Abnormal Responses to Endothelial Agonists in Raynaud's Phenomenon and Scleroderma

ROBERT R. FREEDMAN, REDA GIRGIS, and MAUREEN D. MAYES

ABSTRACT. ***Objective.*** To further specify the site of vascular dysfunction in patients with Raynaud's phenomenon (RP) and scleroderma.

Methods. Ten patients with RP and scleroderma and 11 healthy control subjects received brachial artery infusions of sodium nitroprusside, an endothelium independent vasodilator, bradykinin, and substance P while bilateral finger blood flow was measured with venous occlusion plethysmography.

Results. Both groups showed vasodilation to sodium nitroprusside. However, in response to the endothelium dependent compounds bradykinin and substance P, the controls showed vasodilation, whereas the patients showed vasoconstriction.

Conclusion. The vascular defect in RP and scleroderma does not lie at the site of the muscarinic receptor, but possibly in a distal signaling mechanism. (J Rheumatol 2001;28:119–21)

Key Indexing Terms:
SCLERODERMA RAYNAUD'S PHENOMENON ENDOTHELIUM BLOOD VESSELS

Although Raynaud's phenomenon (RP) occurs in 95% of patients with scleroderma (progressive systemic sclerosis, SSc) and is often the first presenting symptom, its underlying pathophysiology is unknown. Primary Raynaud's disease is characterized by exaggerated α_2-adrenergic vasoconstriction[1], particularly during cooling[2,3], but normal vascular morphology[4] and unimpaired endothelial function[5,6]. In contrast, studies of scleroderma blood vessels have described endothelial injury, adventitial fibrosis, intimal proliferation, and digital artery thromboses[4,7,8].

We recently found evidence of impaired endothelial function in patients with RP and SSc as shown by diminished responses to intraarterial methacholine, an endothelium dependent vasodilator[9]. In this study we further sought to specify the site of endothelial dysfunction by using other endothelium dependent compounds that act through different pathways. Substance P, like cholinergic drugs, acts through an endothelium dependent nitric oxide pathway, but at a different (neurokinin) receptor[10]. Normal substance P responses, along with blunted cholinergic responses, would suggest a defect at the muscarinic receptor. Bradykinin is also an endothelium dependent vasodilator, but acts through a different receptor through a different (pertussis toxin insensitive, G protein dependent) signal transduction pathway[10]. Impaired responses to substance P, bradykinin, and methacholine but normal responses to sodium nitroprusside would suggest a defect other than the muscarinic receptor and possibly distal to the endothelium.

MATERIALS AND METHODS

Subjects. Ten patients with RP and SSc (8 women and 2 men) and 11 healthy volunteers (6 women and 5 men) served as subjects. Eight patients had diffuse SSc and 2 had limited SSc. SSc was defined according to the American College of Rheumatology criteria[11] and RP was defined as episodic, bilateral digital color changes (2 out of 3 colors: blanching, cyanosis, rubor) provoked by cold and/or emotional stress. No patient had flexion contractures; one had active digital ulcers. Nine of 10 patients had a positive antinuclear antibody (ANA) titer. The sole patient who was ANA negative had diffuse skin disease. Of those with positive ANA, only one had a centromere pattern. Scl-70 antibody was not consistently tested in all cases. Pulmonary fibrosis was present in 2 cases, to a mild to moderate extent. No patient was oxygen dependent or had severe fibrosis.

The patients were recruited from a registry of patients with SSc in our local area administered by one of us (MDM) and sponsored by the National Institutes of Health. The controls were recruited using signs posted on our university campus requesting volunteers for research on blood vessels. They were screened by giving a medical history and completing an extensive symptom questionnaire. All patients and controls gave written informed consent and were paid for their participation. All procedures were approved by our Institutional Review Board. All medications were stopped one week prior to study. No patient or control smoked or had received hormone replacement therapy. There were no differences between the patients and controls (mean ± SD) in age (34 ± 11 vs 41 ± 8 years), total cholesterol (177 ± 33 vs 184 ± 36 mg/dl), HDL cholesterol (55 ± 28 vs 44 ± 10 mg/dl), LDL cholesterol (102 ± 24 vs 108 ± 26 mg/dl) or triglycerides (133 ± 45 vs 118 ± 70 mg/dl).

Procedures. Subjects wore street clothing and were tested supine in a room with controlled 24˚C temperature and humidity (RH = 45%). A 20 gauge catheter was inserted percutaneously in a brachial artery using ultrasound guidance. Xylocaine was used as a local anesthetic and the catheter was maintained patent by 0.5 ml/min infusion of 0.9% saline solution. All saline and drug infusions were performed using Harvard Apparatus 901 pumps. Bilateral

From the Departments of Psychiatry and Behavioral Neurosciences, Obstetrics and Gynecology, Internal Medicine (Pulmonary), and Internal Medicine (Rheumatology), Wayne State University School of Medicine, Detroit, Michigan, USA.

Supported by research grants HL-30604 and AR5-2217 from the National Institutes of Health.

R.R. Freedman, PhD, Professor, Department of Psychiatry and Behavioral Neurosciences and Obstetrics and Gynecology; R. Girgis, MB, BCh, Assistant Professor, Department of Internal Medicine (Pulmonary); M.D. Mayes, MD, MPH, Professor, Department of Internal Medicine (Rheumatology).

Address reprint requests to Dr. R.R. Freedman, C.S. Mott Center, 275 East Hancock, Detroit, MI 48201. E-mail: aa2613@wayne.edu

Submitted March 16, 2000 revision accepted July 26, 2000.

finger blood flow was recorded using venous occlusion plethysmography as described[1,2,9]. Blood pressure was recorded using an automatic recorder.

Thirty minutes after placement of the catheter, baseline measurements were recorded for 15 minutes. Then sodium nitroprusside (0.5, 1.0, 2.0, 5.0, 10.0 µg/min), bradykinin (100, 200, 400 ng/min), and substance P (0.5, 1.0, 2.0, 4.0 ng/min) were infused with 20 minute intervals between each drug. Each dose was infused for 3 minutes, after allowing 2 minutes for the drug to take effect.

Data analysis. Finger blood flow signals were digitized at 100 Hz by an analog/digital converter and analyzed by a computer. The tangent to each post-occlusion curve was computed and converted to finger blood flow in ml/100 cc of tissue/min. Blood flow measurements were averaged for the last 5 minutes of each baseline period and for each drug dose.

To control for spontaneous fluctuations in finger blood flow, Duff's method[12] was used. It has been shown that spontaneous blood flow fluctuations occurring in both hands are roughly equal. To control for these fluctuations, the percentages of change from the preceding baseline period are computed for each drug dose, correcting the changes in the infused finger by the corresponding changes in the noninfused finger.

The data were analyzed using 2 way (group × dose) repeated measures analyses of variance and simple effects tests[13]. Research[14] has shown that sodium nitroprusside does not produce dose-response curves for finger blood flow. Therefore, the greatest percentage change in blood flow and the corresponding drug dose were compared for the 2 groups using unpaired 2 sided t tests. The minimum level of significance for all analyses was $p < 0.05$.

RESULTS

During the baseline periods, there were no significant differences in finger blood flow (mean ± SD) between patients and controls (11 ± 10 vs 17 ± 13 ml/100 cc/min), between infused and noninfused hands (15 ± 12 vs 14 ± 12), or among the 3 periods (15 ± 13, 14 ± 12, 15 ± 12). The peak finger blood flow responses to sodium nitroprusside (Figure 1) did not differ between patients and controls (53 ± 21 vs 80 ± 28%) nor did the doses at which these responses were obtained (2.9 ± 1.2 vs 3.7 ± 1.0 µg/min).

Bradykinin produced significant dose related vasodilation in the controls, in contrast to significant dose related vasoconstriction in the patients ($p < 0.001$). The 2 groups differed significantly at all 3 doses (Figure 2). At every dose, substance P produced vasodilation in controls and vasoconstriction in patients. The overall patterns of blood flow change differed significantly ($p < 0.005$) between the 2 groups. The magnitude of blood flow change differed significantly at the first, third, and fourth doses (Figure 3). There were no group differences in blood pressure nor did blood pressure change significantly during any drug.

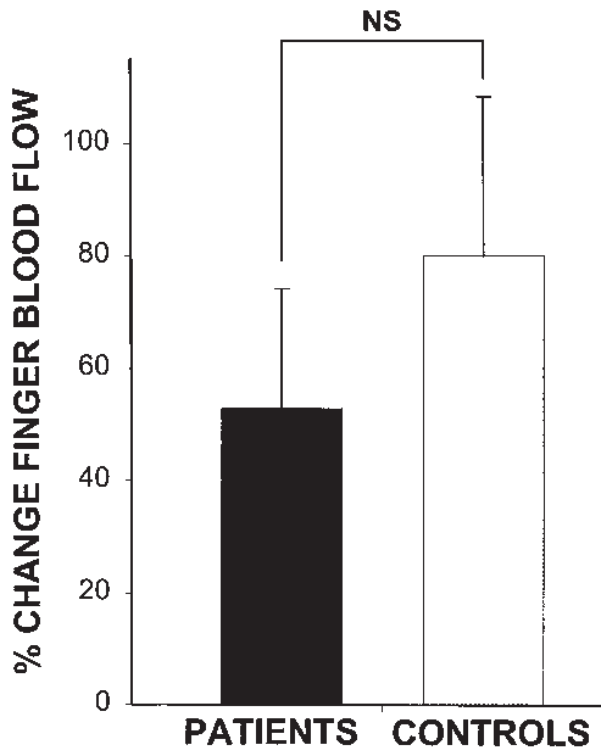

Figure 1. Peak finger blood flow responses to sodium nitroprusside (means ± SE).

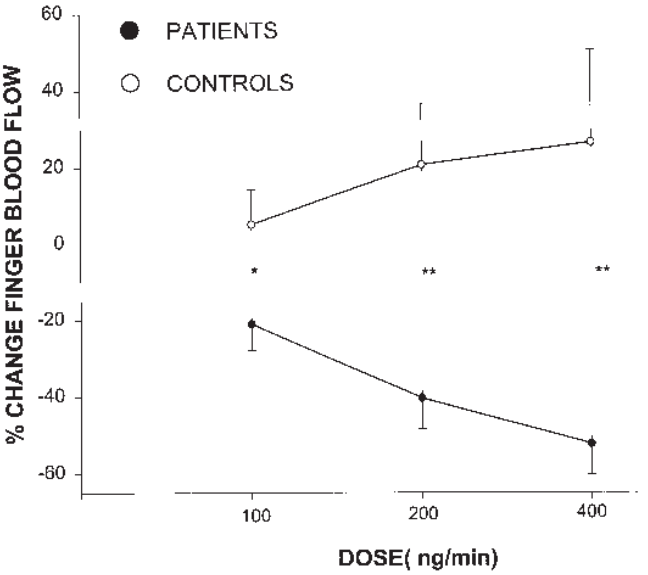

Figure 2. Finger blood flow responses to intraarterial bradykinin (means ± SE). *$p < 0.05$, **$p < 0.005$, ANOVA.

DISCUSSION

We found similar magnitudes of vasodilation in patients with RP and SSc and healthy controls using sodium nitroprusside, an endothelium independent vasodilator. This is in accord with our previous study[9] and suggests unimpaired vascular smooth muscle responsiveness in patients with RP/SSc.

In contrast, we found vasodilation to substance P and bradykinin in the controls and vasoconstriction in the patients. These responses differed significantly between the 2 groups. In a similar study[15], it was found that venous responses to infused substance P were deficient in patients with SSc compared to controls, but that responses to nitroglycerin were similar. Also, patients with SSc did not show pulmonary vasodilation to infused substance P, whereas control subjects did[16].

Taken together with the results of our previous study, we found that patients with RP and scleroderma show impaired

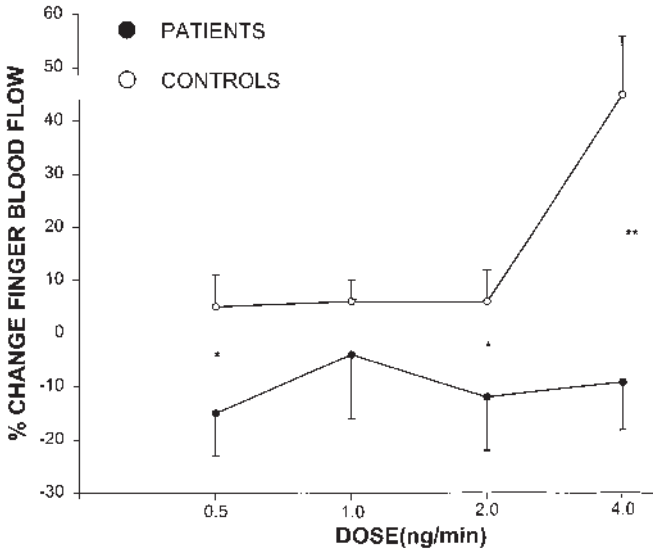

Figure 3. Finger blood flow responses to intraarterial substance P. *p < 0.05, **p < 0.005, ANOVA.

responses to 3 different endothelium dependent vasodilators compared to controls. It is unlikely that these findings are due to defects in 3 distinct receptor types. It is possible that these results are due to a generalized defect at the cellular membrane level, but this is hypothetical.

In a recent investigation[17], we found that intraarterial infusion of L-arginine, the endothelial substrate for nitric oxide formation, significantly reduced RP caused by laboratory cooling in patients with SSc. This suggests, but does not prove, that the mechanism for nitric oxide formation is functioning normally, at least at a level sufficient to reduce symptoms.

It is possible that the impaired responses to methacholine, substance P, and bradykinin are due to a defect in a signal transduction pathway common to these receptors. There is evidence that the protein tyrosine kinase pathway mediates responses to all three[18]. Moreover, this pathway has been implicated in cold induced vascular contraction in 2 animal models[19,20], and it has been hypothesized that this mechanism is involved in RP[20]. Inhibitors of protein tyrosine kinase reversed cold induced contraction of lamb middle cerebral artery, while compounds that potentiate this pathway increased the cold induced contraction[19]. We found similar effects in rat tail artery, which were significantly greater in female compared to male rats[20]. Thus, it is possible that the protein tyrosine kinase pathway mediates cold induced vasoconstriction in RP and SSc. However, considerable research would be needed to verify this hypothesis.

We found that the endothelium dependent compounds substance P and bradykinin produced the expected vasodilation in healthy subjects but vasoconstriction in patients with RP and SSc. The responses to sodium nitroprusside, an endothelium independent vasodilator, were similar in both groups. Together with previous research, these findings suggest that the vascular defect in Raynaud's phenomenon with scleroderma does not lie at the receptor level, but possibly in a distal signal transduction pathway.

REFERENCES

1. Freedman RR, Sabhawal SC, Desai N, Wenig P, Mayes M. Increased alpha-adrenergic responsiveness in idiopathic Raynaud's disease. Arthritis Rheum 1989;32:61-5.
2. Freedman RR, Moten M, Migaly P, Mayes M. Cold-induced potentiation of alpha-2-adrenergic vasoconstriction in primary Raynaud's disease. Arthritis Rheum 1993;36:685-90.
3. Freedman RR, Baer RP, Mayes M. Blockade of vasospastic attacks by alpha-2-adrenergic but not alpha-1-adrenergic antagonists in idiopathic Raynaud's disease. Circulation 1995;92:1448-51.
4. Vajda K, Kadar A, Kali A, Urai L. Ultrastructural investigations of finger pulp biopsies: A study of 31 patients with Raynaud's syndrome. Ultrastruc Pathol 1982;80:175-86.
5. Khan F, Litchfield SJ, McLaren M, Veale DJ, Littleford RC, Belch JJF. Oral L-arginine supplementation and cutaneous vascular responses in patients with primary Raynaud's phenomenon. Arthritis Rheum 1997;40:352-7.
6. Ringqvist A, Jonason T, Leppert J, Ringqvist I. Non-invasive investigation of endothelium-dependent dilatation of the brachial artery in women with primary Raynaud's phenomenon. Clin Sci 1998;94:239-43.
7. Rodnan GP, Myerowitz, RL, Justh GO. Morphologic changes in digital arteries of patients with progressive systemic sclerosis (scleroderma) and Raynaud's phenomenon. Medicine 1980; 59:393-408.
8. Norton WL, Nardo JM. Vascular disease in progressive systemic sclerosis (scleroderma). Ann Intern Med 1970;73:317-24.
9. Freedman RR, Girgis R, Mayes MD. Endothelial and adrenergic dysfunction in Raynaud's phenomenon and scleroderma. J Rheumatol 1999;26:2386-8.
10. Ruschitzka FT, Noll G, Lüscher TF. The endothelium in coronary artery disease. Cardiology 1997;88 Suppl 3:3-19.
11. American Rheumatism Association. Preliminary criteria for the classification of systemic sclerosis (scleroderma). N Engl J Med 1990;323:1486-7.
12. Duff RS. Adrenaline sensitivity of peripheral blood vessels in human hypertension. Br Heart J 1957;19:45-55.
13. Winer BJ, Brown DR, Michels KM. Statistical principles in experimental design. New York: McGraw-Hill; 1991.
14. Coffman JD, Cohen RA. Intra-arterial vasodilator agents to reverse human finger vasoconstriction. Clin Pharmacol Ther 1987; 41:574-9.
15. Matucci-Cerinic M, Pietrini U, Marabini S. Local venomotor response to intravenous infusion of substance P and glyceryl trinitrate in systemic sclerosis. Clin Exp Rheumatol 1990;8:561-5.
16. Cailes J, Winter S, du Bois RM, Evans TW. Defective endothelially mediated pulmonary vasodilation in systemic sclerosis. Chest 1998;114:178-84.
17. Freedman RR, Girgis R, Mayes MD. Acute effect of nitric oxide on Raynaud's phenomenon in scleroderma [letter]. Lancet 1999;354:739.
18. Kitazono T, Ibayashi S, Nagao T, Fujii K, Kagiyama T, Fujishima M. Role of tyrosine kinase in dilator responses of rat basilar artery in vivo. Hypertension 1998;31:861-5.
19. Wagerle LC, Kim SJ, Russo P. Protein tyrosine kinase signaling in cold-stimulated contraction of newborn lamb cerebral arteries. Am J Physiol 1996;270:H645-50.
20. Furspan PB, Freedman RR. Effect of modulators of protein tyrosine kinase activity on gender-related differences in vascular reactivity at reduced temperature. J Cardiovasc Pharmacol 1998;32:728-35.