Nifedipine-induced inhibition of parasympathetic-mediated vasodilation in the orofacial areas of the cat

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Izumi, Hiroshi, and Ikuko Nakamura. Nifedipine-induced inhibition of parasympathetic-mediated vasodilation in the orofacial areas of the cat. Am J Physiol Regulatory Integrative Comp Physiol 279: R332–R339, 2000.—In anesthetized cats, we 1) compared the effects of antihypertensive agents (nifedipine, clonidine, phentolamine, propranolol, and nitroprusside) on the parasympathetic vasodilations elicited by lingual nerve (LN) stimulation in the lower lip and tongue and 2) investigated the mechanisms underlying the inhibitory effect of nifedipine on parasympathetic lower lip vasodilation. At the doses used, each antihypertensive agent reduced systemic arterial blood pressure by ~20 mm Hg; however, the parasympathetic vasodilation elicited by LN stimulation was significantly reduced only by nifedipine. This inhibitory effect of nifedipine was not seen when LN was stimulated during ongoing repetitive stimulation of the superior cervical sympathetic trunk at 1-Hz frequency. This suggests that the ability of lip and tongue blood vessels to relax to parasympathetic stimulation is not directly impaired by this calcium channel blocker and that the inhibitory effects of nifedipine seen here probably resulted from an action on postsynaptic sites in vascular smooth muscle that caused a reduction in preexisting sympathetic vasoconstrictor tone (by inhibiting calcium influx into the vascular smooth muscle cell).

anti hypertensive agents; calcium antagonist; autonomic; blood vessels

THE MAJOR CLINICAL USES OF calcium channel blocking agents lie in the field of therapy for hypertension and vascular insufficiency, particularly cardiac and cerebral (27). The mechanism underlying their antihypertensive effects appears to involve an inhibition of α-adrenoceptor-mediated calcium entry into vascular smooth muscle (7). Thus a reduction in the enhancing (vasoconstrictor) effect on vascular smooth muscle tone elicited by the norepinephrine released spontaneously from the nerve terminals of sympathetic vasoconstrictor fibers seems to be the main mechanism underlying the clinical effects of calcium channel blocking agents. On the other hand, these agents have been reported to elicit unexpected side effects such as headache, flushing, dizziness, peripheral edema, and sexual dysfunction (e.g., impotence and impaired ejaculation) (2–4, 8, 10, 30, 32, 34). These side effects seem to derive not from the drug’s effect on the sympathetic vasoconstrictor mechanism, but rather from effects on parasympathetic- or trigeminal-mediated vasodilator mechanisms (2, 4, 6, 9, 23, 25, 33). However, little is known about the effects of calcium channel blocking agents on parasympathetic-mediated vasodilation.

It is well accepted 1) that the innervation of blood vessels in the orofacial area is very similar to the innervation of those that serve the sex organs (1, 24), 2) that oral tissues, such as the lower lip and tongue, are densely innervated by parasympathetic nerves, and 3) that vasodilation occurs reflexly in such tissues via an activation of parasympathetic nerve fibers (12–19).

With this in mind, we designed the present study to examine whether the calcium channel blocker nifedipine, as well as other antihypertensive agents considered to act via the autonomic nervous system or the smooth muscle of blood vessels, might modulate parasympathetic-mediated vascular responses in orofacial areas in cats (vasodilation in the lower lip and tongue) and to examine the possible mechanisms underlying any inhibitory effect of nifedipine on parasympathetic vasodilation in the lower lip.

In these experiments, we chose doses of the various antihypertensive agents that produced similar falls in systemic arterial blood pressure on intravenous injection. In addition, we measured the actual plasma concentrations of nifedipine reached when it was administered by intravenous infusion or sublingually via a commercial preparation of nifedipine (Adalat).

METHODS

Preparation of animals. Forty-two adult cats, unselected as to sex and of 2.8–4.2 kg body weight, were initially sedated with ketamine hydrochloride (30 mg/kg im) and then anesthetized with a mixture of α-chloralose (50 mg/kg iv) and urethan (100 mg/kg iv). These anesthetics were supplemented if and when necessary throughout the experiment. The anesthetized animals were intubated, paralyzed by intravenous injection of pancuronium bromide (Mioblock; Organon, Teknika, Netherlands; 0.4 mg/kg initially, supplemented with 0.2 mg/kg every hour or so after testing the level

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NaHCO₃ solution was added if necessary (both solutions was continuously infused at a rate of 8 ml/h, and the end-tidal concentration of CO₂ was determined by means of an infrared analyzer (Capnomac Ultima, Datex, Helsinki, Finland), as reported previously (11, 13, 14). Blood pH, PaO₂, and PaCO₂ data were obtained at intervals of 90 min using a blood-gas analyzer (model 148; Ciba-Corning, Medfield, MA), and ventilation was adjusted to keep these parameters within normal limits. Ringer solution was continuously infused at a rate of ~8 ml/h, and 8.4% NaHCO₃ solution was added if necessary (both solutions from Otsuka Pharmaceutical, Tokyo, Japan). Rectal temperature was maintained at 37–38°C using a heating pad.

The criteria for the 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, additional α-chloralose and urethane (i.e., intermittent doses of 5 and 10 mg/kg iv, respectively) were administered. Once an adequate depth of anesthesia had been attained, supplementary doses of pancuronium were given approximately every 60 min to maintain immobilization during periods of

stimulation. All cats were killed at the end of the experiment by an overdose (~150 mg) of pentobarbital sodium.

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

Electrical stimulation of lingual nerve and superior cervical sympathetic trunk. To elicit a parasympathetic reflex vasodilation in the lower lip, the central cut end of the lingual nerve (LN; Fig. 1A) was electrically stimulated at supramaximal intensity (30 V) with pulses of 2-ms duration at a frequency of 10 Hz for 20 s, as described elsewhere (11, 13–15, 20). Tongue blood flow (TBF) changes were evoked in a nonreflex manner by electrical stimulation of the peripheral cut end of the LN (Fig. 1B) using a supramaximal voltage (30 V) with pulses of 2-ms duration at 10 Hz for 20 s, as described elsewhere (14). The magnitude of the parasympathetic vasodilation elicited by LN stimulation was stable and reproducible for at least 6 h when the LN was electrically stimulated centrally or peripherally at 10-min intervals. The peripheral cut end of CST (Fig. 1C) was stimulated using a supramaximal voltage (10 V) and 2-ms pulse duration at various frequencies (0.2–10 Hz for 20 s) when the effects of nifedipine on sympathetic-induced vasoconstriction were to be examined (see Figs. 5–8). In all experiments, the vagi were cut bilaterally in the neck to ensure that the only parasympathetic effects evoked were nonvagal. In addition, the sympathetic trunk on the nonexperimental side was cut in the neck before any stimulation. This preparation is referred to as “vagosympathemized” throughout the text.

Measurement of blood flow in the lower lip and tongue. Changes in blood flow in the lower lip adjacent to the canine tooth and in the tongue were monitored on one side using laser-Doppler flowmeters (LDF; Advance ALF21R, Tokyo, Japan), as described before (11, 13–15, 17). The probe was placed against the lower lip or tongue without exerting any pressure on the tissues. The LDF values obtained in this way represent the blood flow in the superficial vessels in each
tissue. Electrical calibration for zero blood flow was performed for all recordings. Several gains were selectable, and the maximum output of a given level (defined electrically) was taken as 100%. The analog output of the equipment does not give absolute values, but shows relative changes in blood flow. Previous studies have indicated a significant correlation between blood flow changes and those obtained using other well-established methods (5, 31). The magnitude of the LN-evoked blood flow response is expressed as a percentage of the preinjection response (control). Values were obtained 20–25 min after the start of drug administration. LN was stimulated for 20 s at a supramaximal voltage (30 V) at 10 Hz with pulses of 2-ms duration. The number of cats used was 6. Statistical significance was assessed by ANOVA followed by a contrast test.

### Table 1. Effects of nifedipine, clonidine, phentolamine, propranolol, and nitroprusside on SABP and on parasympathetic vasodilation

<table>
<thead>
<tr>
<th>Condition</th>
<th>Decrease in SABP (mmHg)</th>
<th>Parasympathetic Vasodilation % of Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.0</td>
<td>100</td>
</tr>
<tr>
<td>Nifedipine (1.0 μg · kg⁻¹ · min⁻¹)</td>
<td>20.3 ± 1.2a</td>
<td>74.0 ± 5.2a</td>
</tr>
<tr>
<td>Clonidine (10 μg/kg)</td>
<td>15.3 ± 1.4a</td>
<td>99.5 ± 2.2</td>
</tr>
<tr>
<td>Phentolamine (1.0 mg/kg)</td>
<td>23.9 ± 2.6a</td>
<td>94.9 ± 13.1</td>
</tr>
<tr>
<td>Propranolol (1.0 mg/kg)</td>
<td>19.9 ± 3.8a</td>
<td>102.4 ± 13.1</td>
</tr>
<tr>
<td>Nitroprusside (1 μg · kg⁻¹ · min⁻¹)</td>
<td>17.8 ± 5.5a</td>
<td>87.8 ± 10.4</td>
</tr>
</tbody>
</table>

Values are means ± SE shown for the absolute decrease in systemic arterial blood pressure SABP (mmHg) induced by various antihypertensive agents and for the magnitude of the parasympathetic vasodilation in the lower lip elicited by electrical stimulation of the central cut end of the lingual nerve (LN) under these agents. The magnitude of the LN-evoked blood flow response is expressed as a percentage of the preinjection response (control). Values were obtained 20–25 min after the start of drug administration. LN was stimulated for 20 s at a supramaximal voltage (30 V) at 10 Hz with pulses of 2-ms duration. The number of cats used was 6. Statistical significance was assessed by ANOVA followed by a contrast test.

**Measurement of arterial blood pressure.** Systemic arterial blood pressure was recorded from the femoral catheter via a Statham pressure transducer. A tachograph (model AT-610G; Nihon Kohden, Tokyo, Japan) triggered by the arterial pulse was used to monitor heart rate.

**Determination of plasma nifedipine concentration.** As nifedipine was the only one of the antihypertensive agents examined in the present study found to inhibit the parasympathetic reflex vasodilation, the plasma nifedipine concentration was measured to enable us to examine the relationship between plasma nifedipine concentration and the magnitude of the inhibition of parasympathetic reflex vasodilation. The plasma nifedipine concentration was determined as described before (26). Briefly, arterial blood was collected in plastic tubes containing sodium EDTA 20–25 min after the start of intravenous infusions of nifedipine at 0.1 or 1.0 μg · kg⁻¹ · min⁻¹ or the sublingual administration of Adalat (10 mg nifedipine/0.34 ml). Plasma was obtained by centrifugation in a refrigerated centrifuge at 10,000 g for 10 min. A small reverse-phase Sep-Pak C₁₈ cartridge (Waters Associates, Milford, MA) was used to separate nifedipine from the plasma components before injection into HPLC. A Sep-Pak C₁₈ was attached to a 50-ml shaded glass syringe and washed with 30 ml methanol followed by 30 ml water. After the additions of plasma (0.5 ml) and 200 mg internal standard (nisoldipine) to 10 ml of 10 mM HCl in test tubes, the samples were passed through the Sep-Pak C₁₈. The cartridge was washed with 50 ml water and then eluted with 2 ml methanol. The methanol fraction, which contained both nifedipine and internal standard, was freeze-dried using a Centrifugal Freeze Dryer (Yamoto, Tokyo, Japan). Samples (50 μl), after reconstitution of the mobile phase (200 μl; methanol and 10 mM acetate buffer pH 4 (75:25 vol/vol)), were injected into the HPLC system (Tosoh, Tokyo, Japan). This consisted of a UV-8020 UV-Vis absorbance detector, a CCPM-II multi-functional pump, a PX-8020 pump controller, a Chromatocorder 21 integrator, and the HPLC column (a 250 × 4.6 mm ID ODS-80Ts column (Tosoh, Tokyo, Japan)). The whole analysis was carried out in a darkroom to avoid light-induced decomposition of the samples.

**Administration of drugs.** Nifedipine was dissolved in a small amount of ethanol and diluted with 0.9% saline (the
final concentration of ethanol was <1%). This solution was administered by intravenous infusion at either 0.1 μg·kg⁻¹·min⁻¹ or 1.0 μg·kg⁻¹·min⁻¹ using an infusion pump (model 55–5920; Harvard Apparatus, MA). The duration of the infusion was 60 min, and the volume infused was 100 μl/min. For administration of Adalat, a single capsule (containing 10 mg nifedipine in 0.34 ml solvent) was placed under the cat’s tongue and left for 60 min. Clonidine (10 μg/kg), phentolamine (1.0 mg/kg), propanolol (1.0 mg/kg), and nitroprusside (1 μg·kg⁻¹·min⁻¹ by means of an infusion pump) were administered intravenously.

Drugs. The drugs used were nifedipine and dl-propranolol hydrochloride (Sigma, St. Louis, MO), clonidine hydrochloride (Tokyo Case, Tokyo, Japan), phentolamine mesylate (Regitin; Japan Ciba-Geigy, Takarazuka, Japan), sodium nitroprusside (Nacalai Tesque, Kyoto, Japan), nisoldipine (in-ternal standard for measurement of nifedipine by HPLC; kindly supplied by Bayer, Wuppertal, Germany), and Adalat (containing 10 mg nifedipine per capsule in 0.34 ml solvent; Bayer Yakuhin, Osaka, Japan). All other chemicals were of reagent grade and were purchased from commercial sources.

Data analysis. All numerical data are given as the means ± SE. The significance of changes in the test responses was assessed using a paired t-test (Fig. 2; see also Fig. 8) or an ANOVA followed by either a contrast test (Table 1; see also Fig. 4) or a post hoc test (Fisher’s test; see Fig. 6). The raw data were obtained by measuring the height of vascular responses on the chart record (in mm). Differences were considered significant at the level P < 0.05. Data were analyzed using a Macintosh computer with StatView 4.5 and Super ANOVA.

RESULTS

The resting mean arterial blood pressure of the 23 cats used for this part of the study was between 82 and 118 mmHg. The average resting mean arterial blood pressure (±SE) was 96.6 ± 7.3 mmHg, and the heart rate was 194 ± 14 beats/min.

Effects of antihypertensive agents on arterial blood pressure and on parasympathetic vasodilation. The antihypertensive agents tested were nifedipine (1.0 μg·kg⁻¹·min⁻¹, calcium channel blocker), clonidine (10 μg/kg, centrally acting sympatholytic agent), phentolamine (1.0 mg/kg, α-adrenergic sympatholytic agent), propanolol (1.0 mg/kg, β-adrenergic sympatholytic agent), and nitroprusside (1 μg·kg⁻¹·min⁻¹, vasodilator). Table 1 and Fig. 2 show their effects on the parasympathetic vasodilation elicited by electrical stimulation of the central cut end of the LN and on systemic arterial blood pressure (both measured 20–25 min after the start of drug administration). At the doses chosen, nifedipine, clonidine, phentolamine, propanolol, and nitroprusside reduced systemic arterial blood pressure by 20.3 ± 1.2, 15.3 ± 1.4, 23.9 ± 2.6, 19.9 ± 3.8, and 17.8 ± 5.5 mmHg, respectively (6 cats in each case, P < 0.05). On the other hand, the blood flow increase elicited by LN stimulation was reduced significantly only by nifedipine (to 74.0 ± 5.2% of control, n = 6, P < 0.05) and not by clonidine (99.5 ± 2.2%, n = 6, not significant (NS)), phentolamine (94.9 ± 13.1%, n = 6, NS), propanolol (102.4 ± 13.1%, n = 6, NS), or nitroprusside (87.8 ± 10.4%, n = 6, NS).

Effects of nifedipine and Adalat on arterial blood pressure. Intravenous administration of nifedipine at doses of 0.1 and 1 μg·kg⁻¹·min⁻¹ and sublingual administration of Adalat (10 mg nifedipine) produced plasma nifedipine concentrations of 2.28 ± 0.06, 20.78 ± 0.68, and 83.38 ± 15.68 ng/ml, respectively (Fig. 2). These treatments decreased arterial blood pressure by 8.54 ± 0.84, 20.32 ± 1.18, and 45 ± 5.07 mmHg, respectively (Fig. 2).

Effects of nifedipine and Adalat on parasympathetic-mediated vasodilation. Figure 3 shows typical examples of the effects of intravenous infusion of nifedipine at doses of 0.1 and 1.0 μg·kg⁻¹·min⁻¹ and sublingual administration of Adalat on the parasympathetic-mediated vasodilation in the lower lip and tongue elicited by electrical stimulation of, respectively, the central or sympathetic nerves.
peripheral cut end of the LN. Mean data are shown in Fig. 4. Although a significant decrease in arterial blood pressure (8.5 ± 0.8 mmHg) was observed after a continuous intravenous infusion of nifedipine at 0.1 μg · kg⁻¹ · min⁻¹, no statistically significant increase in the basal LBF level and no reduction in the magnitude of the parasympathetic vasodilation were observed at this dose [for lower lip, F(12,60) = 0.53, n = 6, P = NS; for tongue, F(12,48) = 1.99, n = 5, P = NS; all by ANOVA for repeated measurements]. This indicates that a nifedipine-induced decrease in arterial blood pressure is not necessarily accompanied by an inhibition of these parasympathetic-mediated responses. Intravenous infusion of nifedipine at a higher dose (1.0 μg/kg/min) elicited an increase in the basal lower lip blood flow (LBF) level and a marked inhibition of the parasympathetic vasodilations in the lower lip and tongue [for lower lip, F(12,60) = 5.56, n = 6, P < 0.01; for tongue, F(12,48) = 4.05, P < 0.001; both by ANOVA for repeated measurements] (Figs. 3, B and D, and 4). Sublingual application of Adalat elicited a marked increase in the basal blood flow levels in the

Fig. 6. Mean data (±SE) for effect of ongoing sympathetic stimulation on nifedipine-induced attenuation of parasympathetic-mediated vasodilation in lower lip and tongue of cats. Parasympathetic vasodilator responses in the LBF (A) and TBF (B) were evoked by electrical stimulation of either the central (for LBF) or peripheral (for TBF) cut end of the LN in vagosympathectomized cats. The above stimuli were delivered either alone (control) or during the intravenous infusion of nifedipine (1.0 μg · kg⁻¹ · min⁻¹) with or without ongoing repetitive cervical sympathetic trunk (CST) stimulation at a frequency of 1 Hz. Each value is expressed as a percentage of the pretreatment response (control), and each column shows data for 9 animals. Brackets indicate statistical significance [ANOVA followed by post hoc test (Fisher’s test)]. NS, not significant.
Inhibition of parasympathetic vasodilation by nifedipine

DISCUSSION

It has been reported that the lowest therapeutically effective level of nifedipine is ~10–15 ng/ml plasma in human subjects (28). This concentration corresponds quite closely to that produced by an intravenous infusion of nifedipine at 1.0 μg·kg⁻¹·min⁻¹ in the cat (Fig. 2). In this study, we gave intravenous infusions of nifedipine at two doses (0.1 and 1.0 μg·kg⁻¹·min⁻¹), and we also used sublingual administration of Adalat (10 mg nifedipine/0.34 ml), because the sublingual method of administering calcium channel blocking agents is frequently used in hypertensive patients.

As shown in Figs. 3 and 4, it was apparent that both an intravenous infusion of nifedipine at 1.0 μg·kg⁻¹·min⁻¹ and a sublingual application of Adalat increased lower lip and tongue and marked reductions in the parasympathetic vasodilations in both tissues examined (for lower lip, F(12,48) = 7.44, n = 5, P < 0.01 (Figs. 3C and 4A) and for tongue, F(12, 48) = 12.06, n = 5, P < 0.01 (Figs. 3D and 4B); both by ANOVA for repeated measurements). These inhibitory effects of nifedipine and Adalat gradually weakened, but complete recovery to the control level had not occurred by 60 min after the end of drug administration.

Effects of ongoing sympathetic stimulation. Although the blood flow increases in the lower lip (Figs. 3B and 4A) and tongue (Figs. 3D and 4B) elicited by stimulation of the central cut end or peripheral cut end, respectively, of the LN were attenuated by intravenous infusion of nifedipine at 1.0 μg·kg⁻¹·min⁻¹, this inhibitory effect of nifedipine was not seen when the LN was stimulated during ongoing repetitive CST stimulation at a frequency of 1 Hz (P < 0.01, ANOVA followed by contrast test; Fig. 5). Mean data for this effect of ongoing CST stimulation on the nifedipine (1.0 μg·kg⁻¹·min⁻¹)-induced inhibition of lip and tongue vasodilator responses are shown in Fig. 6. This observation indicates that, under nifedipine, the blood vessels could produce a vasodilation of the control size under certain conditions. However, this restoration of the parasympathetic vasodilator response was only seen when a clear sympathetic-mediated decrease in basal blood flow was evoked during the intravenous infusion of nifedipine. In other words, such a restoration did not occur when there was a marked inhibition of the sympathetic-mediated vasoconstriction by a high dose of nifedipine (such as that produced by our use of Adalat; data not shown).

Effects of nifedipine on sympathetic-mediated vasoconstriction. Electrical stimulation of the peripheral cut end of CST elicited a frequency-dependent blood flow decrease in the lower lip [F(5,20) = 48.72, P < 0.001; Fig. 7]. These effects were significantly attenuated during the intravenous infusion of nifedipine (1.0 μg·kg⁻¹·min⁻¹) at all the frequencies examined (0.2–10 Hz) in the present experiments (Figs. 7 and 8).

Fig. 7. Typical examples of the effect of intravenous infusion of nifedipine at 1.0 μg·kg⁻¹·min⁻¹ on sympathetic-mediated vasoconstriction (decrease in LBF). Sympathetic vasoconstriction was evoked by electrical stimulation of the peripheral cut end of the superior CST in cats in the absence (A) or presence (B) of nifedipine. The CST was stimulated where indicated (●) for 20 s at a supramaximal voltage (10 V) with pulses of 2-ms duration at various frequencies (0.2–10 Hz).

Fig. 8. Mean data (±SE) for effects of intravenous infusion of nifedipine at 1.0 μg·kg⁻¹·min⁻¹ on sympathetic-mediated vasoconstriction (decrease in LBF). Sympathetic vasoconstriction was evoked by electrical stimulation of the peripheral cut end of the superior CST in cats. The CST was stimulated for 20 s at a supramaximal voltage (10 V) with pulses of 2-ms duration at various frequencies (0.2–10 Hz).
the basal LBF level and decreased parasympathetic-mediated vasodilation in the lower lip and tongue. At least two possibilities need to be considered before we can begin to assign a mechanism to the inhibitory effect of this calcium channel blocking agent on the parasympathetic-mediated vasodilation in the lower lip. One possibility is that the observed attenuation of the parasympathetic-mediated vasodilations is due to a nonspecific hypotensive effect(s) of antihypertensive agents. As shown in Table 1, all of the antihypertensive agents examined evoked a decrease in systemic arterial blood pressure of ~20 mmHg (at the doses used in the present study). However, a statistically significant reduction in the parasympathetic vasodilation was elicited only by nifedipine; the other antihypertensive agents had no such effect. Captopril (angiotensin-converting enzyme inhibitor), another type of antihypertensive agent, also had no effect on the parasympathetic vasodilation at the lower lip at a dose (1.0 mg/kg iv) that evoked a similar hypotensive effect (data not shown). These results indicate that the attenuation of the parasympathetic-mediated vasodilation seen with nifedipine is not a consequence of the induced fall in arterial blood pressure; i.e., there is no direct causal relationship between the decrease in arterial blood pressure and the inhibition of parasympathetic vasodilation. This is also supported by our other observation that although a significant decrease in arterial blood pressure was elicited by an intravenous infusion of the low dose of nifedipine (0.1 μg·kg⁻¹·min⁻¹), the magnitude of the parasympathetic-mediated vasodilation was unaltered by this dose of nifedipine (Figs. 3 and 4).

The second possibility is that calcium channel blocking agents might modify the calcium influx into vascular smooth muscle cells via an action at postsynaptic sites (7). This possibility is the more plausible because an attenuation of parasympathetic vasodilator responses by nifedipine was not observed during ongoing CST stimulation (Figs. 5 and 6). This result suggested that a parasympathetic-mediated vasodilation of the control size can occur in response to LN stimulation even at clinically used levels of plasma nifedipine (Figs. 2, 5, and 6) if the basal level of basal lip blood flow is made low enough by ongoing CST stimulation (i.e., if the vascular tone is high enough). This result also suggests that the ability of blood vessels to relax in response to parasympathetic stimulation is not directly impaired (to any substantial extent) by the presence of this calcium channel blocking agent. This apparent effect of a change in vascular tone on parasympathetic-mediated vasodilator responses under nifedipine is consistent with our previous observation that the amplitude of the parasympathetic vasodilator response in the cat lip increases as the baseline blood flow level decreases (when adjusted by the use of repetitive stimulation of the CST) (11, 21). We, therefore, suggest that the present effect of nifedipine on parasympathetic vasodilator responses probably resulted from an action on postsynaptic sites in vascular smooth muscle that caused an inhibition of ongoing sympathetic vasoconstriction (i.e., vascular tone), presumably via an inhibition of calcium influx into the vascular smooth muscle cell. In other words, the reduction in the parasympathetic-mediated vasodilation was probably secondary to the main effect of nifedipine, i.e., a lowering of vascular tone. Our finding (Figs. 6 and 7) that sympathetic-mediated vasoconstriction was inhibited by ~50% by an intravenous infusion of nifedipine at 1.0 μg·kg⁻¹·min⁻¹ is consistent with this idea. If our interpretation is correct, the site at which nifedipine acts to inhibit parasympathetic-mediated vasodilation will be postsynaptic (on vascular smooth muscle) rather than presynaptic (on the parasympathetic nerve itself). However, further studies involving, for example, determination of the plasma levels of the vasodilator neurotransmitter [presumably vasoactive intestinal peptide (VIP)] released from parasympathetic vasodilator fibers in response to LN stimulation and a comparison between the levels of VIP present before and after nifedipine administration are needed to reach a definite conclusion on this point.

**Perspectives**

Concerning the mechanism by which calcium channel blocking agents elicit a decrease in arterial blood pressure, the most plausible mechanism appears to be that they inhibit α-adrenoceptor-mediated calcium pathways in the vascular membrane and so cause relaxation of arterial smooth muscle (7). However, it is still unclear whether this mechanism is the one by which calcium channel blocking agents cause side effects such as impotence, impaired ejaculation, headache, and flushing. Indeed, it has been reported that these side effects may be mediated by actions on mechanisms other than sympathetic-mediated ones, possibly parasympathetic- and/or trigeminal-mediated mechanisms (2, 3, 8, 10, 30, 32, 34). The present study suggests that nifedipine reduces the parasympathetic vasodilator response mainly by inhibiting sympathetic vasoconstriction by an action at sites on vascular smooth muscle itself. These results suggest that the inhibitory effects of nifedipine described here may have some relevance to the side effects observed with this agent in the clinical setting. Whether or not this is the case, the present investigation does demonstrate that at least some parasympathetic-mediated responses are weaker under nifedipine, and it should provide an impetus for further studies of the effects of calcium channel blockers on vasodilator responses in orofacial and pelvic regions.

**REFERENCES**


