; 0.6 mg/kg initially, supplemented with 0.4 mg/kg every hour or so after testing the level of anaesthesia; see below), and artificially ventilated via a tracheal cannula with a mixture of 50% air-50% O_2. The ventilator (model SN-480-7; Shinano, Tokyo, Japan) was set to deliver a tidal volume of 8.5–10 cm^3/kg at a rate of 20–23 breaths/min, and the end-tidal concentration of CO_2 was determined by means of an infrared analyser (Capnomac Ultima; Datex, Helsinki, Finland), as reported elsewhere.[15,16,21] Rectal temperature was maintained at 37–38 °C with the use of a heating pad. Before the injection of additional pancuronium bromide, the depth of anaesthesia was checked to be adequate by the absence of flexion response to a noxious stimulus, such as pinching the digit for approximately 2 s. When the depth of anaesthesia was considered inadequate, additional urethane (i.e., intermittent doses of 100 mg/kg, i.v.) was administered.

At the end of the experiment, all rats were killed by an overdose (approximately 100 mg) of pentobarbital sodium. The experimental protocols were approved by the Animal Ethics and Research Committee and were conducted in accordance with the Guidelines for the Care and Use of Laboratory Animals of the Health Sciences University of Hokkaido. All the animals were cared for in accordance with the recommendations in the current National Research Council guide.

2.2. Electrical stimulation of the lingual nerve

The central cut end of the lingual nerve (LN) (Fig. 1a) was electrically stimulated using a bipolar silver electrode attached to an electrical stimulator (model SEN-7103; Nihon Kohden, Tokyo, Japan). For this purpose, the LN was sectioned and stimulated unilaterally under a binocular microscope. The LN was stimulated for 20 s with various voltages (1–30 V) at various frequencies (1–50 Hz) using 2 ms pulse durations. In all experiments, the cervical vagi and superior cervical sympathetic trunk nerves were cut bilaterally in the neck before the stimulation. This ensured that only non-vagal parasympathetic effects were involved in the results reported in the present study.

2.3. Measurement of the blood flow and SABP

Changes in the MBF (Fig. 1b) and LBF (Fig. 1c) were monitored on either side using a laser-Doppler flowmeter (LDF; FLO-C1, Omegawave, Tokyo, Japan), as described elsewhere.[15–17,19,21] The probes were placed against the masseter muscle after making incisions in the cheek skin and lower lip without exerting pressure on the tissues. The masseter muscle was ascertained by the naked eye. The LDF values obtained in this way represent the blood flow in the superficial vessels of the masseter muscle.[23,24] Electrical calibration for zero blood flow was performed for all recordings. Several gain levels could be selected and the maximum output of a particular gain level (defined electrically) was set as 100%. The analogue output of the equipment does not give absolute values but shows relative changes in blood flow (for technical details and an evaluation of the LDF method see Stern et al.[25]). Changes in the CCABF (Fig. 1d) were recorded by means of an ultrasonic blood flowmeter with a wide beam (UBF; SFA211, Advance, Tokyo, Japan). The output from the various devices was continuously displayed on an eight-channel chart recorder (model W5000; Graphtec, Tokyo, Japan) at a speed of 10 mm/min. The blood