Vincento and neural mechanisms of ACh-mediated vasodilation in the forearm cutaneous microcirculation

MARTIN BERGHOFF,1 MADEERA KATHPAL,1 SONJA KILO,1 MAX J. HILZ,2 AND ROY FREEMAN1
1Department of Neurology, Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, Massachusetts 02215; and 2Department of Neurology, University of Erlangen-Nuremberg, 91054 Erlangen, Germany

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Vascular and neural mechanisms of ACh-mediated vasodilation in the forearm cutaneous microcirculation. J Appl Physiol 92: 780–788, 2002; 10.1152/japplphysiol.01167.2000.—The relative contribution of endothelial vasodilating factors to acetylcholine (ACh)-mediated vasodilation in the forearm cutaneous microcirculation is unclear. The aims of this study were to investigate the contributions of prostanoids and cutaneous C fibers to basal cutaneous blood flow (CuBF) and ACh-mediated vasodilation. ACh was iontophoresed into the forearm, and cutaneous perfusion was measured by laser-Doppler flowmetry. To inhibit the production of prostanoids, four doses of acetylsalicylic acid (ASA; 81, 648, 972, and 1,944 mg) were administered orally. Cutaneous nerve fibers were blocked with topical anesthesia. Cyclooxygenase inhibition did not change basal CuBF or endothelium-mediated vasodilation to ACh. In contrast, ASA (972 and 1,944 mg) significantly reduced the C-fiber-mediated axon reflex in a dose-dependent fashion. Blockade of C-fiber function significantly reduced axon reflex-mediated vasodilation but did not affect basal CuBF or endothelium-dependent vasodilation. The findings suggest that prostanoids do not contribute significantly to basal CuBF or endothelium-dependent vasodilation in the forearm microcirculation. In contrast, prostanoids are mediators of the ACh-provoked axon reflex.

endothelium; prostaglandin; nitric oxide; axon reflex; acetylcholine

Cutaneous blood flow (CuBF) is regulated by endothelial, neural, and humoral factors. The interaction between these mechanisms of blood flow control is poorly defined. Recent studies of CuBF have used the technique of laser-Doppler flowmetry in combination with the iontophoretic application of charged vasoactive agents. Acetylcholine (ACh) has been used to induce endothelium-mediated blood flow, and the direct nitric oxide (NO) donor sodium nitroprusside has been used to provoke non-endothelium-mediated blood flow. Despite the widespread use of ACh-evoked vasodilation in studies investigating vascular physiology and pathology, the mechanisms responsible for its effect, particularly in the cutaneous microcirculation, are unresolved. This question has important implications. If prostaglandins do play a major role in endothelium-mediated vasodilation, some effects of acetylsalicylic acid (ASA) therapy, which is extensively used for its prophylactic antplatelet effect in patients with cardiovascular and cerebrovascular disease (17, 27), may be counterproductive. Furthermore, if prostaglandins play a major role in the C-fiber-mediated axon reflex, reduction of this reflex by cyclooxygenase inhibition may attenuate the ability to mount a neurogenic inflammatory response (4, 48). Attenuation of this important protective reflex in patients with peripheral neuropathy impairs wound healing and increases susceptibility to infection, thereby leading to cutaneous ulceration, gangrene, and ultimately amputation (3, 21).

Factors released by the endothelium in response to ACh include NO, vasoactive prostanoids and endothelium-derived hyperpolarizing factor. The relative contribution of these factors to cutaneous vasodilation is unclear (25, 40, 43). Furthermore, the role of axon reflex-mediated changes in blood flow in response to ACh iontophoresis and to iontophoretic current alone is unresolved. Morris and Shore (41) reported that ACh-mediated vasodilation of the forearm microcirculation is not mediated by a prostanoid-dependent mechanism, whereas more recently Khan et al. (33) and Noon et al. (42) suggested that forearm cutaneous vasodilation is mediated mainly by a prostanoid-dependent mechanism. These three studies used different ASA formulations and routes of administration. Furthermore, the studies used different ACh iontophoresis protocols.

There are several possible explanations for these conflicting results. First, the effects of both ACh and ASA are dose dependent. There is evidence that ACh has a biphasic dose-dependent mode of action on blood flow; vasodilation is observed at low concentrations and vasoconstriction at high concentrations (13). Also, ASA-induced cyclooxygenase inhibition is dose dependent, as only high doses of ASA block vascular prosta-
noid synthesis (2). Prior studies have used only a single dose of ASA (600 mg) administered as a dissolved soluble formulation (41), oral enteric-coated ASA once daily for 3 days (33), or as an intravenous bolus (42). The iontophoretic protocols also differed. The maximum iontophoretic charge used to deliver ACh in the respective studies was 4 mC (41), 8 mC (33), or 16 mC (42). We hypothesized that there are differential dose effects of ASA and ACh on the endothelium; that ACh-mediated vasodilation, consistent with the study of Collier and Vallance (13), is attenuated at higher charge densities; and that ASA-mediated cyclooxygenase inhibition would be present only at higher doses.

Second, unrecognized activation of the C-fiber-mediated axon reflex may contribute to the conflicting results. This possibility was suggested by Forst et al. (22), who proposed that the effect of intracutaneous ACh on CuBF is mediated mainly by the axon reflex. The situation is more complex, however, as there is evidence that the iontophoretic current alone may also induce an axon reflex (9). Furthermore, prostaglandins may play a role in the axon reflex. We hypothesized that the conflicting reports on the relative contributions of vasoactive prostanoids were at least in part related to the unrecognized attenuation of the axon reflex by ASA.

The aims of the present study were to isolate the relative contributions of prostaglandins and the axon reflex on CuBF provoked by the iontophoresis of ACh. We therefore inhibited cyclooxygenase with ASA to investigate the effects of prostaglandins on CuBF and measured the direct and axon reflex-mediated blood flow changes in response to ACh. To define the dosage effects of ASA and ACh on the endothelium and the axon reflex, we used both an ASA and an ACh dose-response protocol to identify a potential dose effect on dilating and vasoconstricting prostanoids. To ensure cyclooxygenase inhibition with a single, oral dose of chewable ASA, the maximum dose of ASA administered was 1,944 mg. This dose and formulation have been shown to produce a 90% decrease in serum thromboxane B₂ within 14 min when given orally (19). Finally, to clarify the role played by the axon reflex in mediating CuBF, we used a topical anesthetic agent to block conduction in cutaneous nerves. We thereby minimized the axon reflex response to iontophoresis of ACh and to iontophoretic current alone.

MATERIALS AND METHODS

Two experimental studies were carried out. In protocol 1, the effects of cyclooxygenase inhibition on endothelium-mediated vasodilation and axon reflex-mediated vasodilation were investigated in a dose-response study. In protocol 2, the effects of cutaneous sensory nerve blockade with topical anesthesia on endothelium-mediated vasodilation and axon reflex-mediated vasodilation were studied.

Subjects

All subjects were screened by medical history for the presence of cardiovascular, dermatological, and neurological disease; cardiovascular risk factors; or other major illness. The presence of any of these as well as a history of smoking was a criterion for exclusion from the study. Subjects were asked to refrain from the ingestion of caffeine-containing food and beverages for 2 h before the study. Subjects did not ingest ASA or other nonsteroidal anti-inflammatory medication in the 9 days before each study. None of the subjects was taking any medication known to affect vasomotor or autonomic function apart from one woman who was on an oral contraceptive. All subjects involved in the study were informed of the experimental procedures and gave their written, informed consent. The study was approved by the Beth Israel Deaconess Medical Center Institutional Review Board.

Experimental Protocol 1

Nine healthy subjects (3 men, 6 women) between the ages of 18 and 28 yr (mean 20.9 ± 1.0 yr), body mass index between 21.0 and 26.8 kg/m² (mean 23.4 ± 0.7 kg/m²), participated in this study.

The effect of cyclooxygenase inhibition on endothelium-mediated vasodilation and axon reflex-mediated vasodilation was studied on four occasions separated by a period of at least 9 days. On each of the four study days, the baseline responses to the iontophoresis of ACh (1%) were measured on the ventral aspect of the nondominant forearm while subjects were sitting comfortably in a chair with their arms rested at heart level. Then, chewable ASA (Bayer, Morristown, NJ) in one of four doses (81, 648, 972, 1,944 mg) was given orally in random order. Thirty minutes after the administration of ASA, when the endothelium-derived prostacyclin is maximally inhibited (26), the response to dose-dependent application of ACh (1%) at the corresponding area of the opposite forearm was recorded. All tests were performed after an acclimatization period of at least 30 min. Tests were performed at room temperatures between 22.8 and 25.3°C.

Experimental Protocol 2

Eleven healthy subjects (10 men, 1 woman), between the ages of 25–37 yr (mean 30.3 ± 1.0 yr), body mass index between 21.0 and 29.6 kg/m² (mean 24.0 ± 1.0 kg/m²), participated in the second study. EMLA cream (ASTRA Pharmaceutical Products, Westborough, MA), an eutectic mixture of lidocaine (2.5%) and prilocaine (2.5%), was used to block cutaneous nerve function. In prior studies, EMLA cream has been shown to attenuate the axon reflex-induced increase in CuBF in response to histamine application (23) and electrical stimulation (49). The cream was applied to a 3 × 3-cm area on the ventral aspect of one forearm and covered with an occlusive dressing. After 2 h, the dressing and the cream were removed. To test whether local analgesia was achieved, sensory nerve function for light touch and pinprick was evaluated. While subjects sat comfortably in a chair with arms rested at heart level, either ACh or NaCl was iontophoresed at the EMLA-treated site and at the corresponding untreated, control site of the opposite forearm. These tests were done in random order on two different study days. Tests were performed at room temperatures, which ranged from 23.5 to 26.5°C.

Iontophoresis Protocol

The positively charged endothelium-dependent vasodilator ACh (Penta International, Fairfield, NJ; 1%, dissolved in sterile water) was iontophoresed with anodal current. To investigate to what extent the current alone contributes to an increase in CuBF, NaCl (isotonic solution) was iontophoresed with anodal current. Substances were placed in an ionto-
phoretic drug-delivery electrode that was attached to the direct laser-Doppler probe. A battery-driven power supply provided a constant current (Periiont, Perimed, Järfalla, Sweden). After a baseline recording of 3 min, seven increasing stimuli of varying intensity and duration (from 0.02 mA for 3 s to 0.4 mA for 140 s) were applied every 3 min. Thus, taking into account the area of the drug-delivery electrodes (113 mm²), the following charge densities (mC/mm²) were administered: 5 × 10⁻⁴ (0.02 mA for 3 s), 1 × 10⁻³ (0.02 mA for 6 s), 5 × 10⁻³ (0.02 mA for 28 s), 1 × 10⁻² (0.04 mA for 28 s), 5 × 10⁻² (0.2 mA for 28 s), 1 × 10⁻¹ (0.4 mA for 28 s), and 5 × 10⁻¹ (0.4 mA for 140 s). The same iontophoresis protocol was used for both studies.

**CuBF Measurements**

Cutaneous perfusion was measured with a laser-Doppler flowmeter (Periflux 4001 Master, Perimed) using a laser beam with a wavelength of 780 nm.

Changes in CuBF were measured with two fiber-optic laser-Doppler probes. The blood flow within the stimulated area was measured with a probe placed in a recess in the center of the iontophoresic applicator. A second probe was placed 8 mm outside the edge and proximal to the iontophoretic stimulation site. Data acquired from this probe were used to determine the axon reflex.

The site of EMLA application was marked, and both probes were placed in the marked area. CuBF was recorded continuously during the iontophoresis protocol. The laser-Doppler flowmeter was calibrated against the manufacturer’s motility standard before each study.

**Data Acquisition and Statistical Analysis**

All CuBF data were acquired by using the Windaq data acquisition system (Dataq Instruments, Akron, OH). The data were digitized, recorded, and stored on a personal computer for further analysis. The CuBF data reported are averaged data over 3-min periods for the baseline and subsequent 3-min periods defined by the onset of each iontophoresis stimulus. Basal CuBF values are reported as arbitrary perfusion units. Increases in CuBF are expressed as percentage change over baseline.

The comparison of basal CuBF was done by use of paired t-tests. The dose-response curves were analyzed using analysis of variance (ANOVA). For both protocols, separate analyses were done for each recording site (direct and axon reflex). For protocol 1, separate analyses were done for each of the four ASA groups; the dependent variable in the ANOVA model was CuBF and the independent variables were main effect of level of ACh (1–7) and main effect of group (EMLA treatment or not), and their interaction.

### RESULTS

#### Protocol 1

**Basal CuBF.** Basal CuBF before and after ASA ingestion, measured at the direct stimulation site, and axon reflex recording site, is shown in Table 1. No significant differences were found between the ASA and before-ASA states at either of the recording sites for any level of ASA (paired t-test).

**CuBF response to ACh at the direct stimulation site.** Iontophoresis of ACh produced a significant dose-dependent increase in CuBF measured directly over the iontophoresic site for both the ASA and before-ASA states (ANOVA, main factor effect of level of ACh, P < 0.0001). There were no differences in the dose-response curves between the ASA and before-ASA states (ANOVA, interaction term of ASA and level of ACh). There were no differences in the ED₅₀ before and after ASA ingestion (see Table 2).

**Axon reflex-mediated CuBF response to ACh.** Iontophoresis of ACh also increased CuBF significantly at a distance of 8 mm from the application site (ANOVA, main factor effect of level of ACh, P < 0.0001). After ingestion of 81 and 648 mg of ASA, no differences in the dose-response curves were found. However, after ingestion of 972 and 1,944 mg of ASA, the responses to ACh were significantly reduced compared with the before-ASA state (ANOVA, main effect for ASA = 972, P = 0.014; for ASA = 1,944, P = 0.024). The ED₅₀ before and after ASA ingestion only differed after the administration of 972 mg (paired t-test, P < 0.05; see Table 3).

#### Protocol 2

**Sensory assessment.** Two hours after the application of EMLA, none of the study subjects was able to detect light touch or pinprick in this area. In addition, whereas subjects reported tingling, burning, or itching

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**Table 1. Basal cutaneous blood flow before and after ASA ingestion**

<table>
<thead>
<tr>
<th>Direct Response Recording Site</th>
<th>Before ASA</th>
<th>After ASA</th>
<th>Axon Reflex Recording Site</th>
<th>Before ASA</th>
<th>After ASA</th>
</tr>
</thead>
<tbody>
<tr>
<td>81 mg</td>
<td>6.5 ± 0.7</td>
<td>6.7 ± 0.7</td>
<td>9.4 ± 1.2</td>
<td>7.9 ± 0.6</td>
<td></td>
</tr>
<tr>
<td>648 mg</td>
<td>5.7 ± 0.4</td>
<td>5.7 ± 0.6</td>
<td>11.6 ± 1.5</td>
<td>8.9 ± 1.2</td>
<td></td>
</tr>
<tr>
<td>972 mg</td>
<td>5.8 ± 0.6</td>
<td>6.4 ± 0.8</td>
<td>9.0 ± 1.6</td>
<td>8.8 ± 1.7</td>
<td></td>
</tr>
<tr>
<td>1,944 mg</td>
<td>9.8 ± 1.2</td>
<td>8.0 ± 0.9</td>
<td>10.1 ± 1.4</td>
<td>10.6 ± 1.5</td>
<td></td>
</tr>
</tbody>
</table>

Values are means ± SE given in perfusion units; n = 9. ASA, acetylsalicylic acid.

**Table 2. ED₅₀ before and after ASA ingestion measured at the stimulated area (direct recording site)**

<table>
<thead>
<tr>
<th>Before ASA</th>
<th>After ASA</th>
</tr>
</thead>
<tbody>
<tr>
<td>81 mg</td>
<td>0.017 ± 0.005</td>
</tr>
<tr>
<td>648 mg</td>
<td>0.009 ± 0.002</td>
</tr>
<tr>
<td>972 mg</td>
<td>0.016 ± 0.004</td>
</tr>
<tr>
<td>1,944 mg</td>
<td>0.020 ± 0.007</td>
</tr>
</tbody>
</table>

Values (in mC/mm²) are means ± SE; n = 9.
Table 3. ED_{50} before and after ASA ingestion measured 8 mm from the primary stimulation area

<table>
<thead>
<tr>
<th>Concentration</th>
<th>Before ASA</th>
<th>After ASA</th>
</tr>
</thead>
<tbody>
<tr>
<td>81 mg</td>
<td>0.056 ± 0.016</td>
<td>0.046 ± 0.008</td>
</tr>
<tr>
<td>648 mg</td>
<td>0.066 ± 0.019</td>
<td>0.071 ± 0.010</td>
</tr>
<tr>
<td>972 mg</td>
<td>0.051 ± 0.010</td>
<td>0.078 ± 0.012</td>
</tr>
<tr>
<td>1,944 mg</td>
<td>0.072 ± 0.015</td>
<td>0.089 ± 0.009</td>
</tr>
</tbody>
</table>

Values (in mC/mm^2) are means ± SE; n = 9. *P < 0.05 vs. before ASA.

under the drug-delivery electrode during the iontophoresis of ACh and NaCl at untreated skin, these sensations were not present after EMLA application.

**Basal CuBF.** The basal CuBF at the EMLA-treated and control site is shown in Table 4. EMLA treatment did not affect basal CuBF at the direct or axon reflex recording sites (ANOVA, P = not significant).

**Responses to ACh and anodal current at the direct stimulation site.** Iontophoresis of ACh led to a significant increase in CuBF at both sites (ANOVA, main factor effect of level of ACh, P < 0.0001). The CuBF response to ACh at the EMLA-treated and control sites differed significantly (ANOVA, main effect of EMLA, P = 0.026). The ED_{50} at the EMLA-treated and control sites did not differ significantly (0.015 vs. 0.014 mC/mm^2; paired t-test, P = not significant).

Application of anodal current alone also increased CuBF within the stimulated area (ANOVA, main factor effect of level of anodal current, P < 0.0001). Compared with the iontophoresis of ACh, the threshold required to increase CuBF was higher (5 × 10^{-2} mC/mm^2 vs. 5 × 10^{-3} mC/mm^2, P < 0.01). The CuBF evoked by anodal current was ∼60% less than that evoked by ACh (P < 0.001). Anodal current did not increase CuBF at the EMLA-treated site (see Fig. 3).

**Axon reflex-mediated response to ACh and anodal current.** The axon reflex-mediated responses to ACh and NaCl (anodal current) at the EMLA-treated and control sites were recorded 8 mm from the primary stimulation area (see Fig. 4). ACh application resulted in an increase in CuBF (ANOVA, main effect of level of ACh, in model with main effects of EMLA and level of ACh and interactions, all P < 0.0001). EMLA significantly reduced the ACh-mediated response (ANOVA, main effect of EMLA, P < 0.001; interaction term, P < 0.001). Anodal current did not result in a significant increase in CuBF at the EMLA-treated or control recording sites.

**DISCUSSION**

The results of the present study demonstrate that prostaglandins do not contribute significantly to endothelium-dependent vasodilation in response to ACh. This finding is present with all doses of ASA and ACh. In addition, the endothelium-mediated response to ACh is not significantly affected by neural blockade, indicating that neither the C-fiber-mediated axon reflex nor other neural influences play a major role in ACh-mediated vasodilation. In contrast, the C-fiber-mediated increase in CuBF is partially mediated by cyclooxygenase products. A reduction in this vasodilatory response was found only with the two highest doses of ASA supporting a dose-response effect. The C-fiber-mediated vasodilatory response was attenuated but not blocked by ASA, suggesting incomplete cyclooxygenase inhibition or that in addition to prostaglandins other mediators play a role in this response.

ACh binds to M_{2} muscarinic receptors on the endothelial surface and elicits an increase in CuBF by a direct receptor-mediated effect. Acting via a G-protein-coupled receptor and catalyzed by constitutively expressed endothelial NO synthase, ACh stimulates the production of nitric oxide. NO is a potent mediator of endothelium-dependent vasodilation (25). ACh may also induce the release of other vasodilating substances including prostaglandins and endothelium-derived hyperpolarizing factor (20, 43).

Prostacyclin (PGI_{2}) is the principal vasodilating prostanooid released by the endothelium. It evokes vasodilation through the activation of a cAMP-dependent pathway (44). PGI_{2} is produced from arachidonic acid in a series of reactions catalyzed by enzymes that include cyclooxygenase. ASA is an irreversible acetylator of cyclooxygenase and therefore an inhibitor of prostaglandin production (12, 47). The relative contribution of prostaglandins to endothelium-mediated vasodilation of the cutaneous microcirculation in humans remains unresolved. Conflicting reports have suggested that vasodilating prostanooids play no major role (41) or play the primary role (33, 42) in this response. The three prior studies that have addressed this question used a single 600-mg dose but different ASA formulations and administration routes. ASA has been administered as a single dose of a dissolved soluble formulation (41), as oral enteric coated ASA once daily for 3 days (33), and as an intravenous bolus (42).

To ensure that the failure to demonstrate a prostaglandin contribution to ACh-mediated vasodilation in the study of Morris and Shore (41) was not due to inadequate dosage or absorption, we administered an oral dose of chewable ASA, which is rapidly absorbed and produces a 90% decrease in serum thromboxane B_{2} within 14 min when given orally (19). The maximum dose of ASA administered was 1,944 mg, considerably higher than the doses used in that study (41).

The present study does not support a significant role for prostaglandins in ACh-mediated vasodilation of the
forearm cutaneous microcirculation. We have demonstrated that the application of ACh results in a dose-dependent increase in CuBF within the area of iontophoresis that is not significantly changed by high- or low-dose cyclooxygenase inhibition. Cyclooxygenase inhibition also did not result in a shift to the right of the dose-response curve (see Fig. 1 and Table 2).

Because the endothelium is an important regulator of vascular basal tone (25), we measured basal CuBF after each dose of ASA to exclude the possibility that an effect of ASA on basal CuBF could mask an absolute increase in ACh provoked blood flow. There was no evidence that any dose of ASA altered basal CuBF, suggesting that basal release of prostaglandins is not a significant contributor to resting blood flow in the forearm cutaneous microcirculation (see Table 1). Furthermore, there is no evidence of a dose-dependent release of vasoconstricting prostanooids. These results contrast with recent studies of the forearm macrocirculation in which basal release of vasoactive prostanooids has been shown to play a role in the maintenance of resting forearm blood flow in some (16) although not all studies (8, 18, 34).

A possibility that might explain the conflicting results was raised by Collier and Vallance (13), who demonstrated an endothelium-dependent biphasic response to ACh. These investigators showed that low doses of ACh produce dilation and high doses constriction of dorsal hand veins. We hypothesized that a biphasic response may mask a vasodilator effect of prostanooids, particularly if there were a dose-dependent discordance between the NO and prostanooid production. The studies of Noon et al. (42) and Khan et al. (33), which suggested that prostaglandins contribute significantly to ACh-induced vasodilation, used a limited dose-response protocol (four and two stimuli respectively). To exclude the possibility that the biphasic response to ACh was the reason for these findings, in the present study we extended the dose-response protocol to seven stimuli, including both smaller and larger doses of ACh. There was no evidence of a constrictor response at any ACh dose performed both with and without cyclooxygenase inhibition.

We also examined the role of vasoconstricting prostanooids in the cutaneous microcirculation. The endothelium synthesizes and releases vasoconstricting cyclooxygenase-dependent factors such as thromboxane A2 and prostaglandin F2α (30, 37, 39). Recent human studies have shown that an impaired response to ACh may be due to the enhanced production of cyclooxygenase-dependent vasoconstricting factors in congestive heart failure (31, 32), atherosclerosis (29).
and diabetes (45, 46). This abnormal response can be repaired by cyclooxygenase inhibition. There is some evidence that the enhanced response to ACh after cyclooxygenase inhibition is dose dependent; it is present with high but not low doses of ASA (29). We hypothesized that the conflicting reports on the role of prostaglandins in endothelium-mediated vasodilation may be due to failure to recognize dose-related endothelium-mediated production of vasoconstricting prostanoids. The ASA dose-response study does not provide support for ACh-induced production of vasoconstricting prostanoids in the forearm microvasculature.

The axon reflex, however, appears to be an important contributor to ACh-mediated blood flow. In the present study, ACh provoked a significant axon reflex-mediated increase in CuBF measured 8 mm from the iontophoresic site (see Fig. 2). ACh excites nonmyelinated, sensory primary afferent C fibers (15) and induces axon reflex vasodilation through an unknown mechanism. These sensory nerves, which densely innervate the cutaneous microvasculature, contain neuropeptides such as calcitonin gene-related peptide (CGRP) and substance P (5, 6).

This axon reflex-mediated increase in CuBF was significantly decreased by higher doses of ASA (see Fig. 2). The exact mechanisms through which prostaglandins contribute to the axon reflex are unknown. There is evidence suggesting that PGI₂ and PGE₂ sensitize the primary sensory afferents and increase the basal and evoked release of the neuropeptides CGRP and substance P (11, 28, 38, 50). This mechanism is recognized to play a role in inflammation and wound healing and is most likely responsible for the increase in CuBF as well. Although our study has demonstrated that cyclooxygenase products play a significant role in axon reflex-mediated vasodilation, we are unable to determine whether this response is mediated via a direct prostaglandin effect or whether prostaglandins stimulate or sensitize the sensory nerves.

We also observed an increase in CuBF within the stimulated area after the iontophoresis of NaCl alone. Current-induced vasodilation is well recognized and is most likely secondary to activation of polymodal C-fiber nociceptors (9, 49). The increase in blood flow inside the iontophoretic zone was significantly attenuated by EMLA treatment. This strongly suggests that the effect is secondary to a neurally mediated axon reflex induced by the iontophoretic current (see Figs. 3 and 4).

EMLA cream, an eutectic mixture of the local anesthetics lidocaine and prilocaine, was used to block cutaneous nerve function to minimize the contribution of cutaneous nerves to blood flow. This topical local anesthetic cream induces action potential blockade of...
the peripheral sensory neurons, thereby preventing the axon reflex-mediated release of vasoactive neuropeptides from the sensory nerve terminals. The level of cutaneous analgesia was sufficient to abolish pin and touch sensation, consistent with C-fiber and possibly Aδ- and Aβ-fiber sensory nerve blockade. Previous reports on the effects of local anesthetics on isolated blood vessel segments suggest that there is minimal change in smooth muscle vasomotor tone (36). Our studies, which reveal that there is no change in resting blood flow after EMLA treatment, are consistent with these reports (see Table 4). Although we cannot exclude the possibility that EMLA had a postsynaptic effect on the vascular smooth muscle, prior studies suggest that the response to CGRP is retained after nerve blockade. The possibilities to explain this finding include migration effects of ACh or incomplete neural blockade by EMLA.

In the present study, we observed that axon reflex-mediated CuBF is attenuated by high-dose cyclooxygenase inhibition, suggesting that prostaglandins contribute to this response. In addition, iontophoresis of NaCl with anodal current resulted in an axon reflex-mediated increase in CuBF within the iontophoresed zone. Furthermore, CuBF in response to ACh was reduced after nerve fiber blockade. Taken together, these data suggest that the trend toward a decrease in CuBF within the stimulated area, which was present with high-ASA doses only, was most likely due to ASA-induced attenuation of the axon reflex. None of the prior studies measured axon reflex-mediated changes in CuBF in response to ACh. It is thus possible that the discrepancy between these studies on the contribution of prostaglandins to ACh-provoked CuBF is at least in part secondary to cyclooxygenase inhibition of an unrecognized axon reflex within the stimulated area.

The results of our study may have important clinical implications. The axon reflex has a protective function. Its diminution and the resulting attenuation of neurogenic inflammation is in large part responsible for the high incidence of lower extremity ulceration, infection, and amputation that is associated with the peripheral neuropathy of diabetes and other small-fiber neuropathies (3, 21, 48). The results of the present study suggest that higher doses of ASA may further exacerbate the already decreased ability to mount a neurogenic inflammatory response, thereby increasing the predisposition to this devastating complication of peripheral neuropathy. Such doses of ASA may be used clinically as prophylaxis against cerebrovascular and other atherosclerotic vascular diseases (17, 27).

There are several limitations to this study. It is possible that the rapid metabolism of ASA, or the large volume of distribution associated with the oral ingestion and subsequent gastrointestinal absorption of ASA, attenuated any vascular effect. In addition, our study experimental design did not allow us to exclude the possibility that repeated (33) or intravenous (42) administration of ASA may have produced more complete vascular cyclooxygenase inhibition. Given the clear effect observed on the axon reflex, however, it is unlikely that plasma concentrations of ASA sufficient to attenuate the axon reflex would be insufficient to affect endothelial prostaglandin-mediated vasodila-

Fig. 3. Endothelium-dependent vasodilation in response to the iontophoresis of ACh and NaCl at the EMLA cream-treated and control sites. ACh caused a significant increase in CuBF before and after EMLA treatment (ANOVA, \( P < 0.0001 \)). CuBF at the EMLA-treated and control sites differed significantly (\( P = 0.026 \)). Iontophoresis of NaCl resulted in a significant increase in CuBF (ANOVA, \( P < 0.0001 \)) at the control site; it did not increase CuBF after EMLA treatment. Data are means ± SE.
tion. An additional potential limitation of the study is the possibility that inhibition of prostanoids may have produced compensatory upregulation of NO production. This possibility seems unlikely given the absence of any change in basal CuBF, although we cannot exclude any change in receptor-mediated NO production. The axon reflex was significantly reduced but not abolished by neural blockade. The possibilities to explain this finding include migration effects of ACh or incomplete neural blockade by EMLA. Finally, we are unable to exclude the possibility that nonspecific vehicle-related effects played some role in our results. The vehicle in prior studies assessing the role of prostaglandins in endothelium-mediated vasodilation has included 3% mannitol in sterile water (41), 2% methylcellulose in distilled water (42), and sterile deionized water (33). The relationship of the tonic concentration of the vehicle to the nonspecific response to iontophoresis is unresolved (1, 42).

Our findings are consistent with studies that have reported no role for prostaglandins in thermoregulatory vasodilation of the cutaneous microcirculation (7, 10). Our results do not exclude a possible role for prostaglandins in metabolic and ischemic vasodilation of the cutaneous microcirculation. Furthermore, the role of prostaglandins in endothelium-mediated vasodilation varies among species and among vascular beds within species (14, 24, 35). Finally, we cannot exclude a role for prostaglandins in endothelium-mediated vasodilation in other vascular beds.

In summary, these data suggest that prostaglandins do not contribute to resting blood flow or ACh-provoked vasodilation of the forearm cutaneous vasculature in healthy humans. In contrast to the lack of a significant effect on basal and endothelium-mediated blood flow, our data suggest that the axon reflex, and by inference neurogenic inflammation, is mediated, in part, by prostanoids that enhance local blood flow. The anti-inflammatory effect of cyclooxygenase inhibition is due, in part, to attenuation of this increase in blood flow.

REFERENCES


