

Prolonged acetylcholine-induced vasodilatation in the peripheral microcirculation of patients with chronic fatigue syndrome

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Summary

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Although the aetiology of chronic fatigue syndrome (CFS) is unknown, there have been a number of reports of blood flow abnormalities within the cerebral circulation and systemic blood pressure defects manifesting as orthostatic intolerance. Neither of these phenomena has been explained adequately, but recent reports have linked cerebral hypoperfusion to abnormalities in cholinergic metabolism. Our group has previously reported enhanced skin vasodilatation in response to cumulative doses of transdermally applied acetylcholine (ACh), implying an alteration of peripheral cholinergic function. To investigate this further, we studied the time course of ACh-induced vasodilatation following a single dose of ACh in 30 patients with CFS and 30 age- and gender-matched healthy control subjects. No differences in peak blood flow was seen between patients and controls, but the time taken for the ACh response to recover to baseline was significantly longer in the CFS patients than in control subjects. The time taken to decay to 75% of the peak response in patients and controls was 13.7 ± 11.3 versus 8.9 ± 3.7 min ($P = 0.03$), respectively, and time taken to decay to 50% of the peak response was 24.5 ± 18.8 versus 15.1 ± 8.9 min ($P = 0.03$), respectively. Prolongation of ACh-induced vasodilatation is suggestive of a disturbance to cholinergic pathways, perhaps within the vascular endothelium of patients with CFS, and might be related to some of the unusual vascular symptoms, such as hypotension and orthostatic intolerance, which are characteristic of the condition.

Introduction

Chronic fatigue syndrome (CFS) is a debilitating condition of unknown aetiology. The diagnosis of CFS is made on clinical grounds and patients typically have a variety of symptoms, which can include problems in cognitive performance, sleep quality, skeletal muscle physiology, immune function and hypersensitivity to various stimuli, including prescription medications, along with autonomic nervous system disturbances (Freeman & Komaroff, 1997). It has been proposed that the symptoms of CFS are, in part, cholinergically mediated (Chaudhuri *et al.*, 1997) and two recent studies using brain spectroscopy have revealed metabolic disturbances with significantly elevated choline levels in various regions of the central nervous system (Tomoda *et al.*, 2000; Puri *et al.*, 2002). Such metabolic disturbances might be linked to regional reductions in cortical to cerebral perfusion ratios, which are characteristic findings in many CFS patients (Schwartz *et al.*, 1994; Costa *et al.*, 1995).

In addition, we have recently shown that abnormalities, specific to the cholinergic pathway, also exist in the peripheral microcirculation of CFS patients as demonstrated by enhanced skin vasodilatation to transdermally applied acetylcholine (ACh), but not to the nitrovasodilator sodium nitroprusside (Spence *et al.*, 2000). It is unclear how abnormalities in regional blood flow and choline metabolism in the central nervous system are linked to our findings of enhanced ACh-mediated responses in the peripheral vasculature, but we postulate that our findings might have important implications for vascular integrity in CFS. Indeed, orthostatic intolerance is a recognized feature of many CFS patients (Bou-Holaigah *et al.*, 1995).

There are several possible ways in which increased vasodilatation to ACh could be mediated. To investigate this further, we examined the decay characteristics of the ACh-stimulated blood flow response from its peak back to baseline.

Methods

Thirty patients were enrolled into the study (eight men and 22 women, mean age 43 years, range 18–57 years) from members of local CFS/myalgic encephalomyelitis (ME) support groups (randomly selected from a cohort of 350 volunteer patients). All patients fulfilled the Centre for Disease Control criteria for CFS (Fukada et al., 1994). The mean duration of illness was 11.0 years (range 4–20 years). Patients were not selected or categorized on the basis of symptom severity; all patients were ambulant to minimize the effects of physical deconditioning; patients on non-steroidal anti-inflammatory medications were asked to stop these for 10 days prior to testing because of the possible impact such medications can have on vascular responses to ACh; six patients were on medication: three were taking low dose antidepressants (two amitryptilline, one dothiepin) and we have previously reported that this had no impact on the ACh-mediated blood flow response (Spence et al., 2000), four were on analgesics (three paracetamol based, one opioid), four were taking medication for peptic ulcers or dyspepsia, two were on antihypertensives and one was taking thyroxine (some patients were on more than one medication). Thirty, age- and gender-matched, control subjects (eight men and 22 women, mean age 43 years, range 18–56 years) were also enrolled into the study. The committee on medical research ethics of the University of Dundee approved the study and all volunteers gave written informed consent.

Blood flow responses to ACh were measured on the volar aspect of the forearm using a scanning laser Doppler imager (moorLDI, Moor Instruments Ltd., Devon, UK). Laser Doppler flowmetry has been used successfully for many years to measure microvascular changes in skin and laser Doppler imaging is a recent development of the technique that reduces variations because of spatial heterogeneity by scanning over a region (Essex & Byrne, 1991). The technique utilizes the Doppler principle with a 2 mW helium–neon laser scanning the surface of the skin and recording backscattered light from moving erythrocytes that is shifted in frequency by an amount proportional to their velocity. These Doppler shifts are collected and processed by the instrument. For each scan, the computer builds up a colour-coded image representing skin perfusion in two dimensions. This relative measure of volume flow is called the laser Doppler flux (LDF) and is expressed in arbitrary perfusion units (PU). Iontophoresis was used to transport ACh across the skin within the area described by the iontophoresis chamber (Moor Instruments Ltd., Devon, UK), and according to our previously used protocol (Newton et al., 2001). The iontophoresis chamber consisted of a Perspex ring of internal diameter 20 mm. Experiments were conducted in a temperature-controlled room (22–23°C) with subjects seated and their arms supported at heart level. After a 20 min equilibration period, baseline skin perfusion was measured and this was followed by iontophoresis of 1% ACh for 80 s using a 0.1 mA anodal current delivering an ACh dose of 8 mC. After determining the peak blood flow response to ACh the

subsequent recovery of blood flow from this level back to baseline was recorded for up to 50 min (or longer in those subjects in whom the decay was prolonged) to allow the rate of decay to be established. Two decay points were determined, t_{75} and t_{50} corresponding to the times taken for the blood flow to return to 75 and 50%, respectively, of the peak value to ACh minus the baseline value. Data analysis consisted of an unpaired Student's *t*-test to compare mean values between patients and matched controls, and $P < 0.05$ was chosen as the level of significance.

Results

The basal skin blood flow following the 20 min equilibration period was similar in patients and controls; 12.5 ± 6.4 and 11.7 ± 2.4 PU ($P = 0.44$), respectively. The peak vasodilator response to ACh was not significantly different between the two groups (88.3 ± 16.5 versus 92.2 ± 22.0 PU for patients and controls, respectively, $P = 0.50$), but the time taken for the ACh hyperaemic response to decay was significantly greater ($P = 0.03$) in the patients compared with controls for both t_{75} ; 13.7 ± 11.3 min (95% confidence interval 9.4–17.9; range 6.5–56.0) versus 8.9 ± 3.7 min (95% confidence interval 7.6–10.3; range 4.0–18.0), respectively, and t_{50} decay points ($P = 0.03$); 24.5 ± 18.8 min (95% confidence interval 17.4–31.5; range 7.5–72.0) versus 15.1 ± 8.9 min (95% confidence interval 11.7–18.5; range 4.5–39.0), respectively.

Discussion

The novel finding from this study is that, following stimulation of the skin microvessels with ACh, the time taken for blood flow to return to baseline from the peak vasodilator response is significantly increased in CFS patients compared with age- and gender-matched control subjects. The implication of this is that there is a prolongation of the ACh-mediated vasodilator response in these patients. This result supports our previous finding that cumulative doses of ACh (supplied as a cumulative regimen of 1, 2, 4 and 8 mC) elicited significantly higher peak blood flows in CFS patients than in matched controls (Spence et al., 2000), indicating an abnormally slow clearance of ACh from the vascular endothelium. Given the similarity between peak vasodilator blood flows in CFS patients and controls in the present study, there is no reason to assume that ACh receptor density on the vascular endothelium is abnormal in CFS patients. A possible reason for not observing higher ACh peak responses in the CFS group in the present study is that the dose of ACh delivered was less than that reported by us previously (Spence et al., 2000).

In recent years, the use of ACh in the assessment of endothelial function has played a significant part in the study of various pathological conditions, such as hypertension, diabetes, hypercholesterolaemia and atherosclerosis, all of which are associated with impaired ACh-mediated vasodilatation and, therefore, endothelial dysfunction. Such assessments have often

used somewhat invasive methods, but our group and others have successfully pioneered a combination of iontophoretic drug delivery and laser Doppler imaging to measure blood flow responses in the skin in a variety of conditions (Morris et al., 1995; Khan et al., 1997, 1999, 2000). The method allows very small amounts of drug to be administered non-invasively to a localized area, and is consequently very safe. While studies of the vasodilator properties of ACh are commonplace for assessment of endothelial function, employment of the methodology to study the resolution of the vasodilator response is untested, and so care must be taken in the interpretation of the data in the current study. Recovery of the vasodilator response to cutaneously applied ACh is probably dependent upon various factors, one of which will be the vascular expression of acetylcholinesterase (AChE). In support of this, a link between blood cholinesterase enzymes and the persistence of action of ACh on endothelial receptors has been established. Using the cholinesterase inhibitor edrophonium, the decay of ACh-stimulated hyperaemia in the forearm of normal human subjects was significantly prolonged (Chowienzyk et al., 1995).

Although the aetiology of CFS is unknown, it is commonly associated with viral onset and immunological disturbance sometimes linked to persistent viral infection (Tirelli et al., 1994; Patarca et al., 2000). Speculatively, our findings could be explained by under-expression of AChE on endothelial cells (Kirkpatrick et al., 2001): there is evidence that expression of AChE is inhibited within cholinergically sensitive cells when infected with herpes simplex virus-type 1 (Rubenstein and Price, 1984), and that, in the case of lymphocytic choriomeningitis virus, such inhibition of AChE in neuroblastoma cells persists for years after infection (Oldstone et al., 1997).

The preliminary work described here provides new evidence of disruption to ACh pathways specifically within the peripheral circulation of CFS patients and it should be stressed that speculation about these data is limited to ACh as an endothelial-dependent vasodilator and not its central or peripheral neurotransmitter properties. The significance of this finding in relation to the vascular integrity of this patient group remains to be determined. One of the characteristic signs of CFS is, however, the development of symptoms when upright and this has been designated as a variant of the postural orthostatic tachycardia syndrome (POTS) (Bou-Holaigah et al., 1995; Stewart et al., 1999; Streeten et al., 2000). The pathophysiological mechanism of the chronic orthostatic intolerance associated with CFS has yet to be elucidated and while there have been reports that it is partly mediated by venous pooling in the legs (Streeten et al., 1988; Stewart, 2000) there does not appear to be an increased capacitance or distensibility of leg veins (Stewart, 2002). One recent suggestion is that there is a defect in the regulation of local blood flow, possibly involving the cutaneous circulation (Stewart, 2002), and our findings of a prolonged vasodilatation in response to ACh delivered iontophoretically to skin blood vessels suggests that signalling mechanisms acting on the vascular endothelium might well be implicated in the type of POTS associated with CFS patients. If increased cutaneous blood

flow in response to cumulative doses of ACh is mediated by a prolongation of the response of individual doses in CFS patients, then further study of the actions of ACh as an endothelial-dependent vasodilator is warranted most especially in those CFS patients in whom chronic orthostatic intolerance is a significant symptom.

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