Early vasodilator response to anodal current application in human is not impaired by cyclooxygenase-2 blockade

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Early vasodilator response to anodal current application in human is not impaired by cyclooxygenase-2 blockade. Am J Physiol Heart Circ Physiol 288: H1668–H1673, 2005. First published November 24, 2004; doi:10.1152/ajpheart.00415.2004.—It is generally acknowledged that cutaneous vasodilatation in response to monopolar galvanic current application would result from an axon reflex in primary afferent fibers and the neurogenic inflammation resulting from neuropeptide release. Previous studies suggested participation of prostaglandin (PG) in anodal current-induced cutaneous vasodilatation. Thus the inducible cyclooxygenase (COX) isoform (COX-2), assumed to play a key role in inflammation, should be involved in the synthesis of the PG that is released. Skin blood flow (SkBF) variations induced by 5 min of 0.1-mA monopolar anodal current application were evaluated with laser-Doppler flowmetry on the forearm of healthy volunteers treated with indomethacin (COX-1 and COX-2 inhibitor), celecoxib (COX-2 inhibitor), or placebo. SkBF was indexed as cutaneous vascular conductance (CVC), expressed as percentage of heat-induced maximal CVC (%MVC). Urinalyses were performed to test celecoxib treatment efficiency. No difference was found in CVC values at rest: 14.3 ± 4.0, 11.9 ± 3.2, and 10.9 ± 2.0% MVC after indomethacin, celecoxib, and placebo treatment, respectively. At 10 min after the onset of anodal current application, CVC values were 22.2 ± 4.9% MVC (not significantly different from rest) with indomethacin, celecoxib, and placebo treatment, respectively. Celecoxib significantly depressed the urinary prostacyclin metabolite 6-keto-PGF1α, (P < 0.05 vs. placebo). Indomethacin, but not celecoxib, significantly inhibited the anodal current-induced vasodilatation. Thus, although they are assumed to result from an axon reflex in primary afferent fibers and neurogenic inflammation, these results suggest that the early anodal current-induced vasodilatation is mainly dependent on COX-1-induced PG synthesis. Neurogenic inflammation; neuropeptide release; skin blood flow

ABNORMALITIES OF THE MICROCIRCULATION play a major role in the complication of diabetes (44) and are also observed in coronary heart disease (32). Thus noninvasive methods for assessing peripheral microvascular function are of considerable clinical interest. Continuous monopolar galvanic current application can be used to facilitate the migration of charged molecule across the skin and, thus, allows for the study of endothelium-dependent and non-endothelium-dependent vasodilatation. However, in the human, current induces a cutaneous vasodilatation that could interfere with assessment of microvascular function. Therefore, the study of underlying mechanisms involved in the cutaneous current-induced vasodilatation deserves further investigation.

It is generally acknowledged that the cutaneous vasodilatation in response to monopolar galvanic current application would result from an axon reflex (3, 15) and neurogenic inflammation (3). Indeed, this cutaneous vasodilatation is abolished under local anesthesia and largely decreased after desensitization of C-nociceptive fibers by chronic application of capsaicin cream (8). The axon reflex-related cutaneous vasodilatation relies on the local release, from primary afferent fibers, of neural mediators such as calcitonin gene-related peptide, substance P (16), and prostaglandin (PG) (31). PGs are likely essential for the axon reflex-related cutaneous vasodilatation induced by anodal current application, because the current-induced cutaneous vasodilatation is almost abolished by aspirin treatment (7). Aspirin impairs PG biosynthesis through the irreversible blockade of cyclooxygenase (COX) (41). Two isoforms of COX have been identified: COX-1, a constitutive isoform expressed in most cells, present under physiological conditions, and COX-2, an inducible isoform arising in response to inflammatory stimuli (4). Constitutive COX-1 is the enzyme involved in the basal flow in the uninjured state. On the other hand, the available literature is consistent with the assumption that vasodilatation induced during neurogenic inflammation and/or neuropeptide release depends on the inducible COX-2 isoform (17, 31, 39). In parallel with its effects on COX, aspirin could also impair the anodal current-induced vasodilatation by blocking the vanilloid receptors (VR1) (38) and/or by interfering with the function of acid-sensing ion channels (ASICs) (45), which are possibly involved in the response.

We aimed to confirm that cutaneous vasodilatation induced by anodal current application relies on PG. For this purpose, we studied the effects of the nonselective COX inhibitor indomethacin, which, in contrast to aspirin, has not been shown to have a direct effect on VR1 or ASICs. Then we aimed to test whether anodal current-induced vasodilatation relies on COX-2-dependent PG synthesis. For this purpose, we studied the effects of the COX-2-specific inhibitor celecoxib on anodal current-induced cutaneous vasodilatation.

MATERIALS AND METHODS

Subjects

Sixteen nonsmoking healthy volunteers with no clinical sign of, or risk factors for, vascular disease participated in the study. Anthropo-

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whereas the excretion rate of thromboxane B2 was not statistically different from placebo. However, low oral doses of indomethacin reduce urinary excretion (10, 30), which is correlated with inhibition of PG synthesis. Local cutaneous temperature was measured using a surface thermocouple probe connected to an electronic thermometer (model BAT-12, Physitemp Instruments, Clifton, NJ). The thermocouple was positioned 6 cm from the laser probes. Systemic arterial blood pressure was monitored using a Finapres 2350 (Ohmeda) position on the second or third finger of the hand contralateral to the sites of SkBF measurement. Microvascular investigations were performed in a quiet air-conditioned room with ambient temperature set at 24 ± 1°C. To avoid side effects, position of the probes on the forearm and arm (right or left) was chosen randomly for each microvascular investigation (14). The subjects were placed in a supine position and rested for 15 min before data collection. A resting period of 2 min was recorded at the beginning of each experiment before the start of current application. The current application consisted of transcutaneous delivery of a 0.1 mA anodal current for 5 min. After current application, a 20-min period was recorded. Thereafter, the skin under the active probe was warmed to 44°C for 24 min to attain maximal vasodilatation and limit the total duration of each experiment to <1 h.

Measurement

The laser-Doppler flowmeter technique has been shown to accurately monitor SkBF continuously (18, 34) and is not influenced by underlying muscle blood flow (35). Data were recorded on a computer via an analog-to-digital converter (Biopac System) with a sample frequency of 3 Hz. Because of instantaneous variability of vasomotion, individual laser-Doppler flowmeter signals were averaged over 15-s intervals throughout each experiment. To take into account possible changes in systemic hemodynamic conditions, SkBF was indexed as cutaneous vascular conductance (CVC) calculated as the

![Diagram](http://ajpheart.physiology.org/Downloadedfrom10.220.32.246onNovember10,2017)

**Fig. 1.** Section view of the “active” probe used in experiments designed to allow simultaneous skin blood flow recording and local heating. Current is applied through a patch inserted in a circular chamber below the probe.
ratio of SkBF expressed in arbitrary units to mean arterial blood pressure over the same 15-s interval. Maximal CVC in response to local heating represented the mean CVC values observed over the last minute of the heating period. Then CVC was normalized for each subject, with maximal CVC equal to 100% to better reflect changes in SkBF (20, 29), and results are expressed as percentage of heat-induced maximal CVC (%MVC).

To compare short- and long-term components of the response to current application, four points were analyzed: CVC at rest and at 5, 10, and 25 min, corresponding to the time before current application, the end of current application, and 5 and 20 min after the end of current application, respectively.

*Urinalyses*

A few milliliters of fresh urine were used to assess urinary density and creatine concentration, and 10 ml of urine were stored at −80°C for later urinalyses. We measured 11-dehydrothromboxane (Tx-M) and 6-keto-PGF1α, urinaiy metabolites of thromboxane and prostacyclin, respectively (12). Urine samples were assayed for specific PG with the use of enzyme immunoassay kits (Cayman Chemicals). Values from 11 AM and 1 PM and duplicate enzyme immunoassay analysis for each sample were averaged.

*Statistical Analyses*

SkBF is expressed in arbitrary units, and CVC values are means ± SE expressed as %MVC. CVC comparisons for indomethacin or celecoxib treatment vs. placebo treatment were performed with Student’s unpaired t-test. Comparisons of CVC values at 5, 10, and 25 min with resting values were analyzed with an one-way ANOVA followed by a Newman-Keuls test. Results for urinalyses are presented as means ± SE and expressed as nanograms per millimole creatinine. Statistical comparisons of urinary results between placebo and celecoxib were made by using one-tailed paired t-test, with 95% confidence intervals.

Statistical analyses were performed with Prism (Prism 2.01, Graphpad). P ≤ 0.05 was considered significant in all statistical analyses.

**RESULTS**

*Microvascular Investigations*

Compared with resting values, no significant changes were observed for control SkBF at the reference probe, mean arterial blood pressure, and local skin temperature during each experiment.

**Protocol 1**

A typical recording of the vascular responses to 5 min of anodal current application with indomethacin or celecoxib or placebo treatment in the same subject is presented in Fig. 2. No significant vasodilatation was noted during current application with any treatment (Fig. 2).

After the end of the 5-min anodal current application, a progressive vasodilatation appeared with placebo or celecoxib treatment (Fig. 2, B and C). This vasodilatation lasted throughout the recovery period. In contrast, indomethacin treatment (Fig. 2A) did not allow for development of the vasodilatation.

In the whole group, no difference was found in CVC values at rest: 14.3 ± 4.0, 11.9 ± 3.2, and 10.9 ± 2.0% MVC with indomethacin, celecoxib, and placebo, respectively. At 5 min after the onset of anodal current application, CVC values were 14.5 ± 3.1% MVC [P = not significant (NS) vs. rest] with indomethacin, 21.6 ± 7.3% MVC (P = NS vs. rest) with celecoxib, and 19.7 ± 3.8% MVC (P = NS vs. rest) with placebo. At 10 min after the onset of anodal current application, CVC values were 22.2 ± 4.9% MVC (P = NS vs. rest) with indomethacin, 85.7 ± 15.3% MVC (P < 0.001 vs. rest) with celecoxib, and 70.4 ± 13.1% MVC (P < 0.001 vs. rest) with placebo. At 25 min after the onset of anodal current application, CVC values were 18.9 ± 5.6% MVC (P = NS vs. rest) with indomethacin, 64.5 ± 17.7% MVC (P < 0.001 vs. rest) with celecoxib, and 73.0 ± 15.0% MVC (P < 0.001 vs. rest) with placebo. CVC values recorded with celecoxib or indomethacin treatment compared with placebo treatment at rest and at 5, 10 and 25 min after onset of current application are summarized in Fig. 3.

**Protocol 2**

As observed in protocol 1, after anodal current application, placebo and celecoxib produced a progressive vasodilatation that lasted throughout the recovery period. In the whole group, no differences were found in CVC values at rest: 12.5 ± 7.0 and 17.7 ± 9.0% MVC with celecoxib and placebo treatment, respectively. At 5 min after the onset of anodal current application, CVC values were 32.3 ± 7.9% MVC (P = NS vs. rest) with celecoxib and 30.7 ± 9.9% MVC (P = NS vs. rest) with placebo. At 10 min after the onset of anodal current application, CVC values were 63.3 ± 10.3% MVC (P < 0.001 vs. rest) with celecoxib and 69.2 ± 11.6% MVC (P < 0.01 vs. rest) with placebo. At 25 min after the onset of anodal current application, CVC values were 67.7 ± 10.5% MVC (P < 0.001 vs. rest) with celecoxib and 81.2 ± 8.8% MVC (P < 0.001 vs. rest) with placebo. After anodal current application, the vas-
Cox involvement in axon reflex-related vasodilatation

The major finding of the present study is that the COX-2-specific inhibitor (celecoxib) resulted in no apparent inhibition of the cutaneous vasodilatation observed within 20 min after anodal current application, whereas nonspecific COX blockade with indomethacin significantly decreased this response, compared with placebo.

The unexpected physiological observation that indomethacin, but not celecoxib, interferes with the anodal current-induced cutaneous vasodilatation could be important for clinicians and researchers. To investigate microvascular function, iontophoresis coupled with laser-Doppler flowmetry is largely used. With the use of anodal current application, iontophoresis allows for delivery of positively charged drugs (e.g., acetylcholine) through the skin (1, 22, 27). Unfortunately, anodal iontophoresis through vehicles devoid of vasoactive properties results in a cutaneous vasodilatation. This so-called “nonspecific” current-induced cutaneous vasodilatation could interfere with the study of the microvascular response specifically due to the drug delivered. Indeed, Hamdy et al. (15) showed that the axon reflex-related vasodilatation significantly participates in application, observed with aspirin treatment in a previous study (9) and with indomethacin treatment in the present study, likely results from COX, rather than VR1 or ASIC, blockade.

Several studies have contributed to identification of COX isoforms and their roles. The constitutive isoform COX-1 is reported to be responsible for PG production in basal conditions and in the regulation of basal flow in physiological conditions (2, 6, 42). In contrast, COX-2 is the inducible isoform involved in inflammation (4, 5), notably neurogenic inflammation (17, 43). Although the cutaneous vasodilatation in response to anodal current application is assumed to result from a neurogenic inflammation (3), our results suggest that COX-2 is not essential for this vascular response, at least within 20 min after anodal current application. Because of the time required for synthesis of the inducible enzyme, whether COX-2 may participate later is undetermined and remains to be studied.

It could be suggested that the absence of significant reduction of anodal current-induced cutaneous vasodilatation by the COX-2-specific inhibitor could be due to an insufficient COX blockade. However, the dose used in the present study (200 mg) was in the range used to significantly inhibit COX-2 activity (11, 26). Furthermore, treatment started 3 days before the experiment allowed for a significant decrease in urinary excretion of 6-keto-PGF1α, whereas Tx-M excretion was unchanged. This result confirmed the efficiency of COX-2 inhibition. The early cutaneous vasodilatation induced by anodal current application observed with celecoxib does not exclude the possibility that COX-2 may participate in the underlying mechanisms of this response but suggested that it is mainly a COX-1-dependent phenomenon. This is consistent with recent clinical studies suggesting an important COX-1 contribution to inflammation and pain, showing that inhibition of both COX isoforms is more efficient in achieving an effective analgesia in inflammation than COX-2 blockade alone (24, 25). Although the current delivery was never reported to be painful by any of the subjects, it is well known that stimulation of C-nociceptive fibers in the range of the nociceptive threshold may induce a vascular response, even in the absence of pain perception (23). Whether, during C-nociceptive fiber excitation, COX contribution is different after painful or nonpainful stimulation remains to be confirmed. This would require further experiments.

A specific COX-1 inhibitor is available for in vitro or animal studies (37, 46) but not for oral use in humans. Although our results suggest COX-1 involvement in cutaneous vasodilatation induced by anodal current application, we cannot prove that the response results solely from COX-1 participation.

Fig. 3. Cutaneous vascular response to 5 min of monopolar anodal current application with indomethacin, celecoxib, and placebo treatment. Response was studied at rest and 5, 10, and 25 min after the start of current application. Values are means ± SE, expressed as percentage of heat-induced maximal cutaneous vascular conductance (%MVC).

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**p < 0.01 versus placebo

- placebo

- indomethacin

- celecoxib

**p < 0.01 versus placebo

Time (min)

0 10 20 30

Cutaneous vascular conductance (%MVC)

0 25 50 75 100 125

AJP-Heart Circ Physiol • VOL. 288 • APRIL 2005 • www.ajpheart.org

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the total response to acetylcholine delivered through anodal iontophoresis. Thus the exact part of the microvascular response specifically due to acetylcholine in the total microvascular response after acetylcholine iontophoresis remains difficult to assess. Results of the present study show a difference in sensitivity of anodal current-induced vasodilatation to COX-2-specific and COX-nonspecific inhibitors. This difference should likely be taken into account in the interpretation of studies using anodal iontophoresis for drug delivery.

ACKNOWLEDGMENTS

The authors gratefully acknowledge Dr. Scott Davis for review of the English and Claire Demiot (Pharmacie d’Angers) for technical assistance.

GRANTS

This project was supported by region Pays de la Loire and Centre National de la Recherche Scientifique (UMR 6188) and by grants from Direction Regionale et Departementale de la Jeunesse et des Sport and Projet Hospitalier de Recherche Clinique 2001. The experiments were performed at the University Hospital in Angers. S. Durand is the recipient of a grant from Fondation de Recherche Clinique 2001. The experiments were performed at the Universite Hospital in Angers. S. Durand is the recipient of a grant from Fondation de Recherche Clinique 2001. The experiments were performed at the Universite Hospital in Angers. S. Durand is the recipient of a grant from Fondation de Recherche Clinique 2001. The experiments were performed at the Universite Hospital in Angers. S. Durand is the recipient of a grant from Fondation de Recherche Clinique 2001. The experiments were performed at the Universite Hospital in Angers. S. Durand is the recipient of a grant from Fondation de Recherche Clinique 2001. The experiments were performed at the Universite Hospital in Angers. S. Durand is the recipient of a grant from Fondation de Recherche Clinique 2001. The experiments were performed at the Universite Hospital in Angers. S. Durand is the recipient of a grant from Fondation de Recherche Clinique 2001. The experiments were performed at the Universite Hospital in Angers. S. Durand is the recipient of a grant from Fondation de Recherche Clinique 2001. The experiments were performed at the Universite Hospital in Angers. S. Durand is the recipient of a grant from Fondation de Recherche Clinique 2001. The experiments were performed at the Universite Hospital in Angers. S. Durand is the recipient of a grant from Fondation de Recherche Clinique 2001. The experiments were performed at the Universite Hospital in Angers. S. Durand is the recipient of a grant from Fondation de Recherche Clinique 2001. The experiments were performed at the Universite Hospital in Angers. S. Durand is the recipient of a grant from Fondation de Recherche Clinique 2001.


