Inhibitory effects of excess sympathetic activity on parasympathetic vasodilation in the rat masseter muscle

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Inhibitory effects of excess sympathetic activity on parasympathetic vasodilation in the rat masseter muscle. Am J Physiol Regul Integr Comp Physiol 293: R729–R736, 2007. First published May 30, 2007; doi:10.1152/ajpregu.00866.2006.—The present study was designed to examine the effect of sympathetic tonic activity on parasympathetic vasodilation evoked by the trigeminal-mediated reflex in the masseter muscle in urethane-anesthetized rats. Sectioning of the superior cervical sympathetic trunk (CST) ipsilaterally increased the basal level of blood flow in the masseter muscle (MBF). Electrical stimulation of the peripheral cut end of the CST for 2 min using 2-ms pulses ipsilaterally decreased in a dependent manner the intensity (0.5–10 V) and frequency (0.1–5 Hz) of the MBF. The CST stimulation for 2 min at <0.5 Hz with 5 V using 2-ms pulses seems to be comparable with the spontaneous activity in the CST fibers innervating the masseter vasculature, because this stimulation restored the basal level of the MBF to the presectioned values. Parasympathetic vasodilation evoked by electrical stimulation of the central cut end of the lingual nerve in the masseter muscle was markedly reduced by CST stimulation for 2 min with 5 V using 2-ms pulses in a frequency-dependent manner (0.5–5 Hz). Intravenous administration of phentolamine significantly reduced the vasoconstriction induced by CST stimulation in a dose-dependent manner (0.1–1 mg/kg), but pretreatment with either phenolamine or propranolol failed to affect the sympathetic inhibition of the parasympathetic vasodilation. Our results suggest that 1) excess sympathetic activity inhibits parasympathetic vasodilation in the masseter muscle, and 2) α- and β-adrenoceptors do not contribute to sympathetic inhibition of parasympathetic vasodilation, and thus some other types of receptors must be involved in this response.

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Preparation of animals. Experiments were performed on adult male Wistar rats between 15 and 30 wk of age and weighing 300–450 g. After induction with inhalation anesthesia (ether), urethane (1 g/kg) in a volume of 1 ml/100 g body wt was subcutaneously injected 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 anesthetized animals were

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intubated, paralyzed by intravenous injection of pancuronium bromide (Moblock; 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 anesthesia; see below), and artificially ventilated via a tracheal cannula with a mixture of 50% air-50% O₂. The ventilator (model SN-480-7; Shinano, Tokyo, Japan) was set to deliver a tidal volume of 8.5–10 cm³/kg at a rate of 20–23 breaths/min, and the end-tidal concentration of CO₂ was determined by means of an infrared analyzer (Capnomac Ultima; Datex, Helsinki, Finland), as reported elsewhere (17). Rectal temperature was maintained at 37–38°C with the use of a heating pad. Before the injection of further pancuronium bromide, the depth of anesthesia was checked to be adequate by the absence of flexion response to a noxious stimulus, such as pinching the digit for ~2 s. When the depth of anesthesia was considered inadequate, additional urethane (i.e., intermittent doses of 100 mg/kg iv) was administered.

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

Electrical stimulation of the lingual nerve and superior CST. The central cut end of the lingual nerve (LN) (Fig. 1, A) and the peripheral cut end of the superior CST (Fig. 1, B) were electrically stimulated using a bipolar silver electrode attached to an electrical stimulator (model SEN-7103; Nihon Kohden, Tokyo, Japan). For this purpose, the nerves were sectioned and stimulated unilaterally under a binocular microscope. Electrical stimulation of the CST was delivered for periods of 2 min with various voltages (0.5–10 V) at various frequencies (0.1–5 Hz) using 2-ms pulse durations. The LN was stimulated for 20 s with supramaximal voltage (20 V) at 20 Hz using 2-ms pulse durations (14) either alone, or in combination with CST stimulation. In all of the experiments, the cervical vagi and CST were cut bilaterally in the neck before the stimulation, unless otherwise noted. This ensured that only nonvagal parasympathetic effects were involved in the results reported in the present study.

Measurement of the blood flow and SABP. Changes in the blood flow of the masseter muscle (MBF; Fig. 1, c) and lower lip (LBF; Fig. 1, d) were monitored on both sides using a laser-Doppler flowmeter (LDF; FLO-C1, Omegawave, Tokyo, Japan), as described elsewhere (14, 16–18, 20, 27, 28). The probes were placed against the masseter muscle after making incisions in the cheek skin and lower lip without exerting pressure on the tissue. 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 (8, 22). 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 analog 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. (33)]. The output from the various devices was continuously displayed on an eight-channel chart recorder (model W5000; Graphitec, Tokyo, Japan) at a speed of 10 mm/min. The blood flow changes were assessed by measuring the height of the response. The SABP was recorded from the femoral catheter via a Statham pressure transducer. A tachograph (model AT-610G; Nihon Kohden, Tokyo, Japan) triggered by the arterial pulse was used to monitor the heart rate.

Pharmacological agents. To determine whether the sympathetically-mediated anti-vasodilator effect was mediated via activation of α- and β-adrenoceptors, the central cut end of the LN was electrically stimulated alone, and in combination with electrical stimulation of the peripheral cut end of the CST, before and after administration of phentolamine (0.1–1 mg/kg iv), or propranolol (0.1 mg/kg iv). The magnitude of the response obtained after administration of each agent was expressed as a percentage of the control response recorded before its administration (means ± SE).

Statistical analysis. All numerical data are given as means ± SE. The statistical significance of changes in the test responses was assessed using ANOVA followed by a post hoc test [Fisher’s protected least significant difference (PLSD) test] and a contrast test. Differences were considered significant at P < 0.05. Data were analyzed using a Macintosh computer with StatView 5.0 and Super ANOVA.

RESULTS

Effects of sectioning of the CST and electrical stimulation of its peripheral cut end on the MBF, LBF, and SABP. Figure 2 shows the effects of sectioning of the left CST and electrical stimulation of its peripheral cut end on the MBF, LBF, and SABP. Under the experimental conditions in the present study, similar basal levels for MBF and LBF were recorded before the CST was sectioned (data not shown). Sectioning of the CST resulted in
a continuous (Fig. 2, a1) and a significant increase in the basal level of MBF on the left side, but not of LBF (for MBF, n = 5, P < 0.001; for LBF, n = 5, NS; CSTs in Fig. 3B). Electrical stimulation of the sectioned CST for 2 min with 5 V at 0.5 Hz using 2-ms pulses induced a decrease in both MBF and LBF on the left side (Fig. 2, a2). Figure 3 shows the mean data ± SE for the effects of CST stimulation on both MBF and LBF on the left side when the CST stimulation was delivered for 2 min at various intensities (0.5–10 V; Fig. 3A) and at various frequencies (0.1–5 Hz; Fig. 3B) using 2-ms pulses. Intensity-response relationships were generated using stimulus trains at 5 Hz. Frequency-response relationships were generated using stimulus trains at 5 V. The maximal change in MBF in response to CST stimulation was delivered for 2 min with CST stimulation at 0.1, 0.2, 0.5, 1, 2, and 5 Hz, expressed as percentages of the control response without CST stimulation, were 105.8 ± 13.9 and 117.6 ± 17.5, 73.3 ± 17.4 and 94.1 ± 6.1, 38.3 ± 12.0 and 90.3 ± 8.8, 22.0 ± 7.6 and 95.3 ± 4.2, 12.3 ± 2.8 and 92.4 ± 4.1, and 10.2 ± 4.7 and 87.0 ± 5.7%, respectively. CST stimulation above 0.2 Hz showed a significant inhibitory effect on the increase in MBF evoked by LN stimulation, but this was not observed in LBF (for MBF, F(6,28) = 16.52, n = 5 in each group, P < 0.0001; for LBF, F(6,28) = 0.1, n = 5 in each group, NS; Fig. 5A). These responses in MBF returned almost to the control level 10 min after the cessation of CST stimulation at 5 Hz (Figs. 4 and 5A). We observed a “rebound” increase in MBF after electrical stimulation of the CST was terminated (Fig. 4A). The termination of CST stimulation at levels above 0.5 Hz resulted in a significant effect on the increase in MBF, but this was not observed in the LBF [for MBF, F(5,24) = 33.69, n = 5 in each group, P < 0.0001; for LBF, F(5,24) = 0.8, n = 5 in each group, NS; Fig. 5B]. The mean changes in SABP following LN stimulation were too slight to account for the blood flow changes measured by the present methods (13.1 ± 0.6 mmHg, n = 5; data not shown).

**Effects of pharmacological blocking agents.** Electrical stimulation of the central cut end of the left LN was delivered for 20 s with supramaximal intensity (20 V) at 20 Hz using 2-ms pulses. The peripheral cut end of the left CST was stimulated for 2 min with 5 V at >0.5 Hz using 2-ms pulses. The stimulus frequency (>0.5 Hz) of the CST was chosen to initiate the inhibitory effect on the increase in MBF evoked by LN stimulation. Figure 6 shows the effects of intravenous administration of phentolamine (0.1 and 1 mg/kg; Fig. 6A) and propranolol (0.1 mg/kg; Fig. 6B) on changes in MBF on the left side evoked by LN stimulation alone (a1 and b1), and in
combination with CST stimulation (a2 and b2). The mean data ± SE of the changes in MBF before and 10 min after administration of each agent were expressed as a percentage of the control response recorded before administration (Fig. 6C). The increases in MBF evoked by LN stimulation alone were not affected by phentolamine and propranolol at 0.1 mg/kg but were significantly reduced by pretreatment with phentolamine at 1 mg/kg [F(3,12) = 5.8, n = 4 in each group, P < 0.01; Fig. 6C]. The decrease in MBF elicited by CST stimulation was significantly reduced by phentolamine in a dose-dependent manner (0.1 and 1 mg/kg) [F(3,16) = 13.9, n = 5 in each group, P < 0.0001; Fig. 6C]. The magnitude of the rise in MBF evoked by LN stimulation in combination with CST stimulation was unchanged by pretreatment with either phentolamine (0.1 and 1 mg/kg), or propranolol [F(3,16) = 0.3, n = 5 in each group, NS; Fig. 6C]. The increase in MBF evoked by LN stimulation alone was significantly larger than that in combination with CST stimulation, regardless of the presence or absence of each drug (P < 0.001, ANOVA followed by a contrast test; Fig. 6C). These responses in MBF returned almost to the control level 30–60 min after the administration of each drug (data not shown). The resting mean SABP (means ± SE) before pretreatment was 100.6 ± 6.3 mmHg and 10 min after administration of phentolamine (0.1 and 1 mg/kg); 97.2 ± 5.7 and 64.7 ± 2.7, or propranolol; 109.4 ± 9.9 mmHg, respectively. There was a significant difference in the resting mean SABP before and after the administration of phentolamine at 1 mg/kg [F(3,20) = 8.4, n = 6 in each group, P < 0.001].

**DISCUSSION**

The blood flow changes induced by electrical stimulation of both the peripheral cut end of the CST and the central cut end of the LN in the present study appeared not to be secondary to changes in the SABP because no significant changes in the SABP were observed during stimulation (see RESULTS). In this paper, we therefore refer to the CST-stimulated changes as “vasoconstriction” and to the LN-stimulated changes as “vasodilation.”

Sympathetic outflow to the blood vessels in the skeletal muscles is known to be under tonic control of the arterial and cardiopulmonary baroreflexes (6). In the present study, CST stimulation for 2 min appears to mimic the physiological forms of spontaneous tonic activity in the CST fibers supplying the vasculature in the orofacial area, because vasoconstriction in the masseter muscle and lower lip induced by CST stimulation reached stable levels within <1 min and sustained these levels during the stimulation (Figs. 2, 4 and 6, A and B).

Under our experimental conditions, stimulation of the CST for 2 min at <0.5 Hz with 5 V using 2-ms pulses seems to be comparable with the spontaneous activity in the CST fibers innervating the masseter vasculature, because this stimulation restored the basal level in the MBF to the presectioned values (Figs. 2 and 3B). This observation is consistent with a report that found that the frequency of extracellularly recorded spontaneously active postganglionic units in the rat hindlimb is ~1 Hz (11). Conversely, the LBF was consistently unchanged by sectioning of the CST in all of the animals examined in the present study (Figs. 2 and 3B), suggesting that the CST fibers supplying the vasculature in the lower lip have either none or a very low level of spontaneous activity under physiological conditions. This is consistent with previous observations that the blood flow in the lower lip, palate, submandibular gland, and tongue is largely unaffected by sectioning of the CST in the cat (16).

The parasympathetic vasodilation evoked by LN stimulation in the masseter muscle decreased markedly during CST stimulation for 2 min with 5 V using 2-ms pulses as the frequency increased from 0.5 to 5 Hz and had almost disappeared at a frequency of 5 Hz (Figs. 4A and 5A). This indicates that excess sympathetic activity inhibits the parasympathetic vasodilation evoked by the trigeminal-mediated reflex in the masseter muscle. This inhibitory effect on parasympathetic vasodilation was not observed in the lower lip (Figs. 4B and 5A). The precise mechanism by which excess sympathetic activity inhibits parasympathetic vasodilation in the masseter muscle, but not in the lower lip, still remains unclear. However, the masseter muscle...
seems to differ from the lower lip in sympathetic-parasympathetic interaction, because 1) there is spontaneous tonic activity in the CST fibers supplying the vasculature in the masseter muscle, but not in the lower lip (Figs. 2 and 3B), 2) parasympathetic vasodilation in the masseter muscle is partly sensitive to the antimuscarinic agent atropine (0.1 mg/kg) but not in the lower lip (14, 16–18, 20, 27, 28), and 3) it has been suggested that muscle vasoconstrictor neurons consist of larger sympathetic motor units and innervate a larger volume of vasculature compared with cutaneous vasoconstrictor neurons (9). Considering these different physiological and anatomical observations of sympathetic innervation between the masseter muscle and other orofacial tissue such as the lower lip, it is understandable that the hemodynamics of the masseter muscle could be susceptible to sympathetic activity.

Pretreatment with phentolamine significantly reduced the vasoconstriction induced by CST stimulation in a dose-dependent manner (0.1–1 mg/kg), but pretreatment with either phentolamine or propranolol (0.1 mg/kg) failed to affect the inhibitory effect on parasympathetic vasodilation in the masseter muscle (Fig. 6). The changes in the end-tidal concentration of CO₂ following each drug treatment (from 45 to 40 mmHg) did not account for the blood flow changes measured by the present method (data not shown). These results suggest that α- and β-adrenoceptors do not contribute to the sympathetic inhibition of parasympathetic vasodilation, and thus some other types of

![Diagram](http://ajpregu.physiology.org/)

**Fig. 4.** Typical examples of the effects of electrical stimulation of the peripheral cut end of the left CST on the increases in both MBF (A) and LBF (B) on the left (ipsilateral) side evoked by electrical stimulation of the central cut end of the left LN. The LN was stimulated (●) for 20 s with a supramaximal voltage of 20 V at 20 Hz using 2-ms pulses either alone (control and 10 min after CST stimulation at 5 Hz), or in combination with CST stimulation for 2 min with 5 V at various frequencies (0.1–5 Hz) using 2-ms pulses (hatched bars). Frequencies (0.1–5 Hz) of the CST stimulations are shown at the left above each recording.
receptors must be involved in this response. Although some researchers suggest that, in addition to catecholamine, neuropeptide Y, galanin, and ATP may act as nonadrenergic sympathetic cotransmitters (3, 23, 32), it is still unclear whether these transmitters are involved in this inhibitory response. Further investigations will be necessary to establish the neural mechanisms underlying the sympathetic inhibition of parasympathetic vasodilation.

LN stimulation-induced parasympathetic vasodilation in the masseter muscle was significantly reduced by intravenous administration of phentolamine (1 mg/kg) accompanied by a marked reduction in the resting mean SABP (from 100.6 ± 6.3 to 64.7 ± 2.7 mmHg). The fall in the magnitude of parasympathetic vasodilation caused by phentolamine would not be mediated via α-adrenoceptors because parasympathetic vasodilation during CST stimulation remained the same regardless of the presence or absence of phentolamine (Fig. 6, A and C). It is therefore likely that this response is secondary to a reduction in the resting SABP caused by the administration of phentolamine. This is supported by previous observations that the magnitude of parasympathetic vasodilation in the lower lip of rabbits was reduced by pretreat-

Fig. 5. A: Mean data ± SE for the effects of electrical stimulation of the peripheral cut end of the left CST on the increases in both MBF and LBF on the left (ipsilateral) side evoked by electrical stimulation of the central cut end of the left LN. The LN was stimulated for 20 s with a supramaximal voltage of 20 V at 20 Hz using 2-ms pulses. Electrical stimulation of the CST was delivered for 2 min with 5 V at various frequencies (0.1–5 Hz) using 2-ms pulses. The changes in both MBF (open bars, n = 5 in each group) and LBF (solid bars, n = 5 in each group) evoked by LN stimulation alone (10 min after CST stimulation at 5 Hz) and in combination with CST stimulation are expressed as a percentage of the control response without CST stimulation. B: Stimulus frequency-response relationship for changes in both MBF (●, n = 5 in each group) and LBF (○, n = 5 in each group) on the left (ipsilateral) side in response to termination of electrical stimulation of the left CST after 2 min with 5 V at various frequencies (0.1–5 Hz) using 2-ms pulses. Maximal MBF change after the termination of CST stimulation was taken as 100%. Statistical significance of the differences was assessed by ANOVA followed by a post hoc test (Fisher’s PLSD). *P < 0.05, **P < 0.01, ***P < 0.001 vs. control (A), or basal value after the termination of CST stimulation at 0.1 Hz (B). †P < 0.001, statistically significant difference in the increase between MBF and LBF (ANOVA followed by a contrast test).

Fig. 6. Typical examples of the effects of α- and β-adrenoceptor blocking agents: phentolamine at 0.1 and 1 mg/kg (A) and propranolol at 0.1 mg/kg (B) on the changes in MBF on the left (ipsilateral) side evoked by electrical stimulation of the central cut end of the left LN alone (a1 and b1) and in combination with electrical stimulation of the peripheral cut end of the left CST (a2 and b2). The LN was stimulated (●) for 20 s with a supramaximal voltage of 20 V at 20 Hz using 2-ms pulses. Electrical stimulation of the CST was delivered for 2 min with 5 V at >0.5 Hz using 2-ms pulses (hatched bars). C: mean data ± SE of the increases in MBF evoked by LN stimulation alone (white bars, n = 4 in each group), and in combination with CST stimulation (gray bars, n = 5 in each group), and the decreases in MBF induced by CST stimulation (black bars, n = 5 in each group) before and 10 min after administration of each drug are expressed as a percentage of the pretreatment response. Statistical significance of the differences in this pretreatment response (control) was assessed by ANOVA followed by a post hoc test (Fisher’s PLSD). *P < 0.05, **P < 0.001 vs. control. The significance of the differences between sets of data (†P < 0.001) is indicated above the appropriate square bracket (ANOVA followed by a contrast test).
ment with phentolamine at 1 mg/kg accompanied by a significant decrease in the resting SABP (34). These observations suggest that the magnitude of parasympathetic vasodilation in orofacial tissue may depend on the resting SABP.

A rebound increase in MBF was observed after the completion of CST stimulation for 2 min with 5 V using 2-ms pulses at high frequencies (>0.5 Hz), but not at low frequencies (0.1–0.2 Hz) (Figs. 4A and 5B). This increase disappeared when the vasorestriction induced by CST stimulation was suppressed with the administration of phentolamine (1 mg/kg) (Fig. 6A), suggesting that the rebound MBF increase may be reactive hyperemia in response to changes in local oxygen pressure and metabolite concentration during a period of reduced MBF.

The physiological significance of parasympathetic vasodilatation in the hemodynamics of the MBF is unclear, because it is difficult to activate parasympathetic pathways to the masseter vasculature through natural stimuli (such as pinching the tongue or placing a drop of acid) under our experimental conditions (deeply urethane-anesthetized and artificially ventilated). However, evocation of parasympathetic vasodilatation by pinching, heat, or chemical stimulation (capsaicin, nicotine, or ammonia) has previously been reported in some orofacial areas, such as the lower lip (21) and the nasal mucosa (19). The results were similar to the parasympathetic vasodilatation evoked by electrical stimulation of the LN in anesthetized, artificially ventilated cats. It is therefore likely that evoking parasympathetic vasodilatation in the MBF would involve nociceptive, thermal, and tactile sensations occurring during complex movements such as mastication, speech, and swallowing. Although the underlying mechanisms of MBF disorders are not fully understood, ischemia may play a role in their development because blood flow impairment occurs, even with low levels of activity in the MBF (29). Under such conditions, a blood flow increase to the masseter muscle, which occurs via parasympathetic reflex vasodilatation, could be related to the maintenance of MBF. Thus, the inhibitory effect of excess sympathetic activity on parasympathetic vasodilatation may affect the hemodynamics of the MBF, suggesting an important role in the etiology of MBF disorders.

In conclusion, the present study found that excess sympathetic activity inhibits the parasympathetic vasodilatation evoked by the trigeminal-mediated reflex in the masseter muscle but not in the lower lip, suggesting that the hemodynamics of the masseter muscle are susceptible to sympathetic activity. It is likely that α- and β-adrenoceptors do not contribute to sympathetic inhibition of parasympathetic vasodilatation, and thus some other types of receptors must be involved in this response. Further studies of the neural mechanisms underlying the sympathetically mediated antivasodilator effect would provide data that would enable a better understanding of the etiology of jaw muscle disorders.

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