Transient hyperaemic response to assess vascular reactivity of skin: effect of locally iontophoresed acetylcholine, bradykinin, epinephrine and phenylephrine

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Background. Recently, the transient hyperaemic response (THR) to brief compression (20 s) of the brachial artery has been described as a way to assess vascular reactivity of the forearm skin. We studied the effects of locally iontophoresed vasoactive agents on this response in 20 male volunteers.

Methods. An iontophoresis chamber attached to the anterior forearm permitted simultaneous administration of drugs by iontophoresis and measurement of skin blood flow-flux by laser Doppler probe. Three THR tests were performed before and after iontophoresis by compressing the brachial artery with digital pressure for 20 s and then releasing. The following were iontophoresed: saline 0.9% (iontophoresis vehicle control), acetylcholine, bradykinin, epinephrine and phenylephrine. The THR ratio (THRR) was calculated as F2/F1 where F1 was baseline blood flow-flux immediately before compression and F2 was peak blood flow-flux after release.

Results. When compared with saline 0.9%, acetylcholine and bradykinin increased median F1 from 9.2 (range 5.2–23.8) to 22.1 (8.7–61.5) and from 4.8 (3.0–23.2) to 15.0 (2.5–31.8), respectively, and reduced THRR from 1.26 (1.07–2.2) to 0.99 (0.93–1.04) and from 1.63 (1.06–2.58) to 1.09 (0.93–1.19), respectively. Epinephrine, but not phenylephrine, caused a significant reduction in F1 from 9.2 (5.2–23.8) to 4.0 (1.5–22.3). Neither epinephrine nor phenylephrine had significant effect on THRR.

Conclusions. Iontophoresed acetylcholine and bradykinin significantly increase the flow-flux and impair THR in forearm skin, further validating the concept that THR represents true vasodilatation during arterial occlusion. In addition, iontophoresis of vasoconstrictors does not appear to have any consistent effect.

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Abnormal vascular reactivity is implicated in the symptomatology and pathogenesis of many disease states, both chronic (diabetes mellitus, hypertension) and acute (sepsis). A simple test to assess the response of tissues to disturbance of perfusion may be of benefit in screening, diagnosis, prognosis and treatment. Recently, assessment of vascular reactivity has also been used to predict haemodynamic responses during anaesthesia. This test has several theoretical advantages over the previously described hyperaemic responses following tourniquet application for 3–15 min. Because of a shorter interruption of flow, it would tend to test predominantly myogenic behaviour of the vessels. Also, the test is easily repeatable and is therefore more suitable for research and application in anaesthesia and intensive care.
care. However, there is a paucity of data regarding the effects of different physiological and pharmacological influences on the THR. This knowledge is important; it serves as a point of reference, before the test can be used in the clinical settings.

A previous study has shown that THR was abolished with the iontophoresis of sodium nitroprusside, an endothelium-independent vasodilator.\(^8\) Skin blood vessels also dilate in response to endothelium-dependent substances such as acetylcholine and bradykinin;\(^10\)\(^11\) the effects of these substances on the THR are unknown. The effects of localized vasoconstriction on the THR are also unknown. In addition, iontophoresis itself has a small vasodilatory effect\(^12\) and how that may affect THR is unknown.

We hypothesized that THR would be diminished by alteration of vascular tone with iontophoresis of vasodilators, and that the vasoconstrictors would have the opposite effect. Furthermore, we hypothesized that these effects would be different from those produced by iontophoresis of saline 0.9%.

**Methods**

The Ethical Review Committee of Nottingham University Medical School approved the study. Twenty healthy male volunteers (aged 19–43 years) were studied. Exclusion criteria were systemic or vascular disease, including diabetes mellitus, hypertension, Raynaud’s phenomenon or systemic sclerosis, use of any non-steroidal anti-inflammatory or vasoactive medications, and a history of obesity or smoking. Volunteers were requested to refrain from eating or ingesting caffeine for 2 h before the study. The protocol was explained, and written informed consent was obtained from each volunteer.

The study took place in a quiet room and began after a 20-min period of acclimatization. The participants were asked to keep still and quiet for the period of investigation.

The methodology of the THR test was similar to that described in a previous study.\(^5\) Each volunteer lay supine with one arm outstretched on a pillow at the level of the right atrium. A custom-built Perspex\(^\circledR\) iontophoresis chamber was attached to the volar aspect of the distal forearm using double-sided adhesive tape (Fig. 1). This chamber was specifically designed to allow the simultaneous iontophoresis of drugs and measurement of blood flow-flux by the laser Doppler probe (DR2, Moor Instruments, Axminster, UK). The chamber had two apertures: one to hold the laser Doppler at 90° to the skin and the other for drug administration. The drug chamber contained an inert platinum wire attached to a current generator to serve as the active electrode during iontophoresis. An electrode attached to the forearm 4 cm proximal to the chamber completed the circuit. The chamber was placed on an area of skin far enough away from the antecubital fossa to avoid movement artefacts during THR testing. Placement over obvious superficial veins was avoided. Flow-flux was recorded on a chart recorder running at 0.5 mm s\(^{-1}\). The unit of measurement was the voltage, which was related to flow-flux by an unknown constant.

Arterial pressure was measured continuously using a non-invasive device (Finapress\(^\circledR\)) on a finger of the contralateral arm. Values were recorded every 3 min and changes in mean arterial pressure >10% from baseline were considered significant. Local skin temperature was recorded using a surface probe attached near the iontophoresis site. A change of >0.3°C was considered significant.

**THR tests**

After a steady baseline flow-flux had been recorded for at least 60 s, the brachial artery in the antecubital fossa was identified. This was compressed manually by the investigator for 20 s and then released.

Two practice tests were performed on each volunteer, followed by three control THR tests at 2-min intervals to allow flow-flux to return to baseline. Two min after the last control THR test, saline 0.9% was iontophoresed using a current of 100 \(\mu\)A for 240 s delivered by a constant current generator. One min after completion of iontophoresis, three more THR tests were performed.

The participants were divided randomly into two groups of ten. They received either acetylcholine 1% and epinephrine 1% or bradykinin 0.1% and phenylephrine 1% in random order. All drugs were diluted in saline 0.9% and each was iontophoresed using 100 \(\mu\)A for 240 s. To avoid contamination, a new chamber at a different site on the same forearm was used for each drug. Saline, acetylcholine, epinephrine and phenylephrine were iontophoresed using an anodal current whereas bradykinin was iontophoresed using a cathodal current. A practice THR test was performed after moving the probe to a different site but before iontophoresis to confirm the presence of THR at the new site.

![Fig 1 Iontophoresis and laser Doppler equipment: A, laser Doppler probe; B, iontophoresis chamber and electrode; C, dispersive electrode.](image-url)
Tests were accepted only if: (i) baseline flux was steady before compression; (ii) flow-flux decreased abruptly and consistently at the onset of compression to ‘biological zero’; and (iii) the hyperaemic response was free from movement artefact (Fig. 2).

Data analysis
The following values were recorded for each THR test: the flow-flux immediately before compression (F1), the maximum flow-flux after release (F2), skin temperature and blood pressure (Fig. 2).

THR ratio (THRR) was defined as F2/F1. Doppler signal persisting after complete occlusion (i.e. unrelated to tissue perfusion) was taken as ‘biological zero’. The mean value of each set of three THR tests for each subject was used for analysis.

Previous work gave an estimated coefficient of variation in THRR of 25%. We calculated that a sample size of ten subjects would be sufficient to detect a difference of 25% in THRR for paired comparisons within the groups with a power of 0.8 and α=0.05.

ANOVA was used to assess differences in skin temperature or arterial pressure between experimental conditions. The Mann–Whitney U-test was used to assess differences in baseline flow-flux and THRR before and after iontophoresis with saline 0.9%, and for comparisons between iontophoresis of saline 0.9% and iontophoresis with vasoactive drug.

All analyses was performed using Minitab 13.3 statistical software (Minitab Inc., State College, Pennsylvania, USA).

Results
None of the volunteers experienced a significant change in arterial pressure or skin temperature (mean 32.3°C, SD 0.35) during the study. All of the THR tests were considered suitable for analysis. All data are given as median (range).

Compared with the baseline values of F1 and THRR, saline iontophoresis caused an insignificant change in F1 from 5.4 V (2.6–20.9) to 7.0 V (3.0–23.8), and a significant decrease in THRR from 1.9 (1.23–3.32) to 1.39 (1.06–2.58) (P<0.004). All further comparisons were therefore made between saline and experimental drug iontophoresis. The results for the two groups are given in Tables 1 and 2. Acetylcholine and bradykinin both increased F1 and decreased THRR significantly. Epinephrine but not phenylephrine decreased F1. Neither epinephrine nor phenylephrine had a significant effect on THRR. Individual results for acetylcholine and bradykinin are shown in Figure 3.

There were no adverse events. Some subjects volunteered an awareness of prickling or itching during iontophoresis.

Discussion
We have shown that acetylcholine and bradykinin significantly increase the baseline flow-flux and decrease THRR; epinephrine and phenylephrine have inconsistent effects. In addition, we have shown that iontophoresis with saline 0.9% causes an insignificant change in baseline flow-flux but a significant decrease in THRR. The results confirm that the THR of forearm skin to brief brachial artery compression is impaired when the vascular bed is pre-dilated, as in this study, by endothelium-dependent vasodilators.

Increasingly, vascular research in anaesthesia and intensive care has focused on the dynamic ability of the human system to respond appropriately to disequilibrium rather than more static measures such as degree of vasodilation or vasoconstriction. Disturbances of dynamic function may provide more physiological and prognostic information than simple measures of flow or vascular resistance. This led us to assess whether the THR to brief interruption in blood flow, which has been studied in cerebrovascular context, could be adapted for use in forearm skin, which is an easily accessible organ in almost all patients with critical illness or undergoing anaesthesia and surgery.

Laser Doppler flowmetry can be used to study skin microcirculatory flow. The red-cell flux signal represents the mean from all vessels within a small region of tissue, whose volume cannot be defined precisely. It is thought that the penetration is of the order of 1–2 mm, with a major part of the signal being reflected from sub-papillary vessels and a

![Fig 2](image-url)

Fig 2 Sample traces of transient hyperaemic response (THR) tests. THR ratio is defined as F2/F1. (A) control THR test; (B) THR test after vasodilation with acetylcholine.

<table>
<thead>
<tr>
<th>Table 1 The effects of acetylcholine and epinephrine iontophoresis on baseline flow-flux and transient hyperaemic response ratio (THRR). Data are median (range), n=10, n.s. = not significant vs saline iontophoresis</th>
</tr>
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<tbody>
<tr>
<td>Baseline flow-flux (V)</td>
</tr>
<tr>
<td>------------------------</td>
</tr>
<tr>
<td>Saline 9.2 (5.2–23.8)</td>
</tr>
<tr>
<td>Acetylcholine 22.1 (8.7–61.5)</td>
</tr>
<tr>
<td>Epinephrine 4.0 (1.5–22.3)</td>
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smaller part from capillaries. Consistent with other published work, we found significant variability in baseline flow-flux (range 2.8–20.9 V).\(^{12,7,10}\) This variability was seen despite our attempt to control the factors related to subject selection and study design.\(^{15,16}\) Variability is however inherent in the technique, not least because of the small volume of tissue sampled in comparison with the heterogeneity of skin blood flow and vascular architecture.\(^{15}\) Previously, workers have attempted to reduce this by strict adherence to identical site placement, time of day, laboratory temperature and larger sampling volumes using multiple sites or scanning technology.\(^{15}\) Although these measures resulted in reduced variability, they are of little help in the clinical setting. However, the advantages of laser are that it is non-invasive and portable, and despite large variability in baseline flow-flux, it has been shown to be sensitive and reproducible in detecting changes in response to various stimuli. Because of this, Doppler flowmetry has been used widely in the study of vascular reactivity.\(^{12}\)

The hyperaemic response to the release of brief occlusion of the feeding vessels is thought to be the result of local vasodilatation. There are at least two interacting mechanisms of reactive hyperaemia: (i) myogenic vasodilatation caused by interruption of flow leading to changes in vessel wall tension, compliance and pressure; and (ii) metabolite accumulation during periods of oligemia.\(^{9,17}\)

The mechanisms are time dependent.\(^{18}\) Myogenic relaxation occurs early and is thought to be complete in approximately 20 s. Prolonged oligemia leads to a reduction in oxygen tension and the accumulation of vaso-dilatory metabolites, including hydrogen ions, adenosine, potassium, phosphate, lactate, kinins, peptides, prostaglandins and nitric oxide. These augment and prolong the initial myogenic vasodilatation.\(^{17}\)

Much work has been published regarding the mechanism and characteristics of the response to prolonged ischaemia (2–15 min), which is predominantly a metabolic response.\(^{9,17}\) However, this may not be applicable to research related to anaesthesia and intensive care for two reasons. First, the response is prolonged, and in the awake subject somewhat unpleasant. It is therefore not well suited to repeated testing in rapidly changing conditions. Second, there is evidence that certain conditions such as sepsis alter the myogenic component of vascular control,\(^{3}\) and prolonged ischaemia tests cannot distinguish myogenic from metabolic components. In addition, arterial occlusion for this length of time is achieved using circumferential tourniquets, and associated venous occlusion may have confounding effects on the myogenic hyperaemic response.\(^{9}\)

Previous work has shown that the THR test provokes a much smaller response\(^{9}\) than prolonged occlusion,\(^{9,17}\) but with a similar degree of variability. The response is much shorter, being complete within 20 s rather than over 5 min, making repeated tests more practical. Iontophoresis of sodium nitroprusside, an endothelium-independent vasodilator, caused an increase in baseline flow; it abolished THR.\(^{8}\) This was taken as evidence that the small, transient hyperaemic response after release of compression represents true vasodilatation during the period of compression.

Iontophoresis provides a convenient method of local administration of charged molecules without systemic effects. It uses a small direct electrical current to drive ions across the dermal barrier according to their electrical charge. However, iontophoresis itself can have a non-specific vasodilating effect, dependent on the charge, voltage and ionic vehicle used.\(^{15}\) The appropriate control for iontophoresis is therefore iontophoresis with the carrier vehicle for the substances to be tested. Consistent with previous studies, we found a trend towards increased baseline flow-flux with iontophoresis of saline 0.9%,\(^{12,19}\)

**Table 2** The effects of bradykinin and phenylephrine iontophoresis on baseline flow-flux and transient hyperaemic response ratio (THRR). Data are median (range), n=10, n.s.=not significant vs saline iontophoresis

<table>
<thead>
<tr>
<th></th>
<th>Baseline flow-flux (V)</th>
<th>P-value</th>
<th>THRR</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline</td>
<td>4.8 (3.0–23.2)</td>
<td></td>
<td>1.63 (1.06–2.58)</td>
<td></td>
</tr>
<tr>
<td>Bradykinin</td>
<td>15.0 (2.5–31.8)</td>
<td>0.05</td>
<td>1.09 (0.93–1.75)</td>
<td>0.014</td>
</tr>
<tr>
<td>Phenylephrine</td>
<td>5.1 (1.5–17.2)</td>
<td>n.s.</td>
<td>1.78 (1.01–4.25)</td>
<td>n.s.</td>
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</table>

**Fig 3** The effect of acetylcholine and bradykinin on baseline flow-flux and transient hyperemic response (THR) ratio. Individual data points are shown as dotted lines. Median values are shown as a solid line.
although this did not reach statistical significance, probably because of large inter-subject variability. A small but significant reduction in THR was seen, consistent with the hypothesis that vasodilation from whatever cause will impair the hyperaemic response. The response of baseline flow-flux to acetylcholine was similar to that described previously. In addition, in the present study, vasodilation abolished the THR, again supporting the hypothesis of vasodilation as the mechanism of the THR. Iontophoresis of bradykinin has not been reported before; the mechanism of action is thought to be mainly endothelium dependent, involving nitric oxide production, hyperpolarization of the vascular wall and prostaglandin production. An axonal reflex secondary to nociceptor activation has also been proposed.

The effect of sympathomimetic agents on THR is less clear. Classically, the α-adrenergic effects of epinephrine and phenylephrine would be expected to cause vasoconstriction, which could have variable effects on the THR. In theory, vasoconstriction could have any of three effects on THR. First, vasoconstriction that is proportional across a range of vasodilatory stimuli would not affect THR (peak flow-flux would be affected as much as baseline). Second, marked vasoconstriction would attenuate THR by preventing vasodilation. Third, vasoconstriction that can be overcome by sufficient vasodilatory stimulus would increase THR (peak flow would be ‘normal’ but starting from a lower baseline). This study found no consistent change in either baseline flow-flux or THR.

Previous work has given conflicting results. Some workers have found a neurally mediated vasodilation in response to norepinephrine and phenylephrine that was blocked by local anaesthesia. Others found a vasoconstrictive response to phenylephrine and an attenuation of post-ischaemic hyperaemia (ischaemia induced with tourniquet applied for >2 min) with epinephrine. Baseline flow-flux is low in ‘nutritive’ skin such as the forearm compared with thermoregulatory areas such as the finger pulp. It may be that no consistent vasoconstrictive effect on baseline was seen in our study for this reason. Preliminary studies have shown that the effects of α-adrenergic agonists have been tried by other workers but with limited success. However, using drugs to pre-dilate confounds the pharmacology of the response to α-agonists. Post hoc analysis of the individual data for the α-agonists in the present study suggests that two different types of response may be seen: a vasoconstrictor response which augments THR or a vasodilator response with diminution of THR. Presumably this could reflect differences in baseline sympathetic tone.

In conclusion, this study provides further evidence that vasodilatation can impair THR, validating the concept that THR represents true vasodilatation in forearm skin blood vessels during arterial occlusion. In addition, we have shown that the effects of vasodilators are more pronounced than those seen with iontophoresis of saline 0.9%.

Furthermore, iontophoresis of vasoconstrictors does not appear to have any consistent effects.

References

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