Non-invasive assessment of digital vascular reactivity in patients with primary Raynaud's phenonenon and systemic sclerosis

M.E. Anderson[1], F. Campbell[2], S. Hollis[3], T. Moore[1], M.I.V. Jayson[1], A.L. Herrick[1]

[1]University of Manchester Rheumatic Diseases Centre and [2]R & D Support Unit, Hope Hospital, Salford and [3]Medical Statistics Unit, Lancaster University, Lancaster, United Kingdom.

Abstract

Objective

To examine digital microvascular responses in patients with primary Raynaud's phenomenon (PRP) and systemic sclerosis (SSc), and compare these to the responses in healthy control subjects.

Methods

Digital microvascular responses to repeated episodes of iontophoresis of acetylcholine chloride (endothelial-dependent), sodium nitroprusside (endothelial-independant) and adrenaline were measured using dual-channel laser Doppler in 8 healthy control subjects, 8 patients with PRP and 8 patients with SSc.

Results

There were no significant differences in responses between groups. For each chemical the greatest response was generally seen in period 7 of the protocol (after the third episode of iontophoresis). For acetylcholine chloride in period 7, the age and baseline adjusted ratio of the maximum response of PRP to control was 0.93, 95% CI (0.26, 3.38) and for SSc to control it was 0.60, 95% CI (0.13, 2.81). For sodium nitroprusside in period 7, this age and baseline adjusted ratio of the maximum response of PRP to control was 1.31, 95% CI (0.74, 2.32) and for SSc to control it was 1.35, 95% CI (0.68, 2.67). For adrenaline in period 7, the age and baseline adjusted ratio of PRP to control was 1.51, 95% CI (0.79, 2.89) and for SSc to control it was 2.18, 95% CI (1.01, 4.69).

Conclusion

This study demonstrates the usefulness of iontophoresis of vasoactive chemicals, combined with laser Doppler blood flowmetry, in the non-invasive assessment of dermal microvascular responses. One possible explanation for the lack of difference in responses between groups is that vasoactive chemicals other than those discussed are important in the pathophysiology of primary and secondary Raynaud's phenomenon.

Key words

Iontophoresis, laser Doppler, vascular responses, primary Raynaud's phenomenon, systemic sclerosis, scleroderma.

Marina E. Anderson, MB, ChB; Fiona Campbell, MSc; Sally Hollis, MSc; Tonia Moore, BSc; Malcolm I.V. Jayson, FRCP; Ariane L. Herrick, FRCP.

Please address correspondence to: A.L. Herrick, University of Manchester Rheumatic Diseases Centre, Hope Hospital, Salford M6 8HD, UK. (Reprints will not be available from the author.)

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Introduction

The pathophysiologies of both primary Raynaud's phenomenon (PRP) and Raynaud's phenomenon secondary to systemic sclerosis (SSc) are incompletely understood (1). There is therefore a need to develop and refine methods of studying vascular responses in patients with PRP and SSc. We have already reported our experience with the technique of iontophoresis of vasoactive chemicals with measurement of blood flow responses by dual-channel laser Doppler to examine forearm blood flow responses in patients with PRP and SSc compared to healthy control subjects (2).

Iontophoresis is the name given to the (non-invasive) process whereby ions of a drug or chemical are driven into the skin by the application of a low voltage (3). Our conclusion was that forearm vascular reactivity to acetylcholine chloride (ACh), sodium nitroprusside (Na NP) and adrenaline did not differ between patients and controls. Both endothelial-dependant (ACh) and endothelial-independant (NaNP) vasodilatory responses were assessed because endothelial abnormalities are well-recognised in SSc, including in early disease (4, 5). While there were a number of possible reasons for these negative findings, one was that any dysregulation of neurovascular control mechanisms might be local to the digits rather than a generalised phenomenon. as suggested many years ago by Lewis (6). The aim of this pilot study was therefore to assess digital vascular responses to the same three chemicals in patients with PRP and SSc compared to healthy control subjects. A secondary aim was to assess the reproducibility of the techniques used.

Patients and methods

Patients

Eight patients with PRP (2 male, 6 female; median age 33 years, range 18-50 years), eight with SSc (4 males, 4 female; median age 47 years, range 38-62 years) and eight healthy control subjects (1 male, 7 female; median age 31 years, range 21-42 years) were studied. Patients in the PRP group all had Raynaud's phenomenon for at least two years, and had no clinical nor serological evidence of connective tissue disease. Patients with SSc fulfilled the American Rheumatism Association criteria (7). Four SSc patients had mild digital skin involvement (involved, but able to pinch), two had moderate involvement (unable to pinch, able to move), and two patients had severe involvement (unable to move). In all of these patients, skin involvement of the fingers of both hands was similar. Two of the patients with SSc were on vasodilator therapy (both on nifedipine). One of the control subjects, one patient with PRP and two with SSc were smokers. The study was approved by the Salford Ethics Committee.

Iontophoresis and laser Doppler monitoring

Iontophoresis and laser Doppler blood flow monitoring were performed after 20 minutes' acclimatisation at 23°C in a temperature-controlled room. Patients were asked not to consume caffeine-containing beverages, and not to smoke on the study day. In all participants, each chemical was tested on the same finger (index finger for ACh, middle finger for NaNP and adrenaline) of each hand at the same visit. The testing was always done in the same order.

The iontophoresis controller, iontophoresis chamber and dual-channel laser Doppler blood flow monitor (with two optic probes) were purchased from Moor Instruments Ltd. (Axminster) (Fig. 1). Both probes were carefully calibrated, and at all times the DC signal remained within normal limits. For each study, the iontophoresis chamber (contoured to fit neatly over the finger) was attached to the skin of the dorsum of the proximal phalanx using a double-sided adhesive disc. The Channel 1 laser Doppler probe (which has a platinum electrode at its base) was inserted into the central aperture of the iontophoresis chamber, and Channel 2 (the control probe) was attached to the dorsum of the proximal phalanx of the little finger of the hand being tested. There was one exception to this Channel 2 positioning when, due to little finger amputation, Channel 2 was attached to the dorsum of the ring finger proximal phalanx. The return electrode was strapped to the wrist. For each study, a gel of the chemical being tested was

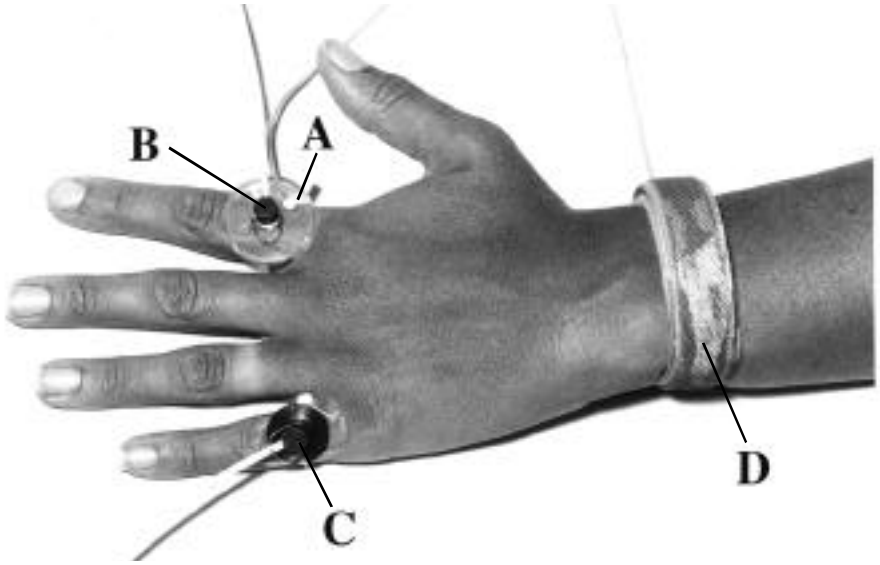

Fig. 1. Iontophoresis chamber (**A**) with Channel 1 laser Doppler probe (**B**), Channel 2 laser Doppler probe (control) (**C**) and return electrode (**D**).

pipetted into the chamber, so the gel filled the small space between the skin surface and the laser Doppler probe base. The reason for using a gel carrier was to prevent leakage from under the probe/electrode, which might have occurred had solution alone been used. Solutions of each of the three chemicals were mixed with 4% methylcellulose aqueous gel (BDH Laboratory Supplies, Poole, England).

The same 12-minute protocol was used for each chemical. The reason for repeating the iontophoresis three times for each chemical was to maximise the opportunity of achieving maximal vasodilation/vasoconstriction. Laser Doppler blood flow was monitored throughout using the following protocol:

Period 1: 60 seconds, baseline blood flow;
Period 2: 30 seconds, iontophoresis at 71 microamps;
Period 3: 120 seconds, resultant blood flow change;
Period 4: 30 seconds, iontophoresis at 71 microamps;
Period 5: 120 seconds, resultant blood flow change;
Period 6: 30 seconds, iontophoresis at 71 microamps;
Period 7: 300 seconds, resultant blood flow change.

ACh iontophoresis. A 1% gel preparation of ACh (Aldrich, Gillingham) was studied on the proximal phalanx of the index finger.

NaNP iontophoresis. A 1% gel preparation of NaNP (David Bull Laboratories PTY Ltd., Victoria, Australia) was studied on the proximal phalanx of the middle finger.

Adrenaline iontophoresis. The increased blood flow induced by NaNP iontophoresis served as an elevated 'baseline' for studying the effects of a 0.5% gel preparation of adrenaline ('Eppy' eyedrops, Chauvin Pharmaceuticals Ltd., Romford).

Typical control subject tracings following the iontophoresis of ACh, NaNP and adrenaline using the above protocol are shown in Figure 2. From the tracings the following measurements were made:

For ACh and NaNP:
- the basal mean blood flow ('Baseline': in perfusion units);
- the maximum blood flow attained in periods 3, 5 and 7 ('Max3', 'Max5' and 'Max7': in perfusion units); and
- the ratio of each maximum to the mean basal blood flow ('Max3/base', 'Max 5/base', 'Max 7/base').

For adrenaline:
- the basal mean blood flow (Baseline: in perfusion units);
- minimum blood flow attained in periods 3, 5 and 7 (Min3, Min5 and Min7: in perfusion units);
- the ratio of each minimum to the mean basal blood flow (Min3/base Min5/base and Min7/base).

The adrenaline baseline was lower than the maximum blood flow after NaNP because of the slow decay in perfusion while the chemical was changed.

Statistical analysis

All data were log-transformed prior to analysis. Repeated measures analysis of variance was used to analyse differences between groups with left/right arm as the within subjects factor, group (control, PRP, SSc) as the between subjects factor, and age and the baseline values as covariates. The data summarised in Table II represent the geometric mean of the left and right values.

Reproducibility was assessed by analysis of variance. Inter- and intra-subject variability in log-transformed responses were determined and back-transformed to give percentages.

Results

On six occasions the flux readings were greater than 1,000 perfusion units and therefore could not be accurately measured. This occurred five times with ACh (in four PRP patients and one SSc patient, always in one finger only) and with NaNP in one PRP patient (one finger only). For statistical purposes, these readings were taken to be 1000 pertusion units. Responses to ACh, NaNP and adrenaline in the three groups are shown in Table I.

Statistical comparisons between groups are adjusted for baseline and for age.

ACh and NaNP

The maximum responses to ACh and NaNP in each period did not differ between the groups. The greatest response was generally seen in period 7. For ACh, the responses were all very variable and showed no clear trend ($F_{2.19} = 0.43$, $p = 0.66$; $F_{2.19} = 0.26$, $p = 0.78$ and $F_{2.19} = 0.27$, $p = 0.77$ in periods 3, 5 and 7 respectively). In period 7, the ratio between PRP and control of the maximum response was 0.93, 95% CI (0.26, 3.38) and for SSc and control it was 0.60, 95% CI (0.13, 2.81).

For NaNP, the ratio of the maximum to baseline geometric mean responses tended to decrease across groups but the dif-

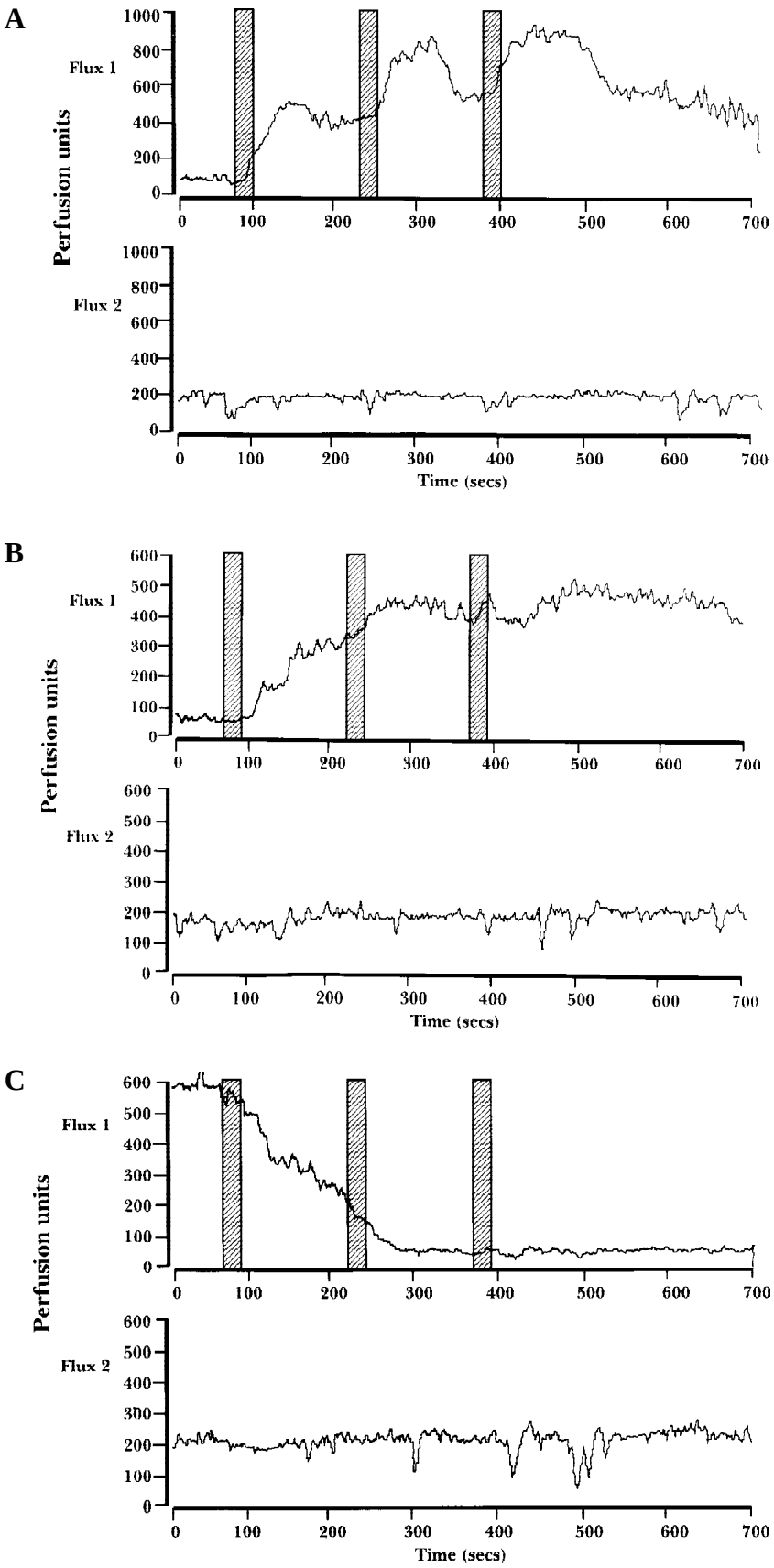

Fig. 2. Typical 'healthy control' blood flow responses (measured in laser Doppler perfusion units) to ACh (**A**); NaNP (**B**); and adrenaline (**C**). Shaded areas represent the timing of iontophoresis. The lower tracing in each figure shows the recording from Channel 2 (control channel).

ferences did not reach statistical significance ($F_{2.19} = 0.10$, $p = 0.91$; $F_{2.19} = 0.06$, $p = 0.94$ and $F_{2.19} = 0.60$, $p = 0.56$ in periods 3, 5 and 7 respectively). In period 7, the ratio of the maximum response of PRP to control was 1.31, 95% CI (0.74, 2.32) and the ratio of maximum response of SSc to control was 1.35, 95% CI (0.68, 2.67).

Adrenaline

The minimum response to adrenaline in all three periods was non- significantly different between groups ($F_{2.19} = 1.20$, $p = 0.32$; $F_{2.19} = 2.74$, $p = 0.09$ and $F_{2.19} = 2.28$, $p = 0.13$ in periods 3, 5 and 7 respectively). In period 7 the minimum blood flow as a proportion of the baseline adjusted for age was approximately 50% higher on average in the PRP group compared to control. The ratio of PRP to control was 1.51, 95% CI (0.79, 2.89) and the ratio of SSc to control was 2.18, 95% CI (1.01, 4.69).

Reproducibility

Results for reproducibility testing between the right and left arms are detailed in Table II. The reproducibility of the method was best for ACh and poorest for adrenaline.

Discussion

The major potential value of the technique of iontophoresis of vasoactive chemicals with concomitant laser Doppler blood flow monitoring is that this provides a non-invasive method of examining neurovascular control mechanisms *in vivo*. This study confirmed the feasibility of applying the technique to patients with PRP and SSc, even when examining digital responses in patients with marked sclerodactyly. As in our previous study (2), none of the subjects tested found the procedure uncomfortable.

Using the protocols described above, digital blood flow responses to ACh and NaNP were similar in patients with PRP or SSc and in healthy control subjects. Responses in the subjects who smoked, and in the two patients on nifedipine, were not obviously different from those of the others studied. Studying adrenaline was more difficult because the baseline blood flow is too low to assess the

Table I. Blood flow prior to and after iontophoresis of acetylcholine chloride, sodium nitroprusside and adrenaline (see text for further explanation). Values are geometric means with 95% confidence intervals

	Controls (n = 8)		PRP (n = 8)		SSc (n = 8)	
Acetylcholine chloride						
Baseline	53.9	(31.3, 92.6)	52.1	(32.1, 84.4)	63.8	(39.0, 104.3)
Max3	290.6	(183.2, 460.9)	324.9	(131.9, 799.9)	265.5	(164.1, 429.7)
Max5	473.4	(315.9, 709.3)	481.5	(241.7, 959.4)	393.8	(227.8, 680.7)
Max7	574.5	(409.7, 805.6)	548.9	(304.6, 989.2)	453.2	(269.5, 762.1)
Max3/baseline	5.40	(3.02, 9.63)	6.23	(3.12, 12.5)	4.16	(2.06, 8.39)
Max5/baseline	8.79	(5.33, 14.5)	9.24	(5.16, 16.5)	6.17	(2.70, 14.1)
Max7/baseline	10.7	(6.77, 16.8)	10.5	(6.16, 18.0)	7.10	(3.04, 16.6)
Sodium nitroprusside						
Baseline	43.2	(24.9, 75.1)	58.6	(35.3, 97.4)	62.0	(41.5, 92.6)
Max3	178.9	(95.6, 334.6)	218.7	(108.9, 439.4)	186.5	(113.0, 307.8)
Max5	291.6	(168.0, 506.0)	343.3	(174.8, 674.3)	294.7	(201.9, 430.1)
Max7	342.4	(202.2, 579.7)	459.5	(260.1, 811.8)	358.5	(252.7, 508.7)
Max3/baseline	4.14	(2.62, 6.55)	3.73	(2.36, 5.90)	3.01	(2.17, 4.17)
Max5/baseline	6.75	(4.11, 11.1)	5.86	(3.14, 10.9)	4.75	(3.02, 7.48)
Max7/baseline	7.93	(4.82, 13.0)	7.84	(3.98, 15.5)	5.78	(3.44, 9.74)
Adrenaline						
Baseline	176.2	(90.6, 342.9)	257.3	(118.4, 559.4)	179.3	(95.4, 336.9)
Min3	67.4	(31.8, 143.1)	131.7	(53.1,326.8)	88.9	(37.8, 208.9)
Min5	35.4	(16.6, 75.7)	70.1	(33.3, 147.7)	68.2	(31.0, 149.9)
Min7	16.9	(8.1, 35.4)	29.5	(16.7, 52.3)	32.4	(21.7, 48.2)
Min3/baseline	0.38	(0.26, 0.56)	0.51	(0.37, 0.71)	0.50	(0.32, 0.77)
Min5/baseline	0.20	(0.10, 0.39)	0.27	(0.18, 0.42)	0.38	(0.26, 0.56)
Min7/baseline	0.10	(0.05, 0.18)	0.11	(0.06, 0.23)	0.18	(0.12, 0.27)

Table II. Results of inter- and intra-subject reproducibility. All data were log transformed before analysis.

	Inter-subject S.D.	Intra-subject S.D.	F-ratio	p-value
Acetylcholine chloride (n = 24)				
Baseline	129%	58%	3.23	0.003
Max3	187%	90%	2.68	0.010
Max5	149%	78%	2.49	0.015
Max7	125%	78%	2.00	0.049
Max3/baseline	199%	79%	3.52	0.002
Max5/baseline	196%	68%	4.34	< 0.001
Max7/baseline	190%	67%	4.33	< 0.001
Sodium nitroprusside (n = 24)				
Baseline	129%	93%	1.58	0.137
Max3	172%	133%	1.40	0.211
Max5	144%	106%	1.53	0.154
Max7	126%	82%	1.85	0.071
Max3/baseline	102%	71%	1.72	0.098
Max5/baseline	141%	72%	2.66	0.010
Max7/baseline	158%	80%	2.58	0.012
Adrenaline (n = 24)				
Baseline	216%	110%	2.40	0.019
Min3	312%	125%	3.07	0.004
Min5	273%	135%	2.37	0.020
Min7	181%	131%	1.52	0.159
Min3/baseline	91%	74%	1.36	0.227
Min5/baseline	147%	96%	1.80	0.080
Min7/baseline	182%	112%	1.91	0.062

adrenaline response directly by looking for a reduction in blood flow. Our approach in this study of measuring the degree of vasoconstriction produced by adrenaline using a 'vasodilated baseline' produced by NaNP iontophoresis proved not to be ideal. This was because by the time the polarity of the electrodes and the gel in the iontophoresis chamber had been changed, this 'baseline' had already fallen substantially (Table I) and therefore blood flow was likely to be falling irrespective of any effect of adrenaline. Nonetheless, adrenaline was vasoconstrictive (Fig. 2) and we believe that our results still suggest that there are unlikely to be important differences in adrenaline responsiveness between groups.

Our rationale for using repeated periods of iontophoresis (three periods each of 71 microamps) rather than one single period, was that this protocol would maximise the vasodilatory/vasoconstrictive effects and might be more likely to detect differences between groups. We believed it important to assess the reproducibility of the technique, which disappointingly had been poor in our forearm study. As in our forearm study, re-

producibility was best for ACh and poorest for adrenaline, and disappointingly reproducibility was even poorer than in the forearm (2). Although reproducibility is poor, by taking both right and left sided measurements the power to detect differences between groups is improved. However, this poor reproducibility is a major limitation and would make us hesitant to use this technique to measure changes in vascular reactivity within an individual over time, or in response to drug treatment. This technique cannot therefore be considered at present to be the long awaited 'gold standard' for the assessment of microcirculatory responses.

The technique of iontophoresis has only been used in a small number of studies of patients with primary and secondary Raynaud's phenomenon. Our results conflict with those of Khan *et al.* and La Civita *et al.* who reported that both ACh- and NaNP-induced vasodilation were reduced in patients with PRP (8) and with SSc (9) compared to healthy control subjects. Methodological differences may be relevant here: the currents used differed between the studies and Khan *et al.* studied the middle (as opposed to the proximal) phalanx (La Civita *et al*. did not state which phalanx was studied). Lindblad *et al.* reported that digital blood flow responses to noradrenaline were similar in patients with Raynaud's phenomenon and control subjects (therefore supporting our own findings with adrenaline) but that phenylephrine, a selective alpha-1 agonist, was less vasoconstrictive in the patients than in the control group (10).

There are several possible reasons for the findings of our study, three of which were discussed previously (2):

1. The iontophoresis protocols were insensitive to differences between groups because the charges used were too large. However, La Civita *et al.* (who reported reduced responses to ACh and NaNP in patients with SSc) used higher currents than in our study.
2. It is the availability rather than the responsiveness to ACh, NaNP and adrenaline which differs between patients with PRP and SSc and healthy controls.
3. The number of patients studied was too small to detect differences between groups. However, no obvious trends were evident from our results.
4. Vasoactive chemicals other than those discussed are important in the pathophysiology of Raynaud's phenomenon (11).
5. Responses were studied in the proximal phalanx rather than more distally (where the most marked ischaemia occurs). In future studies, middle phalangeal responses will be included.
6. Differences in the male: female ratio between the groups may have affected the results.
7. A more accurate estimation of flux readings over 1000 perfusion units might have influenced the results.

In conclusion, we feel that further studies incorporating dose-response curves are necessary before we can be certain as to whether or not digital microcirculatory responses to ACh, NaNP, and adrenaline differ between patients with PRP or SSc and control subjects. The technique of iontophoresis is feasible even in patients with marked sclerodactyly and could therefore be used in further research into the pathophysiology of primary and secondary Raynaud's phenomenon.

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