Parasympathetic Mediated Pupillary Dilation Elicited by Lingual Nerve Stimulation in Cats

Tomohiro Tanaka,[1] *Satoshi Kuchiiwa,*[2] *and Hiroshi Izumi*[3]

Purpose. To determine the autonomic efferent nerve pathways for the reflex pupillary dilation elicited by somatic stimulation in cats.

Methods. Cats anesthetized with a mixture of α-chloralose (50 mg/kg) and urethane (100 mg/kg) were intubated and paralyzed by intravenous injection of pancuronium bromide. The central cut end of the lingual nerve (LN) was stimulated electrically to simulate somatic stimulation, and 1 μL of lidocaine (2%) was microinjected into the Vsp or the EW nucleus to determine its effect on the pupillary dilation induced by LN stimulation. The effect of electrically stimulating the Vsp or sectioning the superior cervical sympathetic nerve (CSN) on the pupillary response was also examined.

Results. Stimulation of the LN or the trigeminal spinal nucleus (Vsp) evoked pupillary dilation in a frequency- and intensity-dependent manner. These responses were not affected by sectioning the ipsilateral or both CSNs. The pupillary responses were markedly suppressed by microinjecting lidocaine into the ipsilateral Vsp or the Edinger-Westphal (EW) nucleus, but not by injection into the contralateral Vsp.

Conclusions. These results indicate that the Vsp and EW nucleus act as bulbar relay centers for pupillary dilation elicited by LN stimulation and suggest that the efferent arc of the response is a parasympathetic pathway. The contralateral pupillary dilation appears to be mediated, at least in part, by fibers projecting from the Vsp to the contralateral EW nucleus. (*Invest Ophthalmol Vis Sci.* 2005;46:4267–4274) DOI:10.1167/iovs.05-0088

We have reported that electrical stimulation of the central cut end of a branch of the trigeminal nerve (e.g., the lingual nerve [LN], or the tooth pulp which is innervated by the trigeminal nerve) elicits different responses mediated by parasympathetic reflex mechanisms.[1–11] These responses include vasodilation in the orofacial area (e.g., lower lip, gingiva, palate, and tongue) and salivary or lacrimal secretions from the submandibular, parotid, and lacrimal glands.

During an experimental study on the effects of somatic stimulation on pupil size in urethane-anesthetized cats, we found that centrally directed LN stimulation consistently dilated the pupil that depended on both stimulus frequency and intensity. This response occurred even though the anesthesia was deep enough to prevent a reflex elevation of systemic arterial blood pressure (SABP) by a noxious stimulus (e.g., pinching the upper lip for approximately 2 seconds).

Somatic stimulation has long been known to induce a reflex pupillary dilation via the efferent autonomic nerve.[12–14] However, the sympathetic and parasympathetic efferent nerve pathways for reflex pupillary dilation induced by somatic stimulation have still not been completely determined, and systematic studies have not been performed to analyze the central or peripheral neural pathways involved in the reflex pupil dilation.

In this study, we used the changes in pupil size and lip blood flow (LBF) elicited by electrically stimulating the central cut end of the LN to study the effect of sectioning the superior cervical sympathetic nerve (CSN), or the microinjection of nonselective, reversible local anesthesia (lidocaine) into the trigeminal spinal nucleus (Vsp) or the Edinger-Westphal (EW) nucleus on the pupillary dilation. The EW nuclei are known to send the preganglionic parasympathetic axons with the oculomotor nerve to the iris.

The purpose of this study was to examine the central and peripheral efferent pathways of the pupillary dilation and increase in the LBF and to determine whether they are sympathetic or parasympathetic pathways. The cat was selected because its pupil is constricted under α-chloralose-urethane anesthesia, which enabled us to obtain measurements of the reflex pupil dilation. The cat was also used because the reflex arcs for somatoparasympathetic reflex vasodilation in the lower lip have been well defined.[15–17]

Methods

Preparation of Animals

The procedure used adhered to the guidelines of the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research and were approved by the Committee of Animal Experimentation, Tohoku University School of Medicine.

Fourteen adult cats approximately 2 to 4 years of age and of both sexes were used. The cats, weighing 2.5 to 4.8 kg, were initially sedated with ketamine hydrochloride (30 mg/kg intramuscularly) and anesthetized with a mixture of α-chloralose (50 mg/kg) and urethane (100 mg/kg). A femoral artery was cannulated for the measurement of SABP. The anesthetized animals were intubated and paralyzed by an initial intravenous (IV) injection of 0.4 mg/kg pancuronium bromide (Mioblock; Organon, Teknika, The Netherlands). The paralysis was maintained by supplements of 0.2 mg/kg approximately every hour after testing the level of anesthesia. The animals were artificially ventilated with a mixture of 50% air and 50% O_2. The ventilator (model SN-480-6; Shinano, Tokyo, Japan) was set to deliver a tidal volume of 10 to 12 cm^3/kg at a rate of 20 breaths/min, and the end-tidal concentration of CO_2 was determined by an infrared analyzer (Capnomac Ultima;

From the [1]Department of Ophthalmology, Tohoku University School of Medicine, Miyagi, Japan; the [2]Department of Neuroanatomy, Field of Neurology, Kagoshima University Graduate School of Medical and Dental Sciences, Kagoshima, Japan; and the [3]Department of Oral Physiology, School of Dentistry, Health Sciences University of Hokkaido, Hokkaido, Japan.

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Corresponding author: Hiroshi Izumi, Department of Oral Physiology, School of Dentistry, Health Sciences University of Hokkaido, Ishikari-Tobetsu, Hokkaido 061-0293, Japan; izumih@hoku-iryo-u.ac.jp.

Datex Co., Helsinki, Finland) and kept at 35 to 40 mm Hg. The rectal temperature was maintained at 37°C to 38°C with a heating pad.

The criterion for an adequate depth of anesthesia was the absence of any significant changes in SABP as reflex responses to a minor noxious stimulus (e.g., a pinch of the upper lip for approximately 2 seconds). If the depth of anesthesia was considered inadequate, additional α-chloralose and urethane were administered intravenously in doses of 5 and 10 mg/kg, respectively. Once an adequate depth of anesthesia was attained, supplemental doses of pancuronium were given approximately every 60 minutes to maintain immobilization during periods of stimulation.

Isolation and Electrical Stimulation of the CSN

An operating microscope (OME; Olympus, Tokyo, Japan) was used for the isolation of the CSN from the vagosympathetic nerve bundle, for the insertion of a bipolar electrode into the peripheral cut end of the isolated nerve, and for the insertion of a guide cannula into the Vsp or EW nucleus. The CSN was excited electrically with a stimulator (model SEN-7103; Nihon Kohden, Tokyo, Japan) for 20-second trains (10 V, 0.1–100 Hz, 2-ms pulse duration).

Measurements of Pupil Size

Photographs of pupils were taken with a digital camera (EOS D60; Canon, Tokyo, Japan). A millimeter scale was placed on the forehead of the cat to measure the pupil size. Photographs were taken from 25 to 30 cm, which was the nearest distance at which both pupils could be viewed on one screen. The photographs were taken before and immediately after the stimulation for each stimulus condition.

The photographs were analyzed by the NIH image program (available by ftp at zippy.nimh.nih.gov/ or at http://rsb.info.nih.gov/nih-image; developed by Wayne Rasband, National Institutes of Health, Bethesda, MD). The areas of pupils were outlined manually on a personal computer, and sizes were calculated automatically in pixels. The pupillary areas were then converted into millimeters by using the scale that had been calibrated in pixels. We calculated and plotted the changes in pupillary size as the size after the stimulation minus the size before the stimulation.

Measurements of LBF and SABP

Changes in the blood flow in the lower lip were measured with a laser Doppler flowmeter (model ALF21D; Advance, Tokyo, Japan), as described elsewhere.[7,18,19] The probe was placed against the lower lip without exerting any pressure on the tissue. The changes in the blood flow were compared by measuring the height (in millimeters) of the response on the polygraph of the flowmeter. The blood flow levels are expressed in arbitrary units.

The SABP was recorded with the femoral catheter connected to a Statham pressure transducer. A tachograph (model AT-610G; Nihon Kohden) triggered by the arterial pulse was used to monitor heart rate.

Electrical Stimulation of LN and Vsp

The central cut end of the LN was stimulated electrically to simulate somatic stimulation (Fig. 1). The routine stimulus parameters were a 20-second train of 2-ms rectangular pulses at a frequency of 10 Hz and at supramaximum intensity, usually 30 V, unless otherwise noted, as described elsewhere.[7,18,19] A bipolar silver electrode connected to a stimulator (model SEN-7103; Nihon Kohden) was used to stimulate the LN.

The animal was mounted in a stereotaxic frame (Narishige, Tokyo, Japan), and after a partial craniotomy, part of the tentorium cerebelli was removed. As shown schematically in Figure 1, a guide cannula (1.00 mm outer diameter) was positioned over either the Vsp (posterior [P], 10–11 mm; lateral [L], 5–6 mm; height [H], 6–7 mm; coordinates of Snider and Niemer[20]) or the EW nucleus (anterior [A], 4–5 mm; L, 0; H, −1 to −2 mm). The guide cannula was held and inserted into the brain with a micromanipulator without removing any part of the brain. A concentric bipolar electrode (Inter Medical, Tokyo, Japan), insulated with enamel except at the tip, was inserted through the guide cannula. The electrode was positioned in the Vsp by lowering the stimulating electrode while delivering electrical stimulation and stopping when the maximum pupillary response was elicited. Small vertical movements of the stimulating electrode (e.g., 0.5 mm) often markedly reduced the responses, indicating that current spread from the electrode tip was not excessive.

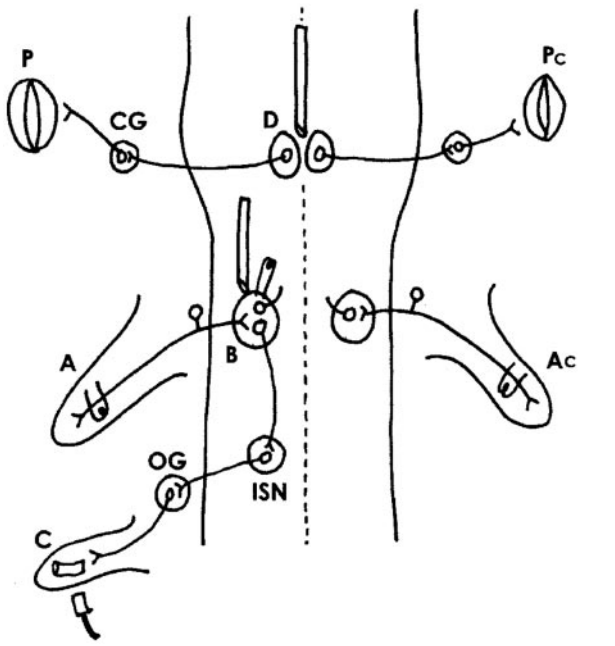

Figure 1. Schematic representation of electrical stimulation, blood flow measurements, and microinjection of lidocaine. The stimulation sites were the central cut end of the LN (A) and the Vsp (B). The blood flow measurement site was the lower lip (C) by laser-Doppler flowmeter. Microinjection sites were the Vsp (B) and the EW nucleus (D). P, pupil; CG, ciliary ganglion; OG, otic ganglion; ISN, inferior salivatory nerve; c, contralateral.

For electrical stimulation of the Vsp, a 20-second train of rectangular pulses generated by a stimulator (model SEN-7103; Nihon Kohden) was delivered through a stimulus-isolation unit (model SS-202J; Nihon Kohden). The usual current was 100 μA, with a pulse duration of 2 ms at a frequency of 10 Hz. The stimulation sites were identified histologically.

Microinjections of Lidocaine

To determine whether the pupillary reflex elicited by LN stimulation was mediated by the Vsp or the EW nucleus, 1.0 μL of lidocaine (2%) was injected into either the Vsp or EW nucleus through an injection cannula (0.50 mm outside diameter) that was inserted through the previously implanted guide cannula. The stimulating electrode was interchangeable with the injection cannula, both were of equal length, and each extended 5.0 mm beyond the tip of the guide cannula. Thus, microinjections and electrical stimulations were performed at the same sites. Saline (1.0 μL) was used for control injections, and the saline injections never induced any significant effect on the LN-evoked pupillary reflex or on the resting cardiovascular parameters. The magnitude of the LN-evoked response obtained after microinjection of a given agent was expressed as a percentage of the control response for LBF or the relative area of the pupillary dilation.

Histologic Examination

At the completion of the experiment, animals were given an overdose of pentobarbital sodium (60 mg/kg) through the ascending aorta fol-

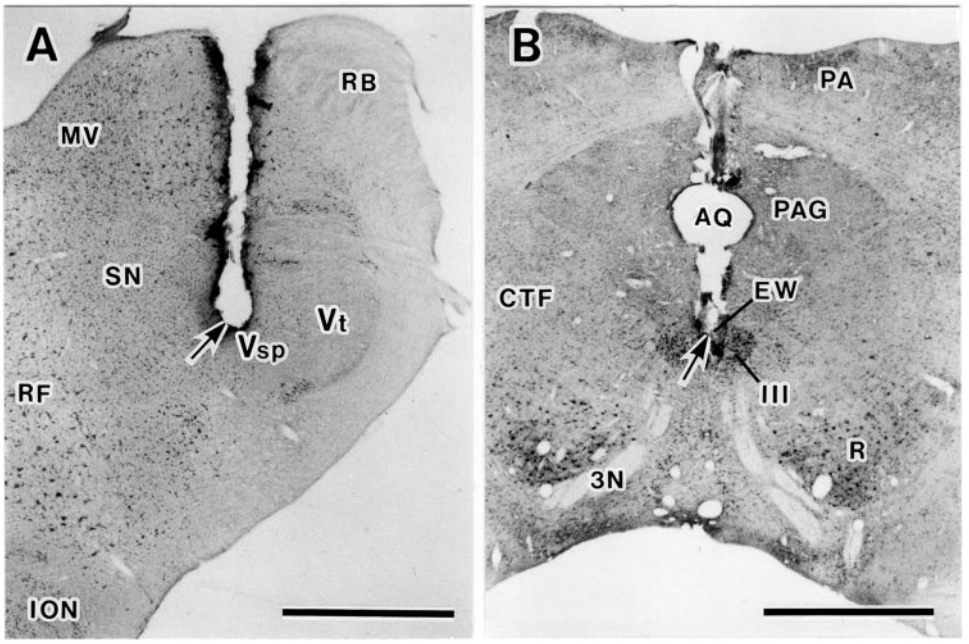

FIGURE 2. Diagram of a control section through the region of the injection site of (**A**) the Vsp and (**B**) the EW nucleus. *Arrow*: site of the tip of the electrode. MV, medial vestibular nucleus; RB, restiform body; SN, nucleus of the solitary tract; RF, reticular formation; Vsp, trigeminal spinal nucleus; Vt, spinal tract of the trigeminal nerve; ION inferior olivary nucleus; PA pretectal region; AQ, cerebral aqueduct; PAG, periaqueductal gray; CTF, central tegmental field; EW Edinger-Westphal nucleus; III, oculomotor nucleus; 3N, oculomotor nerve; R, red nucleus. Scale bar, 5.0 mm.

lowed by 1.0 to 2.0 L of saline (0.9%) and then by 2 L of 10% formaldehyde. The brain stem and upper cervical spinal cord were then removed and stored for 1 to 4 days in buffered 30% sucrose. After storage, 50-μm sections were cut on a freezing microtome and collected in 0.1 M phosphate buffer (pH 7.4). The sections were mounted on gelatin-coated slides and stained with thionin. Photomicrographs of representative coronal sections showing the sites of the electrical stimulation or microinjection of lidocaine into the Vsp or EW nucleus are shown in Figure 2.

Statistical Analysis

All numerical data are given as the mean ± SEMs. Changes in the test response was assessed for significance by analysis of variance (ANOVA), followed by either a post hoc test (the Fisher protected least significant difference [PLSD]) or a contrast test. Differences were considered significant at $P < 0.05$. Data were analyzed on computer (Macintosh; Apple Computer, Cupertino, CA; with StatView 5.0 and Super ANOVA; SAS, Cary, NC).

RESULTS

Pupillary Dilator Responses Induced by Stimulation of the LN

Typical examples of the pupillary dilation and LBF increases elicited by electrical stimulation of the left LN are shown in Figure 3A. The pupillary dilations correlated significantly with the changes in LBF ($r = 0.446$; $y = 0.332x + 7.67$, $P < 0.01$; Fig. 3B). The pupillary dilation elicited by LN stimulation at various intensities at a fixed frequency of 10 Hz (Fig. 4A) and at various frequencies with a fixed intensity of 30 V (Fig. 4B) are shown in Figure 4. These stimulations elicited increases in the pupil sizes bilaterally, and the increases were dependent on intensity (2–30 V) and frequency (0.5–10 Hz). No significant differences were found in the increases of left and right pupil sizes for changes of intensity ($F_{7,70} = 0.006$, not significant) or changes in frequency ($F_{9,81} = 0.177$, not significant) when the LN was stimulated unilaterally.

Effects of Electrical Stimulation of the CSN or of the Section

The responses elicited by electrical stimulation of the peripheral cut end of the left CSN on the left and right pupils are shown in Figure 5A. CSN stimulation elicited an intensity-dependent increase only the left ipsilateral pupil ($F_{3,9} = 99.41$, $P < 0.001$), and had no effect on the right contralateral pupil, indicating a marked difference in the pathways to the ipsilateral and contralateral pupils ($F_{3,18} = 434.0$, $P < 0.001$). The effects of cutting the ipsilateral or both CSNs on the pupillary responses elicited by electrical stimulation of the left LN are shown in Figure 5B. The amplitude of the left and right pupillary responses did not change before and after transecting the CSNs.

Effects of Vsp Stimulation

The effects of electrical stimulation of the left Vsp on pupillary dilation ipsilateral or contralateral to the stimulation site are shown in Figure 6. Vsp stimulation elicited current-dependent (50–200 μA) increases in left and right pupil sizes. Significant increases were observed in the pupil sizes at more than 50 μA. No significant difference was found between left and right pupil sizes ($F_{8,60} = 0.922$, not significant; $n = 6$).

Effects of Lidocaine Injection into the Vsp and EW Nuclei

Typical examples of the effects of microinjection of lidocaine into the left Vsp on the LBF increases and pupillary responses induced by electrical stimulation of the left LN are shown in Figure 7. The pupillary responses and LBF increases were suppressed by lidocaine microinjections and the responses returned in 10 to 50 minutes after injection of lidocaine.

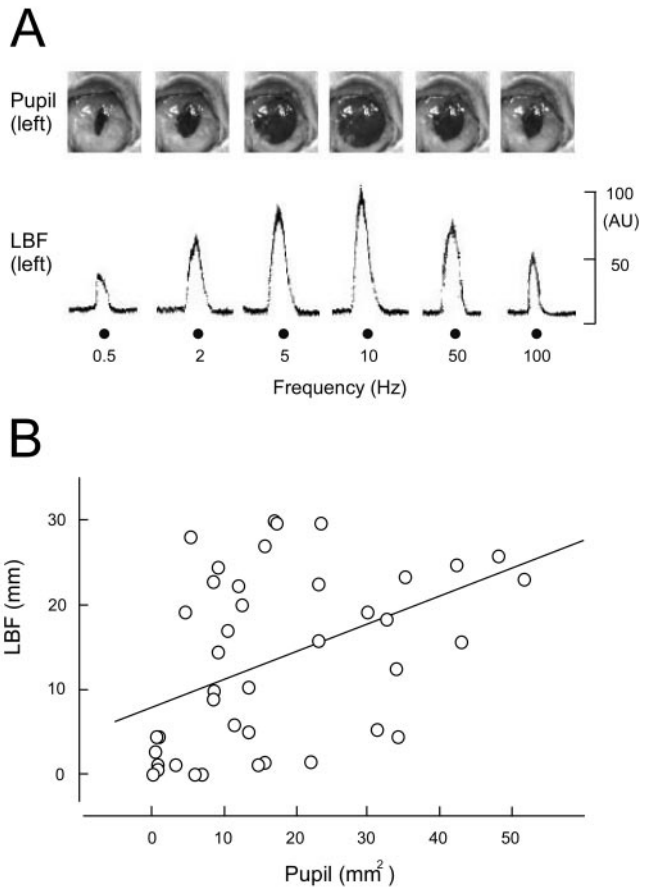

FIGURE 3. (**A**) Photographs of typical patterns of the left pupils (*top*) and the left LBF changes (*bottom*) when the left LN was electrically stimulated at 30 V with a 2-ms pulse duration for 20 seconds with various frequencies from 0.5 to 100 Hz. (**B**) Relationship between pupillary dilation and LBF changes in response to LN stimulation. Regression line for LBF change versus pupillary dilation is $y = 0.332x + 7.67$, $r = 0.446$, $P < 0.01$.

The pupillary responses elicited by left or right LN stimulation after the microinjection of lidocaine into the left Vsp are shown in Figure 8. The left pupillary dilation induced by left LN stimulation was markedly reduced by the microinjection of lidocaine into the ipsilateral left Vsp ($F_{6,42} = 124.26$, $P < 0.001$). In contrast, the left pupillary dilation induced by right LN stimulation was not affected by the microinjection of lidocaine into the left Vsp ($F_{6,42} = 1.567$, not significant).

The effects of the microinjection of lidocaine into the EW nucleus on the pupillary responses in both eyes and the ipsilateral LBF increases induced by electrical stimulation of the left LN are shown in Figure 9. The pupillary responses were suppressed bilaterally for 5 to 50 minutes by a lidocaine injection into the EW nucleus. In contrast, LBF was not affected ($F_{12,36} = 1.197$, not significant), indicating a marked difference between the responses of the bilateral pupils and LBF ($F_{24,132} = 450.1$, $P < 0.001$).

DISCUSSION

The iris contains two muscles: the sphincter pupillae, which is innervated by only the parasympathetic nerves via the oculomotor nerve, and the dilator pupillae which is innervated only by the sympathetic nerves via the cervical sympathetic trunk.[21–27] As a result, pupillary constriction (miosis) and dilation (mydriasis) occur via activation of the parasympathetic and sympathetic nerves, respectively. Earlier reports on the effect of somatic stimulation on pupillary responses have been contradictory. For example, pupillary dilation induced by pain has been reported in one study to occur by inhibition of the parasympathetic nerve,[13] whereas others report that it occurs by activation of the sympathetic nerve.[12,14]

From these reports and our results, three possible mechanisms for mediating the bilateral pupillary dilation induced by stimulation of the central cut end of the LN in cats should be considered: (1) humoral dilation evoked by the release of adrenaline by activation of the sympathetic nerves, (2) neural dilation induced by inhibition or stimulation of the parasympathetic preganglionic cell body (EW nucleus), or (3) neural dilation induced by excitation of the CSN. We will discuss first the possible involvement of humoral substance(s) and sympathetic and parasympathetic nerves as the mechanisms by which LN stimulation evokes the ipsilateral pupillary dilation, and second, the possible pathways of the contralateral pupillary dilation. The latter mechanism is important because mutual neural networks between the Vsp and EW nucleus are still unclear, and it is not known whether an ipsilateral or contralateral mechanism is involved in the trigeminal–parasympathetic reflex pupillary response.

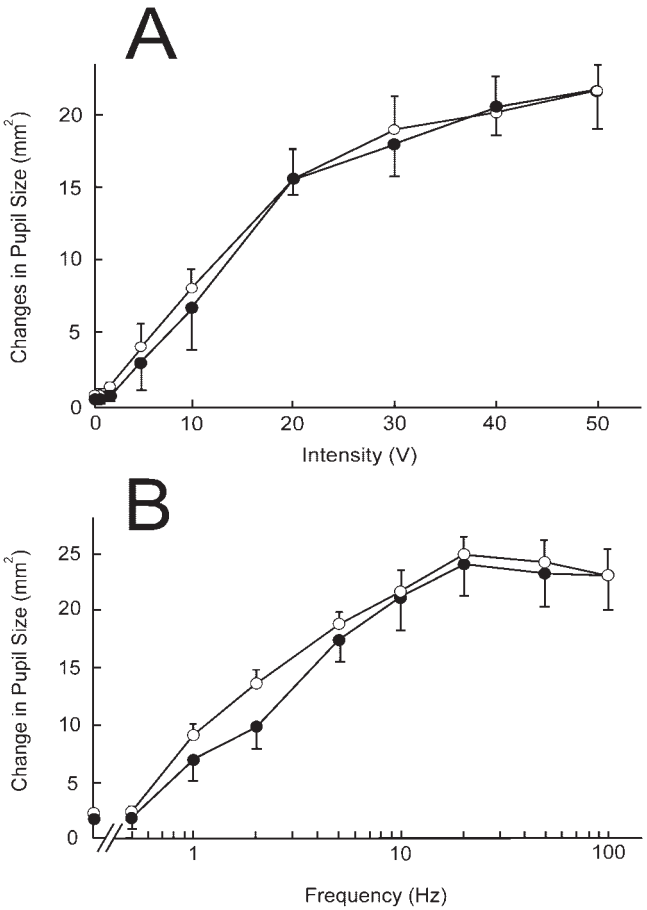

FIGURE 4. Mean changes in pupil size of the two eyes (●; *left*, ○; *right*) elicited by stimulation of the left LN. Electrical stimulations were at (**A**) various intensities from 1 to 50 V at 10 Hz, 2-ms pulse duration for 20 seconds, and (**B**) 30 V, at various frequencies from 0.5 to 100 Hz, with a 2-ms pulse duration for 20 seconds. The data are the mean ± SEM ($n = 6$).

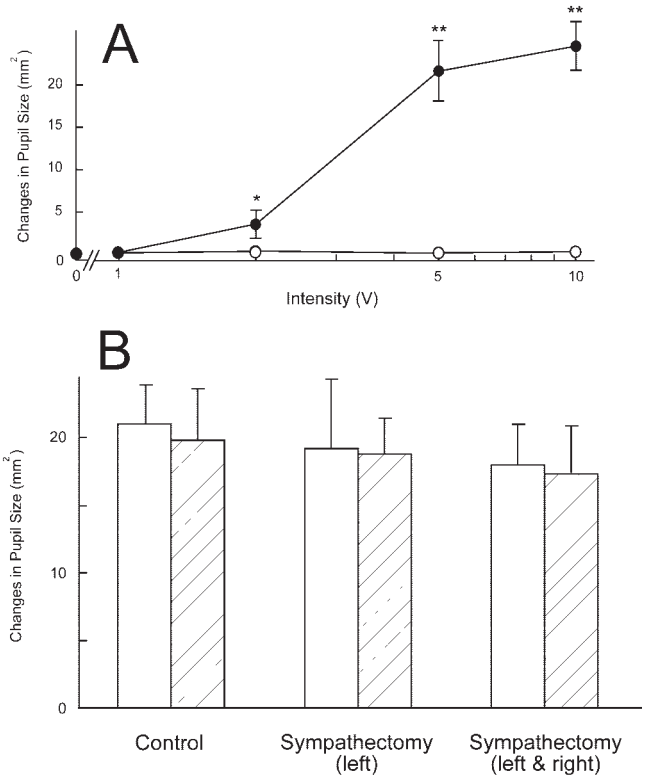

FIGURE 5. (**A**) Changes in pupil sizes elicited by stimulation of the peripheral cut end of the left CSN. The CSN was electrically stimulated at various intensities from 1 to 10 V at 10 Hz, with a 2-ms pulse duration for 20 seconds (●; left pupil, ○; right pupil). Data are the mean ± SEM ($n = 4$). Statistical significance compared with the control (at 0 V) was assessed by means of ANOVA followed by post hoc test (Fisher PLSD). $^{*}P < 0.05$; $^{**}P < 0.001$. (**B**) Effect of sectioning the ipsilateral CSN or bilateral CSNs on the changes in pupil sizes in left eyes (□) and right eyes (▨) elicited by electrical stimulation of the left LN (30 V, 10 Hz, 2-ms pulse duration for 20 seconds) before and after sectioning the left and/or right CSN. Data are expressed as the mean ± SEM ($n = 10$).

Mechanism for Ipsilateral Pupillary Dilation

Pharmacologic analysis has been used to determine whether autonomic nerves are involved in the pupillary response after

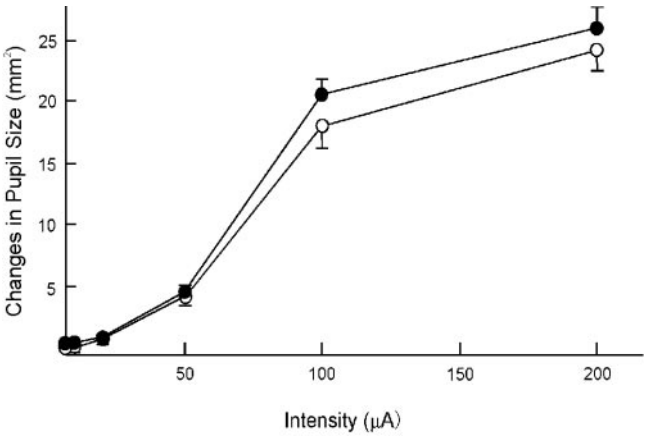

FIGURE 6. Changes in left (●) and right (○) pupil sizes elicited by electrical stimulation of the left Vsp at 10 Hz, with a 2-ms pulse duration for 20 seconds, at various intensities from 10 to 200 μA. Data are expressed as the mean ± SEM ($n = 6$). Statistical significance compared with the control (at 0 A) was assessed by means of ANOVA followed by a post hoc test (Fisher PLSD). $^{*}P < 0.05$; $^{**}P < 0.001$.

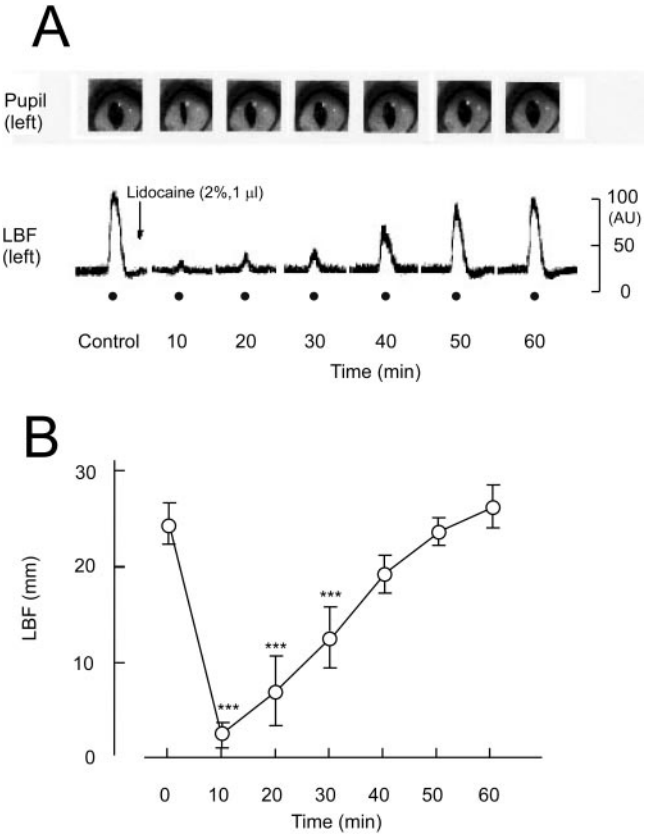

FIGURE 7. (**A**) Typical example of inhibitory effects produced by microinjection of 2%, 1 μL of lidocaine into the ipsilateral Vsp on pupil sizes (*top*) and LBF (*bottom*) elicited by electrical stimulation of the left LN at 10 Hz and 30 V, with a 2-ms pulse duration for 20 seconds. (**B**) Time course of the effects of lidocaine application (2%, 1 μL) into the ipsilateral Vsp on LBF elicited by electrical stimulation of the left LN at 10 Hz and 30 V, with a 2-ms pulse duration for 20 seconds. Data are the mean ± SEM ($n = 6$). Statistical significance compared with the control (before the microinjection) was assessed by means of ANOVA followed by a post hoc test (Fisher PLSD). $^{***}P < 0.001$.

LN stimulation.[1,3,5,16,18,19,28] However, pharmacologic agents such as ganglionic blockers (hexamethonium), antimuscarinic agents (atropine), and antiadrenergic agents (phentolamine and propranolol) should not be used in this type of study, because they have direct actions on the iris sphincter muscle and dilator muscles. Furthermore, the blocking of autonomic ganglionic cells by hexamethonium by itself elicits pupil dilation, presumably by inhibiting spontaneous discharge in the ciliary ganglion. For these reasons, we used microinjections into the Vsp and EW nuclei to produce nonselective, reversible local anesthesia (lidocaine). We also examined the effect of sectioning the CSN to examine the contribution made by the superior CSN.[15–17]

The microinjection of lidocaine into the EW nucleus reduced the pupillary dilation induced by stimulation of the LN significantly, but had no effect on the increase in LBF, whereas microinjection of lidocaine into the Vsp abolished both the pupil dilation and the LBF increases. These results suggest that (1) the EW nucleus is a critical bulbar relay site for the LN-induced pupil dilation, but not for the LN-evoked LBF increase; (2) the neural pathways mediating these two responses diverge after nerve fibers derived from nociceptive trigeminal leave the Vsp, and (3) the LN-induced changes in pupil dilation are not due to humoral substance(s) released by activation of the sympathetic nerve after stimulation of presympathetic brain

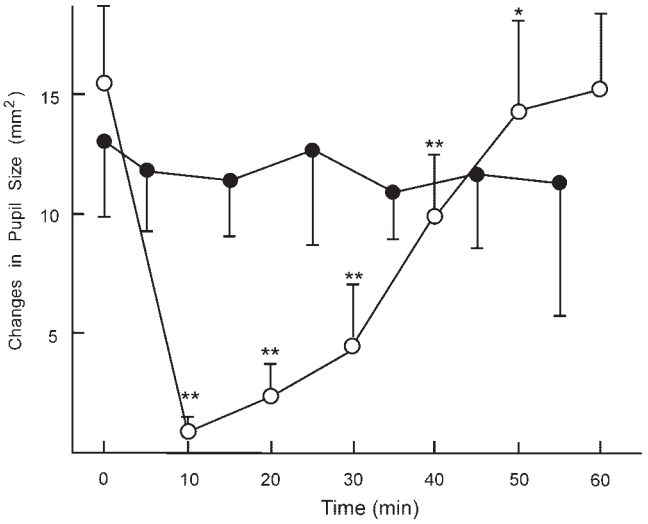

FIGURE 8. Time course of the effect of microinjection of lidocaine into the ipsilateral Vsp on the changes in pupil sizes elicited by electrical stimulation of the left (○) and right (●) LN. Electrical stimulation was applied at 10 Hz and 30 V, with a 2-ms pulse duration for 20 seconds. Data are expressed as the mean ± SEM ($n = 6$). Statistical significance compared with the control (before the microinjection) was assessed by means of ANOVA followed by a post hoc test (Fisher PLSD). $^{*}P < 0.05$; $^{**}P < 0.001$.

stem–spinal neurons. These findings are supported by reports showing efferent connections to the EW nucleus from the Vsp of the cat.[29]

Stimulation of the peripheral cut end of the superior CSN elicited a current intensity-dependent pupil dilation only on the ipsilateral side (Fig. 5A). The pupillary dilation induced by LN stimulation was not affected by sectioning the ipsilateral or both superior CSNs (Fig. 5B). These results suggest that the pupillary dilation evoked by LN stimulation is not mediated by a somatosympathetic mechanism in the cat. However, the pupil dilation was largely abolished by microinjection of lidocaine into the Vsp or the EW nucleus (Figs. 7, 8, 9). These results indicate that the Vsp and EW nuclei participate as

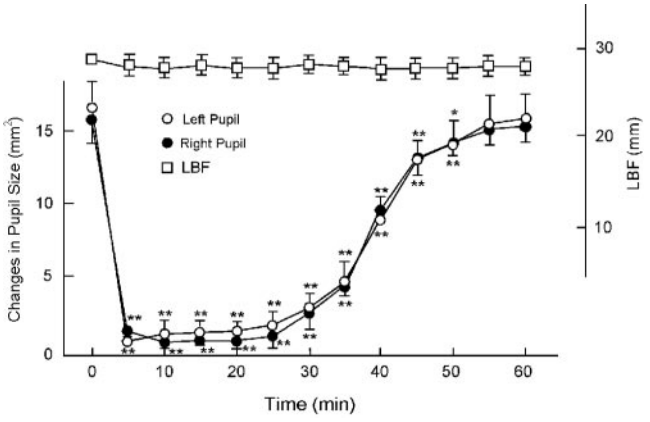

FIGURE 9. Time course of the effects of lidocaine injection (2%, 1 μL) into the EW nucleus on LBF and changes in left and right pupil sizes elicited by electrical stimulation of the left or right LN, respectively. Electrical stimulation was performed at 10 Hz and 30 V, with a 2-ms pulse duration for 20 seconds, before and after the microinjection of lidocaine. Data are expressed as the mean ± SEM ($n = 11$). Statistical significance compared with the control (before the microinjection) was assessed by means of ANOVA followed by a post hoc test (Fisher PLSD). $^{*}P < 0.01$; $^{**}P < 0.001$.

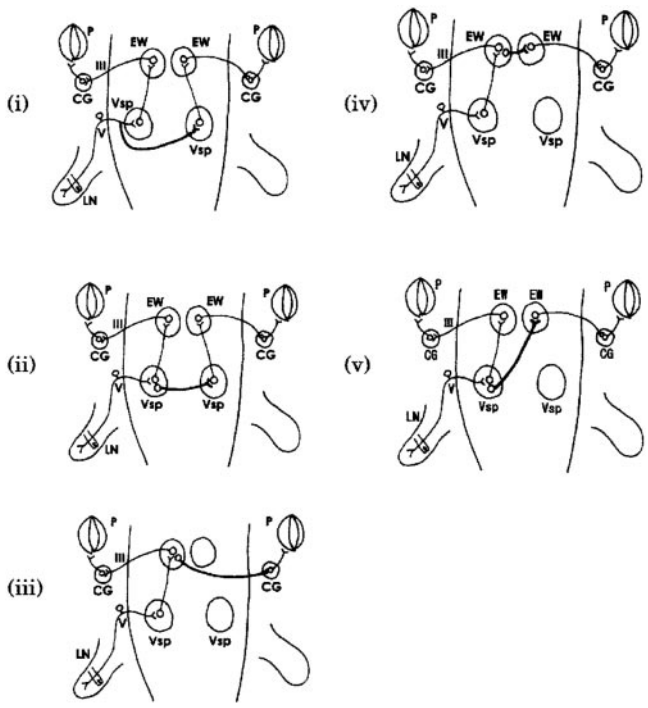

FIGURE 10. Possible pathways by which nerve excitation might evoke pupillary dilation in response to electrical stimulation of the central cut end of the left lingual nerve (LN; site A in Fig. 1) The schematics are described in the Discussion section. *Solid lines:* possible neural pathways. EW, Edinger-Westphal nucleus; III, oculomotor nucleus; Vsp, trigeminal spinal nucleus; P, pupil; CG, ciliary ganglion; V trigeminal nerve.

bulbar relay stations for the pupillary dilation induced by LN stimulation and suggest that the efferent arc of the response is a parasympathetic pathway. These findings are not in accord with the observation of Yu and Blessing[14] who reported that stimulation of the spinal tract of the trigeminal nerve causes a brisk dilation of both pupils that seem to be affected by stimulation of the presympathetic brain stem–spinal neurons in rabbits. These differences may be due to species variation as an earlier study reported a marked species difference in the trigeminal–autonomic reflex responses such as SABP changes and parasympathetic mediated LBF responses.[30]

Mechanism for Contralateral Pupil Dilation

Unilateral application of 2% lidocaine into the Vsp ipsilateral to the LN-stimulated side produced reversible inhibitory effects on the pupillary dilation and the LBF increases on both sides. These results indicate that the ipsilateral Vsp is an essential bulbar relay for the LN-evoked contralateral pupillary dilation. The data also suggest that pupil dilation evoked by LN stimulation depends on the activation of a pathway originating from the Vsp ipsilateral to the stimulated nerve, which crosses over to the contralateral EW nucleus. In addition, the data indicate that the contralateral Vsp is not involved in the LN-induced contralateral reflex pupillary dilation. The microinjection of lidocaine into the EW nucleus abolished the ipsilateral and contralateral pupil dilation in response to LN stimulation, regardless of the side of LN stimulation in sympathectomized cats (Fig. 9).

To judge from the results of our and other studies, at least five possible brain-stem pathways may mediate the contralateral pupil dilation induced by LN stimulation (Fig. 10): (i) the afferent neural input projects directly to the contralateral Vsp,

(ii) there are projections from the ipsilateral Vsp to the contralateral Vsp, (iii) efferent fibers project from the EW nucleus contralaterally, (iv) there are projections from the ipsilateral EW nucleus to the contralateral EW nucleus, and (v) fibers project from the Vsp to the contralateral EW nucleus. We will discuss these possibilities in order.

First, impulses elicited by electrical stimulation of the LN could converge on the contralateral Vsp, because trigeminal afferents that run in the LN pass to the Vsp,[31,32] and unit activity can be recorded in the Vsp caudalis after electrical stimulation of the ipsilateral or contralateral tooth pulp in dogs.[33] In addition, removal of the pulp of all the teeth on one side induces axonal and terminal degeneration bilaterally in the subnucleus interpolaris of the spinal trigeminal nucleus.[27] These facts suggest that primary trigeminal afferents from the tooth pulp converge on the contralateral Vsp. However, as shown in Figure 8, microinjection of lidocaine into the Vsp ipsilateral to the stimulated side significantly reduced contralateral pupillary dilation, suggesting that convergence of the primary afferents in the LN on the contralateral Vsp is unlikely to play a significant role in mediating contralateral pupillary dilation. This conclusion is in good agreement with that reached by Matthews and Lisney,[24] who found no electrophysiologic evidence that tooth pulp afferents project to the contralateral trigeminal nuclei in the cat.

Second, we examined the possibility that fibers project from the Vsp ipsilateral to the stimulated side across to the contralateral Vsp. Microinjection of lidocaine into the Vsp contralateral to the stimulated side did not affect the LN-induced contralateral pupil dilation (Fig. 8). This finding suggests that the contralateral Vsp is not involved in the LN-induced contralateral reflex pupillary dilation.

Third, the possibility that parasympathetic pupil-constrictor fibers project from the EW nucleus contralaterally seems to be unlikely. When horseradish peroxidase is injected into the ciliary ganglion, retrogradely labeled cells are found only in the ipsilateral side (1.0 mm laterally from the midplane) of the lateral border zones of the anteromedian, EW nucleus, somatic oculomotor nuclei, and their ventral continuations of the ventral tegmental areas, including the regions among the oculomotor root fibers.[34]

Fourth, as far as we are aware, no information is available as to whether a connection exists between the parasympathetic preganglionic cells in both hemispheres (e.g., EW nucleus).

Finally, we are left with the fifth possibility: that fibers project from the Vsp to the contralateral EW nucleus. This possibility seems to be the most plausible in view of the present experimental findings: (1) microinjection of lidocaine into the Vsp attenuated the reflex contralateral pupil dilation (Figs. 7, 8), (2) microinjection of lidocaine into the Vsp contralateral to the stimulated side did not affect contralateral pupillary dilation (Fig. 8), and (3) microinjection of lidocaine into the EW nucleus inhibited contralateral pupillary dilation (Fig. 9). On the basis of our data, we suggest that contralateral pupillary dilation is mediated (at least in part) by fibers projecting from the Vsp across the midline to the contralateral EW nucleus. These findings are in accord with our previous observations that neural pathways exist between the Vsp and salivatory nucleus in palate blood vessels.[17]

However, we have not completely ruled out the fourth possibility (that there are projections from the ipsilateral EW nucleus to the contralateral EW nucleus), in view of the following observations. We could not examine the neural pathways between the Vsp and EW nucleus because of technical difficulties. We have reported that the effects produced by a microinjection of lidocaine into the Vsp would be exerted within a 1- to 1.5-mm in radius, judging from our previous histologic examination performed by injecting a histologic marker (horseradish peroxidase) into the brain stem.[15] As shown in Figure 10, the two EW nuclei of cats are not separate, as opposed to those of humans and monkeys, and seemed to be one body, as observed by Loewy et al.[23] and Kuchiiwa et al.[34] Therefore, it seems it would be difficult to elicit the selective inhibition of one side of the EW nucleus by lidocaine microinjections. Furthermore, the site of origin of the oculomotor parasympathetic preganglionic neurons is still not determined. It has been reported in the cat that the oculomotor parasympathetic preganglionic neurons originate from the area immediately ventral to the oculomotor nucleus in the ventral tegmental area[22,34] and that the largest pupilloconstrictor responses elicited by electrical stimulation of the midbrain arise from the area ventral to the EW nucleus, rather than from the nucleus itself.[26]

In addition, we have not conclusively ruled out the possibility that the dilator muscle can be stimulated by activation of the oculomotor parasympathetic nerve followed by the LN stimulation, given that the dilator muscle receives input from acetylcholinesterase-containing fibers that degenerate after removal of the ciliary ganglion.[21] Furthermore, Oono[25] suggested that the efferent pathway for somatosensory stimulation in the conscious condition is different from that in the anesthetized condition. Thus, more work is needed to clarify the neural pathway(s) in the brain stem, although the present level of understanding suggests that the Vsp ipsilateral to the stimulated site and the EW nuclei play critical roles for eliciting pupillary dilation in response to LN stimulation. In addition, it should be clarified whether the contralateral pathways for the LN stimulation-induced pupillary dilation is identical with those concerned with the pupillary light reflex, because it is well known that there are the commissural connections between right and left EW nuclei for the pupillary light reflex.

We do not yet know the nature of the adequate stimulus, or indeed the identity of the sensory receptors, of the LN stimulation-induced pupillary dilation, or the characteristics of the afferent fibers that mediate it. However, C-polymodal nociceptors have been put forward as strong candidates for the primary afferents partaking in the parasympathetic-mediated responses such as LBF increase and salivary secretion, because electrical stimulation at higher intensities, topical application of capsaicin, and radiant heat stimulation to the tongue all cause an increase in ipsilateral LBF[1,35] and because the LBF increases evoked by all these stimuli are significantly attenuated by pretreatment with an autonomic ganglionic blocker, hexamethonium.[1,35] Because these responses can be evoked from all branches of the trigeminal nerve, the afferents are unlikely to arise from a specific cranial organ (e.g., eye, teeth, nasal mucosa, or tongue), and they are not restricted to a specific division of the trigeminal nerve. This suggests that the parasympathetic reflex pupil dilation that occurs in response to trigeminal stimulation does not arise from proprioceptors.

In conclusion, the possibility that fibers project from the Vsp to the contralateral EW nucleus seems to be the most plausible explanation of our results. Further work should clarify the pathways that play roles in eliciting pupillary dilation in response to somatic stimulation.

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