GABA\(_B\) receptors in the NTS mediate the inhibitory effect of trigeminal nociceptive inputs on parasympathetic reflex vasodilation in the rat masseter muscle

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Ishii H, Izumi H. GABA\(_B\) receptors in the NTS mediate the inhibitory effect of trigeminal nociceptive inputs on parasympathetic reflex vasodilation in the rat masseter muscle. Am J Physiol Regul Integr Comp Physiol 302: R776–R784, 2012. First published January 4, 2012; doi:10.1152/ajpregu.00569.2011.—The present study was designed to examine whether trigeminal nociceptive inputs are involved in the modulation of parasympathetic reflex vasodilation in the jaw muscles. This was accomplished by investigating the effects of noxious stimulation to the orofacial area with capsaicin, and by microinjecting GABA\(_A\) and GABA\(_B\) receptor agonists or antagonists into the nucleus of the solitary tract (NTS), on masseter hemodynamics in urethane-anesthetized rats. Electrical stimulation of the central cut end of the cervical vagus nerve (cVN) in sympathetically denervated animals bilaterally increased blood flow in the masseter muscle (MBF). Increases in MBF evoked by cVN stimulation were markedly reduced following injection of capsaicin into the anterior tongue in the distribution of the lingual nerve or lower lip, but not when injected into the skin of the dorsum of the foot. Intravenous administration of either phentolamine or propranolol had no effect on the inhibitory effects of capsaicin injection on the increases of MBF evoked by cVN stimulation, which were largely abolished by microinjecting the GABA\(_B\) receptor agonist baclofen into the NTS. Microinjection of the GABA\(_B\) receptor antagonist CGP-35348 into the NTS markedly attenuated the capsaicin-induced inhibition of MBF increase evoked by cVN stimulation, while microinjection of the GABA\(_A\) receptor antagonist bicuculline did not. Our results indicate that trigeminal nociceptive inputs inhibit vagal-parasympathetic reflex vasodilation in the masseter muscle and suggest that the activation of GABA\(_B\) rather than GABA\(_A\) receptors underlies the observed inhibition in the NTS.

capsaicin; cervical vagus nerve; baclofen; bicuculline; CGP-35348

THE PRESENCE OF PARASYMPATHETIC VASODILATOR FIBERS INNERVATING THE BLOOD VESSELS IN THE JAW MUSCLES has been established both physiologically and histochemically (14, 25, 36), as in the case of other orofacial tissues, such as the lower lip (14, 16–18, 43, 47) and the submandibular gland (16, 24). Recently, we (12) reported that parasympathetic vasodilation in the masseter muscle, evoked by activation of cervical vagal afferents passing to the nucleus of the solitary tract (NTS), a primary target of the central projections of cervical vagal afferents (1, 28), is involved in the increase of blood flow, not only in the masseter muscle, but also in the common carotid artery during the vagal-mediated depressor response. This suggests that parasympathetic reflex vasodilation via vagal-mediated reflex in the masseter muscle represents an important factor in the maintenance of blood flow in the orofacial area during systemic hemodynamic changes. Furthermore, GABAergic inputs in the NTS were found to be involved in the attenuation of vagal-parasympathetic reflex vasodilation in the masseter muscle (12), suggesting that the GABAergic system may exert inhibitory effect on this hemodynamic regulatory pathway.

Disturbation of the hemodynamics of the jaw muscles is prevalent in individuals with a history of orofacial dysfunction associated with chronic orofacial pain, such as temporomandibular disorders and fibromyalgia (5, 6, 19, 21). For example, individuals with a history of chronic jaw muscle pain have slow intramuscular reperfusion during the recovery phase after sustained isometric contractions (6). This implies that orofacial pain affects hemodynamic regulation in the jaw muscles, suggesting an etiology in orofacial dysfunction. Furthermore, some earlier reports (27, 39, 40) have indicated that some nuclei in the brain stem, such as the trigeminal spinal nucleus, NTS, and paratrigeminal nucleus, are strongly activated by noxious stimulation with injection of capsaicin, an irritant that activates C fibers and small-diameter A\(\delta\) fibers (9, 20, 38), into the orofacial area. These nuclei are also suggested to play an important role in the modulation of orofacial reflex functions, such as swallowing reflex during orofacial pain conditions (40). These observations led us to speculate that the central neural responses mediated by trigeminal nociceptive inputs are involved in the modulation of vagal-parasympathetic reflex vasodilation in the jaw muscles.

In the present study, we explored this question by investigating the effects of noxious stimulation to the orofacial area with capsaicin, and by microinjecting GABA\(_A\) and GABA\(_B\) receptor agonists or antagonists into the NTS to study blood flow increase in the masseter muscle (MBF), evoked by the activation of cervical vagal afferents either alone, or in combination with capsaicin injection, in deeply urethane-anesthetized, artificially ventilated, and cervically vago-sympathectomized rats (Fig. 1).

MATERIALS AND METHODS

Preparation of animals. Experiments were performed on 61 adult male Wistar rats between 10 and 16 wk of age, weighing 360–465 g. Urethane (1 g/kg) was subcutaneously injected into the backs of the animals following induction with inhalation anesthesia (ether). One femoral vein was cannulated to allow drug injection, and one femoral artery was cannulated and connected to a Statham pressure transducer to monitor systemic arterial blood pressure (SABP) and heart rate. Anesthetized animals were intubated, paralyzed by intravenous injection of pancuronium bromide (Mioblock; Organon, Teknika, 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

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Modified from Ishii et al. (12).

trigeminal nerve root; IX, glossopharyngeal nerve root; X, vagal sensory root. OG, otic ganglion; VSG, vagal sensory ganglia; TG, trigeminal ganglion; V, pathetic vasodilation in the masseter muscle in response to cVN stimulation.

possible central pathway by which trigeminal nociceptive inputs running in the vagus nerve to the masseter muscle via the sympathetic nervous system is mediated. Figure 1 shows that capsaicin-induced changes in the parasympathetic reflex vasodilation in the masseter muscle were not affected by the tracheal intubation and the vagal nerve section. The results of the present study support the hypothesis that capsaicin-induced changes in the parasympathetic reflex vasodilation in the masseter muscle are mediated by the vagus nerve and the sympathetic nervous system.

Analysis of capsaicin injection and pharmacological blockade.

To examine the effects of capsaicin injection on the parasympathetic reflex vasodilation in the masseter muscle, the central cut end of the cVN was electrically stimulated before and after (10–60 min) the intravenous administration of α-adrenergic blockade using phentolamine mesilate (1 mg/kg; NOVARTIS, Tokyo, Japan), or β-adrenergic blockade using propranolol hydrochloride (0.1 mg/kg; AstraZeneca, Osaka, Japan). The effectiveness of the blockade was assessed by the absence of an inhibitory effect by microinjection of α-blockade and β-blockade using phentolamine and propranolol, respectively.

Central microinjection study.

To determine whether capsaicin-induced changes in the parasympathetic reflex vasodilation were mediated by the GABAergic system in the NTS, the GABA receptor agonist or antagonists, GABA<sub>B</sub> receptor agonist baclofen hydrochloride (10–50 mM; Sigma-Aldrich, St. Louis, MO), GABA<sub>A</sub> receptor antagonist bicuculline methochloride (50 mM; Wako, Osaka, Japan), and GABA<sub>A</sub> receptor agonist CGP-35348 were microinjected into the NTS (100 nl/site) (15). The effectiveness of the antagonists was assessed by the absence of an inhibitory effect by microinjection of α-blockade and β-blockade using phentolamine and propranolol, respectively. Each drug was dissolved in sterile saline.

Measurement of blood flow and SABP.

Changes in blood flow and SABP in MBF (Fig. 1b) were recorded using a laser-Doppler flowmeter (LDF; FLO-C1, Omegawave, Tokyo, Japan), as described elsewhere (10–15; 25, 36). Probes were placed against the masseter muscle after making incisions in the cheek skin without exerting pressure on the tissue. The SABP was recorded before injection.

Blood flow changes were assessed by measuring the height of the response, and vascular conductance in the masseter muscle was calculated by dividing mean flow change by mean SABP. The magnitude of the response was expressed as percentages of the conductance of the control response recorded before injection.
injecting muscimol and baclofen into the NTS on MBF increased evoked by vagal-mediated reflex (data not shown). Animals were mounted in a stereotaxic frame, and a partial occipital craniotomy was performed (12, 13). The dorsal surface of the medulla oblongata was exposed by incising the dura mater and the arachnoid membrane. Each microinjection was performed via an injection cannula (0.3 mm outer diameter) inserted through a guide cannula (0.6 mm outer diameter) positioned within the NTS at stereotaxic coordinates ~0.5 mm rostral to the obex, 1 mm lateral to the midline, and 0.6–0.8 mm beneath the surface of the brain stem (29). These specific coordinates were published previously (12, 13). At the end of the experiments, all animals were given an overdose of pentobarbital sodium (see above) and perfused through the ascending aorta with 200 ml saline (0.9%), followed immediately by 500 ml of 10% formalin. The brain stem and upper cervical spinal cord were removed and stored for 1–3 days in buffered 30% sucrose. After storage, 50-μm-thick sections were cut on a freezing microtome (HM 430; Carl Zeiss, Jena, Germany) and collected in 0.1 M phosphate buffer (pH 7.4). Sections were mounted on gelatin-coated slides and stained with thionin. Transmitted light was employed to identify the sites at which microinjection was delivered to the NTS.

Statistical analysis. All numerical data are presented as means ± SE. The statistical significance of observed changes was assessed using a paired Student’s t-test or ANOVA, followed by a post hoc test (Fisher’s protected least significant difference 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 noxious stimulation to the orofacial area on MBF increases evoked by electrical stimulation of the central cut end of the cVN and SABP. Figure 2A shows the effects of capsaicin injection (20 mM) into the left side of the anterior tongue in the distribution of the lingual nerve on MBF and SABP. Under the present experimental conditions, similar basal levels for MBF were recorded on both sides before capsaicin injection (Fig. 2A). Capsaicin injection resulted in a significant reduction in MBF, and a subsequent “rebound” elevation in MBF, accompanied by a marked increase in SABP. These responses returned almost to control levels just 10 min following capsaicin injection (Fig. 2A). Before the capsaicin injection, electrical stimulation of the left cVN rapidly and markedly increased MBF on both sides (Fig. 2A). Increases in MBF evoked by cVN stimulation were largely abolished 10 min following capsaicin injection (Fig. 2A), whereas vehicle injections consistently failed to produce any significant effect on MBF increases evoked by cVN stimulation, or on resting cardiovascular parameters (Fig. 2B). Mean changes in vascular conductance of the masseter muscle evoked by cVN stimulation following capsaicin or vehicle injection were expressed as percentages of the control response recorded before each injection (Fig. 2C). There were significant differences in the inhibitory effects of capsaicin injection on MBF increases on both sides [left side, F(6,35) = 9.31, n = 6 in each group, P < 0.001; right side, F(6,35) = 5.69, n = 6 in each group, P < 0.001]. These inhibitions occurred in a dose-dependent manner (10–20 mM) (Fig. 2C). These responses recovered in a time-dependent manner and returned to 40–60% of control value just 60 min following capsaicin injection (Fig. 2, A and C). In contrast, there were no significant differences in MBF increases before and after vehicle injection [F(6,35) = 0.0001, n = 6 in each group, nonsignificant (NS); Fig. 2C]. The magnitude of the rise in MBF evoked by cVN stimulation following capsaicin injection into the tongue was significantly smaller than that following vehicle injection 10–40 min after each injection (ANOVA followed by a contrast test; Fig. 2C).

The resting mean SABP (mean ± SE) before capsaicin injection was 99.9 ± 5.7 mmHg, while 10 min after injection, resting mean SABP was 111.8 ± 8.8 mmHg. There was no
significant difference between the resting mean SABP before and after (10 min) capsaicin injection (n = 7 in each group, NS, paired t-test). Capsaicin injection induced a decrease (27 rats; 44%), an increase (26 rats; 42%), or a steady state (8 rats; 14%) of SABP. The mean change in SABP following cVN stimulation before capsaicin injection was 9.41 ± 6.28 mmHg, and 10 min after its injection, −1.28 ± 2.29 mmHg. There was no significant difference between mean SABP before and after cVN stimulation (n = 7 in each group, NS, paired t-test).

Effects of capsaicin injection into the lower lip and foot on MBF increases evoked by electrical stimulation of the central cut end of the cVN and SABP. Figure 3 shows the effects of capsaicin injection (20 mM) into the lower lip (A) and skin of the dorsum of the foot (B) on MBF on the left side and SABP. Changes in MBF and SABP evoked by capsaicin injection into the lower lip were similar to those evoked by injection into the tongue (Fig. 2A). Elevations in MBF evoked by left cVN stimulation markedly attenuated just 10 min following capsaicin injection into the lower lip (Fig. 3A). In contrast, capsaicin injection into the skin of the dorsum of the foot failed to affect MBF increases evoked by cVN stimulation, although its injection significantly increased SABP (Fig. 3B). Mean changes in vascular conductance of the masseter muscle evoked by cVN stimulation after (10–60 min) capsaicin injections into each site were expressed as percentages of the control response evoked by cVN stimulation before capsaicin injection (Fig. 3C). There was a significant difference in the inhibitory effect of capsaicin injection into the lower lip on MBF increases 10–30 min following injection [F(6,35) = 3.21, n = 6 in each group, P < 0.05; Fig. 3C]. These responses recovered in a time-dependent manner and returned to control values ~40 min after capsaicin injection (Fig. 3C). In contrast, there were no significant differences in the observed MBF increases before and after capsaicin injection into the skin of the dorsum of the foot was 113.8 ± 8.8 and 111.8 ± 8.8 mmHg, respectively. There was no significant difference between the resting mean SABP before and after (10 min) capsaicin injection into each site (n = 5 in each group, NS, paired t-test).

Effects of pharmacological blockade on the inhibitory effect of capsaicin injection on MBF increases evoked by electrical stimulation of the central cut end of the cVN and SABP. Figure 4 shows the effects of intravenous administration of phentolamine (1 mg/kg; A) and propranolol (0.1 mg/kg; B) on changes in MBF on the left side evoked by left cVN stimulation, following capsaicin injection (20 mM) into the left side of the anterior tongue in the distribution of the lingual nerve and SABP. Pretreatment with phentolamine or propranolol had no apparent effect on the inhibitory effect of capsaicin injection on MBF increases evoked by cVN stimulation (Fig. 4, A and B). Reduction and rebound increase in MBF, accompanied by marked increase in SABP, following capsaicin injection were attenuated by pretreatment with phentolamine (Fig. 4A). MBF increases evoked by cVN stimulation alone were not affected by either pretreatment (data not shown). Mean changes in the vascular conductance of the masseter muscle evoked by cVN stimulation 10 min following capsaicin injection alone, and in combination with the administration of phentolamine and propranolol, were expressed as percentages of the control response evoked by cVN stimulation before each administration (Fig. 4C). The magnitude of the rise in evoked MBF increase following capsaicin injection remained unchanged by pretreatment with propranolol [F(2,15) = 24.2, n = 6 in each group, P < 0.001], but was marginally increased by pretreatment with phentolamine [F(2,15) = 1.654, n = 6 in each group, NS; Fig. 4C]. However, the inhibitory effect of capsaicin injection on MBF increase evoked by cVN stimulation remained unchanged, regardless of the presence or absence of each drug (NS, ANOVA followed by a contrast test; Fig. 4C). MBF
responses returned to 60–80% of control value just 60 min following administration of each drug (Fig. 4, A and B). Resting SABP (mean ± SE) 10 min after capsaicin injection, in combination with the administration of phentolamine and propranolol, was 59.5 ± 3.4 and 114.1 ± 15.7 mmHg, respectively. There was a significant difference in the resting mean SABP before and after (10 min) capsaicin injection in combination with the administration of phentolamine (n = 5 in each group, P < 0.05, paired t-test).

Effects of microinjecting GABA<sub>B</sub> receptor agonist baclofen into the NTS on MBF increases evoked by electrical stimulation of the central cut end of the cVN and SABP. Figure 5 shows the effects of microinjecting (100 nl/site) baclofen (10 mM; A) into the left NTS at stereotaxic coordinates ~0.5 mm rostral to the obex and 1 mm lateral to the midline, on the MBF increases evoked by electrical stimulation of the central cut end of the left cVN (cVN stim.) for 20 s, with 20 V at 20 Hz using 2-ms pulses, and on SABP following capsaicin injection (20 mM, 20 µl/site) into the left side of the anterior tongue in the distribution of the lingual nerve. cVN stimulation was delivered before (control) and after (5–60 min) its microinjection. C: mean ± SE vascular conductance of the masseter muscle evoked by cVN stimulation before (control, open bar) and 5 min after microinjecting baclofen (10 and 50 mM) into the NTS (solid bars, n = 6 in each group). Each value is expressed as a percentage of vascular conductance of the control response. Statistical significance of the differences from control levels was assessed by ANOVA followed by a post hoc test (Fisher’s PLSD). *P < 0.05 vs. control.

C: micrograph of a representative coronal section through the medulla oblongata stained with thionin showing a typical site (arrowhead) at which microinjection was delivered to the NTS (left), and a summary plot of microinjection sites (right). AP, area postrema; CU, cuneate nucleus; IO, inferior olivary nucleus; VT, spinal trigeminal tract; XII, hypoglossal nucleus; LRN, lateral reticular nucleus; py, pyramid tract; cc, central canal. Scale bar, 500 µm.
on the left side and SABP. Increases in MBF evoked by left cVN stimulation were largely abolished 5 min after baclofen microinjection into the NTS (Fig. 5A). Mean changes in the vascular conductance of the masseter muscle evoked by cVN stimulation 5 min after baclofen microinjection into the NTS were expressed as percentages of the control response evoked by cVN stimulation alone (Fig. 5B). There were significant differences in the inhibitory effects of baclofen microinjections [10 and 50 mM, \( F(2,15) = 4.71, P < 0.05 \) and \( F(2,15) = 7.19, P < 0.05 \), \( n = 6 \) in each group, respectively]. These inhibitions occurred in a dose-dependent manner (10–50 mM) (Fig. 5B). The resting mean SABP (mean ± SE) 10 min after microinjection of baclofen at 10 and 50 mM into the NTS was 122.9 ± 8.9 and 132.3 ± 20.1 mmHg, respectively. There was a significant difference in the resting mean SABP before and after (5 min) baclofen microinjection at 50 mM (\( n = 5 \) in each group, \( P < 0.05 \), paired \( t \)-test). Figure 5C shows a typical microinjection site in the NTS of representative coronal sections through the medulla oblongata stained with thionin and a summary plot of microinjection sites. The microinjection outside the NTS (in the hypoglossal or cuneate nucleus) consistently failed to produce a significant effect on cardiovascular parameters (data not shown).

**Effects of microinjecting GABA receptor antagonists into the NTS on the inhibitory effect of capsaicin injection on MBF increases by electrical stimulation of the central cut end of the cVN and SABP.** Figure 6 shows the effects of microinjecting (100 nl/site) bicuculline (50 mM; A) and CGP-35348 (10 mM; B) into the left NTS (−0.5 mm rostral to the obex and 1 mm lateral to the midline) on MBF changes on the left side evoked by left cVN stimulation following capsaicin injection (20 mM) into the left side of the anterior tongue in the distribution of the lingual nerve and SABP. Microinjection of CGP-35348 into the NTS reversed the inhibitory effects of capsaicin injection on MBF increases evoked by cVN stimulation 10 min following injection, whereas microinjection of bicuculline into the NTS failed to influence this inhibitory effect (Fig. 6, A and B). MBF increases evoked by cVN stimulation were marginally increased by bicuculline microinjection, but were not affected by CGP-35348 microinjection (data not shown). Mean changes in the vascular conductance of the masseter muscle evoked by cVN stimulation 10 min after capsaicin injection alone, and in combination with microinjection of bicuculline and CGP-35348, were expressed as percentages of the control response evoked by cVN stimulation before each microinjection (Fig. 6C). There was a significant difference in the inhibitory effect of capsaicin injection into the tongue on MBF increases evoked by cVN stimulation 10 min after injection [\( F(2,15) = 13.9, n = 6 \) in each group, \( P < 0.001 \)]. The magnitude of the rise in MBF increase following capsaicin injection remained unchanged by pretreatment with bicuculline microinjection [\( F(2,15) = 6.69, n = 6 \) in each group, \( P < 0.01 \); Fig. 6C]. These responses almost returned to control values just 60 min after each injection (Fig. 6A). In contrast, there was no significant difference between the inhibitory effect of capsaicin injection on MBF increase evoked by cVN stimulation before and 10 min after capsaicin injection, in combination with CGP-35348 microinjection [\( F(2,15) = 2.26, n = 6 \) in each group, NS; Fig. 6C]. The magnitude of the rise in MBF evoked by cVN stimulation following capsaicin injection, in combination with CGP-35348 microinjection, was significantly larger than that with capsaicin injection alone (\( P < 0.05 \), ANOVA followed by a contrast test; Fisher’s PLSD). *\( P < 0.01 \), **\( P < 0.001 \) vs. control. Whether differences between sets of data were significant (\( P < 0.05 \)) or NS is indicated above the appropriate square bracket (ANOVA followed by a contrast test).

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and CGP-35348 microinjection, was 108.3 ± 8.2 and 115.4 ± 16.8 mmHg, respectively. There was no significant difference between the resting mean SABP before and after (10 min) each treatment (n = 5 in each group, NS, paired t-test).

**DISCUSSION**

In the present study, electrical stimulation of the central cut end of the cVN rapidly induced a marked increase in MBF bilaterally in sympathectomized rats (Fig. 2). Increases in MBF evoked by cVN stimulation were not dependent on changes in SABP (Figs. 2A and 3A). These observations suggest that the evoked MBF increases as a result of “vasodilation.” Although blood vessels in the masseter muscle are innervated with sympathetic postganglionic neurons derived from the superior cervical ganglion, these neurons did not contribute to the observed MBF increases evoked by cVN stimulation, since bilateral sectioning of the CST in the present study cuts the preganglionic inputs to the superior cervical ganglion, which projects postganglionic fibers to the cranial circulation (see MATERIALS AND METHODS), and electrical stimulation of the CST consistently reduced MBF, as reported by our previous reports (14, 15). Therefore, we hypothesize that the cVN stimulation-induced vasodilation in the masseter muscle is mediated by a mechanism involving a parasympathetic reflex.

Our previous studies on the neural mechanisms underlying parasympathetic vasodilation in the masseter muscle suggest that this vasodilation is mediated by cell bodies in the otic ganglion (Fig. 1) (14, 25). The vagal-parasympathetic reflex vasodilation in the masseter muscle seems to be closely related to the vagal visceral afferents derived from the cardiovascular and/or respiratory systems, rather than those from the abdominal cavity (1), because electrical stimulation of only the abdominal vagus nerve inferior to the diaphragm failed to affect the MBF, as reported by us previously (13).

The MBF increases evoked by cVN stimulation were markedly reduced following capsaicin injection into the anterior tongue in the distribution of the lingual nerve in a dose-dependent manner (10–20 mM) (Fig. 2, A and C). These results were similar to those evoked by capsaicin injection into the lower lip (Fig. 3, A and C). In contrast, capsaicin injection into the skin of the dorsum of the foot had no effect on MBF increases evoked by cVN stimulation (Fig. 3, B and C). These results indicate that trigeminal nociceptive inputs markedly inhibit vagal-parasympathetic reflex vasodilation in the masseter muscle. Furthermore, the inhibitory effect of capsaicin injection into the tongue was stronger and longer than those of capsaicin injection into the lower lip (Figs. 2 and 3), suggesting that trigeminal nociceptive influence may occur differentially in facial and intraoral sites. This is consistent with an earlier report suggesting that deep nociceptive afferents have stronger modulatory effects on the swallowing reflex compared with superficial nociceptive afferents (40).

Capsaicin is also known to enhance adrenal medullary catecholamine secretion, especially adrenaline (44, 45), which is suggested to be important in the modulation of vasomotor responses mediated by α- and β-adrenergic receptors in the masseter muscle, as reported by us previously (10). However, circulating adrenaline appeared not to be involved in the inhibitory effects of capsaicin injection on MBF increases evoked by cVN stimulation, because intravenous administration of either phentolamine or propranolol had no effect on this inhibitory effect (Fig. 4). In the present study, the magnitude of the rise in evoked MBF increase following capsaicin injection was marginally increased by pretreatment with phentolamine (Fig. 4C). This response may be due to α-adrenoceptors in the blood vessels in the masseter muscle, because changes in the SABP evoked by cVN stimulation remained the same, regardless of the presence or absence of phentolamine. Decreases and rebound increases in the MBF, accompanied by marked increases in the SABP, were observed after capsaicin injections (Figs. 2A and 3A), and these responses were attenuated by pretreatment with phentolamine (Fig. 4A). These results suggest that the MBF decreases result from activation of α-adrenoceptors in the blood vessels in the masseter muscle, mediated by circulating adrenaline, and rebound MBF increases may be due to reactive hyperemia in response to reduced MBF. GABAergic transmission in the NTS is thought to be involved in the inhibitory effects of parasympathetic vasodilation via the vagal-mediated reflex in the masseter muscle. Evidence for this includes the fact that microinjection of the GABA_A receptor agonist muscimol into the NTS markedly inhibited MBF increases evoked by cVN stimulation, as reported in our previous studies (12, 13). Binding sites for GABA_B receptors, as well as GABA_A receptors, have been demonstrated to localize in the NTS (3, 46). GABA_B receptor agonist baclofen inhibits neuronal excitability in rat NTS neurons (7, 23, 42, 48). These observations suggest that both GABA_A and GABA_B receptors are involved in the modulation of synaptic transmission within the NTS in response to trigeminal nociceptive inputs. In the present study, the MBF increases evoked by cVN stimulation were largely abolished by microinjection of baclofen into the NTS (Fig. 5). This indicates that not only GABA_A, but also GABA_B, receptors in the NTS neurons are involved in the inhibition of vagal-parasympathetic reflex vasodilation in the masseter muscle. On the other hand, microinjection of the GABA_B receptor antagonist CGP-35348 into the NTS markedly attenuated capsaicin-induced inhibition of MBF increase evoked by cVN stimulation, while microinjection of the GABA_A receptor antagonist bicuculline failed to influence this inhibitory effect (Fig. 6). These results suggest that GABA_B receptors, rather than GABA_A receptors, underlie the observed inhibition in the NTS. GABA_B receptors are G protein-coupled receptors and regulate neuronal activity via the activation of K^+ channels (4, 34), which induces hyperpolarization of the neuronal membrane, causing chronic inhibition of neuronal activity. Therefore, activation of GABA_B receptors in the NTS, via trigeminal nociceptive inputs, could lead to the inhibition of the excitatory NTS neurons that mediate vagal-parasympathetic reflex vasodilation in the masseter muscle.

Trigeminal nociceptive inputs have been shown to modulate orofacial motor functions mediated by GABA_A receptors (33, 40), suggesting an important role in functional disorders of the masticatory system and swallowing abnormalities. However, we have not been able to identify any other studies showing evidence that activation of trigeminal nociceptive inputs in the NTS modulates the reflex vasodilator response in the orofacial area mediated by GABA_B receptors. Consequently, the present study may be considered to be the first published evidence of this mechanism. Compared with GABA_A receptors, little is known about the potential mechanisms that may alter GABA_B receptor function in response to nociceptive inputs. However,
prolonged exposure to GABA (32) and increased nociceptive afferent inputs (22) have been reported to increase the expression of GABAB receptors in the central nervous system, suggesting that synaptic activity can regulate the expression of GABAB receptors. Furthermore, noxious cutaneous stimulation has been reported to increase the proportions of neurons double-labeled for Fos and GABAB receptors in the NTS (31). These observations suggest that an enhanced GABAB receptor function, mediated by central neural plasticity in the NTS, may be involved in chronic disturbance of the parasympathetic reflex vasodilation in the jaw muscles during orofacial pain conditions.

It has been reported that orofacial pain modulates the activity of the jaw muscles (2) and jaw-stretch reflex (2, 30, 37, 41), suggesting that changes in the functional properties of the jaw muscles mediated by trigeminal nociceptive inputs play an important role in the development of functional disorders of the masticatory system, including temporomandibular disorders. The hemodynamics in skeletal muscles, including jaw muscles, are widely recognized as one of the most important factors for maintaining muscle function (8). Thus the inhibitory effect of trigeminal nociceptive inputs on parasympathetic reflex vasodilation in the jaw muscles may affect their hemodynamics, suggesting an important role in the etiology of masticatory dysfunctions.

In summary, our results indicate that trigeminal nociceptive inputs inhibit vagal-parasympathetic reflex vasodilation in the masseter muscle and suggest that activation of GABAB rather than GABA receptors underlie the observed inhibition in the NTS. Further studies on the precise neural pathway and mechanisms for modulation of the GABAergic system involved in the inhibition of parasympathetic reflex vasodilation in the jaw muscles should provide a better understanding of etiology associated with ischemia of the craniofacial area with chronic orofacial pain.

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DISCLOSURES
No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS
H. Ishii conception and design of research; H. Ishii performed experiments; H. Ishii analyzed data; H. Ishii and H. Izumi interpreted results of experiments; H. Ishii prepared figures; H. Ishii drafted manuscript; H. Ishii and H. Izumi edited and revised manuscript; H. Ishii and H. Izumi approved final version of manuscript.

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