

Acetylcholine mediated vasodilatation in the microcirculation of patients with chronic fatigue syndrome

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Abstract

The aetiology of chronic fatigue syndrome (CFS) remains controversial and a number of hypotheses have been put forward to explain it. Research into the condition is hindered by the considerable heterogeneity seen across patients but several reports have highlighted disturbances to cholinergic mechanisms in terms of central nervous system activity, neuromuscular function and autoantibodies to muscarinic cholinergic receptors. This paper examines an altogether separate function for acetylcholine and that is its role as an important and generalized vasodilator. Most diseases are accompanied by a blunted response to acetylcholine but the opposite is true for CFS. Such sensitivity is normally associated with physical training so the finding in CFS is anomalous and may well be relevant to vascular symptoms that characterise many patients. There are several mechanisms that might lead to ACh endothelial sensitivity in CFS patients and various experiments have been designed to unravel the enigma. These are reported here. © 2004 Elsevier Ltd. All rights reserved.

1. Introduction

There are many difficulties surrounding the classification, diagnosis and management of patients with chronic fatigue syndrome (CFS). It is often an extremely debilitating condition with unknown aetiology and pathophysiology and which may include a variety of heterogeneous conditions such as myalgic encephalomyelitis (ME), post-viral fatigue syndrome (PVFS), and chronic fatigue immune dysfunction syndrome (CFIDS). CFS is currently defined exclusively by a number of non-specific symptoms [1] that are common to many conditions but there has been speculation that many of the neurological symptoms might be cholinergically mediated [2]. This conjecture is supported by findings of increased levels of free choline in the central nervous system of CFS patients [3–5]. As well as these neurological findings, however, there has been a recent report of autoantibodies specifically to muscarinic receptors in many CFS patients suggesting that there might well be subgroups within the CFS construct that

are associated with autoimmune abnormalities of cholinergic, muscarinic receptors [6].

Apart from its neurotransmitter functions acetylcholine is, of course, a well established and prominent vasodilator whose action is dependent upon an intact layer of functioning endothelial cells that line the lumen of all blood vessels. Our group has been interested in the vasodilator properties of acetylcholine in CFS patients and this report is a summary of our current findings.

2. Acetylcholine (ACh) but not nitric oxide (NO) sensitivity in CFS patients

A common test of endothelial integrity is the response of blood vessels to both endothelial-dependent vasodilators like acetylcholine and endothelial-independent vasodilators like nitric oxide (NO) via an NO donor like sodium nitroprusside (Fig. 1).

In most medical conditions associated with cardiovascular disease there is a blunted response to acetylcholine. However, we have reported increased responses to a cumulative dose regime of ACh delivered by iontophoresis into the cutaneous microcirculation of 22 CFS patients when compared with 22 age and gender-matched control subjects [7]. In the same patients the

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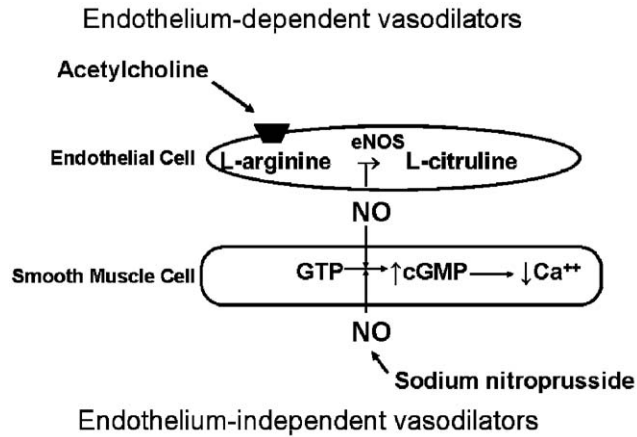


Fig. 1. ACh and NO pathways in the blood vessel endothelium.

response to iontophoretic application of sodium nitroprusside, a donor of NO, was normal compared to controls [7]. There are many possible explanations for endothelial sensitivity to ACh ranging from sensitivity of the G-protein muscarinic (m3) receptor on the surface of the endothelial cell, low levels of the enzyme acetylcholinesterase (AChE) as expressed on endothelial cells and up-regulation of one of many of the post-receptor signaling mechanisms. Since the response to NO was normal we assumed that smooth muscle cell biology was not implicated in the sensitivity to ACh.

3. Testing the sensitivity of the endothelial ACh muscarinic receptor and other similar G-protein receptors, namely, bradykinin (BK) and substance P (SP) in CFS patients

In a further set of experiments, single doses of 1% ACh, 0.06% bradykinin (BK), 0.15% substance P (SP) [all endothelial-dependent vasodilators] and 1% sodium nitroprusside (SNP) [endothelial independent] were delivered by iontophoresis for 80 s using a 0.1 mA anodal current into the forearm skin of 30 well-defined CFS/ME patients and 30 well-matched control subjects. The experiments were carried out under controlled experimental conditions as previously reported by us for each of these vasodilators [8].

The results (Table 1) show that resting skin perfusion was normal and the peak responses to the single doses of ACh, BK and NO were also normal. There was a significantly increased response to SP in CFS patients and this was often accompanied by a spreading flare and localised oedema: a finding not observed in control subjects. It is possible that the increased response to SP is a consequence of a heightened sensitivity to SP in terms of its histamine releasing properties [9]. Indeed, sensitivity to histamine has been implicated in CFS pathogenesis [10,11]. We propose, therefore, that there is

Table 1

Laser Doppler blood flow values (means \pm SD), baseline and peak values after stimulation with ACh, SNP, BK and SP in 30 CFS patients and 30 matched control subjects

Blood flow in arbitrary units (AU)	Control subjects <i>N</i> = 30	CFS patients <i>N</i> = 30	<i>P</i> Value
Baseline blood flow	11.7 \pm 2.4	12.5 \pm 6.4	0.44
Peak ACh	92.2 \pm 22.0	88.3 \pm 16.5	0.5
Peak SNP	53.6 \pm 26.0	57.4 \pm 27.0	0.56
Peak BK	66.2 \pm 29.9	73.4 \pm 24.6	0.24
Peak SP	58.8 \pm 35.7	76.6 \pm 30.1	0.05

Table 2

Blood flow recovery times, blood enzyme and lipid data from 30 CFS Patients and 30 healthy matched controls (Mann Whitney U Statistics) [means (SD)]

Measurement	CFS patients	Control subjects	<i>P</i> Value
<i>t</i> ₇₅ (min)	13.7 (11.3)	8.9 (3.7)	0.03
<i>t</i> ₅₀ (min)	24.5 (18.8)	15.1 (8.9)	0.03
AChE (moles substrate hydrolysed/min per RBC)	0.024 (0.005)	0.023 (0.004)	NS
BChE (U/L)	4990 (979)	5174 (1201)	NS

no widespread upregulation of endothelial G protein receptor function in CFS patients.

4. Prolongation of the acetylcholine-mediated blood flow response and the relation to blood cholinergic enzymes

A further study demonstrated that ACh sensitivity in CFS patients might be explained by prolonged action of the vascular response to ACh [12]. We tested this by recording the dynamics of the ACh-stimulated blood flow response for up to 30 min to allow the rate of decay of the response to be calculated. Two points were determined, *t*₇₅ and *t*₅₀, corresponding to the times taken for the blood flow response to return to 75% and 50%, respectively, of the peak response to ACh minus the baseline response. Also, in the same study, we determined levels of red blood cell acetylcholinesterase (AChE) and plasma butrylcholinesterase (BChE) to check any relationship between blood cholinesterase measurements and the endothelial responses to ACh. The results are listed in Table 2.

The data demonstrated that the dynamics of the ACh-stimulated blood flow response is significantly different in CFS patients compared with control subjects in that the action of ACh is prolonged in the CFS patients—the blood flow recovery half times (*t*₇₅ and *t*₅₀) were 13.7 and 8.8 min and 24.5 and 15.1 min for control subjects and CFS patients respectively (*P* < 0.03 for *t*₇₅ and *t*₅₀). We have postulated that this prolonged ACh response might be related to inhibition of endothelial expression of the enzyme acetylcholinesterase (AChE) [13] possibly

via a viral mechanism since herpes virus is known to inhibit AChE within cholinergically sensitive cells [14] and that lymphocytic choriomeningitis virus can inhibit AChE within cholinergically sensitive cells for years after infection [15].

There were significant correlations between the ACh-stimulated blood flow recovery (t_{50}) and both AChE ($r = 0.4$, $P < 0.03$, i.e. high AChE associated with slower decay of ACh response) and BChE ($r = -0.4$, $P < 0.03$, i.e. high AChE associated with faster decay of ACh response) activity levels in healthy control subjects but not in CFS patients. The range of activity for both of these blood enzymes is great and the values obtained were within normal limits. It is apparent, however, that in control subjects a high BChE activity is normally associated with a quicker decrease in hyperaemic blood flow response but the opposite is true for AChE activity. This may be explained by the fact that AChE is inhibited by its own substrate, ACh, such that large amounts of exogenously delivered ACh may disproportionately swamp local AChE activity. This effect may also be disproportionately greater in those with the highest residual levels of AChE since the maximal rate of hydrolysis of ACh by AChE is obtained at the lowest concentrations of ACh [16]. No such relationship was seen for AChE or BChE activity and recovery of the hyperaemic blood flow response in CFS patients and this is one further indication of disruption to cholinergic pathways in these patients.

5. Acetylcholine sensitivity is specific to a sub-group of patients within the CFS construct

We have recently completed a study looking at ACh and methacholine (MCh) responses in three groups of patients all of whom fulfill criteria for CFS [17]. We had hypothesised that farm-workers who developed a CFS-like syndrome when exposed to organophosphate cholinesterase inhibitors present in sheep dip might also be ACh sensitive. We also speculated that those with Gulf War syndrome who had taken the cholinesterase inhibitor pyridostigmine bromide as a nerve protection agent and who had been exposed to organophosphate de-lousing powder might be ACh sensitive. To test this we studied ACh responses in 46 CFS patients described as patients with myalgic encephalomyelitis (CFS/ME), 24 farmer workers (CFS/OP) and 25 gulf war soldiers (CFS/GWS). All of these patients fulfilled the CDC criteria for CFS [18]. The results demonstrated that only the CFS/ME patients were sensitive to ACh [17].

Our second aim was to test further the hypothesis that vascular hypersensitivity in CFS was due to a reduction in endothelial cholinesterase activity. We tested this hypothesis by assessing the blood flow responses to MCh, a vasodilator that is almost identical to acetylcho-

line, but which is much less influenced by the action of cholinesterase. We predicted that if our ACh results were a consequence of reduced cholinesterase activity we would expect to find no difference between the vascular responses to ACh and MCh. In control subjects MCh responses were indeed significantly greater than those for ACh and this was also true for those with CFS/OP and CFS/GWS conditions. In the CFS/ME group there were no significant differences between ACh and MCh responses. These results point to a problem with AChE under-expression on the vascular endothelium of CFS patients.

6. The fatty acid hypothesis and the ACh vasodilator pathway

While the current data we have might point to under-expression of AChE on the endothelium of CFS patients there are, in fact, several possible pathways for the blood vessel to dilate following stimulation of the ACh muscarinic receptor through to the vascular smooth muscle. These are outlined in Fig. 2.

Under normal circumstances with an intact endothelium it is thought that the calcium activated NO pathway is responsible for the major component of acetylcholine-mediated vasodilatation. Phospholipase A_2 activated prostacyclin (PGI_2) also contributes to vasodilatation and the contribution of this pathway to total relaxation can be determined by blocking with cyclo-oxygenase inhibitors such as aspirin. Under pathological conditions in which the NO pathway is disrupted, however, endothelium-derived hyperpolarising factor (EDHF) may also be a prominent vasodilator [19,20] and this is activated by a cytochrome P450 epoxygenase such as epoxyeicosotrienoic (EET) acid which is a metabolic derivative of arachadonic acid (AA) [21]. What contribution each of these pathways makes to the enhanced sensitivity to ACh in CFS

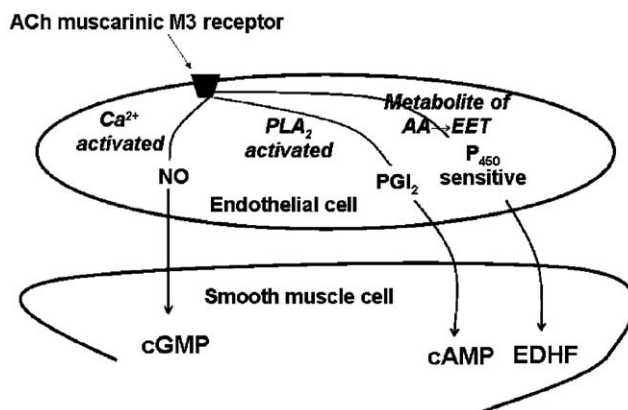


Fig. 2. Pathways for blood vessel vasodilatation from the muscarinic receptor on the endothelial cell through to vascular smooth muscle.

patients is intriguing and needs to be determined. Such research is entirely feasible and urgently required.

7. Conclusion

In three separate studies we have demonstrated abnormalities of the ACh endothelium-dependent vasodilator pathway in CFS patients. Sensitivity to ACh seems to be restricted to those patients within the CFS construct who fit descriptions for ME and PVFS but not those for gulf war syndrome, those exposed to organophosphate compounds or, indeed, those with fibromyalgia [22]. The vascular endothelium is a dynamic organ that is influenced by many biological factors such as age, hormonal status, blood pressure, hyperlipidaemia and cardiovascular disease progression so it is at best a blunt instrument for the assessment of specific disturbances to cholinergic mechanisms. Nevertheless the model used in the studies above has been well tested by us and also by many other international groups of researchers working in vascular medicine [23–25]. We are, therefore, confident that the findings of increased sensitivity to acetylcholine in CFS patients are robust and unusual. Increased sensitivity to ACh is normally associated with trained athletes [26] while CFS patients are characterised by having ‘a substantial reduction in previous levels of occupational, educational, social or personal activities’ [1]. Our results are important in terms of vascular control mechanisms in this patient group and may be relevant to the problems of orthostatic instability that is so evident in most CFS patients. The findings reported here are specific to endothelial cholinergic activity and may or may not be applicable to other more widespread neurotransmitter functions of ACh. Recent evidence in a very small group of patients suggested that CFS might be the consequence of a cholinergic dysautonomia and that treatment with cholinesterase inhibiting agents might well be therapeutic [27]. Such a hypothesis is in direct contrast with the findings reported here so great caution is needed in treating an illness with such obvious heterogeneity. It is important that the mechanisms underlying the pattern of abnormal peripheral endothelial cholinergic activity that we have described in CFS patients is unraveled and that the significance of altered cholinesterase activity, the prostanoid pathway and the role of endothelium-derived hyperpolarising factor (EDHF) is determined.

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References

- [1] K. Fukuda, S. Straus, I. Hickie, The International Chronic Fatigue Syndrome Study Group. Chronic fatigue syndrome: a comprehensive approach to its definition and study, *Ann. Int. Med.* 121 (1994) 953–959.
- [2] A. Chaudhuri, T. Majeed, T. Dinan, P.O. Behan, Chronic fatigue syndrome: a disorder of central cholinergic transmission, *J. Chronic Fatigue Syndrome* 3 (1997) 3–16.
- [3] A. Tomoda, T. Miike, E. Yamada, et al., Chronic fatigue syndrome in childhood, *Brain Dev.* 22 (2002) 60–64.
- [4] B.K. Puri, S.J. Counsell, R. Zaman, et al., Relative increase in choline in the occipital cortex in chronic fatigue syndrome, *Acta Psychiatr. Scand.* 106 (2002) 224–226.
- [5] A. Chaudhuri, B.R. Cindon, J.W. Gow, D. Brennan, D.M. Hadley, Proton magnetic resonance of basal ganglia in chronic fatigue syndrome, *Neuroreport* 14 (2003) 225–228.
- [6] S. Tanaka, H. Kuratsune, Y. Hidaka, Y. Hakariya, et al., Autoantibodies against muscarinic cholinergic receptor in chronic fatigue syndrome, *Int. J. Mol. Med.* 12 (2003) 225–230.
- [7] V.A. Spence, F. Khan, J.J.F. Belch, Enhanced sensitivity of the peripheral cholinergic vascular response in patients with chronic fatigue syndrome, *Am. J. Med.* 108 (2000) 736–739.
- [8] D.J. Newton, F. Khan, J.J.F. Belch, Assessment of microvascular endothelial function in human skin, *Clin. Sci.* 101 (2001) 567–572.
- [9] L.G. Heaney, L.J. Cross, C.F. Stanford, M. Ennis, Substance P induces histamine release from human pulmonary mast cells, *Clin. Exp. Allergy* 25 (1995) 179–186.
- [10] L. Dechene, Chronic fatigue syndrome: influence of histamine hormones and electrolytes, *Med. Hypotheses* 40 (1993) 55–60.
- [11] P. Steinberg, B.E. McNutt, P. Marshall, et al., Double-blind placebo controlled study of the efficacy of oral terfenadine in the treatment of chronic fatigue syndrome, *J. Allergy Clin. Immunol.* 97 (1996) 119–126.
- [12] F. Khan, V.A. Spence, G. Kennedy, J.J.F. Belch, Prolonged acetylcholine-induced vasodilatation in the peripheral microcirculation of patients with chronic fatigue syndrome, *Clin. Physiol. Funct. Imaging* 23 (2003) 282–285.
- [13] C.J. Kirkpatrick, F. Bittinger, R.E. Unger, et al., The non-neuronal cholinergic system in the endothelium: evidence and possible pathobiological significance, *Jpn. J. Pharmacol.* 85 (2001) 24–28.
- [14] R. Rubenstein, R.W. Price, Early inhibition of acetylcholinesterase and choline acetyltransferase activity in Herpes simplex virus type 1 infection of PC12 cells, *J. Neurochem.* 42 (1984) 142–150.
- [15] M.B.A. Oldstone, J. Holmstoen, R.M. Welsh, Alterations of acetylcholine enzymes in neuroblastoma cells persistently infected with lymphocytic choriomeningitis virus, *J. Cell Physiol.* 91 (1997) 459–472.
- [16] R.F. Witter, Measurement of Blood Cholinesterase, *Arch. Environ. Health* 6 (1963) 537–553.
- [17] F. Khan, G. Kennedy, V.A. Spence, D.J. Newton, J.J.F. Belch, Peripheral cholinergic function in humans with chronic fatigue syndrome, Gulf War syndrome, and with illness following organophosphate exposure, *Clin. Sci.* 106 (2004) 183–189.
- [18] G. Kennedy, N.C. Abbot, V.A. Spence, C. Underwood, J.J.F. Belch, The specificity of the CDC-1994 criteria for chronic fatigue syndrome: comparison of health status in three groups of patients who fulfill the criteria. *Ann. Epidemiol.*, in press.

- [19] J. Bauersachs, R. Popp, M. Hecker, E. Sauer, I. Fleming, R. Busse, Nitric oxide attenuates the release of endothelium-derived hyperpolarizing factor, *Circulation* 94 (1996) 3341–3347.
- [20] J. Quilley, D. Fulton, J.C. McGiff, Hyperpolarizing factors, *Biochem. Pharmacol.* 54 (1997) 1059–1070.
- [21] D.R. Harder, W.B. Campbell, R.J. Roman, Role of cytochrome P-450 enzymes and metabolites of arachidonic acid in the control of vascular tone, *J. Vasc. Res.* 32 (1995) 79–92.
- [22] A.W. Al-Allaf, F. Khan, J. Moreland, J.J.F. Belch, Investigation of cutaneous microvascular activity and flare response in patients with fibromyalgia syndrome, *Rheumatology* 40 (2001) 1097–1101.
- [23] S.J. Morris, A.C. Shore, J.E. Tooke, Responses of the skin microcirculation to acetylcholine and sodium nitroprusside in patients with IDDM, *Diabetologia* 38 (1995) 1337–1344.
- [24] F. Khan, S.J. Litchfield, P.A. Stonebridge, J.J.F. Belch, Lipid lowering and skin vascular responses in patients with hypercholesterolemia and peripheral arterial obstructive disease, *Vasc. Med.* 4 (1999) 233–238.
- [25] F. Khan, T.A. Elhadd, S.A. Greene, J.J.F. Belch, Impaired skin microvascular function in children, adolescents and young adults with type 1 diabetes, *Diabetes Care* 23 (2000) 215–220.
- [26] H.D. Kvernmo, A. Stefanovska, K.A. Kirkebuen, B. Østerud, K. Kvernebo, Enhanced endothelium-dependent vasodilatation in human skin vasculature induced by physical conditioning, *Eur. J. Appl. Physiol.* 79 (1998) 30–36.
- [27] Y. Kawamura, M. Kihara, K. Nishimoto, M. Taki, Efficacy of a half dose of oral pyridostigmine in the treatment of chronic fatigue syndrome: three case reports, *Pathophysiology* 9 (2003) 189–194.