Correlation Between Vasodilatation and Secretion in the Lacrimal Gland Elicited by Stimulation of the Cornea and Facial Nerve Root of the Cat

Tomoki Yasui,* Keishiro Karita,† Hiroshi Izumi,† and Makoto Tamai*

Purpose. To determine whether reflex vasodilatation can be elicited in the cat lacrimal gland by electrical stimulation of the cornea, whether the vasodilatation elicited by electrical stimulation of the facial nerve root found to be the efferent arm of the cornea–lacrimal gland reflex pathway correlates with the evoked secretion in the lacrimal gland, and what kind of receptors and which autonomic ganglia are involved in lacrimal vasodilator and secretory responses.

Methods. Electrical stimulation of the cornea or facial nerve root was used to evoke a blood flow increase in the lacrimal gland and tear secretion of the urethane–chloralose-anesthetized, paralyzed, and cervically sympathectomized cat.

Results. The lacrimal vasodilator response depended on stimulus intensity and frequency and correlated well with the tear secretion. Injection of 2% lidocaine solution into the retrobulbar area, where the pterygopalatine ganglion is located, abolished the vasodilator and the secretory responses. Pretreatment with hexamethonium (an autonomic ganglion blocker) greatly attenuated the secretory response, even at a low dose (1 mg/kg given intravenously), although at this dose, the vasodilator response was only slightly affected. Neither phentolamine (an α-adrenoceptor antagonist) nor propranolol (a β-adrenoceptor antagonist), nor a vasoactive intestinal peptide antagonist had any effect on the vasodilator or secretory responses. Scopolamine (a muscarinic receptor antagonist), although having no effect on vasodilatation, had a profound inhibitory effect on the secretory response.

Conclusions. These results suggest that whereas the vasodilator and secretory responses in the lacrimal gland were well correlated, they were mediated by different mechanisms. Invest Ophthalmol Vis Sci. 1997;38:2476–2482.

The lacrimal gland is innervated by parasympathetic and sympathetic nerve fibers. Although lacrimal secretion is known to be induced mainly by activation of the parasympathetic fibers, little is known about parasympathetic effects on lacrimal blood flow. Noxious stimulation of the eye and facial skin elicits a trigemino–parasympathetic reflex lacrimal secretion in rabbits1 and humans.2,3 Stimulation of the lingual nerve, a branch of the trigeminal nerve, induces reflex vasodilatation and salivation in the submandibular gland, which, similar to the lacrimal gland, is a secretory gland in the area of innervation of the facial nerve in the cat.4 Electrical stimulation of the facial nerve also causes a blood flow increase in the lacrimal gland of the cat5 and the rabbit.6 However, it is still unclear whether there is a correlation or indeed a causal relationship between parasympathetic vasodilatation and lacrimation. Furthermore, there is no evidence that parasympathetic reflex vasodilatation in the lacrimal gland can be induced by trigeminal stimulation, as has been observed in facial cutaneous tissues and in the submandibular gland.

The object of the current study was to determine whether reflex vasodilatation can be elicited in the cat lacrimal gland by electrical stimulation of the cornea, which is richly innervated by trigeminal somatosensory nerve fibers; whether vasodilatation elicited by electrical stimulation of the facial nerve root correlates with the evoked lacrimation; and what kind of receptors
and autonomic ganglia are involved in lacrimal vasodilator and secretory responses.

**METHODS**

**Preparation of Animals**

This investigation adhered to the guidelines laid down by the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research and by a local committee on the ethical conduct of animal experiments (Committee of Animal Experimentation, Tohoku University School of Medicine). In addition, we have followed advice issued by The Physiological Society (Journal of Physiology (London), 1996) on the conduct of experiments involving the use of muscle relaxants. Thus, we used an anesthetic regimen (systemic and local) that we have used previously in similar experiments on nonparalyzed cats. In those experiments, it produced adequate anesthesia for at least the same time as the duration of the current experiments (8 hours). In the period when muscle relaxants were used, we regularly examined the adequacy of the depth of anesthesia. During this period, the animal’s blood pressure was stable, and there were no precipitate blood pressure responses to such noxious stimuli as paw-pinching or electrical stimulation of the cornea (Figs. 2, 5). If such criteria had not been satisfied, we were prepared to give additional doses of anesthetic immediately. However, this was never necessary. The experiments were conducted on 25 cats, male and female, weighing 1.8 to 5.0 kg. Cats were initially anesthetized with a mixture of 50 mg/kg α-chloralose and 100 mg/kg urethane, given intravenously, then paralyzed (0.4 mg/kg pancuronium bromide initially, followed by an infusion of 0.4 mg·kg⁻¹·hr⁻¹; Mioblock, Organon, the Netherlands), ventilated with a mixture of 50% air-50% oxygen and placed in a stereotaxic device. Eight cats were decerebrated. Arterial blood pH and gases were monitored twice in each experiment and remained within normal limits. Local anesthesia was always applied to the areas of the skin that were cut. The cephalic vein was cannulated to allow drug injection, and the femoral artery was cannulated and connected to a Statham pressure transducer for the monitoring of systemic blood pressure. The cervical sympathetic trunks were sectioned bilaterally before stimulation to eliminate reflex sympathetic effects on blood flow and secretion. Some animals had previously been decerebrated at the midcollicular level to prevent nociceptive signals projecting to higher centers when the cornea was electrically stimulated. Corneal stimulation was performed only in decerebrate preparations. Rectal temperature was maintained at 36°C to 38°C with a heating pad. At the end of the experiment, the cat was killed with an overdose (200 mg) of pentobarbital sodium.

**Blood Flow Monitoring**

After resection of the frontal skin, the bone of the upper and lateral wall of the orbit was partially removed. The area exposed of the upper surface of the lacrimal gland was kept to a minimum to prevent unnecessary drying of its surface. Blood flow changes in the lacrimal gland were monitored using a laser Doppler flowmeter (Advance ALF21R, Tokyo, Japan; Fig. 1). Electrical calibration for zero blood flow was performed for all recordings. The analogue output of the equipment gives no absolute values, but indicates relative changes in blood flow. The output of the device was continuously displayed on a multichannel chart recorder, alongside that of the blood pressure transducer. In these experiments, blood flow was expressed in arbitrary units. Where appropriate, blood flow changes are expressed as a percentage of the maximum blood flow increase induced by the same type of stimulus (Figs. 3, 4).

**Collection of Tears to Measure Lacrimal Secretion**

As a substitute for the secretory response of the lacrimal gland, we measured the tear secretion, including the secretory responses from all the ocular glands, elicited by electrical stimulation of the facial nerve root. This was collected by placing strips of filter paper 30 mm long and 5 mm wide into the inferior conjunctival cul-de-sac (Fig. 1). Collection was carried out for 2 minutes, from 1 minute before to 1 minute after stimulation. The filter paper was placed as gently as possible to avoid reflex lacrimation caused merely by placing the filter paper. Papers were weighed before and after collection using a balance (Mettler AB54, Switzerland). To minimize the evaporation of tears,
the measurements were made as quickly as possible. The net secretory response was calculated by subtracting the spontaneous tear secretion collected during a 2-minute period from the amount collected when stimulation was performed. Collection of the tear secretion elicited by electrical stimulation of the cornea, however, was not feasible, because the surface of the cornea was always moistened before its stimulation.

**Electrical Stimulation of the Cornea and Facial Nerve Root**

In the decerebrated animals, a bipolar electrode was placed against the center of the corneal surface after it had been washed with saline. The roots of the facial (seventh) and glossopharyngeal (ninth) nerves were exposed at their exits from the brain stem, after an extensive craniotomy. Electrical stimulation of the nerve roots was done intracranially in the nondecerebrated animals. After the roots had been severed, a bipolar electrode was inserted into the distal cut end of the appropriate cranial nerve under visual control, with great care taken because the ninth nerve could otherwise be stimulated together with the seventh, and vice versa, if the cranial nerve roots were not completely isolated (Fig. 1). These sites were stimulated for 10 seconds with pulses lasting 2 msec at 1 to 20 V and 0.5 to 50 Hz frequency, using a Nihon–Koden (Tokyo, Japan) model SEN-7103 stimulator. The interval between stimuli was 10 minutes. To obtain a frequency–response relationship, stimulation was delivered at increasing frequencies from 0.5 to 50 Hz.

**Section and Local Anesthesia of Nerves**

To help trace the peripheral pathways followed by the efferent fibers, reflex vasodilator responses were observed before and after each of the following procedures: The facial nerve was cut close to the medulla, and a 2% lidocaine solution was injected into the retrobulbar area, where the pterygopalatine ganglion is located (Fig. 1).

**Drugs**

To determine whether the lacrimal vasodilator and secretory responses were mediated by activation of the autonomic nervous system, hexamethonium, an autonomic ganglion blocker, was administered (1, 3, or 10 mg/kg given, intravenously), and stimulation repeated 10 minutes later. The responses obtained were expressed as percentages of the response elicited by electrical stimulation (with the same stimulus parameters) of the cornea and facial nerve root before hexamethonium administration. To determine whether the vasodilator and secretory responses were mediated by activation of α- or β-adrenoceptors or of muscarinic cholinceptors, the cornea and facial nerve root were stimulated before and after intravenous administration of 1 mg/kg phentolamine, 0.1 mg/kg propranolol, or 0.1 mg/kg scopolamine. The effect was tested of the vasoactive intestinal peptide (VIP) receptor antagonist, [D-p-Cl-Phe⁶,Leu¹⁷]-vasoactive intestinal peptide on the vasodilator and secretory responses elicited by facial nerve root stimulation to examine whether VIP is a candidate as a transmitter in the relevant vasomotor or secretomotor fibers. The magnitude of the response obtained under each agent was expressed as a percentage of the control response recorded before its administration (mean values ± SE). Test stimuli were delivered 10 minutes after the administration of each drug.

**Statistical Analysis**

The significance of evoked changes in blood flow and secretion was assessed, using paired Student’s t tests, two-way analysis of variance, and correlation coefficients, as appropriate. Differences were considered significant at $P < 0.05$.

**RESULTS**

**Vasodilatation Elicited by Corneal Stimulation**

Blood flow increases in the cat lacrimal gland were elicited by electrical stimulation of the cornea (Fig. 2A). Electrical stimulation of the infra- and supratemporal nerves and the tongue also induced lacrimal vasodilatation, but these responses were less than 50% of the amplitude of the cornea-induced vasodilatations (data not shown). The vasodilator responses induced by corneal stimulation were intensity and frequency dependent (Figs. 3A, 3B). Vasodilator responses induced by corneal stimulation were completely abolished by sectioning of the facial nerve root but not by sectioning of the glossopharyngeal nerve root (Figs. 2B, 2C). Stimulation of the distal cut end of the facial nerve root still evoked a vasodilator response (Fig. 2D).

No reflex vasodilatation caused by placing a filter strip on the conjunctiva was observed.

**Facial Nerve-Induced Vasodilatation and Secretion**

Lacrimal vasodilation and tear secretion induced by electrical stimulation of the facial nerve root were intensity and frequency dependent (Fig. 4). A good correlation was found between the tear secretion and blood flow changes evoked by facial nerve-root stimulation (Fig. 4A [variable intensity]: $r = 0.95$, $P < 0.05$; Fig. 4B [variable frequency]: $r = 0.81$, $P < 0.05$). No changes in lacrimal blood flow and tear secretion were elicited by stimulation of the glossopharyngeal nerve root (data not shown).
FIGURE 2. Decerebrated cat. Effect of section of glossopharyngeal and facial nerve roots on lacrimal vasodilator responses elicited by electrical stimulation of the cornea. Reflex vasodilator response induced by corneal stimulation (30 V, 2 msec, 20 Hz, 10 seconds) (A) before and (B) after section of glossopharyngeal and (C) facial nerve roots. (D) Vasodilator response induced by electrical stimulation (20 V, 2 msec, 20 Hz, 10 seconds) of peripheral cut end of facial nerve root. Blood flow is expressed as a percentage of maximum flowmeter output at the setting used (same setting for each trace).

FIGURE 3. Lacrimal vasodilator responses produced by electrical stimulation of the cornea at different (A) intensities and (B) frequencies. Stimulation parameters: (A) 2 msec, 20 Hz, 10 seconds; (B) 40 V, 2 msec, 10 seconds. Interval between stimuli was 10 minutes. Ordinate: evoked increase in blood flow expressed as a percentage of the maximum increase elicited by corneal stimulation: (A) at 30 V and (B) at 20 Hz. Values are means ± SE. Data are from nondecerebrated cats.

FIGURE 4. Lacrimal vasodilator (line) and secretory (column) responses induced by electrical stimulation of the facial nerve root at different (A) intensities and (B) frequencies. Stimulation parameters: (A) 2 msec, 20 Hz, 10 seconds; (B) 2 msec, 50 V, 10 seconds. Interval between stimuli was 10 minutes. Ordinate: evoked increase in blood flow or secretion expressed as a percentage of the maximum increase elicited by corneal stimulation: (A) at 20 V and (B) at 20 Hz. Values are means ± SE. Data are from nondecerebrated cats.

FIGURE 5. Decerebrate cat. Effect of chemical lesion of the pterygopalatine ganglion (injection of 2% lidocaine solution into the infraorbital plexus) on lacrimal vasodilator responses. Vasodilator responses elicited by electrical stimulation (20 V, 2 msec, 20 Hz, 10 seconds) of (A) the cornea and (B) the facial nerve root before and after injection of lidocaine. Blood flow is expressed as a percentage of maximum flowmeter output at the setting used (same setting for each trace).

Effect of Lidocaine

After an application of 1 ml of 2% lidocaine solution into the infraorbital plexus, where the pterygopalatine ganglion is located, neither the cornea-induced nor facial nerve root-induced vasodilator responses could be evoked (Fig. 5). However, the lidocaine application induced no changes in the pupillary light reflex responses mediated by the ciliary ganglion.
Effect of Hexamethonium

Vasodilator responses elicited by electrical stimulation of the cornea were attenuated by prior treatment with the autonomic ganglion blocker hexamethonium (1, 3, and 10 mg/kg intravenously) in a dose-dependent manner (Fig. 6). Doses greater than 10 mg/kg were not given, to prevent depression of blood pressure. Figure 7 shows the effects of hexamethonium on the vasodilation in the lacrimal gland and tear secretion elicited by electrical stimulation of the facial nerve root. Vasodilator responses in the lacrimal gland and tear secretion were suppressed in a dose-dependent manner by pretreatment with hexamethonium. However, the sensitivity to hexamethonium of the ganglion cells that mediate the lacrimal vasodilator response was very different from that of the ganglion cells that mediate the tear secretion (two-way analysis of variance, \( P < 0.05 \)). At each dose, the vasodilator responses were less affected and, although greatly reduced, were not completely attenuated, even by a large dose (10 mg/kg) of hexamethonium. In contrast, tear secretory responses were greatly attenuated by even a small dose (1 mg/kg) of hexamethonium and were completely abolished by a dose of 10 mg/kg (Fig. 7).

Effects of Adrenergic and Cholinergic Blocking Agents and a VIP Receptor Antagonist

Administration of the \( \alpha \)-adrenoceptor blocker phentolamine (1 mg/kg), the \( \beta \)-adrenoceptor blocker propranolol (1 mg/kg), and the muscarinic cholinoreceptor blocker scopolamine (0.1 mg/kg) induced no changes in the cornea-induced vasodilator responses (Fig. 6). There were also no changes in the vasodilator responses in the lacrimal gland or in the tear secretion elicited by stimulation of the facial nerve root on administration of the \( \alpha \)-adrenoceptor blocker phenolamine (1 mg/kg) and the \( \beta \)-adrenoceptor blocker propranolol (1 mg/kg). In contrast, administration of the muscarinic cholinoreceptor blocker scopolamine (0.1 mg/kg) induced a marked attenuation of the tear secretion but no change in the vasodilator response (Fig. 7).

The VIP receptor antagonist, \([D-p-Cl-Phe^6,Leu^{17}]-\)vasoactive intestinal peptide (0.25 mg/kg intravenously), affected neither the vasodilation nor tear secretion with facial nerve stimulation (Fig. 7).

DISCUSSION

Lacrimal Vasodilator Reflex

Araie\(^7\) reported that, in eliciting reflex lacrimal secretion, electrical or mechanical stimulation of the cornea was more effective than stimulation of other facial structures (the conjunctiva, for example). In our study, vasodilator responses in the cat lacrimal gland were elicited easily when the cornea, innervated mainly by the first branch of the trigeminal nerve,\(^8\)

![Figure 6](http://iovs.arvojournals.org/pdfaccess.ashx?url=/data/journals/iovs/933420/)  
**Figure 6.** Effects of 1, 3, and 10 mg/kg intravenously administered hexamethonium; 1 mg/kg phentolamine; 1 mg/kg propranolol; and 0.1 mg/kg scopolamine on lacrimal blood flow changes evoked by electrical stimulation of the cornea (2 msec, 20 Hz, 20 V, 10 seconds) \(*P < 0.05, **P < 0.01\) by paired Student’s \( t \)-test. Values are means ± SE. The number of experiments is shown within parentheses. Data are from decerebrated cats.

![Figure 7](http://iovs.arvojournals.org/pdfaccess.ashx?url=/data/journals/iovs/933420/)  
**Figure 7.** Effects of 1, 3 and 10 mg/kg intravenously administered hexamethonium, 1 mg/kg phentolamine, 1 mg/kg propranolol, 0.1 mg/kg scopolamine and 0.25 mg/kg of the VIP receptor antagonist, \([D-p-Cl-Phe^6,Leu^{17}]-\)vasoactive intestinal peptide, on lacrimal blood flow (open columns) and secretory changes (hatched columns) evoked by electrical stimulation of facial nerve root (2 msec, 20 Hz, 30 V, 10 seconds). \(*P < 0.05\) by two-way analysis of variance. \( **P < 0.01\) by paired Student’s \( t \)-test. Values are means ± SE. The number of experiments is shown within parentheses. Data are from nondecerebrated cats.
was stimulated electrically. The cornea-induced vasodilator responses were attenuated by prior treatment with the autonomic ganglion blocking agent hexamethonium in our cervically sympathectomized cats (Fig. 6). This indicates that the responses were mediated by a somato-autonomic reflex involving the parasympathetic—that is, a trigeminal–parasympathetic reflex. The reflex vasodilatation was completely abolished by section of the facial nerve root, but not by that of the glossopharyngeal nerve root (Fig. 2), showing that the reflex was mediated through the facial, not the glossopharyngeal, nerve.

Injection of 2% lidocaine solution into the retrobulbar area, where the pterygopalatine ganglion is located, suppressed completely the lacrimal blood flow increase evoked by stimulation of the cornea or facial nerve root (Fig. 5), suggesting that the lacrimal vasodilator reflex is pterygopalatine ganglion-mediated. These results are consistent with reports that lacrimal secretion is mediated by the pterygopalatine ganglion.

The reports that conjunctival stimulation can induce a trigeminal–parasympathetic vasodilator reflex in the human forehead and cheeks and feline lower lip suggest that nociceptive stimulation of the eye, which is innervated by an ophthalmic branch of the trigeminal nerve, may well evoke reflex vasodilatation, not just in the lacrimal gland, but over a wider facial area.

**Lacrimal Vasodilatation Induced by Facial Nerve Root Stimulation**

As shown in Fig. 2D, vasodilator responses similar to those induced reflexively in the cat lacrimal gland by corneal stimulation were elicited by electrical stimulation of the peripheral cut end of the facial nerve root. These results are consistent with the findings of Busija and Heistad that stimulation of the petrosal nerve, a branch of the facial nerve, induces a lacrimal blood flow increase.

Although the reflex vasodilatation was greatly reduced by 3 mg/kg hexamethonium and almost completely abolished by 10 mg/kg (Fig. 6), the facial nerve root–induced response was less strongly affected, and was not completely attenuated, even at the highest dose used (Fig. 7). These results suggest that not only the ganglionic but also the central synapses involved may be only partially sensitive to hexamethonium. However, at this time we have no direct evidence regarding the nature of the residual vasodilatation.

**Correlation Between Lacrimal Vasodilatation and Secretion**

According to previous researchers, lacrimal secretion can be measured by collecting tears. Because the lacrimal gland is the major contributor to tear production, we thought that the volume of lacrimal secretion would be approximately equal to that of tear production, although a small secretion by nonlacrimal gland sources may have contributed to the measurements made in the present experiments.

Electrical stimulation at 20 Hz was maximum for the facial nerve root–induced lacrimal vasodilatation and secretion (Fig. 4). Taking these results together with the report that lacrimal secretion was maximum at 15 Hz when the lacrimal nerve was stimulated and during brain-stem stimulation, maximum responses for lacrimal secretion and vasodilatation seem to be induced by stimulation at similar frequencies. The increase in lacrimal blood flow elicited by facial nerve root stimulation correlated well with the simultaneously evoked lacrimal secretion (Fig. 4), indicating that the blood flow response is a good index of evoked secretory activity in the lacrimal gland. However, the different susceptibilities to hexamethonium of the lacrimal secretion and vasodilatation evoked by stimulation of the facial nerve root (Fig. 7) suggests that the secretory and vasodilator responses were mediated by functionally different parasympathetic ganglion cells. This is consistent with the finding of Karita et al in the submandibular gland of the cat, that the salivary response is more sensitive to hexamethonium than the vasodilator response.

The lacrimal vasodilator or secretory responses elicited by stimulation of the cornea or the facial nerve root were not sensitive to phentolamine (an α-adrenoceptor blocking agent) or to propranolol (a β-adrenoceptor blocking agent), as shown in Figures 6 and 7, suggesting that they were not mediated by activation of sympathetic nerve fibers, which were cut in the neck.

Pretreatment with scopolamine (a muscarinic cholinoreceptor blocking agent) had no effect on the vasodilator responses in the lacrimal gland (Figs. 6, 7). This result is consistent with the findings that the parasympathetic vasomotor fibers to the gingiva, lower lip, and submandibular gland are not cholinergic.

However, the lacrimal secretory responses were abolished by prior treatment with scopolamine (Fig. 7). This result is in good agreement with the report of Elsby and Wilson that the lacrimal secretory response elicited by lacrimal nerve stimulation in the cat is reduced by administration of atropine, a muscarinic blocking agent.

Neither the vasodilator nor the secretory response in the cat lacrimal gland was affected by pretreatment with the VIP receptor antagonist, [D-γ-Cl-Phe⁸,Leu¹⁷]-vasoactive intestinal peptide (Fig. 7). These results suggest that VIP is unlikely to be a transmitter of either the vasodilator or the secretory fibers serving the cat lacrimal gland. This is not consistent with the
reports by Dartt et al.\textsuperscript{19,21} that VIP is a potent secretagoge and that it is coreleased from parasympathetic secretomotor endings along with acetylcholine in the lacrimal gland of the rat and the rabbit. Furthermore, it is not in accord with histologic evidence\textsuperscript{22} that numerous VIP-immunoreactive nerve fibers, in addition to the cholinergic fibers, surround blood vessels in the lacrimal gland of monkeys. We suggest that, in the cat, VIP is not a transmitter in the lacrimal gland, though it may have a role other than that of a neural transmitter in this gland.

In conclusion, our results seem to suggest that although the lacrimal vasodilator and secretory responses are most likely mediated by the same afferent pathway and perhaps by overlapping efferent pathways, they are mediated through different motor transmitters or receptors. Further study will be needed to establish the exact nature of the vasodilator mechanism and the significance of the discrepancy we have identified between the mediation of the secretory and vasodilator responses.

**Key Words**

cat, corneal stimulation, facial nerve-root stimulation, lacrimal blood flow and secretion, parasympathetic vasodilator reflex

**References**


