Digital Cutaneous Vascular Responses to Histamine and Neuropeptides in Raynaud's Phenomenon

Christopher B. Bunker, John C. Foreman, and Pauline M. Dowd
Departments of Dermatology (CBB, PMD) and Pharmacology (JCF), University College and Middlesex School of Medicine, London, U.K.

The pathophysiology of Raynaud's phenomenon is not well defined, but active cutaneous microvascular vasoconstriction and emptying must occur to account for the pallor and are reasons for studying the microvasculature. It has been proposed that there may be a defect in a local histamine vasoconstrictor mechanism. The role of the peptidergic nervous system in Raynaud's phenomenon has not been previously investigated. To study the histaminergic and peptidergic axes in Raynaud's phenomenon, we measured the cutaneous microvascular responses of patients with Raynaud's phenomenon to digital intradermal injections of saline, histamine, the histamine-releasing agent, compound 48/80, substance P, and calcitonin gene-related peptide. We compared these results with those obtained in normal subjects. Intradermal cutaneous microvascular blood flow responses were quantified by planimetry and laser Doppler flowmetry. The results show: a) that in primary Raynaud's phenomenon there is no evidence of local deficiency in histamine release or insensitivity to histamine in the cutaneous microvasculature; and b) that patients with Raynaud's phenomenon react normally to the neuropeptides calcitonin gene-related peptide and substance P, providing a rationale for treating Raynaud's phenomenon with vasoactive peptides. *J Invest Dermatol* 96:314–317, 1991

The pathophysiology of Raynaud's phenomenon (RP) remains poorly understood despite attracting much investigative attention over many years. Although Lewis postulated a...our...“local fault”...in the digital arterioles [1,2], he clearly stated that cutaneous microvascular emptying has to occur for pallor to be observed in the skin [3,4]. A relatively recent hypothesis implicated a “local fault” in a histaminergic vasoconstrictor system argued to be at the level of the cutaneous microvasculature [5].

The importance of the peptidergic nervous system in RP has not been studied. The neuropeptides substance P (SP) and calcitonin gene-related peptide (CGRP) are found in the nerve terminals of unmyelinated sensory afferent fibers in skin and are particularly concentrated in the periphery, where they are considered to subserve nociception [6,7]. CGRP is a novel neuropeptide and an alternative post-transcriptional product of the calcitonin gene; it may play a major role in neurovascular control not only in skin but in many organs and vascular beds [8]. CGRP is a powerful vasodilator that, on intradermal injection, results in a characteristic prolonged flare response [9], and when given intravenously in man, elicits pronounced facial and peripheral flushing [10–12]. These observations suggest that CGRP may be a useful agent in the medical management of severe RP where present therapy is often unsatisfactory.

This study had two aims. The first was to investigate whether digital cutaneous histamine release or microvascular sensitivity was defective in RP. The second was to determine the digital cutaneous responsiveness to neuropeptides in RP in order to explore a role for the peptidergic nervous system in this condition and as a prelude to treating patients with severe RP with intravenous CGRP.

**MATERIALS AND METHODS**

**Subjects** Local Ethical Committee approval was obtained for the study. Twenty patients with Raynaud's phenomenon (RP) were investigated. They consisted of 12 patients with primary RP and eight with RP secondary to systemic sclerosis (SS). There were 19 women and one man, mean age 39, range 15–61 years. RP was diagnosed as episodes of digital ischaemia occurring in response to cold or emotional stimuli and characterized by painful, sequential color changes (white, blue, and red) in the affected parts [13]. Primary RP was diagnosed if patients satisfied the criteria of Allen and Brown [14] (only one of our patients had trophic changes in the digits) and had negative appropriate screening investigations for associated disease. SS was diagnosed according to the criteria of the American Rheumatism Association [15]; all of these patients had trophic changes in the digits. Appropriate investigations directed by symptoms and signs included urinalysis, full blood count, erythrocyte sedimentation rate, urea, electrolytes, calcium, phosphate, total protein, albumen, transaminases, alkaline phosphatase, bilirubin, thyroxine, immunoglobulins, complement C3 and C4, autoantibodies including organ-specific and antinuclear factor and antibodies to extractable nuclear antigens and rheumatoid factor, chest radiography, electrocardiography, barium swallow meal and follow-through, and skin biopsy (histology and immunofluorescence). Ten healthy volunteers were recruited (nine women, one man, mean age 29, range 18–41 years); they had no symptoms of RP or any other cold-related disorder and were normal on physical examination. Patients and subjects gave their signed, informed consent to this study. All abstained from any medication for 3 d prior to the study and neither consumed an alcoholic or caffeinated beverage, or smoked, on the day of the study.

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Dr. Bunker is a Sir Jules Thorn Research Fellow.

Reprint requests to: Dr. Pauline M. Dowd, Department of Dermatology, University College and Middlesex School of Medicine, Mortimer St., London W1N 8AA, UK.

**Abbreviations**

RP: Raynaud's phenomenon
SP: substance P
CGRP: calcitonin gene-related peptide
SS: systemic sclerosis
LDF: laser Doppler flowmetry

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Figure 1. Results for flare measured by planimetry. Flare diameter (means ± SEM) at 2 min (saline, histamine, 48/80, CGRP, and substance P), 20 min (saline — these points are missing because there was no visible flare in any of the subjects to saline at this time point), and 60 min (CGRP) after intradermal injections in the three groups of patients (N, normals; P, primary RP; S, systemic sclerosis).

The experiments took place with the patient or subject equilibrated to an ambient temperature of 21°C in a constant temperature, constant humidity environmental chamber. Intradermal injections of 25 μl volumes of normal saline, 10 μg/ml of histamine (Sigma, Poole, UK), 10 μg/ml of the histamine-releasing agent, compound 48/80 (48/80; Sigma, Poole, UK), 1 μM substance P (SP; Peninsula Labs, Merseyside, UK), and 1 μM calcitonin gene-related peptide (CGRP; human synthetic α CGRP, Celltech, Slough, UK), all endotoxin-free and diluted in endotoxin-free normal saline, were made randomly into dorsal digital skin over the proximal phalanx of either hand. Baseline cutaneous blood flow was measured at these sites by laser Doppler flowmetry (LDF) [16,17] and subsequently at the same sites 2 min after each injection. The flare size was also measured along the longitudinal axis of the finger in millimeters as a modification of a previously employed planimetric method [18]. Further observations were made at the same sites after 20 and 60 min. Any visible flare was measured by planimetry. LDF was measured at the saline site at 20 min (to confirm return to baseline) and at the CGRP site at 60 min.

RESULTS

The results for flare, measured by planimetry, are shown in Fig 1 and those for skin blood flow measured by LDF are shown in Fig 2. There were no apparent differences in baseline skin blood flow between the patient groups, but the limitations of LDF do not make it a suitable objective method for this type of comparison because there are between-subject site-specific and time-specific variables that affect the LDF output [16,17]. LDF is sensitive at detecting within-subject changes where the site and time variables can be controlled. Therefore, the results for LDF are expressed as percentage change from baseline, which has become an accepted derived parameter in laser Doppler flowmetry [19].

All subjects in each group had reacted to all substances injected after 2 min and to CGRP at 20 min and 60 min.

One-way analysis of variance (ANOVA, Kruskal-Wallis) showed no significant differences between the groups of subjects in terms of their responses to each substance injected at 2 min, except for the flare size in response to saline (p < 0.05). This ANOVA difference was found to be attributable (Mann-Whitney U tests) to the significant difference between normals and SS (p < 0.05), because there were no significant differences between normals and primary RP and between primary RP and SS.

Although it appears that there was a tendency, in the SS group, to diminished responses to some of the substances injected, these differences are not consistent between modality (flare and LDF). Although flare and LDF may be measuring different aspects of the vascular response (the factors that determine the propagation of the erythematous response, which is measured by flare, may be different from those that determine the intensity of erythema at a specific site, which is measured by LDF), the apparent differences did not achieve statistical significance when tested (Mann-Whitney U tests), except for the difference between the flare diameter for saline at 2 min compared with normals (p < 0.05, as above). This result, a significant diminution in the non-specific injury response to saline, is likely to reflect the underlying connective tissue and microvascular pathology in SS [20] because a specific pharmacologic unresponsiveness to any of the other substances employed in this study has not been demonstrated.

When the flare responses of agonists, at 2 min, are compared with those of saline within each group of patients (Wilcoxon signed rank tests), all except CGRP in the normal and primary RP groups produced a significantly (p < 0.05) greater response than saline. When the LDF responses of agonists, at 2 min, are compared with those of saline within each group of patients (Wilcoxon signed rank tests), then no significant differences from saline were found in the following instances: histamine in the SS group; 48/80 in the primary RP and SS groups; CGRP and SP in all groups.

The doses of all of the agonists were chosen to give weals and flares at the finger sites that were measurable but that would not be so intense as to exceed the width of the phalanx. This may explain why some of the LDF responses do not appear to be greater than saline. However, when this has happened it does not mean that there is unresponsiveness to a particular agonist. This is exemplified, in the case of 48/80, where the failure to achieve a statistically significant increase in LDF compared with saline in the primary RP group is belied by the visibly obvious and statistically significant greater response measured by flare size.

The visible flare response to saline had disappeared and was not measurable by planimetry when these sites were observed at 20 min and again at 60 min. LDF confirmed that cutaneous blood flow had returned to baseline at 20 min after saline injection. The flare and LDF responses to CGRP were seen to persist in all subjects in all groups at 60 min (with no significant (ANOVA, Kruskal-Wallis) differences between the groups) consistent in size, intensity, and time course with previous reports. Statistical testing in these instances is inappropriate.

In summary, a) there were no consistent differences in the responses to histamine or 48/80 between the control group and those with RP, and b) responses to CGRP at 60 min were as great in
patients with RP as in normal controls. All subjects in all groups manifest an enduring cutaneous erythema in response to CGRP.

DISCUSSION

We have shown using planimetry and laser Doppler flowmetry that patients with primary RP have no overt differences in their cutaneous responses to histamine and other agonists compared with normals.

RP is episodic digital ischemia in response to cold or emotional stimuli. The precise pathophysiology has eluded definition despite much conjecture and investigation [21]. Some of this work has to be interpreted carefully in view of confusion over definitions and terminology. It was Raynaud himself who first suggested that RP may result from faulty neurovascular control and he envisaged a "...local syncope...," due to "...increased irritability of the central parts of the cord presiding over vascular innervation. ..." [22]. The classic experiments of Sir Thomas Lewis led him to propose a "local fault" in the digital arteries leading to vasoconstriction in the cold [1,2], but such a local fault has not been convincingly characterized. Lewis recognized that digital skin pallor occurs in RP (it is a requirement for diagnosis). His own observations had established that pallor in the skin can only be achieved if the capillaries are emptied, and that cutaneous capillaries are capable of independent vasomotion [3]. He reasoned that simple closure of the arterioles would not be sufficient to account for the "dead whiteness..." seen in RP; he said "...there must be in addition an active and strong spasm of the minute vessels themselves..." [4]. We also believe that the cutaneous microvascularity of the digits must be actively involved in the pathophysiology of RP to account for the clinical signs. Therefore, it is the reactivity of these digital cutaneous microvessels that we have studied in these investigations. Defects localized to deeper vascular elements are not excluded by our findings, but these possibilities would not explain, nor allow an interpretation of, the classical pallor of RP.

Interest in the role of histamine in the pathophysiology of RP was generated by Lafferty et al. [5], who proposed that the digital cutaneous vasculature is under a dual system of coarse and fine control, where the coarse control is effected by $\alpha_1$ adrenergic sympathetic vasoconstrictor activity, but release of histamine from perivascular mast cells with resultant vasodilatation provides the fine controlling mechanism. According to this theory, RP occurs because of a "local fault" in either histamine release or vascular sensitivity to histamine after cold exposure has evoked reflex $\alpha_1$ vasoconstrictor. The basis for this hypothesis was a series of experiments that showed an aberrant pattern of blood flow (measured by photoplethysmography) in the hands of patients with RP when the contralateral hand was exposed to rapidly alternating hot and cold stimuli (thermal entrainment) and the simulation of this pattern of response in normal subjects given H1 and H2 antihistamines. Lafferty et al reasoned that the fault most probably was in the local production of histamine from mast cells, rather than at the level of the receptors, because single histaminergic blockade did not affect these results. They acknowledged that the exact site of the proposed local histamine release in post-sympathetic vasodilatation is unclear, but suggested that it is the mast cell population in intimate contiguity with the microvasculature of the skin. Histamine and $48/80$ cause a weal and flare when injected intradermally at other cutaneous sites, but their effect in digital skin has not been previously examined in normal subjects or in disease states. $48/80$ elicits histamine release from mast cells and intradermal injection of $48/80$ is characterized by a weal and flare response very similar to that seen for histamine.

Our experiments show no significantly different digital cutaneous responses to these agents in patients with RP compared to normal subjects. However, we did not test for changes in the dose-response relationship and in the time course of agonist-induced flare or blood flow because the physical dimensions of the finger site do not allow this to be done readily. These findings confirm Lafferty's view that the sensitivity of the cutaneous vessels to histamine is not at fault in RP, but they go against the suggestion that the peri-

crovascular mast cell is a possible primary source of defective histamine release in RP. Although the LDF responses of RP patients to $48/80$ were not significantly different from saline, neither were they significantly different from normals and the flare response to $48/80$ was between three to eight times that of saline. The results for SP, which acts in part by causing mast cell histamine release (see below), are similar. A mast cell defect is unlikely in view of these results. However, what these findings do not exclude (and Lafferty et al have not discussed this possibility) is a defect in neurogenic influences upon the mast cells. But, as one of us has already pointed out, RP has not been reported to occur when patients are given antihistamines even in the relatively high doses common in dermatologic practice. This makes pathologic derangement of a histaminergic vasodilatory axis an unlikely primary situtation in RP [24]. Also, a wider view of active cutaneous vasodilatation [25] is that it is a complex process involving substances other than histamine such as adenosine triphosphate, prostaglandins, activators of kallikrein, SP and, we believe, CGRP, particularly in peripheral skin.

We have shown no overt abnormal cutaneous responsiveness to the neuropeptides SP and CGRP in patients with primary RP. SP was the first neuropeptide to be localized in primary afferent neurons [26]. It has significant central as well as peripheral actions and can be released from nerves in the periphery [27]. There is good evidence that it has a role in neurogenic inflammation in the skin [28]. CGRP is widely distributed and, as a powerful vasodilator, probably contributes to the peptidergic modulation of blood flow in many organs [8], including skin. In skin, unmethylated nerve fibers containing SP and CGRP are found in the superficial dermis and epidermis and in the greatest abundance in fingers and toes. It is considered that CGRP plays an important role as a peptidergic nociceptor transmitter in skin [6,7]. Intradermal injection of CGRP results in a weal and flare response that differs from that seen for histamine or SP. Both weal and flare are initially less pronounced and do not differ markedly from the non-specific response to saline; the weal is short-lived and the erythematous flare, which is thought not to be neurogenic in origin, is small in size and irregular in shape (often with finger-like projections), but the erythema persists for long periods, up to 24 h [9]. It has been observed that subjects given parenteral CGRP manifest marked cutaneous flushing [10,11]. All of these considerations suggest a role for CGRP in the control of peripheral blood flow and the physiologic response to cold and have led to the proposal that there may be a defect in this mechanism in RP. Shawket et al have claimed a suprasensitivity to intravenous CGRP in the hands of patients with RP but provided no data on the fingers, where the clinical changes of RP [12] are seen [29]. Our findings of normal cutaneous responsiveness to CGRP in RP do not preclude an endogenous role of CGRP in perivascular nerves. We have also proposed that CGRP may be a useful treatment for RP, especially in severe cases where peripheral perfusion is critical. Our findings in patients with systemic sclerosis do not point to any specific pharmacologic deficit in this condition. But the results, which show significant enduring erythema with CGRP, strengthen the probability that CGRP may be a useful treatment for severe RP in this condition. We have now conducted pilot studies of intravenous CGRP for this indication, the preliminary encouraging results of which have been reported elsewhere [30,31].

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ANNOUNCEMENT

Creation of The European Tissue Repair Society to promote knowledge and improve contacts between scientists in biology, pharmacology, and clinical care interested in the healing and related reactions of any organ resulting from any pathomechanism is announced. Membership, free of charge for the present year, can be obtained by writing to the Secretary. Beginning in 1991, a fee of 40 ECU for active members and 20 ECU for associate members will be levied.

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