Evidence for Sensory Nerve Involvement in Cutaneous Reactive Hyperemia in Humans

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To study the involvement of local sensory nerves in reactive hyperemia, laser-Doppler measurements of skin blood flow were recorded in locally anesthetized and untreated forearm sites in eight volunteers after 90, 180, and 360 seconds of arrested forearm blood flow. The reactive hyperemia increased in magnitude and duration in response to increasing occlusion periods. However, maximum postocclusion flows in the untreated site of 31±5%, 38±6%, and 49±5% (mean±SEM) flux were significantly greater than the 14±3% (P<.005), 20±4% (P<.005), and 25±5% (P<.001) flux seen in the anesthetized sites. The duration of the hyperemia was also shortened from 139±26 seconds in the untreated site to 61±17 seconds (after the 360-second occlusion, P<.02) in the anesthetized sites. The anesthesia did not alter the increase in local blood flow induced by intradermally injected calcitonin gene-related peptide. Topically applied capsaicin induced a localized increase in blood flow that was unaffected by anesthesia and a surrounding flare that was abolished by the treatment. The results show that local anesthesia can significantly inhibit reactive hyperemia by a mechanism that does not alter the vasodilation induced by exogenous calcitonin gene-related peptide or the localized capsaicin-induced release of vasodilators from sensory nerves. Indomethacin was also found to be effective in suppressing reactive hyperemia. The evidence suggests that postocclusion reactive hyperemia in human forearm skin is mediated by a local reflex involving sensory nerves and a cyclooxygenase product, probably a vasodilator prostaglandin. (Circulation Research 1993;73:147-154)

KEY WORDS • humans • reactive hyperemia • local anesthesia • sensory nerves • axon reflexes • neuromodulation • vascular tone

The reperfusion of functionally intact vascular beds is characterized by a period of hyperemia in which increased blood flow rectifies the nutritional debit that occurs in tissues during circulatory arrest. The mechanisms controlling this reactive hyperemia are incompletely understood. Formerly, the response was thought to be regulated principally by the ischemia-induced release of vasodilator metabolites from local tissues, the response of vascular smooth muscle to reduced oxygen tension, or myogenic vasorelaxation triggered by reduced transmural pressure in the static microcirculation. Recent studies of reactive hyperemia in the myocardial and skeletal circulation after selective damage to the endothelium or in the presence of nitric oxide synthesis inhibitors suggest that endothelium-derived relaxing factors may partially contribute to the response. There is also some evidence for a neural component. Immunohistochemistry shows extensive perivascular localization of small-diameter sensory nerve fibers containing the neuropeptides calcitonin gene-related peptide (CGRP) and substance P in the skin and cardiovascular system of several species, including humans. Our previous studies have shown that CGRP and substance P are very potent vasodilators, causing prolonged increases of blood flow in peripheral and coronary circulations in humans. Peripheral activation or antidromic stimulation of sensory C fibers leads to the release of these neuropeptides, which is associated with local vasodilation and increased blood flow. Chronic denervation or treatment with capsaicin, which depletes unmyelinated sensory C fibers and significantly reduces CGRP- and substance P–like neural immunoreactivity, can abolish vasodilation induced by antidromic stimulation and reduce active hyperemia in response to exercise. Such nerve depletion can also reduce postocclusive reactive hyperemia in the rat paw by more than 60% and cerebral hyperemia in cats by more than 50% after trigeminal axotomy, suggesting that vasodilator mechanisms dependent on sensory nerve activity may be involved in reactive hyperemia. Since the mechanisms regulating the microvasculature during reperfusion are unclear, as is the physiological significance of vasodilation induced by antidromic nerve activation, we used local anesthesia in the skin of human volunteers to investigate the possible role of local sensory nerve reflexes in reactive hyperemia in humans.

Materials and Methods

Subjects
The studies were approved by the hospital’s local ethical committee, and volunteers gave informed consent after explanation of the procedure by one of the investigators. The subjects were normal men, aged 24 to 32 years and weighing between 65 and 80 kg. All subjects were healthy nonsmokers on no medication.
Cutaneous Blood Flow Measurements

Skin blood flow changes were measured in a clinical laboratory where room temperature was thermostatically controlled between 22° and 25°C and maintained within ±1°C for each study. Temporal alterations of red blood cell flux, taken as an index of skin blood flow, were determined simultaneously at two sites using two Perimed II laser-Doppler flowmeters (Perimed, Stockholm, Sweden) set to a gain of 10. With the subjects supine, the laser probes were secured at right angles to the skin at two sites on the volar surface of the forearm by 3-cm-diameter self-adhesive guides, and the flows were recorded continuously using a MacLab computer. Detailed descriptions of the technique have been published previously by ourselves and others. Spatial variations in skin blood flow were measured using a Lisa lds laser-Doppler scanner (Moor Instruments, Axminster, England). With the subjects seated, the laser-Doppler probe head was positioned 20 cm above the volar surface of the forearm, and light from the laser was scanned step by step over a 4 to 8-cm² area of the skin by a motor-driven mirror system. Computer processing of the backscattered light from each scanned point then yielded a color-coded image showing the spatial distribution of tissue perfusion.

Reactive Hyperemia After Local Anesthesia

The effects of a eutectic mixture of local anesthetics (EMLA [lignocaine and prilocaine], 5%; Astra Pharmaceuticals, UK) in postocclusion reactive hyperemia were studied in eight subjects. EMLA cream was applied to approximately 4 cm² of either a proximal or distal forearm skin site and covered with an occlusive dressing (Tagederm, 3M). The dressing and cream were removed after 1 hour, and the laser probes were applied to the EMLA-treated site and an untreated site. At the EMLA-treated site, the subjects were unable to sense the pain stimulus of a needle point but could still discern touch, heat, and cold. After measurements of basal flow, 90-, 180-, and 360-second intervals of suprasystolic pressure occlusion of forearm blood flow were regulated with a sphygmomanometer cuff around the upper arm. Between each occlusion period, skin blood flow from both sites was allowed to return to basal rates. Spatial responses were observed on separate occasions in the eight subjects. After a scan of basal perfusion, EMLA cream was applied to 4 cm² of skin within the scan area. The cream and dressing were removed after 1 hour, and scans were repeated before, during, and immediately after a 360-second period of occlusion. Differences in regional blood flow in treated and untreated areas of the skin were determined from the average flow observed in 1 cm² of the corresponding perfusion image.

Reactive Hyperemia After Indomethacin Pretreatment

In seven subjects, the separate and combined effects of locally injected indomethacin and topically applied EMLA were also studied. After 45 minutes of pretreatment with EMLA, the cream was removed. Indomethacin (Indocid PDA, 1×10⁻⁶ mol per site; Merck Sharp & Dohme) and
control vehicle (0.9% saline) were then injected intradermally in 50-μl volumes using a 27 standard wire gauge needle into separate areas of the EMLA-pretreated skin and into untreated sites elsewhere on the volar surface of the arm. The EMLA cream was reapplied for a further 15 minutes. A laser probe was applied to the vehicle-injected site, and simultaneous recordings of the resting and hyperemic responses were taken in randomized order at each of the other treated sites after repeated periods of 360 seconds of arterial occlusion.

**CGRP- and Capsaicin-Induced Increase in Skin Blood Flow**

The effects of EMLA on increased skin blood flow induced by CGRP and capsaicin were studied in six subjects. After basal blood flow measurements were taken, a forearm skin site on each subject was pretreated with EMLA for 1 hour. After removal of the cream, 1×10⁻¹¹ mol sterile pyrogen-free human αCGRP (Bachem, UK) was injected intradermally in 50 μL vehicle (50:50, 0.9% saline and Hesperan) into the anesthetized area and an additional untreated skin site. One hour later, the blood flow response to the injection of CGRP was compared in the two sites. On a separate occasion, the effect of 1% capsaicin (pelargonic acid vanillylamide, Fluka Chemika, Switzerland) mixed in Diprobase cream (Schering-Plough, UK) and applied topically to 25 mm² of the skin for 45 minutes was compared at a site pretreated with EMLA for 1 hour and at an untreated site. On six occasions, the effects of

**FIG 3.** Left panels: Scanning laser images of cutaneous blood flow in a region of the forearm pretreated with a eutectic mixture of local anesthetics (EMLA). The images were taken immediately before a 360-second occlusion of forearm blood flow (top left panel) and immediately after reperfusion (bottom left panel). Darker tones (blues) indicate low flows; lighter tones (yellows and reds) indicate high flows. Before occlusion, the skin blood flow is uniformly low. After occlusion, the lighter areas of the image indicate the increased skin flow associated with reactive hyperemia. The darker circular area in the center of the image corresponds to the region of skin treated with EMLA, where the hyperemic response is markedly attenuated, with the exception of a small patch where the EMLA cream had not been applied to determine the effect of the dressing alone. Bar, 1 cm. Right panel: Graph showing the effect of EMLA on the maximal hyperemic response observed using the scanning laser. Data were obtained by computer averaging of the percent flux in 1-cm² regions of skin within and outside the EMLA-treated area. Maximum hyperemic flows were significantly higher than basal rates in both sites, but the responses in the EMLA-treated sites were significantly lower than in untreated sites (% flux, mean±SEM, n=6; *P<.05, **P<.01).
the occlusive dressing applied for 1 hour, with and without a vehicle cream, were tested.

**Data and Statistics**

Cutaneous blood flows are expressed as percent flux, and inhibition is expressed in terms of the percent reduction in the EMLA-treated sites compared with the response observed in the untreated sites. Blood flow changes during the postocclusive reactive hyperemic phase were measured in terms of the time taken to reach the maximum response (seconds) and the recovery of preocclusion flow (seconds), the maximum flow (percent flux), and the index of total flow during the hyperemic phase (integration of the area shown diagrammatically in Fig 1). Statistical comparisons were performed by analysis of variance with data expressed as mean±SEM and significance accepted at values of $P \leq 0.05$.

**Results**

**Effects of Local Anesthesia on Postocclusive Reactive Hyperemia**

Transient arterial occlusion of forearm blood flow induced postocclusion reactive hyperemia in the untreated skin sites of all subjects. The hyperemia increased significantly in magnitude and duration with increasing periods of occlusion. Simultaneous measurements of cutaneous blood flow in the EMLA-treated sites showed significant reductions in the postocclusion hyperemic responses throughout the treated areas of skin (Figs 2 and 3).

There were no significant differences between the basal blood flow rates in the EMLA-treated (5.2±2% flux) and untreated (7.1±1% flux) sites and no differences in the basal rates in the periods after the recovery from the 90-, 180-, and 360-second occlusions (Fig 4A). During occlusions, flow rates fell below 1% flux. The maximum postocclusion hyperemic blood flow was observed between 9 and 20 seconds after the cuff deflation, with no significant differences in the response time in the treated and untreated sites. At the maximum, flow increased to 31±5%, 38±6%, and 49±5% flux in the untreated sites, whereas the significantly lower responses of 14±3% ($P < 0.005$), 20±4% ($P < 0.005$), and 25±3% ($P < 0.001$) flux in the EMLA-treated site indicated inhibitory effects of 54±5%, 46±8%, and 49±5%, respectively. This inhibitory effect of EMLA was also observed when using the scanning laser: the maximum response of 74±15% in a 1-cm² region of the untreated skin after a 360-second occlusion was significantly greater than in an adjacent EMLA-treated site where flow reached 28±16% ($P < 0.01$).

In the untreated sites, the intervals required for the hyperemic blood flow to return to basal rates after the 90-, 180-, and 360-second occlusions were 66±18, 96±20, and 139±26 seconds, respectively. Significantly shorter intervals of 23±5 ($P < 0.05$), 31±6 ($P < 0.005$), and 61±17 ($P < 0.02$) seconds in the EMLA-treated sites showed that the duration of the hyperemic phase was reduced by 40±12%, 64±6%, and 52±9% after the respective occlusion periods (Fig 4B). Accordingly, the total blood flow during the hyperemic phase, determined by integration of the area of the postocclusion blood flow tracing, was significantly lower in the EMLA-treated sites when compared with the untreated (Fig 4C) or saline-injected sites (Fig 5). The total blood flow in the hyperemic phase after 360-second occlusions was inhibited by 72±7% in the EMLA-treated site. In these experiments, the effect of intradermally injected indomethacin was also determined. Indomethacin reduced the total blood flow response to occlusion by 59±8% (Fig 5). Combining EMLA with indomethacin reduced the blood flow response by no more than EMLA alone, i.e., 71±8%. After all of the 360-second occlusion periods, three of the 180-second occlusions, and one of the 90-second occlusions, oscillations of blood flow (5% to 10% flux) at a frequency of approximately 7 cycles per minute (0.12 Hz) were observed during the return of blood flow to basal values in the untreated sites (see Fig 2). In the corresponding EMLA-treated sites, these oscillations were not observed.

**Effects of Local Anesthesia on Increased Skin Blood Flow Induced by CGRP and Capsaicin**

The intradermal injection of $1 \times 10^{-11}$ mol CGRP increased cutaneous blood flow from 5±1% to 83±9% in control sites and from 4±1% to 79±12% in the EMLA-

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**Fig 4.** Panel A: Graph showing maximum hyperemic blood flow responses after increasing intervals of forearm blood flow occlusion in untreated skin sites (○) and skin sites treated with a eutectic mixture of local anesthetics (EMLA, ●). Increasing intervals of occlusion were associated with greater maximal responses, which were inhibited in the EMLA-treated sites (mean±SEM, n=8; $*P < 0.005$, $**P < 0.001$ for differences after respective occlusion periods). Symbols joined by dashed lines indicate the resting skin blood flow rate in the two sites immediately before each occlusion. Panel B: Graph showing time taken for return to basal flow rates after increasing intervals of occlusion. Recovery times increased with longer occlusion periods but were shortened significantly in EMLA-treated sites ($*P < 0.05$, $**P < 0.005$). Panel C: Graph showing total flow during the hyperemic phases. Total hyperemic blood flow was increased after longer occlusion periods but was markedly reduced in sites treated with EMLA.
pretreated sites (Fig 6A). During the 360-second occlusion, flow in the CGRP-injected site fell rapidly to less than 1% flux and then returned to 86±10% at 40 to 60 seconds after the release of the cuff pressure, without evidence of a superimposed reactive hyperemic phase.

The topical application of capsaicin induced local erythema, increasing blood flow from 10±2% to 72±5%, and extensive surrounding flare, which caused 55±13% increased flux at a distance of 2 cm from the central erythema (Fig 6B). In the EMLA-treated site, there was no flare associated with the capsaicin treatment, but local erythema corresponding precisely to the area of capsaicin application caused an increase of flow from 9±1% to 73±9%. Neither the occlusive dressings or the Diprobase cream carrier had any significant effect on cutaneous blood flow.

Discussion

Extensive studies have shown the widespread distribution, release, and vasoactivity of sensory neuropeptides localized in peripheral sensory nerve terminals associated with mammalian blood vessels. The physiological role of these peptides and the mechanisms that can stimulate their release remain unclear. Previous studies have shown that the release of CGRP and substance P may be important in the inflammatory response, where axon-reflex-mediated flare and local erythema are characteristic features. In the present study, we provide evidence that sensory nerve axon reflexes, and hence their constituent neuropeptides, are involved in the mechanisms promoting reactive hyperemia after arterial occlusion in human skin.

The principal findings of this study are as follows: (1) Increasing periods of arterial occlusion were associated with increases in the magnitude and duration of postocclusive reactive hyperemia in the microvasculature of the skin measured by laser Doppler flow metering. (2) Pretreatment of the skin with the local anesthetic EMLA, sufficient to cause the loss of pain sensation, reduced the magnitude and duration of the reactive hyperemia. (3) Locally injected indomethacin suppressed reactive hyperemia. (4) The local anesthesia did not affect the local increase in cutaneous blood flow induced by intradermally administered CGRP or topically applied capsaicin, but it abolished the surrounding flare induced by capsaicin. From these observations, we postulate that topically applied EMLA blocks action potential conduction in local sensory nerves, including those participating in axon-reflex neurotransmission and, in conjunction with local prostanoid formation, contributes to the observed reduction in postocclusive hyperemia.

Duff and Shepherd's plethysmographic observations of forearm blood flow in patients with severely impaired sensation resulting from brachial plexus injury showed that postocclusive hyperemia could be reduced by up to 38% in the involved limb. They thought this may be attributed to a loss of vasodilator substances associated with the chronically denervated limb. In trigeminal nerve-sectioning experiments in the cat, Moskowitz et al. noted that rhizotomy preserved peripheral axons and normal cerebral hyperemia after occlusion of the supply vessels. However, at sites where peripheral axons were degenerated after ganglionectomy, the hyperemia response was attenuated by more than 50%, which agrees closely with the extent of inhibition reported in the present study. In Lembeck and Donnerer's study, chronic denervation and capsaicin pretreatment, known to deplete peripheral sensory nerves, also reduced reactive hyperemia in the rat hind limb through mechanisms thought to involve substance P. The subsequent discovery of the potent vasodilator activity of CGRP in the microvasculature and its localization in sensory nerves suggest that it too may be involved in neural
regulation of the vasculature. More recent work by Macfarlane et al. has shown that the attenuation of the hyperemic response in the cat cerebral circulation after sensory denervation is unlikely to involve the endothelium-dependent vasodilator substance P. They reported a loss of endothelium-dependent vasodilator responses after occlusion, suggesting that the non-endothelium-dependent vasodilator effects of CGRP are more likely to be those responsible for the neural component of reactive hyperemia. In the present study, we cannot determine which of the sensory nerve neuropeptides is involved. However, previous observations show that substance P-induced vasodilation undergoes rapid tachyphylaxis after infusion into the forearm circulation. We have not observed significant alterations in the hyperemic responses after repeated episodes of occlusion, suggesting that mediators that undergo tachyphylaxis are not involved.

The suggestion that local sensory nerve reflexes are involved in the responses observed in the present study depends on the selectivity of this type of anesthetic for local sensory nerve conduction rather than direct effects on other neurons, transmitter release, or vascular smooth muscle tone. In the present study, we confirmed the specific action of EMLA with several observations. The efficacy of local anesthetics in selectively blocking local nerve action potential conduction is largely dependent on the access of the drug to exposed sections of nerve axon and the diameter of the nerve fiber. It is established that unmyelinated small-diameter nerve fibers are more readily blocked and that different critical lengths of fiber have to be exposed to anesthesia to achieve blockade in functionally different nerve types. With their shorter critical lengths and small diameter, the sensory C fibers are preferentially blocked in a fashion that is reflected by the loss of sensory function in a predictable order: pain sensation is lost first, followed by the sensations of cold, warmth, touch, and deep pressure. When tested at the site of EMLA treatment, our subjects did not sense pain in response to a needle point but retained other sensations, indicating that selective sensory C fiber blockade was achieved.

Capsaicin, the pungent principle of peppers, selectively activates peripheral endings of sensory C fibers, causing membrane depolarization and the release of vasoactive neuropeptides including CGRP and substance P by mechanisms similar to the peptide release that can be induced by antidromic nerve stimulation. The response to administration of capsaicin in animal skin is characterized by increased microvascular blood flow that can be partially inhibited by the Fab fragment of anti-CGRP antibodies or the CGRP antagonist CGRP-A. In the present study, we exposed the skin to capsaicin to test whether the characteristic release of sensory nerve neuropeptides was affected by local anesthesia. The local anesthesia did not modify the increase in skin blood flow induced by capsaicin at the site of its application but abolished the surrounding flare reaction. This indicates that EMLA did not alter the release of endogenous vasodilators from sensory nerve terminals, although the flare response mediated by axon-reflex mechanisms was blocked.

Therapeutic doses of local anesthetics stabilize cell membrane potential in excitable tissues including muscle. However, they do not have any significant direct effects on vascular smooth muscle tone. Studies of the effects of procainamide and lidocaine in human and animal isolated blood vessel segments and blood flow studies in vivo have shown that local anesthetics generally have no effect, or only cause small degrees of vasorelaxation, in some precontracted blood vessels. To confirm this in the present study we injected CGRP to determine whether the vasodilator response of skin microvasculature was altered by the local anesthetic. The EMLA pretreatment had no significant effects on basal skin blood flow rates. Further, EMLA had no effect on the vasodilation induced by CGRP, suggesting that the anesthetic had little effect on resting blood vessel tone and did not alter postsynaptic mechanisms of vasodilation in response to the sensory neuropeptide.

We observed that EMLA abolished the oscillations of blood flow during the reactive hyperemia resulting from longer occlusion periods. These oscillations are reported elsewhere and appear to be coordinated by a diffuse common pacemaker system perhaps of neural origin. The oscillations and vascular responses mediated by axon reflexes can be inhibited by stimuli that cause sympathetic activation in the skin, suggesting that axon-reflex modulation of postocclusion blood flow can be modulated by other nerve interactions.

The study by Lembeck and Donnerer also showed that pretreatment with compound 48/80, which depletes mast cells, had an inhibitory effect on reactive hyperemia in the rat, suggesting an involvement of histamine release from mast cells. Although EMLA and other local anesthetics block histamine-induced flare in human skin, they do not modify the direct vasodilator effects of histamine in the microcirculation. Furthermore, studies in isolated rat mast cells show that the release of histamine induced by compound 48/80 was only inhibited when relatively high concentrations of nonionized local anesthetics were used. EMLA is ionized and buffered to a near neutral pH after absorption through human skin, and in the present study, the selective blockade of pain sensation alone confirms that only low doses of anesthetic penetrated the skin. Therefore, it is unlikely that the EMLA altered histamine release from mast cells.

Combining the evidence that the local anesthetic is primarily selective for C fibers with our observations that EMLA causes local analgesia and inhibition of flare responses but does not appear to alter neurotransmitter release or vascular responses to vasodilators, we suggest that the inhibitory action of EMLA in this study is mediated by action potential conduction blockade in peripheral sensory neurons. Such blockade would prevent sensory nerve terminal excitation and the release of vasodilator neuropeptides in axon-reflex-mediated responses.

The link between vascular occlusion and stimulation of sensory nerves has not been determined. Ischemia resulting from arterial occlusion is associated with the local accumulation of several mediators known to stimulate sensory nerves. These include histamine, potassium, and prostaglandins, and several studies have shown that these stimuli can excite sensory neurons to release vasodilator neuropeptides. Ischemia and bradykinin, but not hypoxia, can induce the outflow of CGRP or substance P from the isolated guinea pig heart, whereas decreasing pH can cause neuropa-
tide release from bladder tissue. In the rabbit ear, thermal stimulation, capsaicin, and potassium can cause the release of substance P from local peripheral nerve terminals associated with vasodilation. Although the low metabolic demands of the skin may only cause relatively low concentrations of ischaemia-induced metabolites to accumulate during occlusion, their stimulatory effects on sensory nerves may be enhanced by the sensitization of the neurons. Geppetti et al. have shown that arachidonic acid and bradykinin induce the release of CGRP from the guinea pig heart by mechanisms sensitive to indomethacin and capsaicin treatment. This suggests that products of arachidonic acid metabolism can lead to neuropeptide release, and the authors postulate that eicosanoids can activate sensory neurons. In the present study, we show that reactive hyperemia is partially dependent on mechanisms sensitive to indomethacin. This may be because of the direct vasodilator activity of arachidonic acid metabolites, e.g. prostaglandins E₂ and I₃, on the microvasculature. Alternatively, the arachidonic acid metabolites may stimulate or sensitize sensory nerves by analogy with the effect of prostaglandins in inducing hyperalgesia.59,60

In summary, we propose that mediators which accumulate during ischemia induced by arterial occlusion can stimulate sensory nerves to release vasodilators, perhaps including CGRP and substance P, to mediate part of the subsequent postocclusive reactive hyperemia. The present study shows the important contribution of sensory nerves in the regulation of vascular tone.

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References


44. Altura BM, Altura BT. Effects of local anesthetics, antihistamines, and glucocorticosteroids on peripheral blood flow and vascular smooth muscle. Anesthesiology. 1974;41:197-214.


49. Lewis T, Grant RI. Vascular reactions of the skin to injury, II: the liberation of a histamine-like substance in injured skin: the underlying cause of factitious urticaria and of wheals produced by burning, and observations upon the nervous control of certain skin reactions. Heart. 1924;11:209-265.


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