

The Effect of Iontophoresis on the Cutaneous Vasculature: Evidence for Current-Induced Hyperemia¹

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Received November 30, 1994

Combining laser-Doppler blood flux measurements of the skin microcirculation with iontophoresis of vasoactive agents is a promising noninvasive tool for pharmacological studies. However, preliminary observations in our laboratories suggested significant current-associated vasodilation when an expected vasoconstrictor (N⁰-monomethyl-L-arginine acetate) was iontophored. The present study was designed to define nonspecific current-related versus specific pharmacological effects of iontophoretically administered ions on the cutaneous vasculature. Dose-response studies to a series of anions (nitrite, chloride, acetate, and bicarbonate) and cations (sodium, lithium, and acetylcholine) were carried out in six healthy volunteers (three male) by iontophoresis to the forearm skin on separate days. Laser-Doppler flux was measured at the same sites. All ions caused dose-dependent vasodilation. There was no difference in the response between chloride, bicarbonate, or acetate and nitrite, the nitric oxide donor. The acetylcholine dose response was shifted rightward after atropine pretreatment. Cutaneous vascular responses to iontophoresis comprise nonspecific, current-induced hyperemia and specific effects of the administered agent. Acetylcholine appears to cause muscarinic and current-induced dilatation. Nitrite may cause current-induced hyperemia alone. Current-induced hyperemia should be considered in interpreting the acute cutaneous vascular responses to iontophoretically administered agents in humans. © 1995 Academic Press, Inc.

INTRODUCTION

Cutaneous iontophoresis of pharmacologically active agents, with simultaneous laser-Doppler measurement of dermal blood flux at the site of administration, is a promising noninvasive tool for studying the physiology and pathophysiology of the skin microcirculation in humans [1-3].

Laser-Doppler flowmetry is based on the frequency shift of low-power laser light caused by moving red blood cells in the capillaries and is used as an index of blood flow. It is specific to skin and is not influenced by underlying muscle blood flow [4].

Iontophoresis is defined as the introduction, by means of electrical current, of ions of soluble salts into the tissues of the body [5]. The technique is noninvasive and avoids the potential confounding effects of systemic drug administration. The extent of drug absorption into the skin is proportional to the magnitude and duration of current applied

¹ Data from this study were presented at the Clinical Research Meeting of the American Federation for Clinical Research, Baltimore, MD, 1994.

[6, 7] so that the current · time product is an index of drug dose. For example, we have shown that single doses of acetylcholine and sodium nitrite led to the same skin vascular response as the same dose nested in the dose–response curve [8].

Studies of the dermal microcirculation can provide useful experimental information regarding, for example, the physiology of cutaneous thermoregulation and microvascular pathophysiology in disorders such as hypertension and diabetes mellitus [9]. Lindblad *et al.* [10] first described the combination of laser-Doppler flowmetry and cutaneous iontophoresis of norepinephrine. Kellogg *et al.* [11, 12] combined iontophoretical pretreatment with bretylium tosylate (to block noradrenergic vasoconstrictor nerves in the skin) and laser-Doppler measurement of skin blood flow under heat stress and prolonged exercise. Westerman *et al.* [13] observed apparently impaired cutaneous dilator responses to endothelium-dependent (acetylcholine) and endothelium-independent (sodium nitrite) vasodilators in diabetic patients with peripheral neuropathy compared to healthy controls.

Care must be taken in interpreting the response to iontophoretically administered drugs. First, the concentration of drug at the site of action is unknown [14]. Even if the amount of residual drug in the delivery reservoir can be assayed, degradation within the reservoir, the extent of dermal penetration, and the rate of removal of drug by metabolism and by washout in the microcirculation are unknown quantities. Analysis of the “dose” response to an administered drug may overcome these uncertainties and provide a reasonable basis for quantifying drug effect. Provided that a wide range of response can be provoked from threshold to near maximum, and that a reproducible surrogate of drug dose is available (in this case the current · time product), dose–response studies can be performed without knowledge of the actual dose administered or concentration achieved. This may be particularly useful when studying the actions of putative antagonists. Second, since iontophoresis inevitably causes electrochemical disturbances in the current path, independent of any pharmacological effects of the drug, any observed response could be the net outcome of both processes. It has been assumed that the pharmacological effects predominate, but this has never in fact been confirmed experimentally.

The present study tests the hypothesis that cutaneous dilation after iontophoresis represents a combination of nonspecific, current-mediated vasodilation and the specific pharmacological effect of the administered drug. The impetus was our unexpected observation that direct current iontophoresis of N^G-monomethyl-L-arginine acetate (L-NMMA), a false substrate for nitric oxide biosynthesis [15], led to marked local cutaneous vasodilation in human volunteers (unpublished). Given that basal release of nitric oxide in the forearm arterial circulation maintains vasodilation under resting conditions [16] we had expected L-NMMA to reduce dermal blood flow.

The study was designed to compare responses to a series of anions and cations, including the endothelium-dependent and -independent vasodilators acetylcholine and sodium nitrite. The monovalent cations sodium and lithium were selected for comparison with acetylcholine. The monovalent anions chloride, bicarbonate, and acetate were selected for comparison with nitrite. Additionally, we speculated that bicarbonate and acetate might buffer current-induced dermal acidosis and provide some indirect evidence for or against pH change as a possible mechanism of iontophoretic vasodilation.

METHODS

Subjects

Six healthy subjects (three male) participated. Their average (\pm SD) age was 38 ± 5 years, height was 174 ± 3 cm, and weight was 70 ± 3 kg. The study was approved

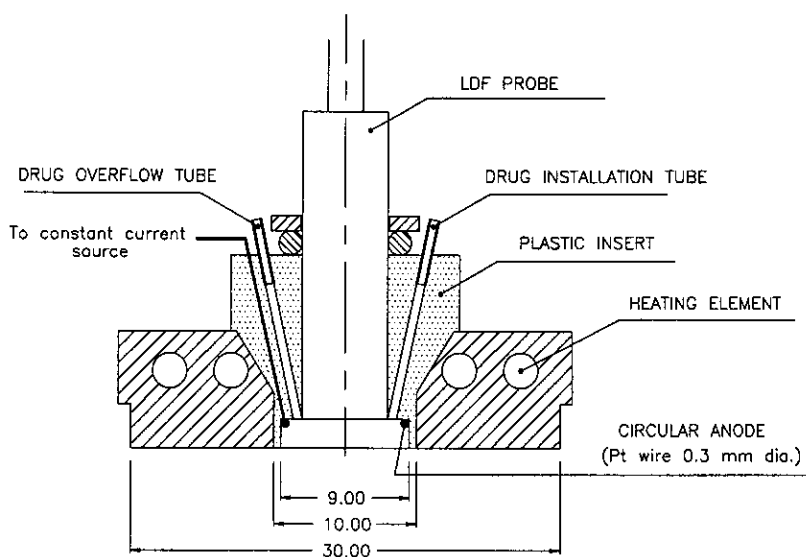


FIG. 1. Cross-sectional view of the iontophoresis heater-laser-probeholder. Dimensions are in millimeters.

by the University of Texas Health Science Center Institutional Review Board. All subjects gave informed written consent to participate.

Subjects were nonsmokers and in normal health by history and physical examination. Medications other than acetaminophen were prohibited in the 2 weeks prior to the study.

Laser-Doppler Flowmetry and Iontophoresis

A customized aluminum probeholder designed and constructed in our laboratories was used to permit the simultaneous administration of drugs by iontophoresis, measurement of cutaneous laser-Doppler flux (LDF), and control of the skin temperature (Fig. 1). Multifiber laser probes were used (MBF3D laser blood flow monitor, Moor Instruments Ltd., Devon, England). The plexiglass iontophoresis chamber had a diameter of 9 mm (area, 0.64 cm^2) with a platinum ring electrode recessed 2 mm from the skin surface. The indifferent electrode, a 4×2.5 -cm copper plate padded with cotton gauze and moistened with electrode gel, was mounted at the volar surface of the same wrist. Both electrodes were connected to an isolated constant current source delivering up to $350 \mu\text{A}$ (Texpol, San Antonio, TX). Current duration was controlled manually. Skin temperature was recorded by a thermocouple between probeholder and skin and maintained constant at $34 \pm 0.1^\circ$ by servo control of the heating elements in the probeholder block.

Mean arterial pressure (MAP) was measured continuously from the left middle finger (Finapres BP monitor, Ohmeda, Englewood, CO). The laser-Doppler flowmeter was calibrated with a Latex suspension (Moor Instruments Ltd.). The units of LDF are arbitrary.

Drugs

The following salts were used: sodium acetate, sodium bicarbonate (Fischer Scientific Co., Fair Lawn, NJ), sodium nitrite, sodium chloride, lithium chloride, acetylcho-

TABLE 1
Current–Time Schedule of the Iontophoresis

Current (μA)	Duration (sec)	CTP ($\mu\text{A} \cdot \text{sec}$)	CTP/A ($\mu\text{A} \cdot \text{sec}/\text{cm}^2$)	Time of administration (min)	
				Cations	Anion
5	20	100	156	00:00	00:00
5	60	300	469	01:30	04:00
5	180	900	1406	04:00	12:00
15	180	2700	4219	09:00	20:00–22:00 ^a
45	180	8100	12656	14:00	28:00–32:00 ^a
90	270	24300	37969	19:00	36:00–42:00 ^a

Note. CTP, current time product; A, area of skin treated (0.64 cm^2).

^a Depending on the time to onset of a plateau of response.

line chloride, and atropine sulfate (all from Sigma Chemical Co., St Louis, MO). Direct current iontophoresis was performed with 10 mM solutions of these salts in nonconducting propylene glycol (1,2-propanediol, Sigma Chemical Co.).

Protocol

All experiments were performed between 9:00 and 11:00 AM. Subjects rested supine at least 20 min prior to data collection. The room temperature was $24 \pm 1^\circ$. The left forearm rested on a rigid arm support. Two probeholders were attached to the volar aspect of the left upper forearm skin with double-stick discs (3M Medical Device Division, St. Paul, MN). Dose responses were constructed for each agent according to the schedule indicated in Table 1. The schedule was based on preliminary experiments [9] and reflects the times required to reach steady-state response after each dose.

In one set of experiments a 0.64 cm^2 area of the forearm skin was pretreated by iontophoretically applying atropine sulfate at a dose of $400 \mu\text{A} \cdot \text{cm}^{-2}$ for 35 sec to block cutaneous muscarinic receptors. An initial vasodilation lasting up to 70 min was seen at the atropine-treated sites. Acetylcholine dose responses were carried out at these sites after skin blood flow had returned to baseline. We have shown that the current \cdot time dose selected for atropine abolishes the sudomotor response to hyperthermia and to acetylcholine iontophored at $31 \mu\text{A} \cdot \text{cm}^{-2}$ for 45 sec [17].

Data Acquisition and Analysis

The data acquisition system and temperature servo control were based on an IBM-compatible personal computer and virtual instrumentation developed in our laboratory within LabVIEW for Windows (National Instruments, Austin, TX).

Since no plateau of response was seen at the highest doses in some experiments, reliable estimates of the conventional dose–response parameters [E max (the maximum response) and ED₅₀ (dose to achieve half E max)] could not be obtained by nonlinear curve fitting. Two-way repeated measures analysis of variance was therefore performed to determine vascular effects of iontophoresis with main factors of dose and drug iontophored. When an interaction was found, planned contrasts were performed to determine individual paired differences. All data are presented as means and SEM. The alpha level for statistical analysis was set at 0.05.

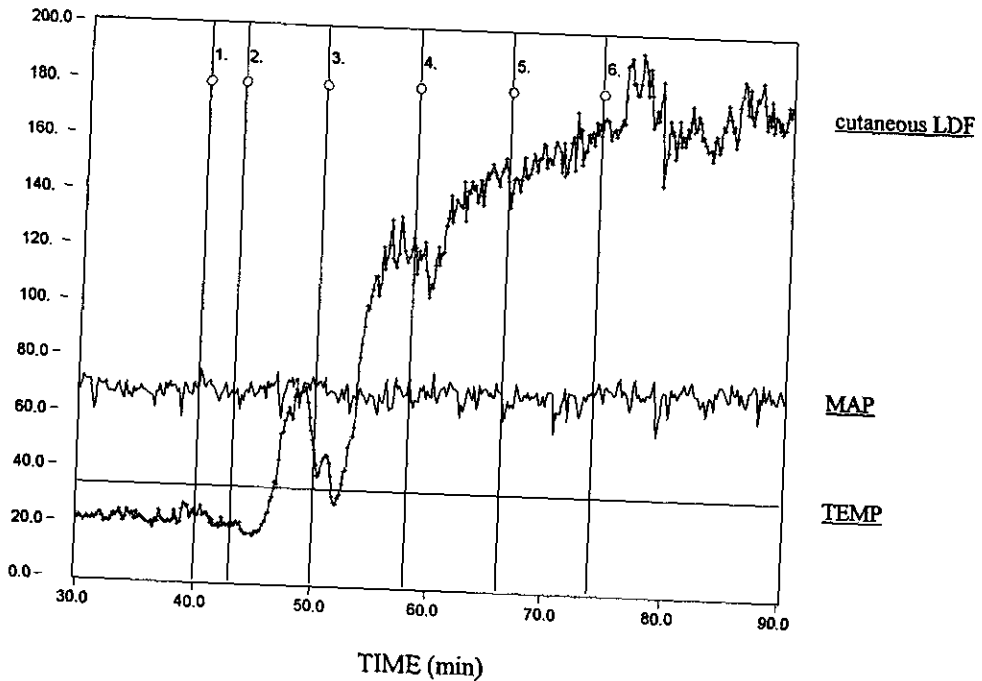


FIG. 2. Representative example of the response of cutaneous laser-Doppler flux (LDF), finger mean arterial pressure (MAP) and skin temperature (TEMP) after iontophoretic administration of increasing doses of nitrite. The flux units are arbitrary, MAP is in millimeters of mercury, and TEMP is in degrees centigrade. Periods of iontophoresis are indicated: 1, $100 \mu\text{A} \cdot \text{sec}$; 2, $300 \mu\text{A} \cdot \text{sec}$; 3, $900 \mu\text{A} \cdot \text{sec}$; 4, $2700 \mu\text{A} \cdot \text{sec}$; 5, $8100 \mu\text{A} \cdot \text{sec}$; 6, $24300 \mu\text{A} \cdot \text{sec}$.

RESULTS

Independent of their polarity, all anions and cations given in this study led to significant increases in LDF. Figure 2 illustrates the time course of the LDF response to the iontophoresis of the anion, nitrite, in one subject as a representative example. The vertical lines indicate the beginning of the iontophoretical application of each dose of nitrite, beginning with $100 \mu\text{A} \cdot \text{sec}$ (dose 1) up to $24,300 \mu\text{A} \cdot \text{sec}$ (dose 6). LDF increased after the second dose of nitrite, $300 \mu\text{A} \cdot \text{sec}$. Similar response patterns were seen with all other anions, which provoked dose-dependent increases in LDF. The responses to chloride, bicarbonate, and acetate were not significantly different from those to nitrite (Fig. 3). No change in blood pressure was seen in any subject.

Figure 4 shows dose responses to the cations, sodium, lithium, and acetylcholine. Iontophoresis of sodium and lithium led to a significant increase of LDF at doses higher than $900 \mu\text{A} \cdot \text{sec}$. There were no significant differences in response between sodium and lithium. Responses to the second and subsequent doses of acetylcholine were significantly higher than the responses to sodium or lithium.

After local iontophoretic pretreatment with atropine the acetylcholine dose response was significantly lower compared to the untreated site (Fig. 5) at all but the first dose. There was a significant increase in LDF after the first dose of acetylcholine without pretreatment. The atropine-pretreated site showed a significant increase after the third dose.

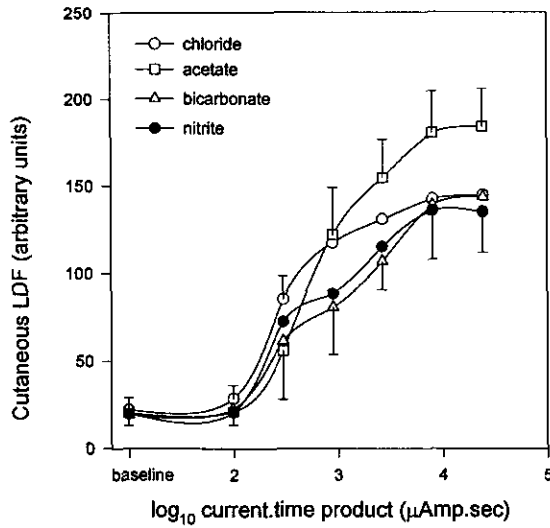


FIG. 3. Dose-response curves of cutaneous laser-Doppler flux (LDF) after chloride, acetate, bicarbonate, and nitrite iontophoresis. Error bars are SEM. Some error bars have been omitted for clarity.

DISCUSSION

All chemicals iontophored in this study increased cutaneous laser-Doppler flux, suggesting a nonspecific vasodilator effect of iontophoretic current. Since iontophoresis by definition introduces charged particles, any of which may have direct effects on membrane potential or ion fluxes, the present study does not directly prove the hypothesis that iontophoretic current *per se* causes hyperemia. Indeed, most of the salts given in this study exhibit vasoactivity. Sodium gradients influence calcium transients and myogenic

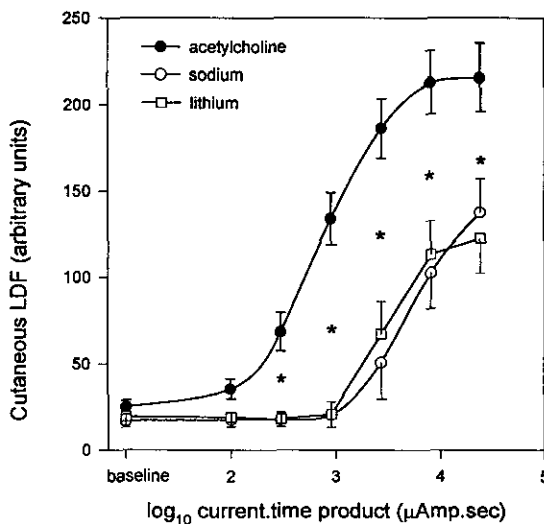


FIG. 4. Dose-response curves of cutaneous laser-Doppler flux (LDF) after sodium, lithium, and acetylcholine iontophoresis. Error bars are SEM. * $P < 0.05$ for acetylcholine vs each other cation.

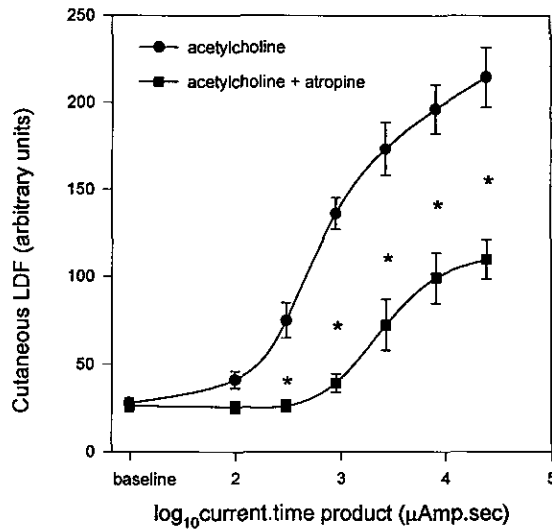


FIG. 5. Dose-response curves of cutaneous laser-Doppler flux (LDF) after acetylcholine iontophoresis, with and without atropine pretreatment ($8890 \mu\text{Amp}\cdot\text{sec}$). Error bars are SEM. * $P < 0.05$.

tone in vascular smooth muscle [18, 19]. Sodium chloride causes relaxation of rabbit coronary arteries, perhaps due to membrane hyperpolarization [20]. Low concentrations of lithium enhance endothelin-1-induced vasoconstriction [21] but we know of no evidence in the literature for direct lithium-induced vasodilation. Bicarbonate may cause vasodilation through activation of $\text{Na}^+\text{-H}^+$ exchange [22]. Acetate has been shown to vasodilate by complexing ionized calcium [23].

Application of direct current to the skin induces pH changes: acidosis under the anode and alkalosis under the cathode [24]. pH changes in both directions are known to cause vasodilation [25, 26]. Bicarbonate and acetate are expected to buffer pH changes. Nevertheless, vasodilation was observed with both ions, suggesting that pH changes were not solely responsible for vasodilation. However, we did not measure dermal pH in the present noninvasive study and therefore cannot discount pH change as a contributory mechanism. Future studies employing cutaneous microdialysis may shed further light on this question.

The anions generally produced greater responses than the cations sodium and lithium at lower doses (up to $2700 \mu\text{A}\cdot\text{sec}$). This could reflect a "solvent drag" phenomenon, in which particle flux through pores and hair follicles is enhanced. This is reported to be greater in anionic iontophoresis [27].

An important finding of this study was that dilation after nitrite was no greater than after the other anions. This suggests that the response to sodium nitrite iontophoresis represents a current-associated effect of nitrite, rather than a pharmacological effect of nitrite-derived nitric oxide. This casts doubt on the validity of nitrite iontophoresis as a model of nitric oxide-mediated, endothelium-independent vasodilation.

Of the cations, acetylcholine led to the highest vasodilation, suggesting an additional specific pharmacological effect beyond that due to current. Acetylcholine stimulates the release of nitric oxide, prostacyclin, and a hyperpolarizing relaxant factor from the vascular endothelium [28–31]. Atropine shifted the acetylcholine dose-response curve rightward in a fashion that suggests noncompetitive muscarinic antagonism.

This form of antagonism contrasts with classic theory, which predicts competitive antagonism [32], but has been reported for nicotinic responses [33]. Since the local concentrations of acetylcholine and atropine are unknown, since only one dose of atropine was given, and since we cannot exclude the possibility of current-mediated vasoactivity accompanying atropine iontophoresis, we must interpret the acetylcholine/atropine interaction with some caution. The shift in the acetylcholine dose response appears to represent specific pharmacological antagonism by atropine. Since we used only one dose of atropine, we cannot say whether or not higher doses might have attenuated the acetylcholine response more. It is possible that atropine at the dose given fully antagonized the specific effect of acetylcholine, and that the remaining dilation seen in Fig. 5 is solely a nonspecific current-mediated response. That the atropine-pretreated acetylcholine dose-response curve overlays the sodium and lithium curves lends support to this suggestion.

The precise mechanism of the nonspecific vasodilation induced by iontophoresis remains unknown but the phenomenon should be taken into consideration in interpreting responses to iontophoretically applied chemicals and drugs. The effect of iontophoretically applied acetylcholine suggests a substantial pharmacological component. Acetylcholine iontophoresis may therefore be a useful tool for studying the vascular endothelium in conditions such as hypertension and diabetes. Given the extent of current-associated hyperemia that we have seen, the combination of iontophoresis of vasodilator agents with laser-Doppler flowmetry must be used and interpreted with care.

ACKNOWLEDGMENTS

This study was supported by a grant from the North Atlantic Treaty Organization (NATO) through the German Academic Exchange Service (M.G.). M.J.J. is recipient of a Pharmaceutical Research and Manufacturers of America (PhRMA) Foundation Faculty Development Award in Clinical Pharmacology. D.L.K. is a Merck AFAR fellow.

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