Transient hyperaemic response to assess skin vascular reactivity: effects of heat and iontophoresed norepinephrine

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Background. Forearm skin vascular reactivity may be assessed using a transient hyperaemic response (THR) after 20 s of brachial artery compression. THR has been manipulated by iontophoresis of vasodilators, but not vasoconstrictors, possibly because of low baseline blood flow. The effects of vasoconstrictors on vascular reactivity of pre-dilated blood vessels are unknown. We have investigated this using locally applied heat to vasodilate the skin microcirculation before iontophoresis of norepinephrine.

Methods. Active and control laser Doppler probes measured forearm skin blood flow-flux. Three THR tests were performed before and after heating skin for 5 min, and then after iontophoresis of norepinephrine 0.1%. Iontophoresis was pulsed using 45 s periods of 75 mA and 0 current over 10 min. Three temperatures were used: unheated skin, skin at 35°C, and skin at 42°C. Baseline flow-flux was measured for 60 s before each set of THR tests. THR ratio (THRR) was calculated by comparing baseline flow-flux immediately before arterial compression (F1) with the maximum after release (F2): THRR=F2/F1. The average values of each group of THRR results, and baseline data, were compared using the Kruskal–Wallis test.

Results. Iontophoresis of norepinephrine caused significant decreases in flow-flux (P<0.005). Unheated skin and skin heated to 35°C showed significant decreases in THRR after norepinephrine. THRR was abolished by heating to 42°C and partially restored by iontophoresis of norepinephrine.

Conclusions. Iontophoresed norepinephrine causes vasoconstriction, and it partially restores vascular reactivity in the heat-induced vasodilated skin. This may be of benefit when norepinephrine is used in clinical situations.


Keywords: blood, flow; blood, flow, forearm; blood, flow, skin; sympathetic nervous system; sympathetic nervous system, norepinephrine

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Many acute and chronic medical conditions are known to disrupt autoregulation and vascular reactivity; these include diabetes, hypertension, heart failure, and sepsis.1–4 Accurate assessment of the degree of any disruption may be beneficial in terms of prognosis and diagnosis, and may also prove useful in guiding treatment.4

We have previously described a non-invasive method of assessing cutaneous vascular reactivity using laser Doppler flowmetry to measure the transient hyperaemic response (THR) in forearm skin.5 Other methods used to measure changes in vascular reactivity commonly involve the provocation of maximal hyperaemic responses to stimuli such as prolonged ischaemia or pharmacological vasodilatation.6 7 Forearm THR requires brief (only 20 s) arterial compression and has proved to be well tolerated and easily repeatable with no prolonged recovery time between tests.5

Previously THR has been shown to be impaired after the application of vasodilators such as iontophoresed sodium nitroprusside (SNP), acetylcholine (ACH), and
local anaesthetics. It is less clear how vasoconstrictors might affect vascular reactivity as measured by THR.

A previous study using iontophoresed phenylephrine and epinephrine failed to show any consistent effects on THR. One suggestion is that the cutaneous microcirculation is relatively vasoconstricted in its resting state and is unlikely to be dramatically affected by the further application of a vasoconstricting stimulus. Other studies have shown that iontophoresed phenylephrine can reverse the vasodilatation of the forearm skin microcirculation seen after immersion in warm water. We hypothesized that localized heating of forearm skin will result in vasodilatation and a reduction in THR. In addition, we hypothesized that iontophoresis of norepinephrine to the skin will reverse the heat-induced changes in flow-flux and THR.

Methods

The Ethical Review Committee of the University of Nottingham approved the study. Three groups of 15 healthy volunteers were studied (aged 20–51 yr). Any volunteer with a history of cardiovascular disease was excluded, as was anyone taking regular medication. Specific exclusion criteria included a history of diabetes, peripheral vascular disease, hypertension, Raynaud’s phenomenon, systemic sclerosis, morbid obesity, or smoking. Damaged forearm skin or a history of allergy to adhesive dressings also precluded entry into the study. Volunteers were also asked to refrain from consuming caffeine-containing substances for a minimum of 2 h before being studied. Written, informed consent was obtained from each volunteer.

Subjects were allowed to acclimatize in a quiet room for 20 min where they were asked to sit comfortably in a chair with one arm resting on a table. They were asked to remain still throughout the period of investigation.

The methodology of the THR test has been described in previous studies and was only modified slightly to allow for localized heating to be applied. A custom-built iontophoresis chamber containing an inert platinum electrode was attached to the anterior aspect of the forearm using adhesive tape, 5–10 cm away from the antecubital fossa. An electrode attached to the forearm near the wrist completed the iontophoresis circuit. The iontophoresis chamber had two apertures; the larger central well held a combined heater and temperature probe in close proximity to the skin. When required, a quantity of drug could be placed in the well before insertion of the heater, and this could be topped-up via the smaller aperture. Iontophoresis was controlled using a MIC1-e system (Moor, Axminster, UK). Blood flow-flux was measured using a laser Doppler needle probe which passed through the centre of the heater. Localized heating was provided by an SHO2 servo-controlled heater (Moor) which allowed skin temperature to be heated to a maximum of 45°C with an accuracy of 0.1°C. A control probe with a combined temperature sensor and laser Doppler was placed on the forearm distal to the iontophoresis chamber. Flow-flux was measured using a DRT4 laser and recorded using dedicated software (Moor).

There were three separate protocols, one for each group of volunteers, with each volunteer acting as his/her own control. In each protocol, iontophoresis of norepinephrine took place at different skin temperatures: unheated skin, skin at 35°C, and skin heated to 42°C. Flow-flux and THR were measured before and after every period of heating or iontophoresis (Table 1). The skin was heated to the set temperature for 5 min, during which time a plateau of Doppler flux was reached. Iontophoresis of epinephrine 0.1% was performed using a pulsed cathodal current, which involved 14 45 s periods alternating between 75 μA and no current. This pattern of iontophoresis is well tolerated and avoids skin polarization. Pilot studies demonstrated a consistent reduction in skin blood flow-flux to minimal values using this protocol. Arterial pressure was recorded before and after iontophoresis to ensure that locally applied norepinephrine had no systemic effects.

The choice of temperatures was decided after an initial pilot study which showed that between 35°C and 41°C, the flow-flux increased and the THR decreased in a dose–response manner.

THRR has an estimated coefficient of variation of 25%. In order to detect a 25% change in THRR in each paired comparison with a power of 0.8 and α of 0.05, we calculated a requirement for 15 volunteers in each group allowing for multiple comparisons.

Baseline flow-flux and THRR were measured for both active and control lasers. A 60 s period of baseline Doppler flow-flux followed by three THR tests was recorded before and after every intervention. Each THR was separated by a pause of 60 s. THR was elicited by a 20 s manual compression of the brachial artery at a level midway along the upper arm. Confirmation that the artery was being compressed was achieved by observing the presence of a minimal response representing biological zero on the Doppler signal (F0). THR ratio (THRR) was calculated by comparing a 10 s average of the baseline flow-flux immediately before arterial compression (F1) with the maximum increase after release (F2) such that THRR=F2/F1. The mean of each set of three THR results for each subject was used for analysis. Temperature readings taken at the same time as THR tests were treated in the same fashion.

Changes in the values of flow-flux and THRR were compared using the Kruskal–Wallis test and statistically

<table>
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<tr>
<th>Table 1 Iontophoresis protocols. Each group consisted of 15 volunteers. Flow-flux and THRR were measured before and after every period of heating or iontophoresis. Each period of heating lasted 5 min before any measurements were made.</th>
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<tr>
<td>Group 1 Iontophoresis in unheated skin</td>
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<td>Group 2 Heating to 35°C Iontophoresis at 35°C</td>
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<td>Group 3 Heating to 42°C Iontophoresis at 42°C</td>
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Results

There were no significant differences in resting arterial pressure or unheated skin temperature between the groups, and arterial pressure did not change significantly throughout the course of the experiment (Table 2).

All the experimental protocols were well tolerated by the volunteers and there was minimal discomfort with no adverse reactions. Some volunteers commented upon an itchy sensation associated with iontophoresis. No volunteers reported awareness of any sensation associated with heating. All volunteers completed the study. Some volunteers developed minor skin blanching after the application of norepinephrine which persisted for 1–2 h.

Control laser measurements did not change significantly in any of the groups of subjects throughout the course of the experiment. The control measurements of flow-flux and THRR were similar to the baseline measurements from the active probe.

All THR were considered suitable for analysis. Heating skin to 35°C did not significantly change either flow-flux or THRR (Table 3). Heating skin to 42°C resulted in a marked increase in flow-flux from a median value of 11.8 (arbitrary units) to 116.6 (P<0.001). The vascular reactivity at this temperature was abolished; THRR decreased significantly from a median value of 3 to 0.96 (P<0.001).

At 42°C, the increased flow-flux of vasodilated skin took longer to reach biological zero once arterial pressure was applied, and the level of biological zero was appreciably higher than during THR tests at ambient temperature. THR tests at 42°C did not result in a classic hyperaemic response (as reflected in THRR results), but instead resulted in a return to baseline (F1) levels with little or no hyperaemic peak. In fact, the flow-flux after arterial release was often below baseline (F1), decreasing still further before gradually increasing once more to baseline values (Fig. 1).

In unheated skin, and in skin at 35°C, the iontophoresis of norepinephrine led to a significant decrease in flow-flux, and it significantly decreased the value of THRR. However, the values of THRR remained >1.00, suggesting that some vascular reactivity was still preserved.

At 42°C, the iontophoresis of norepinephrine restored the flow-flux close to baseline by decreasing the median value from 116.6 to 13.7. In addition, it moderately restored the vascular reactivity by increasing the THRR from 0.96 to 1.62, although not to the baseline value of 3.0 (Table 3).

Discussion

We have shown that local heating of skin produces a vasodilatory response, as evidenced by changes in flow-flux, which led to the loss of THR at 42°C. In addition, we have shown that iontophoresis of norepinephrine 0.1% caused consistent decrease in flow-flux at all skin temperatures. At relatively normal skin temperatures, norepinephrine significantly decreased vascular reactivity, but did not abolish it. At 42°C, when the vascular reactivity was abolished, norepinephrine partially restored it.

Most techniques of assessment of vascular reactivity involve a one-off measurement of the maximum degree of vasodilatation obtainable by a hyperaemic insult. THR offers the ability to examine changes in the dynamic function of blood vessels which occur over time, or in response to potential treatments. This may be of benefit in diseases which are known to affect vascular reactivity. Hyperaemic responses may be affected by disease or by vascular tone:
fully vasodilated vessels may be normal but have no hyperaemic response.

Forearm skin is readily accessible in most patients and its microcirculation can be studied using laser Doppler flowmetry which measures red cell flux to a depth of 1–2 mm. Changes in cutaneous microcirculation may be a reasonable surrogate marker for changes elsewhere within the body failure, yet there is a paucity of data concerning factors which affect it.

It is unclear why norepinephrine has affected THR in this study when previously THR had been unaffected by phenylephrine and epinephrine. Changing the agent and the iontophoresis regimen may have resulted in better delivery of the vasoconstrictor. The amount of drug delivered by iontophoresis is affected by many factors, including strength of current, length of current-time, degree of skin polarization, and any ionic charge held by the drug.

One of the criticisms of this technique is that the exact quantity of drug iontophoresed remains unknown, although the individual Doppler traces indicated a maximal reduction in flow-flux occurred in response to norepinephrine midway through the iontophoresis regimen. We did not conduct a dose–response study and different iontophoresis protocols may have produced different results. Despite this reduction in flow-flux, THR was not abolished, only reduced. Norepinephrine also partially restored vascular reactivity after it had been temporarily abolished by heating skin to 42°C. These findings are supported by animal models of sepsis where organ blood flow is measured, and by clinical studies using gastric tonometry as a marker of splanchnic blood flow where moderate doses of norepinephrine appear to preserve autoregulation.

Other studies suggest that any preservation of autoregulation caused by norepinephrine might also occur in the skin microcirculation. The skin of critically ill patients exhibits diminished reactive hyperaemia. However, the skin microcirculation of patients with septic shock requiring norepinephrine was still responsive to vasodilation triggered by the iontophoresis of ACh and SNP, albeit to a lesser extent than the microcirculation of healthy volunteers.

Previous studies have indicated that hyperaemia associated with prolonged ischaemia can be enhanced by local warming which would suggest the presence of different vasodilatory mechanisms. Studies using iontophoresed bretylium to abolish sympathetic vasoconstrictor control have suggested that the reflex vasodilatory effects of localized heating are most likely mediated via vasoconstrictor withdrawal. Assuming this to be the case, the application of exogenous vasoconstrictor would effectively reverse any changes seen. Exogenous vasoconstrictor in amounts over and above that required to replace endogenous vasoconstrictor might result in the microcirculation becoming resistant to vasodilation. This could explain why in our experiment norepinephrine returned blood flow in heated skin to near-normal values, but only partially restored THR. Unfortunately, the effects of localized heating upon skin blood vessels is not as clear cut as this explanation might suggest as denervated skin is known to be capable of reacting to both heat and hyperaemic stimuli.

Our study is limited that it does not explore the underlying mechanisms of changes in skin vascular behaviour during heat, iontophoresed norepinephrine, or the interaction between the two. The results, however, provide insight into how different factors may interplay to alter vascular reactivity that may have implications if the tests to assess vascular reactivity (such as THR test) are to be used and interpreted in clinical situations. Although the results obtained from healthy volunteers in this study cannot be translated into clinical scenarios, we believe that the data will serve as an important point of reference for future studies in clinical settings.

**Funding**

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**References**

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