Responses of the skin microcirculation to acetylcholine and sodium nitroprusside in patients with NIDDM

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Summary The mechanisms involved in the pathogenesis of microangiopathy occurring in non-insulin-dependent diabetes mellitus (NIDDM) are unclear. In the present study, blood flow responses to the vasodilators acetylcholine (which acts via the endothelium) and sodium nitroprusside (a smooth muscle relaxant) were evaluated in this patient group. In 14 male patients with NIDDM, treated with either diet alone ($n = 6$) or diet plus insulin, (mean age 59 years) and 14 age-pair-matched control subjects, forearm skin perfusion following multiple doses of iontophoretically applied 1 % acetylcholine and 0.01 % sodium nitroprusside was recorded by laser Doppler perfusion imaging. Basal skin blood flow was not significantly different in the diabetic group compared with the control group. The following results are expressed as drug-minus-vehicle response. Acetylcholine significantly increased forearm skin perfusion ($p < 0.001$, analysis of variance) in all subjects, but the vasodilatation was attenuated in the patient group compared with control subjects (0.86 ± 0.09 vs 1.36 ± 0.14 arbitrary units of volts (V) respectively, at the fifth measurement point, mean $\pm$ SEM, $p < 0.01$). Skin perfusion significantly increased following sodium nitroprusside ($p < 0.001$) but was lower in patients than control subjects (0.12 ± 0.05 vs 0.45 ± 0.11 V, respectively, at the fifth measurement point, $p < 0.01$). These data suggest that endothelial and/or smooth muscle function may be impaired in the skin microcirculation of patients with NIDDM. [Diabetologia (1995) 38: 1337–1344]

Key words Endothelium, microcirculation, non-insulin-dependent diabetes mellitus, skin, vascular smooth muscle.

It has been proposed that haemodynamic factors are involved in the pathogenesis of diabetic microangiopathy [1]. In support of this hypothesis both capillary pressure [2] and capillary filtration coefficient [3] are increased in patients with insulin-dependent diabetes mellitus (IDDM) particularly in those at risk of diabetic nephropathy. The prevalence of microvascular

complications associated with non-insulin-dependent diabetes (NIDDM) differs from those associated with IDDM, raising the possibility that the functional evolution of microangiopathy is different [4]. In support of this hypothesis Shore et al. [5] found no evidence of an increase in capillary pressure in normotensive patients with NIDDM under resting conditions; neither was capillary filtration coefficient increased [6]. The ability to maximally dilate the vasculature has however been shown to be reduced in both NIDDM [7] and in subjects with impaired glucose tolerance [8].

Although it is not known whether this profoundly impaired vasodilatation reflects structural and/or functional changes it has been suggested that endothelial dysfunction may play an important role in the pathogenesis of diabetic microangiopathy in experi-

Table 1. Characteristics of the subjects studied

	NIDDM patients	Healthy control subjects
n (Males)	14	14
Age (years)	59.0 ± 2.3	58.9 ± 2.6
Duration of diabetes (years)	9.1 ± 1.9	
Mean blood pressure (mmHg)	89 ± 3	95 ± 2
Body mass index (kg/m^2)	25.9 ± 0.7	26.2 ± 1.0
HbA$_{1c}$ (%)	6.5 ± 0.2	4.8 ± 0.1[a]
Skin temperature (°C)	32.8 ± 0.4	33.3 ± 0.3
Warm sensory threshold (°C)	2.2 ± 0.7	1.2 ± 0.4
Cold sensory threshold (°C)	0.7 ± 0.2	0.4 ± 0.1
Vibration sensory threshold (V)	5.4 ± 0.4	4.4 ± 0.4[b]
Complications		
Retinopathy	7 subjects no evidence of retinopathy 6 subjects background retinopathy 1 subject proliferative retinopathy	
Nephropathy	11 subjects no evidence of nephropathy 3 subjects raised urinary albumin : creatinine ratio	
Neuropathy	12 subjects no evidence of neuropathy 1 subject autonomic and peripheral neuropathy 1 subject peripheral neuropathy	

Data are mean ± SEM
Significant difference between diabetic and control groups [a] $p < 0.001$, $n = 14$; [b] $p < 0.04$, $n = 14$

mental diabetes [9, 10,], IDDM [11–14] and NIDDM [15, 16]. As well as its role in transport, haemostasis and cell growth, the endothelium is an influential mediator of vascular tone, capable of synthesising nitric oxide (NO), prostaglandins and vasoactive peptides [17]. It has been proposed that NO is important in the acute response to acetylcholine and the delayed response to inflammatory stimuli in forearm skin microcirculation [18]. The latter action of NO may be of clinical relevance to NIDDM during the formation and healing of foot ulcers, common in this patient group [19].

The aim of this study was therefore to investigate the responses of the skin microcirculation to the iontophoretic application of acetylcholine, an endothelium-dependent vasodilator, and sodium nitroprusside, an NO donor, in patients with NIDDM. Heterogeneity of skin blood flow [20] makes interpretation of responses of the skin microcirculation to physiological and pharmacological interventions difficult, if perfusion is only measured at one site on the skin. The recently developed technique of laser Doppler perfusion imaging, which allows the mapping of tissue perfusion over a large number of immediately adjacent sites on the skin [21], was therefore used in this study to assess the microvascular responses.

Subjects and methods

Subjects. A total of 14 male NIDDM patients (defined as such when diagnosed at 33 years old or older with no ketones at time of diagnosis, and initially established on treatment with

oral hypoglycaemic drugs or by diet alone) and 14 healthy control subjects, pair-matched for age, gave their fully informed witnessed written consent to this study which was approved by the local Medical Research ethics committee. The diabetic patients were controlled by either diet alone ($n = 6$) or diet plus insulin ($n = 8$). Subject characteristics are summarised in Table 1. None of the subjects were taking any additional vasoactive agents. 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.

Experimental protocol. These studies were carried out in a temperature controlled room (21.5–22.5 °C) with the subjects lying in the supine position. During the 30-min acclimatization period the flexor aspect of the right forearm (the left in one subject) was gently cleaned with an alcohol wipe and then swabbed with deionised water. A thermocouple (Comark Electronic, Littlehampton, Sussex, UK) was then fixed to the volar aspect of the wrist in order to measure skin temperature. Blood pressure was determined by the mean of five readings taken at 1-min intervals using the oscillometric method and a semi-automatic blood pressure recorder (Dinamap, Critikon, Tampa, Fla., USA). Vibration Sensory Threshold was determined on the first and fourth fingers of the left hand using a biothesiometer (Biomedical Instrument Co., Newbury, Ohio, USA). A computerised thermal testing system (Middlesex Hospital, London, UK) was used to assess warming and cooling thermal sensory thresholds on the palm of the left hand. Blood glucose concentrations were measured in the diabetic patients at the beginning and end of the study (BM test 144, Refloflux II Meter; Boehringer Mannheim, Mannheim, Germany). HbA$_{1c}$ (normal range, 4.0–6.0 %) measured by HPLC and renal function (plasma creatinine concentrations, normal range 45–125 μmol/l) were measured in all subjects from a venous blood sample collected at the end of the study. Following acclimatization the skin erythrocyte flux was measured using laser Doppler perfusion imaging after application of acetyl-

choline and sodium nitroprusside on the right forearm (the left in one subject) and maximum blood flow was measured on the left forearm (see relevant sections below).

Measurement of skin erythrocyte flux responses to acetylcholine and sodium nitroprusside by laser doppler perfusion imaging. A laser Doppler perfusion imager (LDPI) (Lisca PIM 1.0; Lisca Development AB, Linköping, Sweden) was used to map skin perfusion. (A 1-mW Helium-Neon laser beam (wavelength 633 nm) sequentially scans an area of tissue. The laser beam penetrates the tissue and a fraction of the light is backscattered by mobile erythrocytes and undergoes a frequency shift according to the Doppler principle, generating a signal proportional to tissue perfusion). This procedure is repeated at each measurement site (up to 4096 sites) producing a colour-coded image of skin erythrocyte flux on a computer monitor. The area of drug delivery could be clearly identified on the image as a coloured circular area against a grey background (black tape on the electrode chamber, see below). Iontophoresis was used to apply the substance being tested across the skin of the forearm. This is a non-invasive method of drug application which allows the local transfer of charged substances across the skin using a small electric current [22]. The current strengths chosen for this study were not felt by the majority of subjects and in the remainder only a mild prickling sensation was perceived. A perspex direct electrode chamber (30 mm total diameter × 3 mm height, inner (drug) chamber 10 mm diameter) was used to deliver the drugs (Moor Instruments, Axminster, Devon, UK). The outer ring of the chamber was covered with non-reflective black tape in order to minimise artefacts on the LDPI's recordings and also to identify the direct site of drug delivery on the image produced. The chamber was attached to the forearm by means of a double-sided adhesive disc and an indifferent electrode was attached to the volar aspect of the wrist of the subject to complete the circuit. The position of the chamber was chosen in order to avoid hair, freckles and broken skin. The chamber was filled with the solution under test and covered with a glass cover slide to prevent reflection artefacts from the solution in the chamber. The head of the LDPI was positioned approximately 13 cm above the centre of the chamber and a black cloth placed over the LDPI and the forearm in order to minimise optical interference with the LDPI's signal. This experimental setup allowed the non-invasive measurement of skin erythrocyte flux directly over the site of drug application.

A battery-powered iontophoresis controller (MIC 1, Moor Instruments) was used to provide a direct current for drug iontophoresis. Acetylcholine (1 %) was delivered using an anodal current; 7 × (0.1 milliamps (mA) for 20 s), followed by 1 × (0.2 mA for 20 s), with 60 s interval between each dose (total charge 18 millicoulombs (mC)). Pilot studies demonstrated that a 60-s interval between each iontophoresis period was required to achieve the plateau of the response following each delivery of acetylcholine. The responses to acetylcholine vehicle (3 % mannitol in water for injection) and acetylcholine were measured at one and two sites on the forearm, respectively. Forearm skin erythrocyte flux was recorded immediately before the start of iontophoresis and after each period of drug application with the LDPI set to scan 32 × 32 measurement sites, covering an area approximately 4 × 4 cm. The intrasubject reproducibility of this protocol was measured in eight healthy volunteers. The coefficient of variation of the maximum response to acetylcholine (determined as the point of maximum vasodilatation to acetylcholine when there was negligible vehicle response) was 16.3 ± 3.7 %, at two or three measurement sites. Day-to-day reproducibility

was 21.7 ± 6.4 %, determined in 4 subjects on two to four occasions.

The 0.01 % sodium nitroprusside was delivered using a cathodal current; 2 × (0.1 mA for 20 s), followed by 1 × (0.2 mA for 20 s) with 180-s intervals between each dose (total charge 8 mC). Pilot studies showed that the response to sodium nitroprusside required longer to develop than that to acetylcholine. The responses to sodium nitroprusside vehicle (deionised water) and sodium nitroprusside were measured at one and one or two sites on the forearm, respectively. Forearm skin erythrocyte flux was recorded immediately before the start of iontophoresis and at 0, 60, and 120 s following each period of drug application. Due to technical reasons the responses to sodium nitroprusside were only measured in 25 of 28 subjects. The intrasubject reproducibility of this protocol was measured in eight healthy volunteers. The coefficient of variation at the fifth point of measurement was 10.9 ± 5.2 %, at two measurement sites. Day to day reproducibility was 31.0 ± 13.2 %, determined in three subjects on two to three occasions.

At the end of each study, biological zeroes (that is, the flux value without arterial in-flow) were measured at both an untreated and a vasodilated site on the forearm during arterial occlusion of the upper arm by an inflated cuff. Day-to-day reproducibility of biological zero was 7.2 ± 3.1 % determined in three subjects on two to five occasions.

Analysis of the colour-coded images of erythrocyte flux obtained with the LDPI was carried out using the Pim 2.3 software package Lisca Development ab, Linköping Sweden. The responses to the direct application of acetylcholine and sodium nitroprusside were calculated as the mean of the erythrocyte flux of 80 ± 2 points, over a 0.78-cm^2 area of the forearm.

Measurement of maximum blood flow. This method is described in detail elsewhere [23]. In brief, an area of skin on the flexor aspect of the left forearm was heated to 42 °C with a small brass heater (Moor Instruments) attached to the skin surface by means of a double-sided adhesive disc. After 30 min the maximum microvascular hyperaemic response (termed maximum blood flow) was measured using laser Doppler fluximetry (Periflux Pf2, Perimed, Stockholm, Sweden) (30 s to each of eight heated sites) and calculated as the mean erythrocyte flux of the eight skin sites.

Drugs. Acetylcholine (Miochol) was obtained from IOLAB (Bracknell, Berks. UK); the vehicle for acetylcholine, 3 % mannitol in water for injection, was prepared by the Royal Devon and Exeter Hospital Pharmacy (Exeter, Devon, UK); sodium nitroprusside (Nipride), from Roche (Welwyn Garden City, Herts UK), was dissolved in sterile deionised water (sodium nitroprusside vehicle) obtained from Baxter Healthcare Ltd. (Thetford, Norfolk, UK). All drugs were freshly prepared immediately prior to administration and sodium nitroprusside was kept in the dark.

Statistical analysis

To remove any influence of vehicle on the analysis of the drug responses the results are expressed as the response to acetylcholine and sodium nitroprusside minus their respective vehicle responses. Assessment of drug-minus-vehicle responses were made using two-way analysis of variance for repeated measures (ANOVA) using the first five scans only as the vehicle response overwhelmed the sodium nitroprusside response at later iontophoresis periods. The same conclusions were

reached whether statistical analyses were performed on the absolute responses to acetylcholine or sodium nitroprusside (statistical analysis not shown) or on the absolute drug responses minus respective vehicle responses. Comparisons between subject groups for biological zeroes, maximum blood flow and subject characteristics were made by Wilcoxon rank sum test. Paired Student's t-tests were used to compare the change in flux from baseline following drug and vehicle administration. Spearman rank-correlation coefficients (R_s) were calculated were appropriate. Results are expressed in the text as percentage increase from basal flux, absolute values in arbitrary units of volts (V) and responses to acetylcholine and sodium nitroprusside after subtraction of their respective vehicle responses (V). Data are presented in the text as mean ± SEM.

Results

Basal forearm skin perfusion. Basal forearm skin perfusion was not significantly different in the NIDDM patients compared with the control subjects.

Response to acetylcholine. The responses of forearm skin erythrocyte flux to the iontophoresis of acetylcholine and acetylcholine vehicle in both NIDDM patients and control subjects are shown in Figure 1. Acetylcholine vehicle elicited a small but significant increase in the erythrocyte flux, from the fourth iontophoresis period onwards. This increase was similar in both NIDDM and control (e.g. $6.7 \pm 2.0\%$ and $9.9 \pm 4.0\%$ increase following the fourth iontophoresis from basal flux respectively; $p < 0.161$, $n = 14$) but negligible in comparison to the corresponding acetylcholine response (e.g. $109.2 \pm 11.9\%$ and $162.1 \pm 14.2\%$; increase from basal flux respectively). To remove any influence of changes in tissue perfusion due to vehicle, the following results are expressed as erythrocyte flux to acetylcholine minus the vehicle response (Fig. 2). Forearm tissue perfusion significantly increased following the iontophoresis of acetylcholine ($p < 0.001$), but the vasodilatation to acetylcholine was lower in the NIDDM group than the control subjects ($p < 0.01$).

Response to sodium nitroprusside Figure 3 shows the responses of forearm skin erythrocyte flux to the iontophoresis of sodium nitroprusside and sodium nitroprusside vehicle in 12 matched pairs of NIDDM patients and control subjects. The vasodilatory response to sodium nitroprusside vehicle (delivered with a cathodal current) was much more marked than to acetylcholine vehicle (delivered with an anodal current). For example, in control subjects the increase in flux from baseline following the second iontophoresis period was $76.9 \pm 22.5\%$ to sodium nitroprusside vehicle compared with $4.4 \pm 2.1\%$ to acetylcholine vehicle. Hence the following results are expressed as erythrocyte flux to sodium nitroprusside minus the vehicle response (Fig. 4). Forearm tissue
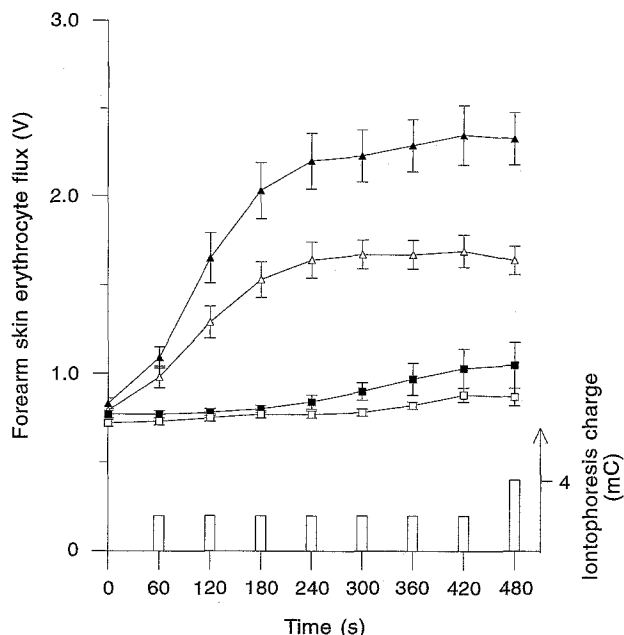

Fig. 1. Absolute forearm skin erythrocyte flux (Volts, mean ± SEM, $n = 14$) following the iontophoresis of acetylcholine in NIDDM patients (△) and healthy control subjects (▲) and acetylcholine vehicle in NIDDM patients (□) and healthy control subjects (■)
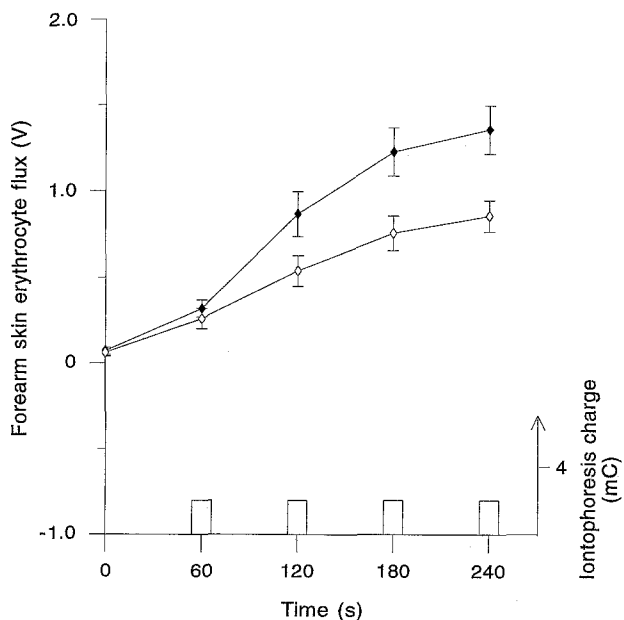

Fig. 2. Response of forearm skin erythrocyte flux (Volts, mean ± SEM, $n = 14$) following the iontophoresis of acetylcholine minus the acetylcholine vehicle response in NIDDM patients (◇) and healthy controls (◆). The vasodilatation to acetylcholine was significantly lower in the NIDDM patients than the control subjects ($p < 0.01$)

perfusion significantly increased following the iontophoresis of sodium nitroprusside ($p < 0.001$). The vasodilatation to sodium nitroprusside was lower in the NIDDM group than the control subjects ($p < 0.01$).
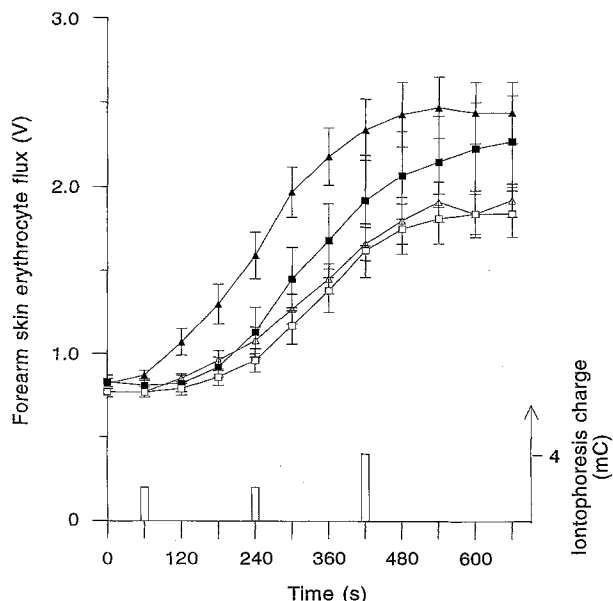
Fig. 3. Absolute forearm skin erythrocyte flux (Volts, mean ± SEM, $n = 12$) following the iontophoresis of sodium nitroprusside in NIDDM patients ($\triangle$) and healthy control subjects ($\blacktriangle$) and sodium nitroprusside vehicle in NIDDM patients ($\square$) and healthy control subjects ($\blacksquare$)

Fig. 5. Forearm skin maximum blood flow in the forearm of NIDDM patients and healthy control subjects (Volts). Maximum blood flow was significantly lower in the NIDDM patients than the control subjects ($p < 0.021$, $n = 14$)
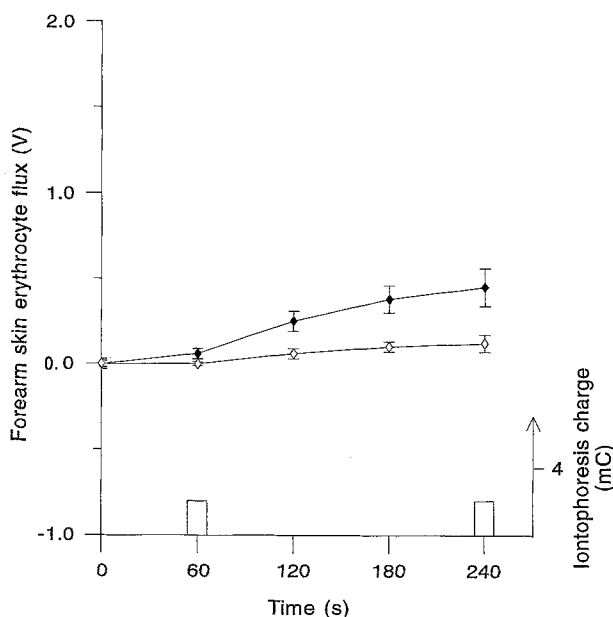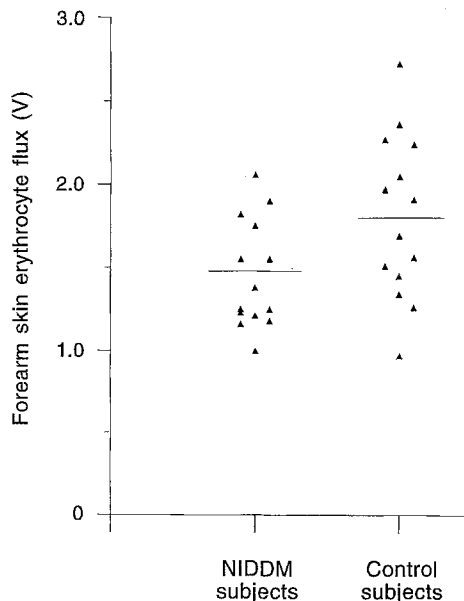

Fig. 4. Response of forearm skin erythrocyte flux (Volts, mean ± SEM, $n = 12$) following the iontophoresis of sodium nitroprusside minus the sodium nitroprusside vehicle response in NIDDM patients ($\Diamond$) and healthy control subjects ($\blacklozenge$). The vasodilatation to sodium nitroprusside was significantly lower in the NIDDM patients than the control subjects ($p < 0.01$)

Biological zero. There was no significant difference between biological zero measured at an untreated site and a vasodilated site on the forearm, in both NIDDM subjects (0.65 ± 0.02 vs 0.65 ± 0.02 V, respectively; $p < 0.959$, $n = 13$) and pair-matched control

subjects (0.68 ± 0.02 vs 0.72 ± 0.03 V, respectively; $p < 0.311$, $n = 12$). Mean biological zero was not significantly different between the NIDDM patients (0.65 ± 0.02 V) and the control subjects (0.69 ± 0.02 V; $p < 0.300$, $n = 14$).

Maximum blood flow. The maximum microvascular hyperaemic response was attenuated in NIDDM patients compared to control subjects (1.45 ± 0.09 vs 1.81 ± 0.13 V, $p < 0.031$, $n = 14$) (Fig. 5). In the NIDDM patient group maximum blood flow correlated with HbA_{1c} values ($R_s = -0.608$, $p < 0.021$), but not with the responses to acetylcholine and sodium nitroprusside or duration of diabetes.

There were no significant differences in either the responses to acetylcholine and sodium nitroprusside or the maximum skin blood flow response in patients with microvascular complications and those free of complications.

Discussion

This study suggests that the skin microvascular vasodilatory responses, assessed by laser Doppler perfusion imaging, to iontophoretically applied acetylcholine (which acts via the endothelium) and sodium nitroprusside (an NO donor, which acts directly on smooth muscle) are impaired in patients with NIDDM, treated with either diet alone or diet plus insulin, compared with age- and sex-matched control subjects.

The techniques of laser Doppler perfusion imaging and iontophoresis were combined, enabling the re-

producible measurement of skin blood flow responses to vasoactive agents. Although iontophoresis provides a non-invasive technique to introduce charged substances across the surface of the skin, offering advantages over the use of intradermal injections, the study revealed the scope for misinterpretation with this technique. It was demonstrated, for example, that current alone may affect tissue perfusion. Vasodilatation to drug vehicle was observed with both anodal and cathodal currents, although the increase in erythrocyte flux to a cathodal current was observed after a lower total iontophoresis charge and was much more pronounced than that produced by an anodal current. The major pathway of iontophoretically applied substances across the skin is through areas of low electrical resistance such as sweat ducts, hair follicles and broken skin [22]. Sweat glands contain vasoactive substances such as kinins [24] which may possibly be iontophoresed along with the substance being tested, depending on their charge and size [25]. Transcutaneous electrical nerve stimulation has been utilised to stimulate axon reflex vasodilatation in the skin [26], although this method, in contrast to this study, uses painful stimuli it indicates that electrical charge alone may have a direct action on vascular tone. A combination of the above effects of iontophoresis could account for the observed response to current alone and the differences in the extent of vasodilatation to anodal and cathodal currents. These considerations make it essential for experiments using iontophoresis to control for both polarity of current and charge effects.

Our observations on the skin microcirculation are in agreement with those of McVeigh et al. [16] who demonstrated blunted endothelium-dependent and endothelium-independent forearm blood flow responses in patients with NIDDM, using venous occlusion plethysmography. These findings may implicate a role for abnormal endothelium and/or smooth muscle function in the pathogenesis of diabetic microangiopathy in NIDDM, and a number of contender mechanisms exist through which this impairment may be expressed.

Acetylcholine elicits vasodilatation through a complex sequence of events: when applied to blood vessels acetylcholine binds to muscarinic receptors on the surface of endothelial cells, which activates specific G proteins, resulting in the production of NO from L-Arginine (catalysed by NO synthase). NO then diffuses across the intercellular gap, through the interstitial basement membrane and binds to the cytosolic guanylate cyclase of the smooth muscle cells. This induces a rise in cyclic guanosine monophosphate and consequently relaxation [17]. In addition to eliciting NO production acetylcholine also stimulates the release of the vasodilators prostacyclin and the putative endothelium-derived hyperpolarising factor [27] as well as vasoconstrictor prostanoids

[28] from the endothelium. Acetylcholine also inhibits the release of noradrenaline from nerves [29]. Therefore, the attenuated acetylcholine response of the skin microcirculation observed in NIDDM patients in this study may be attributed to abnormalities in one or several of these steps.

Although there is some evidence for abnormal muscarinic receptors or specific G protein function in IDDM [14] there has been little investigation of these factors in NIDDM. However, some preliminary data suggests that there are differences in the responses to acetylcholine and bradykinin in isolated resistance arteries from NIDDM patients, indicating that abnormalities of muscarinic receptors or specific G protein function may exist [15]. Observations in experimental diabetes indicate that specific receptor abnormalities are not responsible for the abnormal acetylcholine response in diabetes as impaired vasodilatation has been demonstrated to other endothelium-dependent vasodilators such as histamine which do not act via muscarinic receptors [30]. Although care should be taken when applying information from animal models to man, these findings suggest that other mechanisms may be responsible for the impaired acetylcholine response observed in NIDDM.

A decrease in NO production could contribute to the attenuated vasodilatation following stimulation with acetylcholine, found in the NIDDM group. This mechanism was examined by McVeigh et al. [16], who were unable to demonstrate any difference in NO production, following acetylcholine stimulation, between NIDDM patients and healthy control subjects. They assessed NO production through the inhibition of NO synthesis with L-NMMA, a competitive inhibitor of NO synthase. However, their study did not demonstrate a vasoconstrictor effect of L-NMMA on basal blood flow in either control subjects or diabetic patients which is in contention with previous findings [31] questioning the sensitivity of the experimental methods used. Therefore, an attenuated production of NO causing a reduced response to acetylcholine in NIDDM cannot be ruled out.

The vascular endothelium plays an important role in the control of vascular tone through the balanced release of both vasodilators, NO, prostacyclin and endothelium-derived hyperpolarising factor and vasoconstrictors, prostaglandins and endothelin. Evidence from experimental diabetes suggests that diabetes may be characterised by increased production or release of prostanoid constrictors which could act to attenuate the vasodilatory impaired endothelial-dependent responses to acetylcholine [32, 28], although this is not a universal finding [33].

Another potential mechanism through which the response to acetylcholine may be diminished in NIDDM is via the impairment of NO diffusion from the endothelium to the underlying smooth muscle,

and indeed a number of candidates have been proposed to effect this pathway in diabetes. The accumulation of advanced glycation end products, formed by the nonenzymatic glycation reaction between glucose and collagen, is heightened in diabetic basement membrane [34]. These products have been shown to impair endothelial-dependent relaxation in experimental diabetes through the quenching of NO [35]. HbA_{1c} levels of which are raised in diabetes, has also been reported to quench NO and therefore blunt NO-dependent responses [36]. Although this study did not find any correlation between HbA_{1c} levels and the response to acetylcholine or sodium nitroprusside in either the NIDDM group or control group this does not exclude an effect of advanced glycation end products as their accumulation will depend on long-term control and may not be adequately reflected in a single HbA_{1c} level. Increased free radical activity has been associated with diabetic microangiopathy [37], and may contribute to blunted endothelial-dependent responses in diabetes [38] as NO is degraded rapidly by these highly reactive species. Levels of oxidised low-density lipoproteins, raised in diabetes [39], may also cause defective endothelium-dependent responses in diabetes by inactivating NO [40].

The reduced response to acetylcholine in the NIDDM group observed in this study may not necessarily indicate endothelial dysfunction but could result from abnormalities of the smooth muscle function. This study has demonstrated a reduced response to the NO donor sodium nitroprusside in the skin microcirculation in NIDDM patients and implies that endothelium dysfunction may not solely be responsible for the blunted acetylcholine response observed. This finding in the skin microcirculation is in agreement with observations from the forearm skeletal muscle bed in NIDDM [16].

As discussed earlier one of the main routes of drugs applied by iontophoresis is through the sweat glands. Acetylcholine, as well as directly initiating endothelium-dependent vasodilatation, also binds to cholinergic receptors on the sweat glands in the skin leading to sweat production. As kinins [41], histamine [42] and prostaglandin-like activity [43] have all been reported to be present in sweat, it is possible that the tissue perfusion response observed to acetylcholine may be contributed to, at least in part, by these vasodilators. Even if this was the case these vasodilators all act via the endothelium and ultimately the measured response to acetylcholine would still be endothelium-dependent. As the group of patients investigated was relatively free of complications and the majority had no evidence of diabetic neuropathy, abnormalities of sweat production in response to iontophoresised acetylcholine are unlikely to explain the different perfusion responses observed in this study.

The observation that maximum blood flow in the forearm is reduced in NIDDM when compared to age- and sex-matched control subjects is consistent with previous data from the foot, although in this study the difference between the patients and control subjects is less marked [7]. Thickness of basement membrane increases with the distance away from the heart, an effect attributed to exposure to increased hydrostatic pressure [44]. Such a difference in basement membrane thickness may be a potential mechanism for the altered maximum blood flow responses between the forearm and feet. Maximum blood flow did not correlate with either the measured response to acetylcholine or sodium nitroprusside, which suggests that complex structural and functional mechanisms determine the microvascular maximum hyperaemic response.

In summary, laser Doppler perfusion imaging provides a non-invasive, reproducible method to assess the responses of the skin microcirculation to iontophoretically applied acetylcholine and sodium nitroprusside. The results of this study suggest that the function of the endothelium and/or smooth muscle may be abnormal in NIDDM. The mechanisms involved remain to be elucidated.

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