La désensibilisation lors d'une hypérémie locale par échauffement est un phénomène reproductible

THESE

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Résumé de la thèse:

But: L'échauffement local augmente le flux sanguin cutané (SkBF) en induisant une vasodilatation. Cette réponse de la microcirculation dermique est dénommée hyperémie thermique. Dans une précédente étude, nous avons montré qu'un stimulus thermique local appliqué une première fois atténue la réponse hyperémique à un second stimulus appliqué ultérieurement sur le même site cutané, un phénomène que nous appelons désensibilisation. Cependant, d'autres études n'ont pas mis en évidence une désensibilisation dans des conditions similaires. Le but du présent travail est d'explorer les raisons de cette discordance qui pourrait relever d'une différence au niveau de l'instrumentation.

Méthode: vingt-huit jeunes hommes en bonne santé sont étudiés. Deux paires de corps de chauffe, l'une faite sur mesure (notre étude) et l'autre produite commercialement (autres groupes d'investigateurs), sont fixées sur l'avant bras. SkBF est mesuré par fluxmétrie laser Doppler ponctuelle (LDF) (780 nm) sur une paire, et par imagerie laser Doppler (LDI) (633 nm) sur l'autre paire. Des paliers de température de 34 à 41 °C sont appliqués pendant 30 minutes et reproduits après deux heures.

Résultats: Durant le second échauffement, le plateau SkBF est plus bas que lors du premier stimulus thermique et ce phénomène est observé avec chacune des quatre combinaisons entre différents instruments de mesure du SkBF et de chauffage (p< 0.05 pour chacune des conditions, différence de -9% à -16% de la valeur initiale).

Conclusion: La désensibilisation lors d'une hyperémie thermique n'est pas spécifique à des conditions opératoires particulières.
Desensitization of Thermal Hyperemia in the Skin is Reproducible

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ABSTRACT

Objective: Local heating increases skin blood flow SkBF (thermal hyperemia). In a previous study, we reported that a first local thermal stimulus could attenuate the hyperemic response to a second one applied later on the same skin spot, a phenomenon that we termed desensitization. However, other studies found no evidence for desensitization in similar conditions. The aim of the present work was to test whether it was related to differences in instrumentation.

Methods: Twenty-eight healthy young males were studied. Two pairs of heating chambers, one custom-made (our study) and one commercial (other groups), were affixed to forearm skin. SkBF was measured with single-point laser-Doppler flowmetry (LDF) (780 nm) in one pair, and laser-Doppler imaging (LDI) (633 nm) in the other. A temperature step from 34 to 41°C, was applied for 30 minutes and repeated after two hours.

Results: During the second thermal challenge, the plateau SkBF was lower than during the first thermal and was observed with each of the four combinations of SkBF measurement techniques and heating equipment (p < 0.05 for all conditions, range -9% to -16% of the initial value).

Conclusion: Desensitization of thermal hyperemia is not specific to peculiar operating conditions.

Key words: adaptation, physiological, hot temperature, humans, hyperemia, male, microcirculation/physiology, nitric oxide, regional blood flow, skin/blood supply, temperature, vasodilation/physiology, young adult

Abbreviations used: SkBF, skin blood flow; LDI, laser-Doppler imaging; LDF, single-point laser-Doppler flowmetry; PU, perfusion unit; T0, time of protocol start; T2, two hours after T0.

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INTRODUCTION

In nonglabrous human skin, a local rise in temperature is a powerful stimulus for local vasodilation, mediated by neurogenic reflexes and locally released substances [12,13,15,16]. The mechanisms implicated in this so-called thermal hyperemia remain incompletely defined. In contrast with thermoregulatory skin vasodilation, it is not mediated by central reflexes because it is unaffected by regional nerve block [17] and is preserved in grafted skin [5]. The response of skin blood flow (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 minutes response [3,10-12,16]. The plateau seems to depend on the local, non-neurally mediated release of nitric oxide (NO), because it is suppressed by inhibitors of NO synthase [11,12,16] and insensitive to local anesthesia [16]. In contrast, the early peak shows little dependence on NO, and is largely mediated by the stimulation of nociceptive C-fibers that trigger vasodilation through an axon reflex [13]. Accordingly, it is diminished by local anesthesia [7,16,21]. In short, the prevailing view [15] is that the early part of thermal hyperemia is due to the transient activation of an axon reflex, which progressively gives way, as heating is pursued, to a non-neural, NO-dependent mechanism.

Thermal hyperemia can easily be recorded in the skin in a non-invasive fashion, using laser-Doppler flowmetry to evaluate SkBF. Indeed, thermal hyperemia has been proposed as a test of microvascular function. This test has
been used to document microvascular dysfunction in diabetes [1,22,23] and other conditions [14,19].

In a previous study, we found that the repetitiveness of a local thermal stimulus on the same skin patch was associated with a reduction in the elicited vasodilatory response, a phenomenon hereafter termed desensitization [3]. This result is of some practical importance, for example, if thermal hyperemia is to be used as an end point in acute interventional trials. However, other groups [4,20] found no evidence for desensitization, when recording two thermal hyperemia either one or two hours apart on the same skin site, as we had done. The aim of this study was to understand the reasons for this apparent discrepancy and, more specifically, to test whether it was related to differences in instrumentation. We had measured SkBF with laser-Doppler imaging (LDI) at a wavelength of 633 nm [3], whereas the cited studies used single-point laser-Doppler flowmetry (LDF) at 780 nm [4,20].

In comparison with 633 nm, the latter wavelength has greater skin penetration, and thus the potential to explore different vessels. In addition, the heating chambers used in our study were custom-made, as opposed to the commercial equipment employed by these other authors. We therefore test the contributory role of desensitization to thermal hyperemia occurred under four sets of conditions, i.e., measuring SkBF with LDI or LDF, and heating the skin with our custom-made or with commercially available chambers.

**METHODS**

**Subjects**

Twenty-eight healthy male subjects, aged from 18 to 32 years, were included. They were all non-smokers, had no personal history of hypertension, diabetes, or hypercholesterolemia, and no dermographism. None took any drugs related to diabetes [1,22,23] and other conditions [14,19].

The present study aimed at comparing results obtained with the four different systems for the local heating of the skin. The first one, custom-made, had been used in our previous study [3]. It comprised a stainless steel, temperature-controlled, ring-shaped chamber with inner diameter, outer diameter, and thickness of 8, 25, and 8 mm, respectively, affixed to the skin with a double-sided tape [3,7].

The second system was commercially available (Perimed). It comprised a thermistor probe holder (PF450; Perimed), which is a ring-shaped chamber, whose visible part is in plastic with inner diameter, outer diameter, and thickness of 6, 32, and 12 mm, respectively, and is also affixed to the skin with a double-sided tape. The chamber was connected to an analog dual-channel temperature controller with adjustable set point (Peritemp 4005 Heater; Perimed).

The present study aimed at comparing results obtained with each of the four combinations of measurements (LDF or LDI) and heating devices (commercial or custom-made). The required adaptations are described below (see Figure 1).

**Assessment of Skin Microvascular Reactivity**

Measuring devices. To investigate forearm SkBF, we used two different laser-Doppler measuring devices. The first one was a laser-Doppler imaging system (LDI; Moor Instruments, Axminster, UK) and the second one, a single-point dual-channel laser-Doppler flowmeter (PF6001; Perimed, Jarfalla, Sweden).

Laser-Doppler imaging system (LDI). The LDI system used a beam of coherent red light generated by a 633-nm-helium-neon laser. In this system, the beam is directed by a moving mirror whose rotations around two perpendicular axes are controlled by a computer, allowing the scanning of a delimited area. The analysis of the backscattered Doppler-shifted light results in a computer-generated, color-coded image of the spatial distribution of microvascular blood flow over the scanned area. No direct contact with the skin is required. The scanned area can be chosen in a range from a few mm² to a complete body part such as the hand or thorax, depending on angular amplitudes of mirror movements and distance of the latter to the skin.

In the present study, the scanned area was about 3 × 7 cm, and the distance travelled by the incident laser beam from the device shutter to the skin was set at 41 cm. SkBF was expressed in perfusion units (PU).

Single-point fiber-optic laser Doppler (LDF). The LDF system used infrared light produced by a 780-nm-helium-neon laser. In this system, two optical fibers are embedded in a probe placed in contact with the skin surface. One fiber is used to transmit a laser beam and the other to detect the back-scattered light. The measurement depth varies according to the distance between the fibers. The probes used in this study (PF408; Perimed) had a diameter and a fiber separation of, respectively, 6 and 0.25 mm. SkBF was expressed in vols.

Assessment of thermal hyperemia response. We used two different systems for the local heating of the skin. The first one was custom-made, which had been used in our previous study [3]. It comprised a stainless steel, temperature-controlled, ring-shaped chamber with inner diameter, outer diameter, and thickness of 8, 25, and 8 mm, respectively, affixed to the skin with a double-sided tape [3,7].

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In preliminary experiments, a small-size thermistor (length and diameter of 0.3 cm and 0.01 cm, calibrated with a mercury thermometer) was used to check the temperature underneath each chamber at settings of 34°C and 41°C. This thermistor (custom prepared from a recycled 2F Swan-Ganz catheter, Edwards, Irvine, United States) was placed between the skin and the double-sided tape within a tub of heat-conducting paste.

The sequence for inducing thermal hyperemia was as follows. The temperature was set at 34°C during about three minutes to ensure thermal stability. Then, SkBF was recorded for five minutes at 34°C, after which the temperature was raised to 41°C and maintained at this level for the next 30 minutes [3]. The time required to reach the final temperature was slightly shorter with the commercial device.

For the measurements with the LDF device, probes were fitted into either a custom-made or a commercial chamber. An adaptor was required to hold the PF408 probe in the custom-made chamber (Figure 1C). In preliminary experiments, a small-size thermistor (length and diameter of 0.3 cm and 0.01 cm, calibrated with a mercury thermometer) was used to check the temperature underneath each chamber at settings of 34°C and 41°C. This thermistor (custom prepared from a recycled 2F Swan-Ganz catheter, Edwards, Irvine, United States) was placed between the skin and the double-sided tape within a tub of heat-conducting paste.

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remained quiet in that position for about 15 minutes before taking of any recordings.

On the ventral surface of one forearm (dominant or not), two sites (A, B) were selected, distant from each other by 2–3 cm and excluding visible veins. The site A received the custom-made chamber, which was filled with saline and overlaid with a transparent glass cover slip (Figure 1A). Site B, was placed an empty commercial chamber, overlaid with a transparent glass cover slip, too (Figure 1B). It was not feasible to fill this chamber with water, as it was not water-tight. SkBF was measured by LDI, simultaneously in two sites (A, B) were selected, distant from each other on the ventral surface of the other forearm, to receive the custom-made chamber, which was filled with saline and using the two channels of the Periflux PF4001. Care was taken that the probes did not exert any pressure on the skin. With this experimental design, the conditions of our previous study [3,20] were sufficiently reproduced on site A, and those of site D were analogous to those used by Cracowski et al. or Shatzy et al. [4,20].

At T0 (time zero), the temperature of the four chambers was raised from 34°C to 41°C and maintained at this level for the next 30 minutes. At T0 +30 minutes (time zero plus 30 minutes), the heating was turned off. The chambers on sites A and B were uncovered, and saliine was spilled from the chamber located on site A. Blood pressure and heart rate were measured on the arm on which SkBF was measured by LDF, simultaneously on sites C and D, using the two channels of the Periflux PF4001. Care was taken to measure SkBF to avoid any pressure on the skin. With this experimental design, the conditions of our previous study [3,20] were sufficiently reproduced on site A, and those of site D were analogous to those used by Cracowski et al. or Shatzy et al. [4,20].

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Data Processing

The raw flow images generated by the LDF device were processed with the image analysis software provided by the manufacturer (Moor LDI Image Review, V5.0). Each image contained two areas of non-zero flow, corresponding to the custom-made and the commercial chamber, simultaneously exerted as described above. Separate regions of interest were defined around each of these areas, to calculate in each, the spatial average of non-zero pixels. The software has a facility to exclude zero-valued pixels from the computation, so that the result is insensitive to small variations in the shape of the region of interest. The thermal hyperemia elicited by each chamber is thus reduced to a series of average flow values, separated by time intervals of one minute (as scans are repeated at a rate of 1/minute).

The PF4001 laser-Doppler flowmeter generates analog DC output voltages proportional to the detected flow, which were digitized at a sampling frequency of 40 Hz and stored on computer disk, using the PowerLab 8/35 hardware and the Labchart V5.0 software by ADInstruments (Spechbach, Germany). These signals were then time-averaged over successive, contiguous periods of one minute.

In this fashion, whether evaluated with LDI or LDF, all thermal hyperemias were expressed in time series of identical format. The last step in data reduction was then the calculation of the following variables: baseline flow (average of five values corresponding to the five minutes preceding the rise in local temperature), early peak response (maximal flow during the 10 minutes following the rise in temperature, minus baseline flow), nadir response (minimal flow from the time of early peak to the 15th minute of recording, minus baseline flow), and plateau response (mean of the last five flow values, recorded from 25th to 30th minute following the rise in temperature minus baseline flow).

Data Analysis

As measurements obtained with the two laser-Doppler techniques are not in the same units (i.e., volts vs PU), statistical analysis was carried out separately for LDI and for LDF data. Baseline flow, early peak response, nadir response, and plateau response were tested with analysis of variance for repeated measures. The model included time (T0 or T2), chamber type (custom, commercial), and their interaction as repeated factors. The alpha level of all tests was set at 0.05. Data are presented as the mean and SD, unless specified otherwise.

RESULTS

The 28 subjects were healthy men, aged 19–32 years. Fifteen of them were lean (BMI <25 kg/m²) and the others were overweight, but not obese (BMI 25–29 kg/m²). The mean skin temperature measured in the immediate vicinity of sites A, B, C, and D was 32.8 ± 0.8°C.

Between T0 +30 and T2 +30 minutes, HR did not change (65 ± 8 vs 64 ± 7 beats/minute), but the mean BP slightly increased (from 80 ± 7 to 87 ± 6 mmHg, p < 0.001), a difference that may be explained by the discomfort induced by lasting bilateral arm immobilization, as expressed by several subjects.

Figure 2 shows the mean time courses of SkBF responses to local heating, observed in the four experimental conditions. As expected, the general shape was biphasic with an early peak of SkBF occurring between 0 and 5 minutes after the onset of local heating, followed by a nadir during about five minutes and later a secondary progressive increase, which stabilised between 25 and 30 minutes (plateau). The most obvious feature is a decrease in the plateau SkBF contrasting with a slight increase in the early peak, from T0 to T2. These changes were consistently observed in the four conditions. Figure 3 shows the mean values of baseline, early peak, nadir, and plateau SkBF. The visual impression conveyed by Figure 2, was confirmed by the statistical analysis of these parameters. From T0 to T2, the mean decrease in the plateau SkBF and increase in early peak were, respectively, -9% to -16% and +10% to +18% of the value at T0 (p values below 0.05 for all conditions). As occurred with

Figure 3. Summary variables derived from thermal hyperemia responses recorded on the same skin site carried out on two occasions separated by two hours (T0, T2), using two different heating chambers (custom-made, commercial) and two methods for the measurement of skin blood flow (LD, laser-Doppler imaging; LDF, laser-Doppler flowmetry). P values are mean of 28 subjects. Error bars are SE rather than SD for graphical convenience.

* p < 0.05, ** p < 0.01, *** p < 0.001 T2 vs T0. * P < 0.05, ** P < 0.01, *** P < 0.001 commercial vs custom-made chamber.
the plateau, the nadir tended to be lower at T2 than at T0 in the four conditions tested, but the difference reached statistical significance only in the case of the custom-made chamber probed with LDL. Finally (and not obvious in Figure 2), the baseline SkBF, in all conditions, was slightly and significantly lower at T2, in comparison with T0.

At T2, the higher peak response was associated with a higher mean BP and therefore could reflect a change in perfusion pressure rather than in vascular tone. Against this interpretation, cutaneous vascular conductance (i.e., SkBF divided by mean BP) consistently increased from T0 to T2 (LDF custom-made chamber: from 4.7 ± 1.5 to 5.8 ± 1.9 PU/mmHg, p < 0.001; LDL custom-made chamber: from 4.0 ± 2.0 to 5.3 ± 2.6 PU/mmHg, p < 0.001; LDF custom-made chamber: from 6.8 ± 4.0 to 8.3 ± 4.7 mV/mmHg, p < 0.001; LDL custom-made chamber: from 6.2 ± 2.7 to 7.7 ± 3.4 mV/mmHg, p = 0.001).

Finally, the plateau response was somewhat lower with the custom commercial, when compared with the custom-made chamber. Although statistically significant, this effect was minor and could have been related to small differences in heating rate and temperature reached.

**DISCUSSION**

The present study confirms our previous observation that the repeated application of a local thermal stimulus on the same skin patch, at least when carried out within two hours, leads to a reduction in the elicited vasodilatory response. However, two studies [4,20] have not noticed this phenomenon. Therefore, the questions that must be asked are whether there is an apparent discrepancy and whether differences in methods could be involved. The major difference relates to the equipment, both for measuring SkBF (LDF vs LDI) and for local heating (commercially available vs custom-made chambers, which may not have the same surface area and heating rate). Any of these factors could have contributed to the discrepancy between our previous observations [3] and those made by these other groups [4,20]. However, in the present study, desensitization clearly occurred in all tested conditions, supporting its independence from the measuring equipment and heating system used in the experiment.

In the work by Shary et al., 10 subjects participated, five men and five women. The laser-Doppler flowmeter (PF 5010; Perimed) was single point at 780 nm, based on exactly the same technology as the less recent Perimed 4003 used in the present study. The local heaters (also by Perimed) were set at 42°C until SkBF had reached a plateau and then turned off. SkBF values were allowed to return to baseline (in about one hour) and the test was repeated [20] with a plateau response somewhat lower than the first one (94%), a difference that was not statistically significant.

In the protocol by Czaczek et al. [4], six subjects were enrolled, three men and three women. The laser-Doppler flowmeter (MoorLAV; Moor Instruments, Devon, UK), was also single point at 780 nm, and associated with integrated local heaters (SHI02; Moor Instruments). Heating was carried out to 42°C until SkBF reached a plateau (30 minutes), on two occasions separated by two hours [4].

Thus, the set of conditions in the present study essentially included those used by both authors, in terms of equipment and timing. And nevertheless, desensitization of the plateau response was systematically observed.

The major remaining difference is the much larger size of our study, compared with these others. It must be underscored that the primary aim of these two studies was not to test the reproducibility of thermal hyperemia. Rather, they were powered to detect effects of locally administered pharmacological agents, with sites that were either untreated [4] or treated with placebo [20] used as controls. The data just cited from these two studies exclusively concern the control sites.

With relatively few subjects, the desensitization effect could have been missed, considering the variability of SkBF measured with LDI, which is much higher than with LDL, as clearly demonstrated by Rosuth et al. [18]. Indeed, we carried out a preliminary analysis of our data after the inclusion of the first 12 subjects (not shown), with results qualitatively similar to those observed in the present study and statistical significance for desensitization attained on sites evaluated with LDL (p = 0.001), but not with LDI (p = 0.13). Power calculations then induced us to include at least 16 more subjects to have a greater power to detect relatively small effects. Desensitization to NO could account for the observed modification of baseline SkBF if, in these thermal conditions (i.e., 34°C), NO actually contributed to lower dermal microvascular tone, as suggested by some [12,16], although not all studies [18,19]. More difficult to understand in this context is the increase in the early peak response observed from T0 to T2.

As the early peak is not caused by NO, it should not be affected by removing or attenuating (by desensitization) the action of this mediator. One might argue that the basal level of NO-dependent vasodilation (i.e., in normothermia, prior to heating and during the first few minutes of heating, when it would remain unaffected) might still modulate the early peak. In that case, however, the expected result of desensitization to NO would be a decrease, not an increase of the initial vasodilatory response to the thermal stimulus. Similar reasoning would lead to this same conclusion even in the presence of NO-dependent vasodilation by other mechanisms leading to enhanced activity of eNOS, vasoconstrictor on the other hand through a direct action on vascular smooth muscle [8,9]. Importantly, the local thermal challenge could desensitize in a much larger number of subjects to local heating slightly differed in that respect. In our preliminary checks, the temperatures achieved by each system were verified by placing a thermistor probe underneath the adhesive tape affixing the chamber to the skin, i.e., not on the exact sites where SkBF was measured (see Methods). At these sites, a small systematic temperature difference between heating systems therefore cannot be formally excluded.

In summary, we confirmed that the hyperemic response of skin microcirculation to local heating is subject to desensitization, at least in young men and with protocols in which temperature is increased rapidly. Desensitization was observed with two different methods of measuring skin blood flow and two different equipments for carrying out local heating, making it likely that our observations reflect a general physiological phenomenon.

**PERSPECTIVE**

Although its mechanisms remain to be defined, desensitization should be taken into account by studies using thermal hyperemia to probe the physiology or pharmacology of microcirculation in human skin.

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