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Escape of Parasympathetic Vasodilatation from Sympathetic Attenuation in Oro-Facial Areas in the Cat

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Murakami, M., Date, H., Karita, K. and Izumi, H. *Escape of Parasympathetic Vasodilatation from Sympathetic Attenuation in Oro-Facial Areas in the Cat.* Tohoku J. Exp. Med., 1999, **188** (2), 153-160 —— We examined the effects of concurrent repetitive stimulation of the cervical sympathetic trunk (CST) on the parasympathetically mediated reflex blood flow increase in the orofacial area of cats. In urethane plus α-chloralose anaesthetized cats, parasympathetic reflex vasodilatation in the ipsilateral lower lip was elicited by electrical stimulation of the central cut end of the lingual nerve (LN). This blood flow increase was attenuated in a frequency-dependent manner when CST was stimulated concurrently at 0.5-10 Hz for 10 minutes. When we applied repeated LN stimulation (using identical parameters, each time) at intervals during a 30-minutes period of 10 Hz CST stimulation, the attenuation of the blood flow increase gradually weakened in a time-dependent manner even though the direct vasoconstrictor effect of CST stimulation showed no such decline. ———— parasympathetic reflex vasodilatation; sympathetic-attenuation; orofacial; cat

A prolonged sympathetically mediated attenuation of the cardiac effects of vagal activity has been reported to occur in cats and dogs, probably via the release of neuropeptide Y (NPY) or galanin from the sympathetic nerves (Potter 1985; Revington et al. 1990; Moriarty et al. 1992; Ulman et al. 1992, 1993). Recent studies using cats and dogs have further suggested that substances other than noradrenaline (such as NPY or galanin) are released during a 3-minute period of stimulation of the sympathetic nerves, and that these substances attenuate not only the vagal effect on the heart, but also the parasympathetically evoked vasodilatation in the nasal mucosa (Lacroix et al. 1994a,b). The latter effect (in

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the nasal mucosa) was reported to persist for nearly 1 hour after the cessation of sympathetic stimulation. In a recent study, in which blood flow changes were measured by laser Doppler flowmetry, we demonstrated a clear sympathetically mediated attenuation of reflexly evoked parasympathetic vasodilatation in the lower lip (Izumi and Ito 1998). However, we could not detect any such attenuation 5–10 minutes after cervical sympathetic trunk (CST) stimulation had ended. It is unclear whether this discrepancy is due to the different methods used to activate the parasympathetic vasodilator fibers (direct electrical stimulation of the peripheral efferents in the earlier studies and indirect reflex activation in our study) or the different vascular beds studied.

In the study mentioned above, we observed that the attenuation of reflex parasympathetic vasodilator responses in the lower lip and palate was dependent on the frequency of the concurrent stimulation of the CST (Izumi and Ito 1998; Izumi 1999b). We also noticed that magnitude of the CST-induced attenuation of the reflex (lingual nerve [LN]-evoked) lower lip blood flow (LBF) increase seemed to depend on when the LN was stimulated during a period of CST stimulation. Therefore, in the present study we examined in more detail the time-dependence of the attenuating effect of CST stimulation on the LN-induced LBF increase.

Materials and Methods

Preparation of animals

Fifteen adult cats, unselected as to sex and of 2.7 to 3.8 kg body weight, were initially sedated with ketamine hydrochloride (30 mg/kg, i.m.) and then anesthetized with a mixture of α-chloralose (50 mg/kg, i.v.) and urethane (100 mg/kg, i.v.). These anesthetics were supplemented when necessary throughout the experiment. The anesthetized animals were intubated, paralyzed by intravenous injection of pancuronium bromide (Mioblock, Organon; 0.4 mg/kg initially, supplemented with 0.2 mg/kg every hour or so after testing the level of anaesthesia; see below) and artificially ventilated via the tracheal cannula with a mixture of 50 % air-50% O_2. The ventilator (Model SN-480-6, Shinano, Tokyo) was set to deliver a tidal volume of 10–12 cm^3/kg at a rate of 20 breaths/minute. The end-tidal concentration of CO_2 was determined by means of an infrared analyzer (Capnomac Ultima, Datex Co., Helsinki, Finland) as described before (Izumi and Karita 1992; Izumi et al. 1997; Izumi and Ito 1998). Rectal temperature was maintained at 37–38°C using a heating pad.

The criteria for maintenance of an adequate depth of anesthesia were the persistence of miotic pupils and the absence of a reflex elevation of heart rate and arterial blood pressure during stimulation of the central end of the lingual nerve. If the depth of anesthesia was considered inadequate, on the basis of the above criteria, additional α-chloralose and urethane (i.e., intermittent doses of 5 mg/kg and 10 mg/kg i.v., respectively) was administered. Once an adequate depth of

anesthesia had been attained, supplementary doses of pancuronium were given approximately every 60 minutes to maintain immobilization during periods of stimulation.

In all experiments, the vagi and sympathetic trunks were cut bilaterally in the neck prior to any stimulation. This avoided the involvement of the cervical sympathetics in any reflex effect and ensured that the only parasympathetic effects involved in the present study were non-vagal. All cats were killed at the end of the experiment by an overdose (about 150 mg) of sodium pentobarbitone.

The experimental protocols were reviewed by the Committee on the Ethics of Animal Experiments in the Tohoku University School of Medicine, and carried out in accordance with the Guidelines for Animal Experiments issued by the Tohoku University School of Medicine, and the Law (No. 105) and Notification (No. 6) of the Japanese Government.

Electrical stimulation of CST and LN

The present experiments involved electrical stimulation of the central cut end of the LN (A in Fig. 1) or the peripheral cut end of the CST (B in Fig. 1). The

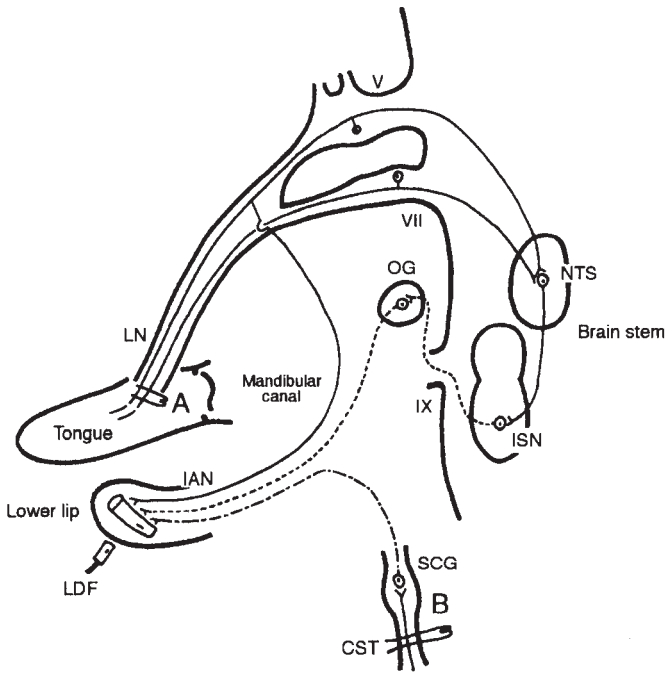

Fig. 1. Schematic representation of the sites of electrical stimulation. Stimulation sites: The central cut end of the lingual nerve (A), and the peripheral cut end of the cervical sympathetic trunk (B). The broken lines indicate the parasympathetic vasodilator fibers from the inferior salivatory nuclei. The solid lines indicate trigeminal and facial sensory inputs to the brain stem. The sympathetic supply, via the superior cervical ganglion, is shown at the bottom. Abbreviations: IAN, inferior alveolar nerve; ISN, inferior salivatory nucleus; LDF, laser-Doppler flowmeter; LN, lingual nerve; NTS, nucleus of solitary tract; OG, otic ganglion; CST, cervical sympathetic trunk; SCG, superior cervical ganglion; V, trigeminal nerve root; VII, facial nerve root; IX, glossopharyngeal nerve root.

CST was stimulated for 10 or 30 minutes at a supramaximal voltage (10 V) using 2 milliseconds pulses at various frequencies (0.5–10 Hz). To elicit a parasympathetic reflex vasodilatation in the lower lip, the LN was stimulated for 20 seconds at a supramaximal voltage (30 V) using 2 milliseconds pulses at 10 Hz as described before (Izumi and Karita 1994, 1995; Izumi et al. 1997; Izumi and Ito 1998; Izumi 1999b). LN stimulation was delivered alone or during on-going repetitive CST stimulation and begun between 2.5 and 7.5 minutes after the start of a period of repetitive sympathetic stimulation, unless otherwise noted.

Measurement of LBF and of systemic arterial blood pressure

Changes in blood flow at sites in the lower lip adjacent to the canine tooth were recorded on either side using laser Doppler flowmeters (LDF; ALF21R, Advance, Tokyo) as described before (Izumi and Karita 1992, 1993; Izumi 1999b). Output from the devices was continuously displayed on an 8-channel chart recorder (Model W5000, Graphtec, Tokyo) at a speed of 10 mm per minutes. The blood flow changes were assessed by measuring the height of the response.

Systemic arterial blood pressure was recorded from the femoral catheter via a Statham pressure transducer. A tachograph (Model AT-610G, Nihon Koden, Tokyo) triggered by the arterial pulse was used to monitor heart rate.

Statistical analysis

All numerical data are given as the mean $\pm$ S.E. The significance of changes in the responses was assessed using either a paired Student's t-test, or an analysis of variance (ANOVA) followed by a contrast test. Differences were considered significant at the level $p<0.05$. Data were analysed using a Macintosh Computer with StatView 5.0 and Super ANOVA.

Results

Fig. 2 shows a typical example of blood flow responses in the lower lip evoked when central cut end of the LN was stimulated during on-going CST stimulation at frequencies of 0.5–10 Hz. It can be seen that the reflex parasympathetic LN-evoked blood flow increase in the lower lip was reduced by concurrent CST stimulation in a manner that was frequency-dependent. The order in which the different frequencies were presented made no statistically significant difference to the magnitude of this attenuating effect on the LN-evoked responses.

Fig. 3 shows a typical example of the effect of elapsed time on the magnitude of the sympathetic attenuation of the reflex LBF increases. In experiments of this type, LBF increases were evoked by LN stimulation at 5-minutes intervals (using identical parameters each time) during a 30-minutes period of CST stimulation at 10 Hz. Averaged data are shown in Fig. 4. It can be seen that the CST-induced attenuation of the LN-evoked LBF increase weakened ("escape") in a time-dependent manner ($F[7,32]=11.923$, $n=5$, $p<0.001$ for ANOVA for

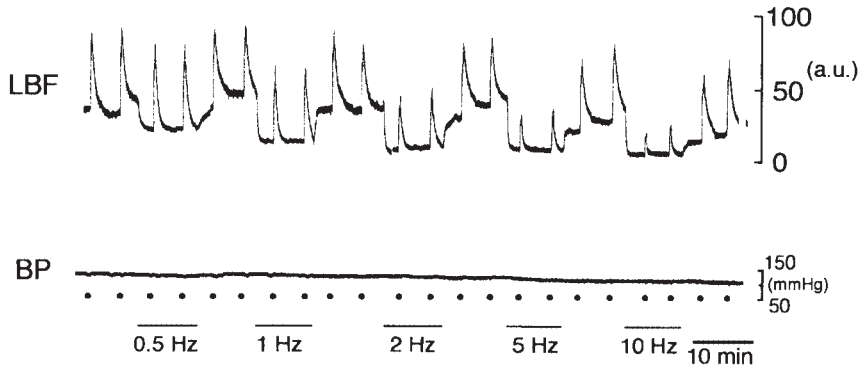

Fig. 2. Sympathetic modulation of the effect of electrical stimulation of the central cut end of the LN on the lower lip blood flow (LBF). The LN stimuli were delivered alone or during on-going repetitive CST stimulation in anesthetized vago-sympathectomized cats. The LN was stimulated where indicated by dots for 20 seconds at a supramaximal voltage (30 V) at 10 Hz with pulses of 2 milliseconds duration. Horizontal lines indicate 10-minutes periods of CST stimulation (2 milliseconds; 10 V) at various frequencies (0.5–10 Hz). Abscissa, time (minutes). Ordinates, LBF in arbitrary units (a.u.) and systemic arterial blood pressure (BP, mmHg).

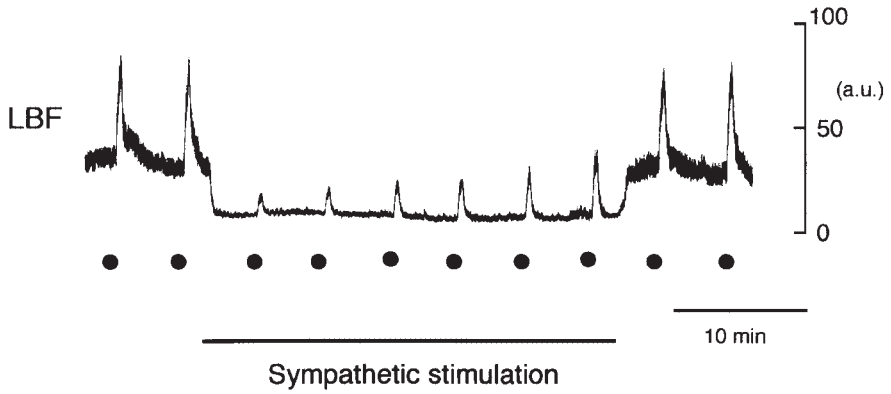

Fig. 3. Recording from an anesthetized vago-sympathectomized cat showing changes in blood flow in the lower lip following electrical stimulation of the central cut end of the LN either alone or during on-going repetitive CST stimulation. The LN was stimulated where indicated by dots for 20 seconds at a supramaximal voltage (30 V) at 10 Hz with pulses of 2 milliseconds duration. Horizontal bar indicates 30-minutes period of CST stimulation (2 milliseconds; 10 Hz; 10 V). Abscissa, time (minutes). Ordinate, LBF in arbitrary units (a.u.).

repeated measurements). About 5–10 minutes after the cessation of a 30-minutes period of CST stimulation, the LN-evoked LBF increase was fully restored to the magnitude seen before sympathetic stimulation.

Discussion

It was apparent (Fig. 2) that the basal blood flow level in the lower lip decreased in a frequency-dependent manner when the CST was stimulated at 0.5–10 Hz for 10-minutes periods. However, the basal level of blood flow in the lower

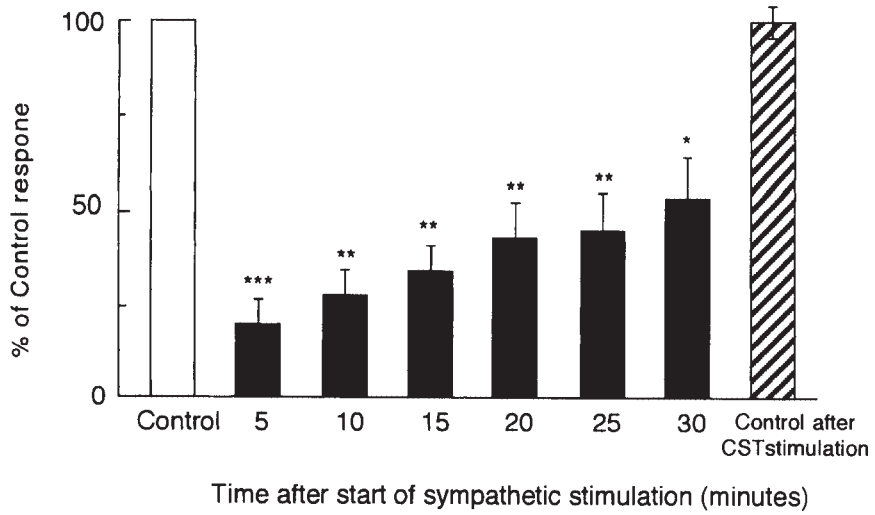

Fig. 4. Averaged data showing time-dependence of sympathetic modulation of effect of electrical stimulation of the central cut end of the LN on the LBF. LN stimulation was delivered either alone or during a 30-minutes period of repetitive stimulation of the CST in anesthetized vago-sympathectomized cats. Open and closed columns show, respectively, the control response to LN stimulation alone (10-minutes before CST stimulation) and the responses to LN stimulation during the CST stimulation at various times after the start of CST stimulation. Hatched column shows the response to LN stimulation alone 5–10 minutes after the end of the CST stimulation. Experimental conditions were as in Fig. 3. Lip blood flow responses ($n=5$) are expressed as a percentage of the control response recorded before the period of CST stimulation. Statistical significance was calculated by means of ANOVA followed by a contrast test. $^{*}p<0.05$, $^{**}p<0.01$, $^{**}*p<0.001$ vs. the control response recorded before CST stimulation. Values shown are means ± S.E.

lip was almost constant throughout a 30-minutes period of CST stimulation at 10 Hz (Fig. 3), suggesting that the direct vasoconstrictor effect of CST stimulation did not weaken over a 30-minutes period. The "escape" phenomenon described above occurred against this background of an apparently constant level of vasoconstriction. It is known that the vasoconstrictor effect elicited by CST stimulation is mediated via a release of noradrenaline from the sympathetic fibers, since the α-adrenoceptor blocking agent phentolamine largely abolishes it (Izumi and Karita 1990). Our data suggest that a release of noradrenaline occurred steadily throughout the period of CST stimulation.

The cause of the time-dependent decline in the attenuation of the reflex LBF increase is still obscure. One possible explanation might be that some as yet unknown type of desensitization was caused by a substance(s) released from the sympathetic nerves. Indeed, powerful desensitizing effects of galanin and NPY on the cardio inhibitory actions of the vagus have been demonstrated by other investigators (Hall et al. 1990; Ulman et al. 1992, 1993). Another possibility might be that repetitive stimulation of the CST caused a progressive depletion of the factor(s) mediating the attenuation. If it is true that noradrenaline is released during such repetitive sympathetic stimulation at a constant rate (see

above), it would have to be a substance(s) other than noradrenaline that was undergoing depletion. However, it is also possible that noradrenaline has two separate actions: A direct action on vascular smooth muscle (vasoconstriction) and an unknown action causing an attenuation of parasympathetically mediated effects. Our results could be explained if the latter action is more prone than the former to time-dependent decline.

Hall et al. (1990) were the first to observe that the long-lasting potentiation of contractions (which were evoked either by noradrenaline or by transmural nerve stimulation) induced by high frequency sympathetic nerve stimulation was abolished or at least reduced when trials were repeated in the rabbit ear artery. Nevertheless, it should not be taken for granted that the "escape" seen in the present experiment reflects a peripheral phenomenon. It is conceivable, for example, that on repeated electrical stimulation of the central cut end of the LN, there could be time-dependent changes in the number of central synapses activated (or in their pattern of activation), leading to a progressive enhancement of the reflex vasodilator response. However, we think this is unlikely to be explanation for our present results since LN-evoked reflex LBF increases remain remarkably stable for a long time (5 hours) during periods of repeated LN stimulation (in the absence of CST stimulation) (Izumi 1999a). Clarification of the exact mechanism underlying the "escape" described here must await further experiment.

Acknowledgments

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