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## Local heating of human skin causes hyperemia without mediation by muscarinic cholinergic receptors or prostanoids

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**Golay, Sandrine, Christian Haerberli, Anne Delachaux, Lucas Liaudet, Paul Kucera, Bernard Waeber, and François Feihl.** Local heating of human skin causes hyperemia without mediation by muscarinic cholinergic receptors or prostanoids. *J Appl Physiol* 97: 1781–1786, 2004. First published July 9, 2004; doi:10.1152/jappphysiol.00814.2003.—Local changes in surface temperature have a powerful influence on the perfusion of human skin. Heating increases local skin blood flow, but the mechanisms and mediators of this response (thermal hyperemia response) are incompletely elucidated. In the present study, we examined the possible dependence of the thermal hyperemia response on stimulation of muscarinic cholinergic receptors and on production of vasodilator prostanoids. In 13 male healthy subjects aged 20–30 yr, a temperature-controlled chamber was positioned on the volar face of one forearm and used to raise surface temperature from 34 to 41°C. The time course of the resulting thermal hyperemia response was recorded with a laser-Doppler imager. In one experiment, each of eight subjects received an intravenous bolus of the antimuscarinic agent glycopyrrolate (4 µg/kg) on one visit and saline on the other. The thermal hyperemia response was determined within the hour after the injections. Glycopyrrolate effectively inhibited the skin vasodilation induced by iontophoresis of acetylcholine but did not influence the thermal hyperemia response. In a second experiment, conducted in five other subjects, 1 g of the cyclooxygenase inhibitor aspirin administered orally totally abolished the vasodilation induced in the skin by anodal current but also failed to modify the thermal hyperemia response. The present study excludes the stimulation of muscarinic receptors and the production of vasodilator prostaglandins as essential and nonredundant mechanisms for the vasodilation induced by local heating in human forearm skin.

regional blood flow; skin blood supply; skin temperature; laser-Doppler flowmetry; receptors; vasodilation; prostaglandins

IN NONGLABROUS HUMAN SKIN, a local rise in temperature is a powerful stimulus for local vasodilation. Indeed, a test of microvascular functional integrity has been proposed based on the laser-Doppler measurement of skin blood flow (SkBF) increase elicited by mild local heating (hereafter termed “thermal hyperemia response”). This test has been used to document microvascular dysfunction in diabetic patients (2, 21).

The mechanisms implicated in the thermal hyperemia response remain incompletely defined. In contrast with thermoregulatory skin vasodilation, the cutaneous vascular response to local heating is not mediated by central reflexes because it is unaffected by regional nerve block (15, 18) and is preserved in grafted skin (7). The response of SkBF to a step increase in local temperature is biphasic, with an early peak occurring

within minutes, followed by a nadir, and then a late phase with a progressive rise to a plateau in 20–30 min (11, 15). The late phase seems to depend on the local, nonneurally mediated release of nitric oxide (NO), because it is suppressed by inhibitors of NO synthase (11, 15) and insensitive to local anesthesia (15). In contrast, the early peak shows little dependence on NO, has no precisely known mediator, and was found to be diminished by local anesthesia in one study (15). A role for acetylcholine (ACh) originating from terminal nerve endings of the active cholinergic vasodilatory system (10) seems unlikely, because depletion of their neuromediator stores by intradermal administration of botulinum toxin had no more effect on the early peak than on the late phase, and neither did local pretreatment of the skin with atropine, an antagonist of muscarinic cholinergic receptors (12). However, this latter study tested the response to local heating shortly after a heat stress applied to the whole body. In the skin, such stress can activate cholinergic mechanisms of vasodilation (19), which, even if rapidly dissipated on cessation of body heating, may lead, at least in theory, to desensitization of cholinergic receptors (3, 9). Therefore, we decided to verify the lack of influence of cholinergic blockade on the thermal hyperemia response in the basal state.

It is likely that the sensitivity of the early peak of thermal hyperemia response to local anesthesia reflects, at least in part, the stimulation of C-fiber nociceptors, which trigger vasodilation through an axon reflex (14). Vasodilator prostanoids have been implicated in several axon reflex-dependent cutaneous vasomotor responses, such as the hyperemia induced by repeated pulses of anodal current (6) or the vasodilation observed at some distance (a few millimeters) from the local application of ACh (4). Therefore, the second aim of the present study was to test whether inhibition of prostanoid synthesis attenuated the axon reflex-dependent part of the thermal hyperemia response. Finally, it is of interest that the synthesis of NO, which, as mentioned, is an essential mediator of the thermal hyperemia response, can be modulated by products of the cyclooxygenase (COX) pathway (5, 20). Thus our third aim was to examine the effects of blocking COX on the NO-dependent part of the thermal hyperemia response.

### METHODS

#### Subjects

Sixteen male healthy subjects, aged from 20 to 30 yr, were included. They were all nonsmokers and had no personal history of hypertension, diabetes,

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or hypercholesterolemia. Subjects known to have an allergy or an intolerance to aspirin were excluded. None of the subjects took any vasoactive or anti-inflammatory drug for the 10 days before the start of the study. The volunteers were not allowed to drink caffeine-containing beverages on the day of the experiments. They were fully informed about the protocol of the study and gave their written consent. The study was conducted according to the principles outlined in the Declaration of Helsinki, and its protocol was approved by the institutional Ethics Committee.

#### Assessment of Skin Microvascular Reactivity

**Measurement of SkBF.** To investigate the forearm skin microvascular blood flow, we used a laser-Doppler imaging system (LDI; Moor Instruments, Axminster, UK) as previously described (13, 17). In contrast with the more traditional single-point laser-Doppler flowmetry, the imaging system does not require direct contact of an optical fiber with the site of SkBF measurement. Total SkBF was expressed in perfusion units according to the principles of laser-Doppler flowmetry.

**Assessment of the thermal hyperemia response.** A stainless steel, temperature-controlled, ring-shaped chamber with inner diameter, outer diameter, and thickness of 8, 25, and 8 mm, respectively, was affixed to the skin with double-sided tape, filled to the rim with deionized water, and overlaid with a transparent glass coverslip. The skin underneath the coverslip and water was thus accessible to LDI. The device was programmed to repetitively scan the area comprising the chamber every 30 s, each scan being accomplished in 25 s.

The chamber was connected to an analog temperature controller with adjustable set point. Temperature was set at 34°C until a stable blood flow reading was obtained, and then it was raised to 41°C in 60 s and maintained at this level for the next 11 or 23 min. The short and long durations were used in *experiments 1* and *2*, respectively (see below).

The temperature controller was designed to provide a transition entirely free of overshoot. Preliminary experiments were carried out to check this dynamic behavior in situ, with water temperature measured with a rapid response thermistor.

**Assessment of vasodilation induced by ACh.** When required to document the efficiency of pharmacological blockade of muscarinic receptors (*experiment 1*), the response of SkBF to the local application of ACh was also determined. This test was carried out as previously described (13) except that a dose-response curve was constructed in lieu of using a single dose of ACh. Briefly, ACh was administered transdermally by iontophoresis by means of a custom-made ring-shaped chamber made of neoprene, fitted with an electrode connected to an iontophoresis controller (MIC1-e, Moor Instruments), filled with a 1% solution of ACh in distilled water, and overlaid with a glass coverslip to allow the measurement of blood flow with LDI in the exposed skin, as described for the assessment of the thermal hyperemia response. The LDI device was programmed to repetitively scan the area comprising the chamber every 60 s, with each scan being accomplished in 50 s. Three different doses (iontophoresis with total current charge densities of 1.4, 7, and 28 mC/cm<sup>2</sup>) were administered, each in a pulsed fashion over 7 min, with 1 min interposed between each dose. The skin was pretreated with an anesthetic cream (EMLA cream 5%, Astra Pharmaceutica, Dietikon, Switzerland) applied for 1 h under an occlusive dressing (Tegaderm, 3M Health Care) to prevent current-induced, axon reflex-mediated vasodilation (4, 22) as described (13) and so retain a vascular response strictly dependent on the stimulation of cholinergic receptors. The exposure time to EMLA of 1 h was chosen because, in our experience, a longer exposure (2 h) induces baseline hyperemia in some subjects. A 1-h exposure did not have this drawback and consistently abolished touch sensation, although a prick test was not performed, and in a previous study from our laboratory (13) was sufficient to prevent any vasodilation in response to the current doses used here.

**Assessment of SkBF response to anodal current.** When required to document the efficiency of pharmacological blockade of COX (*experiment 2*), we determined the response of SkBF to the local application of anodal current, which has been shown to be prostanoid dependent (6). The aforementioned iontophoresis chamber was affixed to the forearm skin, filled with deionized water, overlaid with a coverslip, and attached to the positive outlet of the MIC1-e iontophoresis controller. Two current pulses were administered, separated by 5 min. Each pulse had a current amplitude of 100  $\mu$ A and a duration of 60 s (charge 6 mC, charge density 8 mC/cm<sup>2</sup>). SkBF was monitored as above. This current protocol was taken from the work of Durand et al. (6), who found in healthy volunteers that the second pulse caused a large rapid increase of SkBF, which could be abolished by 1 g of aspirin taken orally.

#### Protocols

On the days of the experiments, the subjects were asked to report at our research facility at 8:00 AM. The investigations were carried out in a quiet room with air conditioning. Ambient temperature was systematically measured and ranged from 21 to 23.5°C. Skin temperature was also systematically measured using a cutaneous probe (G. Mettraux, Crissier, Switzerland). The subjects were examined in the supine position with the arm supported by a cushion. The distance traveled by the incident laser beam from the laser aperture to the skin was set at 41 cm. Blood pressure and heart rate were measured serially during the course of the study using an automated oscillometric device (Datascope Accutorr 1A, MS Cardio-Medical, Brunnen, Switzerland). The study was subdivided into two experiments.

**Experiment 1.** This experiment was designed to verify the lack of effect of muscarinic receptor blockade on the thermal hyperemia response. Furthermore, we needed to check the presence of a neurally mediated component of heat-induced vasodilation in our precise conditions, which somewhat differed from those used in the original demonstration (15), potentially leading to variations in sensory stimulation [present study: heating chamber filled with water and measurement of SkBF with a LDI device; study by Minson et al. (15): heating chamber surrounding a laser-Doppler optical probe in direct mechanical contact with the skin]. Therefore, we carried out recordings of the thermal hyperemia response both with and without suppression of the axon reflex by means of surface anesthesia.

Eight subjects were studied on two occasions 3–10 days apart. On arrival, surface anesthesia in the form of an EMLA patch was applied on two sites located on the proximal anterior face of the right forearm: one for the assessment of thermal hyperemia response in absence of axon reflex and one for ACh iontophoresis as described above. A third site was chosen on the volar face of the same forearm for assessment of native thermal hyperemia response (i.e., intact axon reflex, no surface anesthesia). On the first visit, the exact location of each site was marked on a transparent acetate film, together with the anatomic outline of the forearm, taking care to precisely reproduce the position of the distal skinfold marking the limit of the hand. In this way, the same skin sites could be probed on the second visit, thus removing one source of experimental variation.

An intravenous injection of either glycopyrrolate on one visit (4  $\mu$ g/kg given in 5 min) or an equivalent volume of isotonic saline on the other visit was carried out. The order of administration was randomized in a balanced fashion, as appropriate for a crossover design. Glycopyrrolate nonselectively and competitively inhibits all major subtypes of muscarinic receptors (8). This drug was preferred to atropine in view of its expected superior tolerance by human subjects; the chosen dose is in accordance with current clinical practice and should ensure substantial muscarinic blockade starting within minutes and lasting at least 1 h (1, 16). Within this time lapse, the following sequence of recordings was accomplished: response to iontophoresis of ACh, thermal hyperemia response on anesthetized skin, and finally thermal hyperemia response on skin not exposed to EMLA cream.

In these experiments, SkBF was recorded in baseline and for the next 11 min, which followed the temperature change. In preliminary tests, we found that, in the conditions used here, the late plateau of the thermal hyperemia response was almost attained in this time frame, which was expected to contain the transient, neurally mediated component.

**Experiment 2.** This experiment was carried out after termination of *experiment 1* and was designed to test the effects of COX inhibition on the thermal hyperemia response.

Eight subjects were studied according to a protocol similar to that described for *experiment 1*, with the following differences: 1) oral intake of 1 g of acetylsalicylic acid (aspirin) dissolved in 125 ml of orange juice on one visit and the same volume of orange juice alone on the other visit; 2) visits separated by 1–3 wk; 3) use of two unanesthetized sites (the same on both visits, one on each forearm) for the determination of thermal hyperemia response 30 min and 2 h after aspirin or placebo intake; and 4) use of two additional unanesthetized sites (the same on both visits, one on each forearm) for the determination of SkBF response to anodal current 60 min and 2.5 h after aspirin or placebo intake. The sites for exposure to anodal current were chosen in close proximity to those used for local heating. To avoid potential interferences, the two types of SkBF responses were recorded sequentially rather than simultaneously.

The dose of aspirin and timing of measurements were chosen in view of two studies in human volunteers. Thirty minutes after the oral intake of 972 mg of aspirin, there was a partial inhibition of the SkBF response recorded at a distance from the site of ACh iontophoresis (4). Two hours after the ingestion of 1 g of aspirin, the SkBF response to anodal current was abolished (6).

In *experiment 2*, SkBF was recorded in baseline and for the next 23 min, which followed the temperature change. The longer time frame compared with *experiment 1* was chosen because of concerns that 11 min of heating might have been insufficient to reach a plateau of SkBF.

#### Statistical Analysis

Data are presented as means  $\pm$  SD. To take into account the biphasic nature of thermal hyperemia response, this response was summarized as 1) the peak increase in SkBF above baseline in the first 5 min after the change in the setting of the temperature controller and 2) the average blood flow increase in the last 2 min of measurement. The response to ACh iontophoresis or to anodal current were summarized as the blood flow increase above baseline observed at end iontophoresis of each current dose.

Statistical analysis of *experiment 1* was carried out with univariate analysis of variance using a model that allowed for repeated measures in the same subject. The factors included in the model were skin anesthesia, glycopyrrolate administration, and their possible interaction. The same model was used to compare the global time courses of thermal hyperemia response between conditions by means of multivariate analysis of variance. The analysis of *experiment 2* was analogous, except that measurement timing (30 min or 2 h) replaced skin anesthesia as a repeated factor.

The alpha level of all tests was set at 0.05. All computations were performed with JMP software version 3.2.2 (SAS Institute, Cary, NJ).

## RESULTS

In *experiment 1*, as in *experiment 2*, baseline conditions that might have contributed to the variation in SkBF responses, namely arterial blood pressure, skin temperature in the vicinity of measurement sites, and SkBF before application of local heating, were similar on administration of either the treatment drug or its inactive control (Tables 1 and 2). In each of the tested experimental conditions, the time course of thermal hyperemia response was biphasic, with an early peak of SkBF

Table 1. Skin blood flow responses to saline and glycopyrrolate administration

	Saline	Glycopyrrolate
Arterial blood pressure		
Systolic, mmHg	118 $\pm$ 8	118 $\pm$ 9
Diastolic, mmHg	65 $\pm$ 5	64 $\pm$ 7
Skin blood flow response in presence of local anesthesia		
Skin temperature before probe placement, °C	31.4 $\pm$ 1.0	31.6 $\pm$ 1.1
Baseline skin blood flow, PU	55 $\pm$ 13	56 $\pm$ 11
Peak early response, PU	270 $\pm$ 96	247 $\pm$ 59
Late response, PU	325 $\pm$ 97	277 $\pm$ 81
Skin blood flow response without local anesthesia		
Skin temperature before probe placement, °C	31.3 $\pm$ 1.6	31.9 $\pm$ 1.2
Baseline skin blood flow, PU	68 $\pm$ 15	71 $\pm$ 19
Peak early response, PU	347 $\pm$ 97*	372 $\pm$ 100*
Late response, PU	333 $\pm$ 66	336 $\pm$ 90

Values are means  $\pm$  SE of  $n = 8$  subjects. PU, perfusion unit. \*  $P < 0.01$ , anesthetized vs. unanesthetized skin. Intravenous glycopyrrolate had no statistically discernible effect on any of the measured variables.

occurring between 2 and 4 min after the onset of local heating, followed by a nadir around *minute 5* and later a slight secondary progressive increase (see Figs. 2 and 4). Both the baseline SkBF and the amplitude of thermal hyperemia response were somewhat lower on unanesthetized skin in *experiment 1* than in *experiment 2*, a fact that may have been related to a slightly higher skin temperature (as measured before thermocontrolled chamber placement) in the latter experiment (compare Tables 1 and 2).

#### Experiment 1

As shown in Fig. 1, the iontophoresis of ACh dose-dependently increased SkBF, with a maximal effect attained at a current charge density of 7 mC/cm<sup>2</sup>. Vasodilation induced by this and the lowest dose (1.4 mC/cm<sup>2</sup>) was markedly diminished by intravenous treatment with glycopyrrolate, an inhibition that could only be overridden by a largely supramaximal amount of agonist (i.e., 28 mC/cm<sup>2</sup>). Such observations are in keeping with the competitive nature of the antagonism exerted by glycopyrrolate. They indicate that this drug, as applied in the present study, substantially blocked the muscarinic cholinergic mechanisms of vasodilation in the skin.

In contrast to the inhibition of ACh-mediated vasodilation, glycopyrrolate had no effect on either the time course (Fig. 2) or the derived parameters of thermal hyperemia response (Table 1).

Surface anesthesia blunted the early peak by 20–30% ( $P = 0.001$ ) but had no discernible effect on the late component of thermal hyperemia response (Table 1).

#### Experiment 2

In three of the eight enrolled subjects, there was no response of SkBF to current application in the absence of aspirin treatment. Because in these subjects it was not possible to ascertain the efficiency of COX inhibition, they were excluded from the study. In the remaining five volunteers, the second pulse of anodal current triggered a substantial increase of SkBF, which, as expected (6), was abolished by the oral intake of 1 g of aspirin whether recordings were taken 1 or 2.5 h after

Table 2. Skin blood flow responses to placebo and aspirin

	Placebo	Aspirin
Arterial blood pressure		
Systolic, mmHg	114 ± 8	114 ± 9
Diastolic, mmHg	60 ± 5	61 ± 7
Skin blood flow response at 30 min		
Skin temperature before probe placement, °C	33.1 ± 0.7	32.7 ± 0.7
Baseline skin blood flow, PU	87 ± 8	88 ± 19
Peak early response, PU	437 ± 47	442 ± 51
Late response, PU	489 ± 60	511 ± 117
Skin blood flow response at 2 h		
Skin temperature before probe placement, °C	NA	NA
Baseline skin blood flow, PU	90 ± 16	104 ± 38
Peak early response, PU	391 ± 93	396 ± 83
Late response, PU	476 ± 83	452 ± 89

Values are means ± SE of  $n = 5$  subjects. NA, not available. Time intervals of 30 min and 2 h are with respect to aspirin intake. Oral aspirin had no statistically discernible effect on any of the measured variables.

drug administration (Fig. 3). In contrast, aspirin had absolutely no effect on the thermal hyperemia response whether examined after 30 min or after 2 h (Table 2, Fig. 4).

## DISCUSSION

To date, the mechanisms of thermal hyperemia response remain incompletely understood. In particular, there is only scant information on the possible role of cholinergic stimulation or mediation by prostanoids. We present clear evidence against a major, nonredundant role of either mechanism in humans.

The time course of thermal hyperemia response recorded with the laser-Doppler imager featured an early transient peak, later followed by a progressive rise of SkBF (Figs. 2 and 4), in agreement with observations made by others with single-point laser-Doppler flowmetry (11, 12, 15, 18). Notably, surface

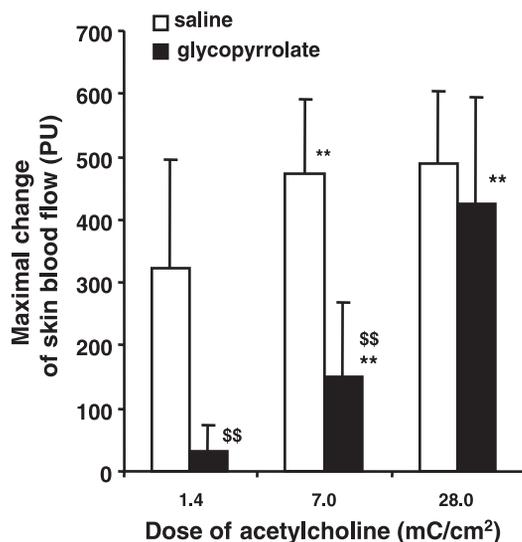


Fig. 1. Effects of intravenous glycopyrrolate on the skin vasodilation induced by local administration of acetylcholine. Acetylcholine was administered by iontophoresis at each of 3 doses expressed as current charge density in millicoulombs (mC) per cm<sup>2</sup>. Data are expressed in perfusions units (PU) and reported as means ± SD of 8 subjects. Significant difference between glycopyrrolate and saline: \* $P < 0.05$ ; \*\* $P < 0.01$ ; \$\$\$ $P < 0.001$ . Significant difference vs. lower dose of acetylcholine: \*\* $P < 0.01$ .

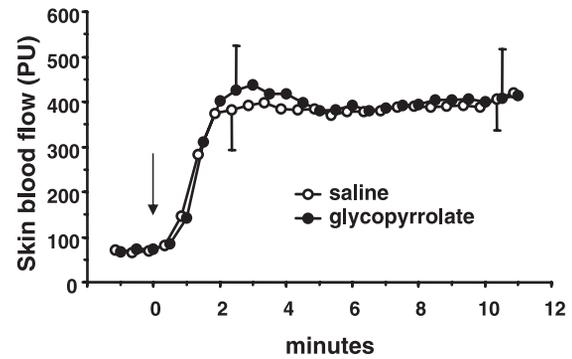


Fig. 2. Effects of intravenous glycopyrrolate on the time course of skin vasodilation induced by local heating. Shown is the response of skin blood flow to local heating on unanesthetized skin, as recorded 30 min after an intravenous bolus of either saline (○) or glycopyrrolate (●) (4 μg/kg). Starting at the arrow, skin temperature was raised in 1 min from 34 to 41°C and kept at that latter value for the rest of the recording. Data are means ± SD of  $n = 8$  subjects.

anesthesia selectively blunted the early component of thermal hyperemia response, consistent with the data reported by Minson et al. (15). These authors found that such intervention, while not affecting the late component, was associated with a 70% reduction in the amplitude of the early peak. The corresponding figure in our study is closer to 30% (Table 1). This discrepancy could reflect differences in the degree of local anesthesia, which we acknowledge was not tested in our experiments. Indeed, Minson et al. applied the EMLA cream for two consecutive periods of 60 min each, i.e., twice the duration used in our study. Differences in sensory stimulation to unanesthetized skin could also have existed, if only because heat application was mediated in part by water in our experiments but not in those of Minson et al.

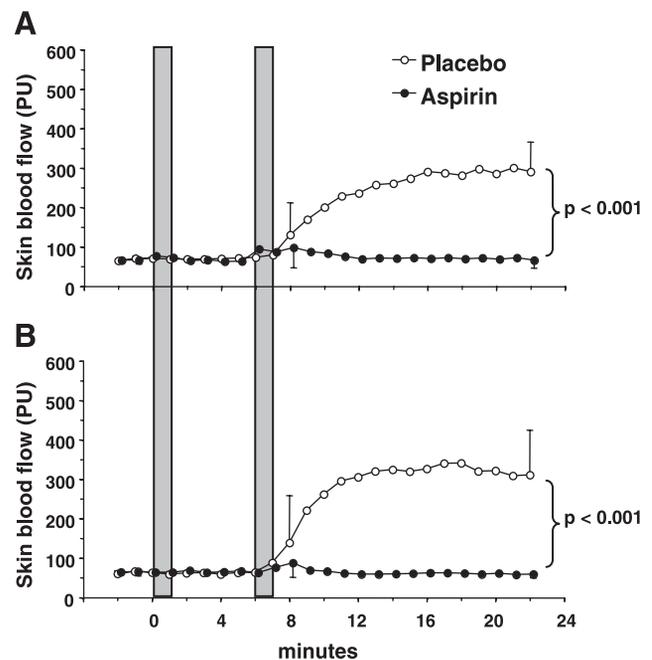


Fig. 3. Effects of oral aspirin (1 g) on the response of skin blood flow to anodal current. Response of skin blood flow to 2 successive pulses of anodal current (each amounting to 8 mC/cm<sup>2</sup>, rectangular marks) was determined after 1 (A) and 2.5 h (B) after the intake of either placebo or aspirin.

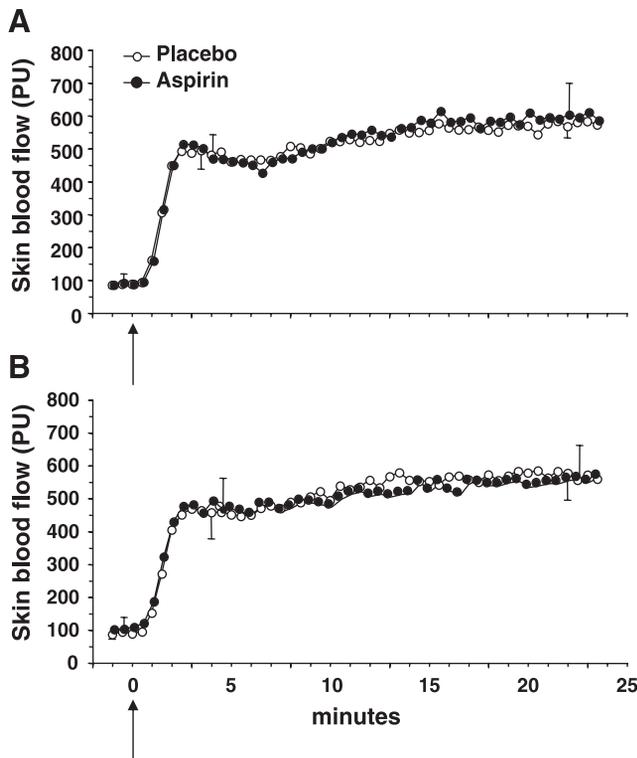


Fig. 4. Effects of oral aspirin (1 g) on the time course of skin vasodilation induced by local heating. Response of skin blood flow to local heating was determined on unanesthetized skin 30 min (A) and 2 h (B) after the intake of either placebo or aspirin. Starting at the arrow, skin temperature was raised in 1 min from 34 to 41°C and kept at that latter value for the rest of the recording. Data are means  $\pm$  SD of  $n = 5$  subjects.

Whatever their dissimilarities, both studies fundamentally concur to indicate that a substantial part of thermal hyperemia response, notably its late component, cannot be blocked by surface anesthesia and therefore must depend on mechanisms other than the activation of an axon reflex. Kellogg and coworkers (11) have examined the effects of NO synthase inhibition on the thermal hyperemia response by elegantly using microdialysis to locally administer the L-arginine analog *N*<sup>ω</sup>-methyl-L-arginine within the area subjected to local heating in human forearm skin. This experiment has also been carried out in the Minson et al. study cited above (15). Both groups reported that NO synthase inhibition profoundly depressed the late response, while more slightly affecting the early peak, i.e., a pattern of effects inverse to that just discussed for surface anesthesia, indicating an essential role of NO in the nonneurally mediated component of thermal hyperemia response.

Cholinergic stimulation is widely known to upregulate the production of NO by the vascular endothelium. One motivation for the present study was the paucity of factual information regarding a possible cholinergic mechanism of thermal hyperemia response. To our knowledge, the only relevant data have been presented a few years ago by Kellogg and coworkers (12). Their study primarily aimed at examining the role of the active cholinergic vasodilatory system in the cutaneous vasodilation induced by an increase in core temperature (heat stress), as opposed to local heating of the skin. Cholinergic mechanisms were blocked in discrete areas by local administration of either botulinum toxin, which depletes cholinergic nerve endings of

all neuromediators, or atropine, which inhibits postsynaptic muscarinic receptors. For normalization purposes, the protocol included recordings of a thermal hyperemia response starting 5 min after the end of the heat stress. In such conditions, the thermal hyperemia response was insensitive either to botulinum toxin or to atropine. However, it must be considered that these observations were obtained quite shortly after a major upheaval of skin neurovascular homeostasis (i.e., the heat stress). Our data show that they are generalizable to the basal state.

In human skin, products of COX metabolism are implicated in several vasodilatory responses to local stimuli, e.g., the intradermal administration of captopril, an angiotensin converting enzyme inhibitor (23), or the dermal hyperemia induced by electrical stimulation (6). In the same vascular bed, prostanoids may play a role in axon reflex-dependent vasodilation (4), which underlies the early, neurally mediated component of the thermal hyperemia response. Finally, the late component of this response depends on NO (11, 15), the synthesis of which may be modulated by products of the COX pathway (5, 20). These considerations provide a sturdy rationale for testing the involvement of COX products in the thermal hyperemia response, but to our knowledge this has not been done. One possible exception is the study by Durand et al. (6), who were primarily interested in the mechanisms of dermal vasodilation induced by electrical stimulation and indeed were the first to report the abolition by aspirin of the SkBF response to anodal current, an observation that we confirmed (Fig. 3). For normalization purposes, Durand et al. elicited maximal vasodilation in the skin area previously exposed to anodal current by locally heating to a temperature of 44°C and reporting in these conditions values of SkBF that were unaffected by aspirin. However, this result may have been confounded by the previous electrical stimulation, the vascular effects of which were still to be seen at the time of heating (see Fig. 6 in Ref. 6). Furthermore, a temperature of 44°C can induce nociceptive stimuli not present with more modest heating and so modify the mechanisms of vasodilation, possibly through the release of neuropeptides. Indeed, Minson et al. (15) reported that the SkBF increase caused by sustained local heating above 41°C was usually associated with burning pain and was then not dependent on NO, which was in complete contrast with observations made using a milder thermal stimulus. In our study, the thermal hyperemia response was tested on sites not recently exposed to other forms of stimuli by means of the heating step used by Kellogg et al. (Ref. 11; i.e., from 34°C at baseline to 41°C), which did not elicit pain and was associated with NO-dependent vasodilation. The data clearly rule out a predominant role of COX products in these conditions (Figs. 3 and 4).

Some limitations of the present work must still be mentioned. In *experiment 1*, the thermal hyperemia response was recorded for only 11 min, which in other studies (11, 15) was quite insufficient to attain a steady state of vasodilation. Indeed, Minson et al. (15) as well as Kellogg et al. (11) reported that establishing a true plateau could take as long as 30–40 min and that the value of SkBF at that time was largely above the early peak. As recorded in our conditions, the time course of thermal hyperemia response departed from this pattern, with a clear plateau reached by 20 min of heating, which only slightly exceeded either the early peak or the value recorded at

11 min (Fig. 4). Therefore, we believe it extremely unlikely that *experiment 1* (limited to 11 min; Fig. 2) would have come out differently if the recording had been prolonged. The reasons for the variation in the time courses of the thermal hyperemia responses recorded in different laboratories are unclear. In particular, the important determinants of heat-induced nociceptive stimulation, which are the rate of rise and amplitude of temperature step (14), were similar (0.1°C/s and 7°C, respectively) between the present study and that by Minson et al. (15). As already mentioned, heat was transmitted to the skin in part by water in our experiments but not in those by other authors (11, 15), which could potentially lead to some differences in sensory stimulation. Finally, the time courses of SkBF presented in Figs. 2 and 4 have been recorded with a LDI device, which entails no mechanical contact of the measurement device with the skin, whereas the other studies used the single-point technique, which necessitates the affixing of an optical fiber to the probed area.

In summary, the data strongly oppose a predominant role of either muscarinic receptor stimulation or COX product generation in the thermal hyperemia response, although they do not rule out either mechanism as possible parts of a redundant system. Further studies resorting to the simultaneous blockade of more than one pathway are necessary to resolve this point.

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