ARCHIVES OF ORAL BIOLOGY 54 (2009) 909–916

available at www.sciencedirect.com

journal homepage: www.intl.elsevierhealth.com/journals/arob

Involvement of vasoactive intestinal polypeptide in the parasympathetic vasodilatation of the rat masseter muscle

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ARTICLE INFO

Article history:
Accepted 29 June 2009

Keywords:
Vasoactive intestinal polypeptide
Masseter muscle
Vasodilatation
Parasympathetic nerve

ABSTRACT

The parasympathetic vasodilatory fibres are known to innervate vessels in a rat masseter muscle via both cholinergic and non-cholinergic mechanisms. However, the non-cholinergic mechanisms are still unclear. Recently, vasoactive intestinal polypeptide (VIP) was convincingly shown to be involved in the parasympathetic vasodilatation in orofacial areas, such as submandibular glands and lower lip. However, very little is known about the rat masseter muscle. The present study was designed in the rat masseter muscle to assess (1) whether the parasympathetic nerve innervating vessels have VIP immunoreactivities, (2) whether intravenous administration of VIP induces the vasodilatation, and (3) effects of selective VIP receptor antagonist ([4Cl-D-Phe^{6}, Leu^{17}] VIP) in the presence or absence of atropine on the parasympathetic vasodilatation. The VIP immunoreactivities were found at two sites of the parasympathetic otic ganglion and nerve fibres located around vessels. The intravenous administration of VIP induced the vasodilatation, and [4Cl-D-Phe^{6}, Leu^{17}] VIP markedly decreased the vasodilatation evoked by VIP administration. The parasympathetic vasodilatation was not inhibited by [4Cl-D-Phe^{6}, Leu^{17}] VIP. However, treatment with [4Cl-D-Phe^{6}, Leu^{17}] VIP markedly decreased the parasympathetic vasodilatation when [4Cl-D-Phe^{6}, Leu^{17}] VIP was administered together with atropine. These results suggest that (1) VIP exists in the postganglionic parasympathetic nerve innervating the vessels in the masseter muscle, (2) the intravenous administration of VIP induces the vasodilatation in the masseter muscle, and (3) VIP may be involved in the parasympathetic vasodilatation in the masseter muscle when muscarinic cholinergic receptors are deactivated by either atropine or the suppression of the ACh release.

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1. Introduction

The rat masseter blood vessels are innervated by both parasympathetic nerves that synapse in the otic ganglion (OG)[1] and sympathetic nerves that synapse in the superior cervical ganglion.[2] The activations of these parasympathetic and sympathetic nerves elicits the vasodilatation[1] and vasoconstriction,[3] respectively, in the rat masseter muscle.

The parasympathetic-mediated vasodilatations in orofacial regions such as the salivary glands of rat,[4] lower lip[5] and gingiva[6] of cats are regulated largely by non-cholinergic mechanisms since pretreatment with atropine, a non-selec-

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Abbreviations: FG, fluorogold; ip, intraperitoneal injection; LN, lingual nerve; MBF, masseter blood flow; OG, otic ganglion; RCA-I, ricinus communis agglutinin I; SABP, systemic arterial blood pressure; VAChT, vesicular acetylcholine transporter; VIP, vasoactive intestinal polypeptide.

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doi:10.1016/j.archoralbio.2009.06.009

tive muscarinic cholinergic blocker, has no effect on the responses. However, the parasympathetic vasodilatation of the rat masseter muscle is significantly, but not completely, inhibited by the pretreatment with atropine.[1] This result suggests that the vasodilatation of the rat masseter muscle would occur via the cholinergic and non-cholinergic mechanisms, and that the neural regulatory mechanism in the vasodilatation of rat masseter muscle seems to differ from that in other orofacial regions.

Vasoactive intestinal polypeptide (VIP) has been suggested to be a plausible candidate for a neurotransmitter of parasympathetic non-cholinergic vasodilator fibres, because (1) an abundant VIP immunoreactivity is found in the parasympathetic autonomic ganglia of the rat[7,8] and in the nerve fibres innervating vessels in the rat lower lip,[9] and (2) a VIP antiserum and VIP receptor antagonist inhibit the parasympathetic-mediated vasodilatations in guinea-pig's carotid artery,[10] cat's submandibular gland,[11] and cat's[12] and rat's[13] brain. However, it remains unknown whether the blood vessels in the rat masseter muscle are innervated by VIP-containing fibres.

Lundberg et al.[14–16] reported the existence of atropine sensitive muscarinic autoreceptors that may negatively regulate the VIP release from the nerve endings of the postganglionic parasympathetic nerves, since an administration of atropine increases the VIP release evoked by electrical stimulation of the parasympathetic nerve in both the nasal mucosa[14] and submandibular gland[15,16] in cat, and suggested that there is an interaction between ACh and VIP with the regulation of the parasympathetic vasodilatation.

The present study investigated (1) whether or not the postganglionic parasympathetic nerve innervating the vessels of the masseter muscle has VIP immunoreactivity, (2) whether or not intravenous administration of VIP induces the vasodilatation of the masseter muscle, and (3) the effects of selective VIP receptor antagonist in the presence or absence of atropine on the parasympathetic vasodilatation evoked by trigeminal-mediated reflex of the rat masseter muscle to examine the interaction between ACh and VIP with regulation of the parasympathetic vasodilatation.

2. Materials and methods

2.1. *Animals*

Adult male Wistar rats (260–640 g) were kept in a 12:12 h light:dark schedule with food and water *ad libitum*. The experimental protocol conformed to the Guidelines for the Care and Use of Laboratory Animals in Health Sciences University of Hokkaido, and was approved by the Animal Ethics and Research Committee. All the animals were cared for in accordance with the recommendations in the current National Research Council guide.

2.2. *Fluorescent double-labelling method of retrograde transport of fluorogold combined with VIP or vesicular acetylcholine transporter immunohistochemistry*

The rats were anaesthetized by an intraperitoneal injection (ip) of sodium pentobarbital (50 mg/kg), and a small incision was made in a cheek skin to expose a surface of the masseter muscle. One microlitre of 2% fluorogold (FG: a retrograde tracer, Biotium, Hayward, CA, USA), dissolved in a sterile saline, was injected (0.1 μl/min) directly into the masseter muscle between a facial nerve and parotid duct using a Hamilton microsyringe with a needle inserted perpendicular to the muscle. After the injection, the needle was left in place for 2 min to avoid backflow of FG.

After 48–72 h, the rats were deeply anaesthetized (sodium pentobarbital, 50–100 mg/kg, ip) and were perfused with 250 ml of saline followed by 500 ml of 4% paraformaldehyde in 0.1 M sodium phosphate buffer at pH 7.4. The OG was removed and immersed in a cold buffer containing 30% sucrose. The OG was cut in 40 μm sections with a freezing microtome (HM430; Carl Zeiss, Jena, Germany), and all sections of the OG were immunohistochemically stained with VIP- and vesicular acetylcholine transporter-antibodies (VAChT, a cholinergic marker) according to the methods described below. After staining, the OG sections were mounted on gelatin-coated glass slides, and the FG-labelled neurons, VIP, and VAChT immunoreactivity was examined with a fluorescence microscope (TSC NT; Leica Wetzlar, Germany) as described.[17,18] Six to nine sections were made from one OG tissue. The number of neuronal cell bodies labelled by both FG and antibodies in either VIP or VAChT were counted from each OG section, and were expressed as percentages to total amount of the FG-labelled neurons in the OG.

2.3. *Immunohistochemical staining of VIP and/or VAChT*

The OG and masseter muscle were removed and immersed in a cold buffer containing 30% sucrose. The sections of the OG and masseter muscle (100 μm) were rinsed in phosphate buffered saline and incubated (at least 3 h) with a blocking solution containing 1% non-immune donkey serum, 0.3% Triton X-100, and 0.3% bovine albumin. The sections of the OG and masseter muscle were then incubated with VIP- (rabbit antibody, 1:3000; DiaSorin, Stillwater, MN, USA) and VAChT-antibodies (goat antibody 1:1000; Chemicon, Temecula CA), dissolved in the blocking solution for 2 days at 4 °C. After rinsing with phosphate buffered saline, the sections were incubated with donkey anti-rabbit IgG antibodies labelled with Alexa-488 and/or donkey anti-goat IgG antibodies labelled with Alexa-568 (1:1000; Molecular Probes, Eugene, OR, USA) dissolved in the blocking solution for 1 day at 4 °C. Some of the sections of the masseter muscle were immunohistochemically stained with the VIP antibodies and donkey anti-rabbit IgG antibodies labelled with Alexa-568. After staining, the sections were incubated with fluorescein ricinus communis agglutinin I (RCA-I; 20 μg/ml) for 30 min at 4 °C. RCA-I has been previously reported to the marker for rodent vessel walls.[19] After rinsing, the sections were mounted on the gelatin-coated glass slides. Fluorescence was examined under a confocal laser scanning microscopy (TSC NT; Leica Wetzlar, Germany, Ex, 488 or 568 nm; Em, 510–530 or 600–650 nm). Control preparations were performed by omitting either primary or secondary antibodies from the incubation solutions.

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