

The effect of acetylcholine on finger capillary pressure and capillary flow in healthy volunteers

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1. Constitutive nitric oxide (NO) synthase has been demonstrated in human skin microvascular endothelial cells; however, the physiological significance of this finding is not known. The aim of this study was to investigate the effects of acetylcholine (ACh), which stimulates the release of NO from endothelial cells, on skin capillary pressure, capillary pulse pressure amplitude (CPPA) and capillary red blood cell velocity (CBV) in healthy volunteers.
2. Finger nailfold capillary pressure was measured in five healthy volunteers. CBV was measured in capillaries of the dorsal middle phalangeal area of the finger in six subjects using a recently developed capillary anemometer. In each case the responses to iontophoretically applied ACh and vehicle were measured on two separate fingers on the left hand.
3. Application of vehicle did not significantly change either capillary pressure, CPPA or CBV. ACh significantly increased capillary pressure (from 15.8 ± 2.2 mmHg under basal conditions to 27.7 ± 3.8 mmHg at the plateau of the ACh response; $P < 0.008$), CPPA (from 2.4 ± 2.4 mmHg at baseline to 8.4 ± 2.4 mmHg at the plateau of the drug response; $P < 0.013$) and CBV (from 0.54 ± 0.22 mm s⁻¹ at baseline to 2.46 ± 1.12 mm s⁻¹ after ACh; $P < 0.008$).
4. The increases in capillary pressure, CPPA and CBV following the application of ACh suggest that the overall effect of ACh was to induce a reduction in the pre- to postcapillary resistance ratio.

The transport and exchange of substances such as nutrients, waste products, fluids and proteins across the microvascular bed is crucial for maintaining tissue homeostasis. Fluid exchange is governed by Starling's forces (the differences between osmotic and hydrostatic pressures between the blood and the tissue). Capillary pressure is the most variable of these forces and is therefore likely to have a major influence on transcapillary fluid exchange (Michel, 1983). The level of capillary pressure is determined by the pre- to postcapillary resistance ratio as well as by arterial and venous pressures. Capillary pressure may be protected from small increases in systemic blood pressure (Shore, Sandeman & Tooke, 1993) and to a lesser extent from changes in venous pressure (Mahy, Tooke & Shore, 1995). It is believed that these protective measures may be brought about through regulatory changes in the pre- to postcapillary resistance ratio. However, little is known about the mechanisms involved in the regulation of capillary pressure in man.

The presence of constitutive nitric oxide (NO) synthase has been demonstrated in human cutaneous microvascular endothelial cells (Bull, Hothersall & Dowd, 1995). The physiological significance of this finding is not fully understood, although it suggests that NO may play a role in

the control of the microcirculation. One of the actions of acetylcholine (ACh) is to stimulate the production of NO, through the conversion of L-arginine by NO synthase, from the vascular endothelium in many varieties of tissues and species (Rubanyi, 1991).

The aim of this study was therefore to investigate the effects of the iontophoretic application of ACh on the control of the skin microcirculation, specifically its actions on finger nailfold capillary pressure, capillary pulse pressure amplitude (CPPA) and finger capillary red blood cell velocity (CBV) in nutritive capillaries.

METHODS

Subjects

Finger nailfold capillary pressure and finger CBV were measured in two different groups of subjects, with one subject common to both groups. Capillary pressure was measured in five healthy volunteers (3 female, 2 male), aged 33.8 ± 8.2 years. CBV was measured in six volunteers (4 female, 2 male), aged 27.5 ± 4.8 years. Six of the females were taking the oral contraceptive pill, but none of the subjects were taking any other vasoactive medication. Subjects were asked to refrain from smoking on the day of the study and not to consume caffeine-containing drinks for at least 2 h before the start of the measurements. All the volunteers gave their fully

informed, witnessed, written consent to this study, which was approved by the South Western Local Research Ethics Committee.

Experimental protocol

Capillary pressure and CPPA (an indicator of precapillary resistance) were measured in nailfold capillaries. Pilot studies demonstrated that it was not technically possible to measure CBV responses to the iontophoresis of ACh in nailfold capillaries. This was due to the fact that CBV analysis by standard techniques requires the application of a translucent layer (e.g. nail varnish and paraffin oil) to the nailfold in order to produce a high-resolution microscopic image; in this study it was impossible to use such a translucent layer as it would impede drug delivery by iontophoresis. Until recently, CBV measurements have been restricted to the nailfold area (Flynn, Williams & Tooke, 1989), but the newly developed capillary anemometer CAM1 (KK Technology, Axminster, UK) allows the measurement of CBV in perpendicular skin capillaries. Therefore the CBV responses to ACh were measured in capillaries of the dorsal middle phalangeal area of the finger, which are anatomically similar to nailfold capillaries (Zook, 1990).

All studies were performed in a temperature-controlled room (21.5–22.5 °C) with the subjects lying in the supine position, following a 30 min acclimatization period. ACh (1%) and ACh vehicle (3% mannitol in water for injection) were administered using iontophoresis, a technique which allows the local transfer of charged substances across the skin by means of a small electric charge (Harris, 1967). Due to differences in the thickness and size of the area of skin undergoing iontophoresis at the nailfold and dorsal middle phalangeal area of the finger, it was necessary to use slightly different iontophoresis protocols at each of these sites. In both cases the protocol was designed to elicit a maximum response to ACh.

At the finger nailfold, a Perspex direct electrode chamber (17 mm length × 14 mm width × 2 mm height; inner (drug) chamber, 7 mm length × 4 mm width; Moor Instruments, Axminster, Devon, UK) was used to deliver the solutions. The chamber was attached to the finger by means of a double-sided adhesive disc and a thin layer of Plasticene so that the drug chamber was positioned directly over the nailfold. An indifferent electrode was attached to the wrist of the subject to complete the circuit and the chamber was filled with the solution under test. A battery-powered iontophoresis controller (MIC 1, Moor Instruments) was used to provide a direct current for drug iontophoresis. The substances were delivered using an anodal charge: 7 × 0.05 mA for 20 s followed by 1 × 0.1 mA for 20 s with a 60 s interval between each dose.

At the dorsal middle phalangeal area of the finger nailfold, a larger Perspex direct electrode chamber (30 mm total diameter × 3 mm height; inner chamber, 10 mm diameter) was used. The solutions were delivered using an anodal charge: 5 × 0.1 mA for 20 s with a 60 s interval between each dose.

In each subject, the responses to ACh and ACh vehicle were measured on separate fingers on the left hand. The application of drug and vehicle was randomized and the operator was blind to the nature of the solution under test.

Measurement of capillary pressure

The method of capillary pressure measurement has been described in detail elsewhere (Wiederhielm, Woodbury, Kirk & Rushmer, 1964; Shore, Sandeman & Tooke, 1995). In brief, the cuticle and upper layer of opaque stratum corneum around the nailfold of two fingers on the left hand were carefully pared away using a scalpel

blade. A thermocouple (Comark Electronic, Littlehampton, Sussex, UK) was then fixed to the finger in order to measure skin temperature, and an electrocardiogram (ECG) was recorded throughout the study. Finger nailfold capillary pressure was measured following direct cannulation at the apex of the capillary loop with glass micropipettes, using an electronic resistance feedback servonulling system, which enables the dynamic recording of capillary pressure. Following filtering and digitization, capillary pressure and ECG were stored on computer for later off-line analysis of mean capillary pressure and CPPA. CPPA was obtained from an average capillary pressure waveform derived by computerized superimposition of at least three cardiac cycles. Using the simultaneously recorded ECG to define the start of each beat, CPPA was calculated as the difference between the diastolic trough and the systolic peak. Capillary pressure was measured in at least three different capillaries under basal conditions and then during the 60 s period following each dose of ACh or ACh vehicle. Due to technical difficulties, it was not always possible to measure capillary pressure following each of the iontophoresis periods. The measurement of capillary pressure is highly reproducible (Shore *et al.* 1995). The coefficient of variation between nine different capillaries across the nailfold in five individuals was $5.4 \pm 2.0\%$. Day-to-day reproducibility was $5.2 \pm 3.6\%$, determined in six subjects on three occasions.

Measurement of capillary red blood cell velocity

During the acclimatization period, the dorsal middle phalangeal areas of two fingers on the left hand were gently cleaned with an alcohol wipe and then swabbed with deionized water. Skin temperature was measured using a thermocouple (Comark Electronic), which was fixed to the finger. The finger was stabilized using Plasticene and the fingernail was attached to a solid holder using artificial nail glue in order to minimize fine pulsation movements.

CBV in single capillaries was measured at the dorsal middle phalangeal area of the finger using the CAM1 capillary anemometer system. A thin layer of clear lubricating jelly (Lloyds, Tamworth, UK) was placed on the finger in order to aid visualization of the capillaries. The finger was illuminated by a 100 W halogen lamp (Euromex, Arnhem, The Netherlands) with an 8 mm diameter fibre-optic guide. The light was passed through a blue-green filter which served to both enhance the visualization of the red blood cells and reduce the heating effect of the light source. The column of red blood cells within the capillary was visualized by means of a television microscopy system equipped with a ×10 magnification microscope objective (KK Technology) and a high-resolution camera (monochrome XC-75 C half-inch CCD camera, Sony, Kanaga-ken, Japan), which provided an approximately ×450 magnified image on a television monitor (monochrome, SSM-121CE, Sony). CAM1 utilizes a through-the-objective low-power near-infrared laser (0.8 mW at 780 nm), positioned using a micromanipulator, which is focused to a spot about 10 μm in diameter on the arterial side of the apex of a single capillary. The positioning of the laser beam on the arterial side of the apex of a capillary is achieved using both the capillary microscope and the audible Doppler signal, which is amplified through a loudspeaker. The laser beam is positioned so that the maximal strength signal is obtained, i.e. the beam is focused on the column of red blood cells in the capillary limb; the direction of flow is assessed visually. The laser light is reflected by moving red blood cells at the focal point perpendicular to the skin surface and the Doppler shift is detected by the CAM1. The signal is then processed and stored on a PC for off-line analysis using CAM1 software.

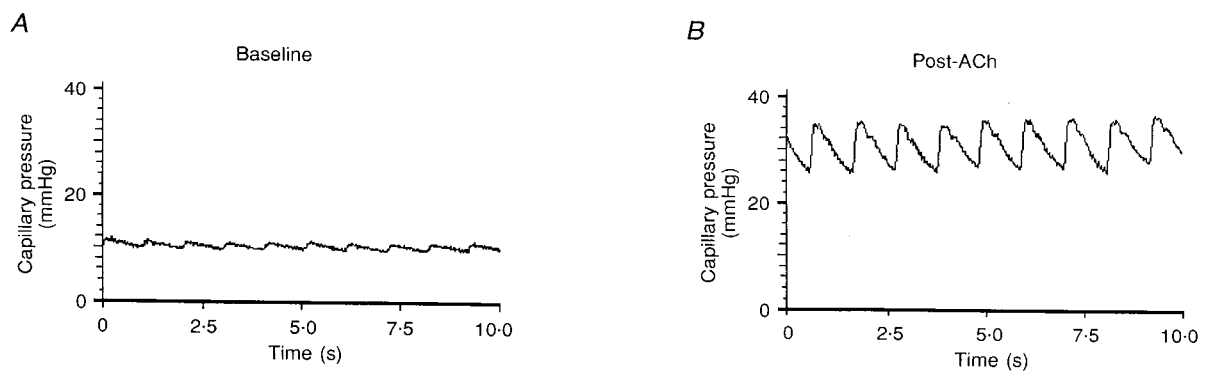


Figure 1. Original finger nailfold capillary pressure tracings obtained in one individual at baseline (A) and following the iontophoresis of ACh (B)

Both capillary pressure and capillary pulse pressure amplitude were increased from baseline following ACh administration.

The calculated values of CBV are expressed as millimetres per second, assuming that the signal arose from a vessel perpendicular to the skin surface. Variations of capillary angle would influence the recorded CBV value, but the error is small (e.g. at an angle of 18 deg from the perpendicular, the CBV value would be reduced by 5%). The CBV values obtained directly from CAM1 are given in the text; although values may differ slightly from 'true' values, changes in velocity will be accurately reflected.

CBV was recorded for at least 20 s in six different capillaries at baseline (both fingers) and then at the end of the entire iontophoresis protocol for the application of ACh (one finger) or ACh vehicle (one finger).

Although to our knowledge, this is the first experimental use of the CAM1, the techniques of laser Doppler are well established and laser Doppler anemometry has been previously validated both *in*

vivo (Riva, Ross & Benedek, 1972) and *in vitro* (Einav, Berman, Fuhro, DiGiovanni, Fridman & Fine, 1975). As this is the first experimental use of the CAM1, confirmation of the previously observed relationship between skin temperature and CBV was also investigated in ten healthy volunteers (aged 30.0 ± 7.2 years).

Drugs

ACh (Miochol) was obtained from IOLAB (Bracknell, Berkshire, UK). The vehicle for ACh, 3% mannitol in water for injection, was prepared by the Royal Devon & Exeter Hospital Pharmacy, Exeter, UK. ACh was prepared immediately prior to administration.

Statistical analysis

The effects of ACh and ACh vehicle on capillary pressure, CPPA and CBV were assessed using paired Student's *t* test to compare basal measurements with either the plateau of the drug responses (capillary pressure and CPPA) or the post-iontophoresis response

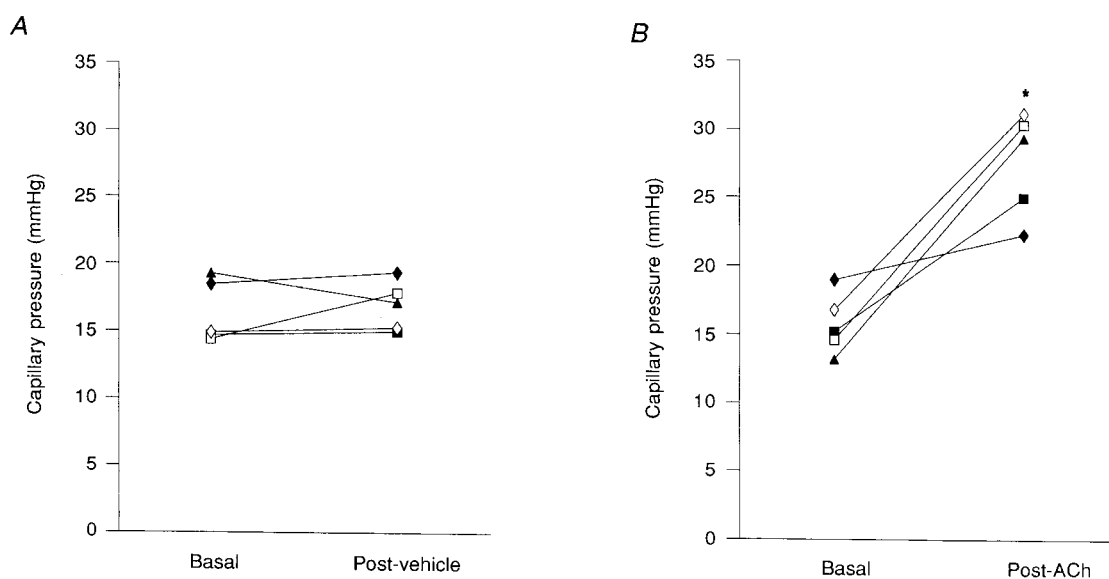


Figure 2. The effect of ACh on finger nailfold capillary pressure in each of the 5 healthy volunteers

The iontophoresis of vehicle (A) did not change capillary pressure from baseline, but ACh (B) elicited a significant increase in finger nailfold capillary pressure ($*P < 0.008$). Different symbols have been used for each of the 5 individuals, which correspond to those used in Fig. 3.

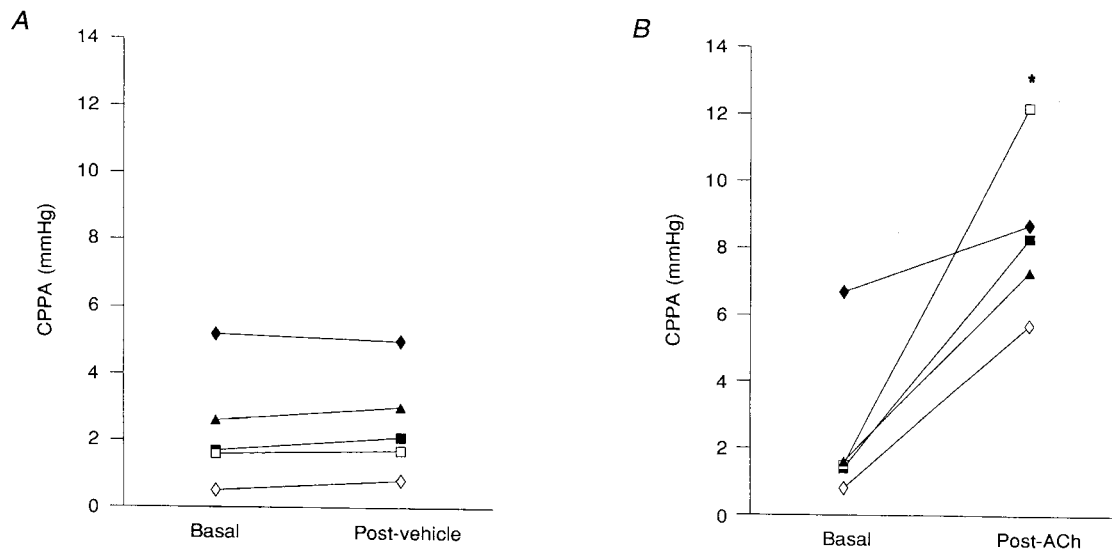


Figure 3. The effect of ACh on finger nailfold capillary pulse pressure amplitude (CPPA) in each of the 5 healthy volunteers

The iontophoresis of vehicle (A) did not change CPPA from baseline, but ACh (B) elicited a significant increase in CPPA (* $P < 0.013$). Different symbols have been used for each of the 5 individuals, which correspond to those used in Fig. 2.

(CBV). Spearman rank correlation coefficients (R_s) were used to assess the relationship between temperature and CBV. Data are presented in the text as the mean \pm standard deviation (s.d.) with a P value < 0.05 considered to be statistically significant.

RESULTS

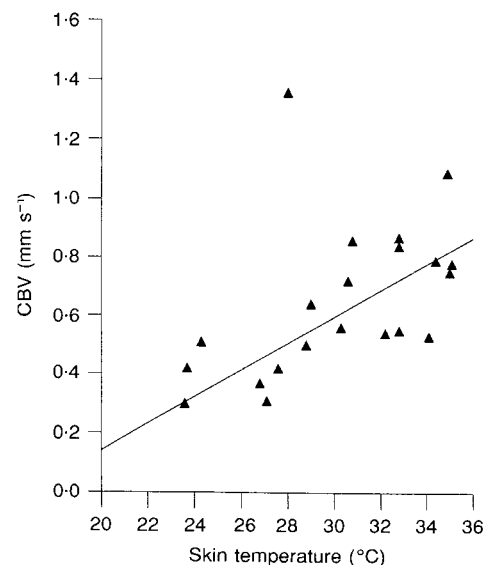
Effect of ACh on capillary pressure and capillary pulse pressure amplitude

Figure 1 shows original finger nailfold capillary pressure tracings obtained in one individual at baseline and following the iontophoresis of ACh. In five healthy volunteers,

application of ACh vehicle did not significantly change either capillary pressure (16.4 ± 2.3 mmHg at baseline and 15.0 ± 1.8 mmHg following vehicle application) or CPPA (2.3 ± 1.8 mmHg at baseline and 2.5 ± 1.6 mmHg following vehicle iontophoresis). Capillary pressure was significantly increased following the iontophoresis of ACh (from 15.8 ± 2.2 mmHg under basal conditions to 27.7 ± 3.8 mmHg at the plateau of the drug response; $P < 0.008$) (Fig. 2). ACh significantly increased CPPA from 2.4 ± 2.4 mmHg at baseline to 8.4 ± 2.4 mmHg at the plateau of the drug response; $P < 0.013$ (Fig. 3).

Figure 4. The relationship between finger capillary red blood cell velocity (CBV) and skin temperature

Measurements of basal skin CBV and skin temperature were recorded in 10 healthy subjects. Basal skin CBV was significantly related to skin temperature ($R_s = 0.66$, $P < 0.001$).



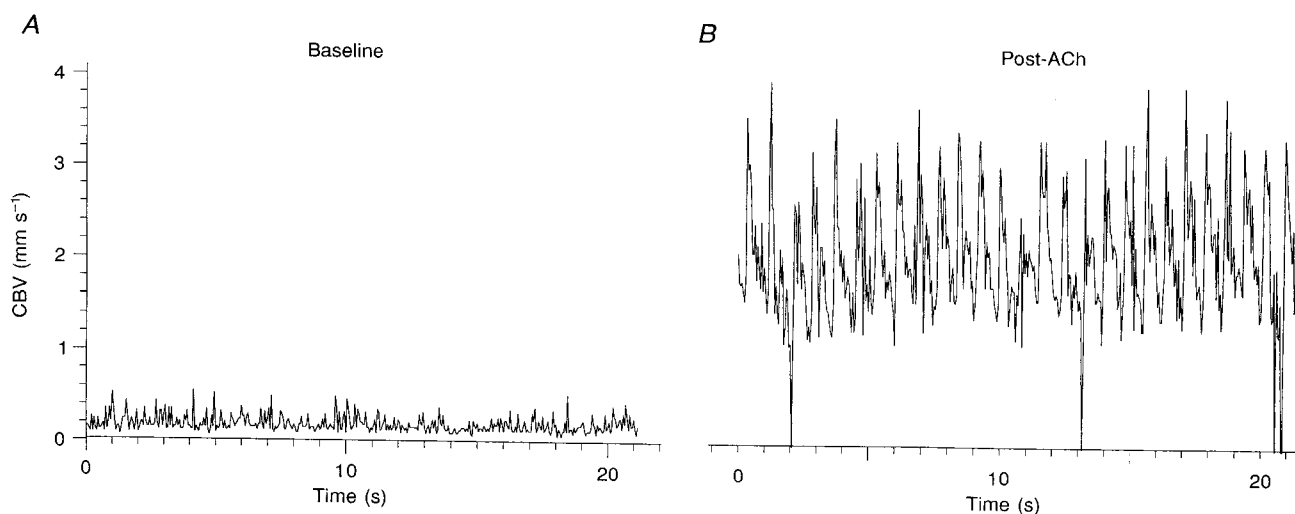


Figure 5. Original finger CBV tracings obtained in 1 individual at baseline (A) and following the iontophoresis of ACh (B)

CBV was increased from baseline following ACh administration.

Effect of ACh on capillary red blood cell velocity

Basal CBV ($0.66 \pm 0.26 \text{ mm s}^{-1}$) measured using the recently developed CAM1 was similar to that measured using traditional techniques (Bollinger & Fagrell, 1990) and, as anticipated, was significantly related to skin temperature ($30.4 \pm 3.8 \text{ }^\circ\text{C}$) ($R_s = 0.66$, $P < 0.001$) (Fig. 4). Original finger capillary red blood cell velocity tracings obtained in one individual at baseline and following the iontophoresis of ACh are shown in Fig. 5. In six healthy volunteers, CBV was not significantly changed following the iontophoresis of ACh vehicle, but the application of ACh elicited a

significant increase in CBV from $0.54 \pm 0.22 \text{ mm s}^{-1}$ at baseline to $2.46 \pm 1.12 \text{ mm s}^{-1}$ after ACh; $P < 0.008$ (Fig. 6).

DISCUSSION

This study demonstrates for the first time that the effects of iontophoretically applied vasoactive agents on skin nailfold capillary pressure and CPPA may be measured in man. This study also suggests that the CAM1 may potentially be a suitable tool for the measurement of CBV in single perpendicular finger skin capillaries. The range of resting

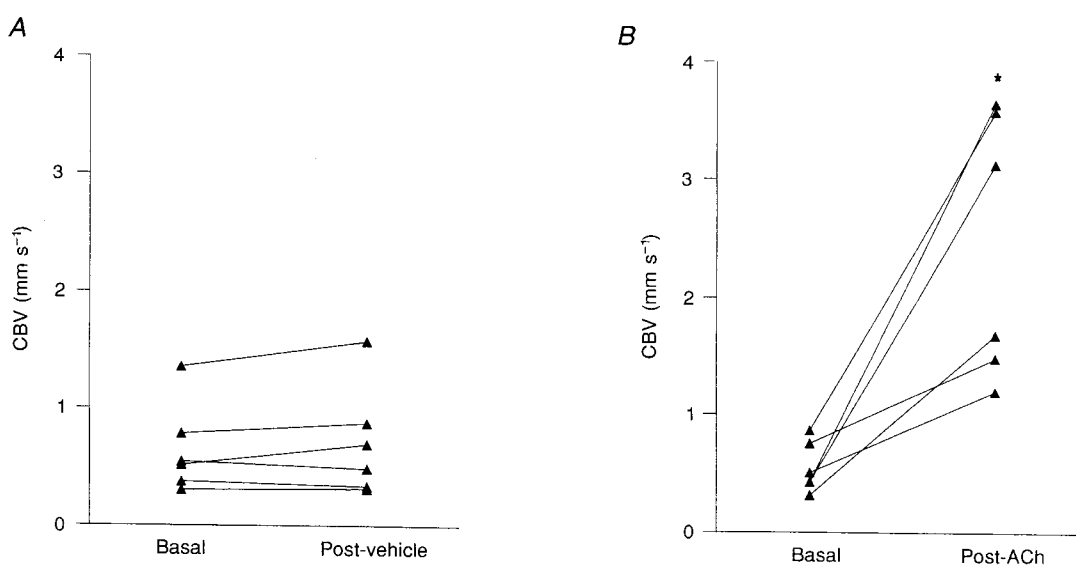


Figure 6. The effect of ACh on finger CBV in each of the 6 healthy volunteers

The iontophoresis of vehicle (A) did not change CBV from baseline, but following ACh iontophoresis (B), CBV was significantly increased (* $P < 0.008$).

CBVs obtained with the CAM1 are comparable to those obtained by established methods of measuring nailfold CBV, such as frame-by-frame analysis, flying spot and cross-correlation techniques (Bollinger & Fagrell, 1990). CAM1 has several advantages over traditional methods; for example, capillary velocity can be measured in capillaries away from the nailfold, and it is able to measure considerably greater velocities.

These data demonstrate that the iontophoresis of ACh elicits an increase in finger skin capillary pressure, CPPA and CBV in healthy volunteers. The increase in CBV in single capillaries following the iontophoresis of ACh is in accordance with previous findings of the overall vasodilatory effect of this agent on the skin microcirculation (capillaries and larger vessels) measured by laser Doppler fluximetry techniques (Westerman, Widdop, Low, Hannaford, Kozak & Zimmet, 1988; Morris, Shore & Tooke, 1995). The observed increase in capillary pressure in association with the rise in CPPA suggests that the precapillary resistance was reduced following application of ACh. As CBV was also augmented, this suggests that postcapillary resistance was either unchanged or lowered following stimulation with ACh. These observations combined suggest that the overall effect of ACh was to induce a reduction in the pre- to postcapillary resistance ratio.

Little is known about the control of the human skin microcirculation. Basal release of NO has been demonstrated to be important in the control of microvascular blood flow in areas of skin involved in temperature regulation but not in regions of the skin containing primarily nutritive vessels (Noon, Haynes, Webb & Shore, 1996). This study examined the effects of ACh on capillaries involved in nutritive perfusion and indicates that the stimulated release of endogenous vasoactive substances may be important in the regulation of the microcirculation.

ACh may elicit vasodilatation through a number of different mechanisms. It may stimulate the release of the vasodilators NO, prostacyclin, the putative endothelium-derived hyperpolarizing factor (Rubanyi, 1991), as well as vasoconstrictor prostanoids (Teschfariam, Jakubowski & Cohen, 1989), from the endothelium. ACh also inhibits the release of noradrenaline from nerves (O'Rourke & Vanhoutte, 1992) and, in addition, causes an increase in skin perfusion in sites adjacent to the area of drug application through the activation of the axon reflex (Westerman *et al.* 1992).

Studies of the forearm skin microcirculation in healthy subjects suggest that ACh is likely to exert its vasodilatory effects predominantly through the production of NO (Warren, 1994), thus the increase in skin blood flow associated with the intradermal injection of ACh was attenuated when the NO synthase inhibitor L-NMMA was co-injected. In contrast, indomethacin, a cyclo-oxygenase

inhibitor, at a dose which inhibited the vasodilatory response to arachidonic acid, did not significantly alter the response to ACh, suggesting that prostaglandins do not play a major role. In support of these findings, NO has also been demonstrated to be a major mediator of ACh-induced relaxation of isolated perfused, transverse para-umbilical human skin flaps (Kreidstein, Pang, Carlson & Xu, 1992). However, this mechanism of action of ACh in the skin microcirculation is not a universal finding. A preliminary study by Noon and colleagues has suggested that the major action of iontophoretically applied ACh to the forearm skin of control subjects is through the release of vasodilator prostanoids (Noon, Hand, Jordan, Simpson, Walker & Webb, 1995), although we could not confirm these findings (author's unpublished observations).

The mechanisms responsible for the actions of ACh were not investigated in the present study due to the extreme technical difficulties of measuring the effects of iontophoretically applied agents on capillary pressure and CPPA. However, these present findings in combination with the demonstration of the presence of constitutive NO synthase in the human cutaneous microvasculature (Bull *et al.* 1995) may suggest that, at least in part, ACh actions on capillary pressure, CPPA and CBV may be mediated through NO.

In summary, these data suggest that ACh may effect the control of the finger skin microcirculation in healthy volunteers. The mechanisms involved remain to be determined.

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