Diminished Flare Response in Neuropathic Diabetic Patients

Comparison of Effects of Substance P, Histamine, and Capsaicin

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SUMMARY

The flare response in skin largely depends on an intact primary sensory fiber, the C-fiber. We measured the flare response to the intradermal injection of substance P, histamine, and capsaicin in control subjects and in diabetic patients with and without clinically obvious polyneuropathy. The neuropathic diabetic patients had a reduced flare response to substance P, histamine, and capsaicin, compared with control and nonneuropathic diabetic subjects. The smaller flare response in the neuropathic diabetics after capsaicin administration suggested a dysfunction of the peripheral component of the C-fiber. Alternatively, dysfunction of the mast cell or vascular reactivity may contribute to the diminished flare. Because C-fibers participate in nociception in addition to the flare response, the findings of this study, by a method that permits a quantifiable measurement of the function of peripheral sensory neurons in diabetic subjects, has potential usefulness in evaluating sensory neuropathy in diabetic patients.

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Diabetes mellitus is often accompanied by peripheral sensory neuropathy, a complication often difficult to evaluate clinically. Subjective assessments (i.e., pin prick and light touch) cannot be easily quantified, and measurement of sensory nerve function by sural nerve–conduction tests may not correlate with perceived abnormal findings on sensory examination (1). Slowly conducting, unmyelinated C-fibers are thought to convey much of the sensory perception of pain from the periphery to the spinal cord (2). These fibers contain an 11–amino acid peptide, substance P, that is present in peripheral cutaneous endings and in the axon terminals of C-fibers in the dorsal horn of the primate spinal cord (3–9). Substance P is a leading mediator of pain sensation and is a participant in another property of the C-fiber, the axon response (10–18). Exposure of skin to noxious agents is thought to stimulate the local release of transmitters (including substance P) from sensory cutaneous fibers (10). This chemical release is followed by an immediate vasodilation, an increase in vascular permeability, and histamine secretion from mast cells, resulting in a visible cutaneous flare and wheal. Several studies since 1930 have investigated the wheal response in diabetic patients after intradermal application of histamine to assess the presence of vascular insufficiency (19–21). The association of another component of the axon response, the flare, which depends on the intactness of the peripheral nerve more than the wheal, has not been studied in detail. Our hypothesis is that, in the presence of peripheral neuropathy, the release of substance P from the C-fiber is diminished and that this alteration is manifested clinically as a reduction in the flare response. We therefore examined the flare response to the intradermal injection of substance P, histamine, and capsaicin (a drug that releases substance P from primary afferent neurons) in control and diabetic subjects without and with clinically obvious polyneuropathy (22).

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MATERIALS AND METHODS

Subjects. Patients with either type I (insulin-dependent) or type II (non-insulin-dependent) diabetes mellitus were studied at the University of Alabama at Birmingham School of Medicine (UAB), Birmingham, Alabama, or the Metabolic Research Unit at the University of Massachusetts Medical School (UMMS), Worcester, Massachusetts. The patients were classified as neuropathic if they had both symptoms and signs of diabetic neuropathy. Age- and sex-matched nonneuropathic volunteers were recruited at both medical centers. Treatment of the diabetic patients included diet, oral hypoglycemic agents, and insulin.

TABLE 1
Clinical characteristics of subjects (University of Alabama at Birmingham)

	Control subjects	Diabetic subjects	
		Nonneuropathic	Neuropathic
n	11	11	33
Sex (M/F, %)	64/36	64/36	61/39
Age (yr)	51.1 ± 3.6	51.1 ± 3.5	51.2 ± 1.8
Duration of diabetes (yr)		10.2 ± 2.6	9.1 ± 0.9
Treatment		7 insulin, 2 oral agents, 2 diet	28 insulin, 3 oral agents, 2 diet
Symptoms (%)			
Pain			94
Tingle			39
Numbness			78
Dyck neuropathy score		6.5 ± 2.0	23.6 ± 1.6*†
Signs			
Absent Achilles reflex (%)			78
Autonomic symptom score		0.6 ± 0.3	6.5 ± 2.0*
Nerve conduction velocity (m/s)			
Median motor	53.4 ± 0.9	50.1 ± 0.7‡	46.8 ± 0.9*†
Median sensory	44.9 ± 0.7	46.8 ± 2.5	45.4 ± 1.2
Peroneal motor	46.3 ± 0.7	38.7 ± 2.2§	36.5 ± 0.9*
Sural sensory	36.0 ± 0.6	30.4 ± 1.3‡	26.2 ± 0.4*

*$P < .001$, ‡$P < .025$, §$P < .005$ vs. controls.
†$P < .01$ vs. diabetic controls.

Chemicals. Substance P synthesized for human use by Sigma (St. Louis, MO; cat. no. S-6883, lot no. 124F-59201) was used. The peptide was diluted in sterile isotonic saline to a concentration of 3 ng/20 μl. Histamine phosphate (Lilly, Indianapolis, IN) was made to a final concentration of 10 ng/20 μl in isotonic saline, and capsaicin (Sigma, cat. no. M-3264) was diluted to 100 ng/20 μl in isotonic saline. Each chemical was handled as follows: dilutions were made in bacteriostatic sterile saline (Abbott, N. Chicago, IL; NDC-0074-1966-07) and filtered by a 0.22-μm Millex filter (Millipore, Bedford, MA, cat. no. SLG-S02050 S). The solutions were placed into 10-ml sterilized Wheaton-serum vials, and the vials were capped. The duration of bioactivity of these solutions to produce a flare of similar size was at least 3 mo for substance P and histamine and 12 mo for capsaicin. The vials were stored at 4°C.

Experimental design. The same experimental design was used at both medical centers. Patients were clinically evaluated for evidence of neuropathy (by R.S.C. at UAB; by N.A. at UMMS), and a Dyck neuropathy score was estimated (22). In addition, at UMMS, all subjects had plasma HbA_{1c} measured at the time of testing, and at UAB, all subjects had nerve-conduction studies. Diabetic and normal subjects received 20 μl intradermal injection of substance P (3 ng), histamine (10 ng), capsaicin (100 ng), or normal saline vehicle in the medial forearm. Only one chemical was injected per forearm per visit. Most subjects received one or two series of injections in the course of the study.

The flare response was visualized, the borders were marked, and the maximal flare was traced onto clear plastic. The area of the flare was cut out and weighed. Responses were compared as centimeters squared of flare. In all cases, the flare appeared as a red reticular pattern extending over the medial surface of the forearm.

Statistics. All data are expressed as means ± SE. Statistical analysis of differences between groups was by analysis of variance and two-tailed Student's *t* test or Student-Newman-Keuls test.

RESULTS

The clinical characteristics of all participants in this study are summarized in Tables 1 and 2. The control subjects (by definition) had no evidence of peripheral or autonomic neuropathy. The nonneuropathic diabetic patients at UAB had slight but statistically significant abnormal physical findings consistent with diabetic polyneuropathy, although they were asymptomatic. The two nonneuropathic patients studied at UMMS had neither symptoms nor signs of diabetic neurop-

TABLE 2
Clinical characteristics of subjects (University of Massachusetts Medical School)

	Control subjects	Diabetic subjects	
		Nonneuropathic	Neuropathic
n	9	2	8
Sex (M/F, %)	50/50	50/50	38/62
Age (yr)	40 ± 4	56, 62	44.5 ± 6.3
Duration of diabetes (yr)		2, 4	14.5 ± 0.4
Treatment		2 oral agents	7 insulin, 1 oral agent
HbA_{1c} (%)	5.5–7.5	10.3, 11.9	10.2 ± 0.9
Dyck neuropathy score			13.8 ± 3.5

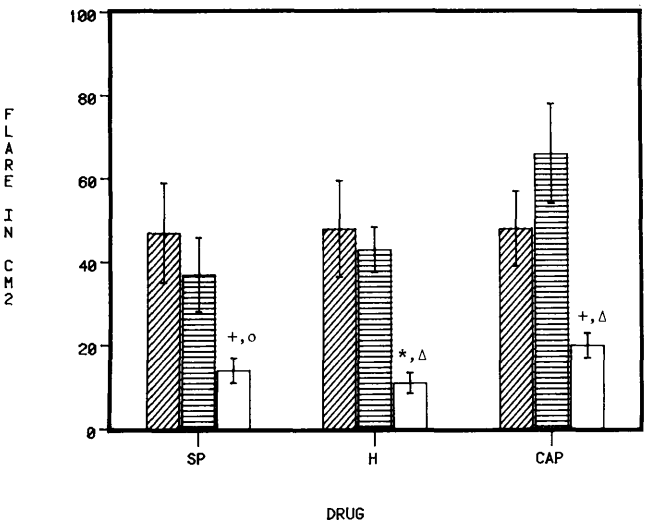

FIG. 1. Flare response to intradermally injected substance P, histamine, and capsaicin at University of Alabama at Birmingham. Substance P (SP) 3 ng in 20 μl, histamine phosphate (H) 10 ng in 20 μl, and capsaicin (CAP) 100 ng in 20 μl were intradermally injected, and area of flare was measured. *Hatched bars*, control subjects (n = 11); *horizontal-lined bars*, nonneuropathic diabetic patients (n = 11); *open bars*, neuropathic diabetic patients (n = 33). $^{+}P < .025$ vs. control, $^{\circ}P < .025$ vs. nonneuropathic diabetic patients, $^{*}P < .005$ vs. control, and $^{\Delta}P < .001$ vs. nonneuropathic diabetic patients.

athy (Table 2). There was a generalized slowing of nerve-conduction velocities in both the nonneuropathic and the neuropathic diabetic patients studied at UAB, although the extent of these abnormalities was considerably more severe in the patients with clinical neuropathy (Table 1). The neuropathic subjects at UMMS had a significantly lower Dyck neuropathy index (13.8 ± 3.5) than did neuropathic diabetic subjects at UAB (23.6 ± 1.6, $P < .025$), although their index was slightly higher than that of the nonneuropathic patients at UAB.

Flare responses. The neuropathic diabetic patients had a reduced flare response to substance P, histamine, or capsaicin compared with nondiabetic control and nonneuropathic diabetic subjects at both UMMS and UAB (Figs. 1 and 2). The flare responses of control and neuropathic and nonneuropathic diabetic subjects were greater at UMMS than at UAB, but the neuropathic diabetic subjects consistently had smaller flare responses to substance P and capsaicin than control subjects at both locations: 36% (UAB) and 54% (UMMS) after substance P injection and 43% (UAB) and 50% (UMMS) after capsaicin administration. During the study, we noted that the areas of flare in response to all substances appeared to be largest in fair-skinned individuals and smallest in darker-skinned people. Because all subjects studied at UMMS were fair skinned and because those studied at UAB were largely darker skinned, we speculate this may have accounted for the differences in flare areas between the two centers. Note, the flare response to a 20-μl saline injection (n = 8) was either undetectable or barely detectable, <2% of substance P or capsaicin values.

DISCUSSION

A reduction in the flare response in the neuropathic diabetic patients suggests a dysfunction of the peripheral component of the C-fiber. Because C-fibers participate in both nociception and flare responses and because the reduction in the flare was most evident in neuropathic patients, our findings support prior studies with histamine that showed a diminished axon response in some diabetic patients (19–21). Additionally, our observations suggest that the reduced flare response involves a functional impairment of the cutaneous primary afferent system in neuropathic diabetic patients.

The actual cause of the abnormality in the flare response is difficult to delineate. The flare response probably depends on multiple, coordinated activities: the intactness of the cutaneous fiber, sufficient neurotransmitter (including substance P), responsive mast cells near the neural fibers, and cutaneous blood vessels that can vasodilate. A model for the mechanisms of the flare proposes that secretion of substance P causes histamine release from mast cells, which in turn promotes further substance P release (18). The flare pattern follows the cutaneous innervation, because substance P also elicits vasodilation of vessels near the fiber endings. Consistent with this model, the flare response to substance P and capsaicin is decreased after antihistamine administration (15). However, altered mast cell function in diabetic skin has not been demonstrated. The wheal component in diabetic patients with diminished peripheral blood circulation is decreasd after histamine application (21), although a relationship of the wheal to the presence of neuropathy has not been made.

Support for a possible neuropathic origin of the reduced flare is derived from studies showing that depletion of substance P in peripheral nerve endings after application of capsaicin also reduces the flare response (15). Therefore, an altered content or releasability of substance P from peripheral nerve endings would be expected to impair the flare. Recent observations in the rat indicate that sensory fibers contain the peptides neurokinin A and calcitonin-gene–related peptide in addition to substance P (23). Neurokinin A,

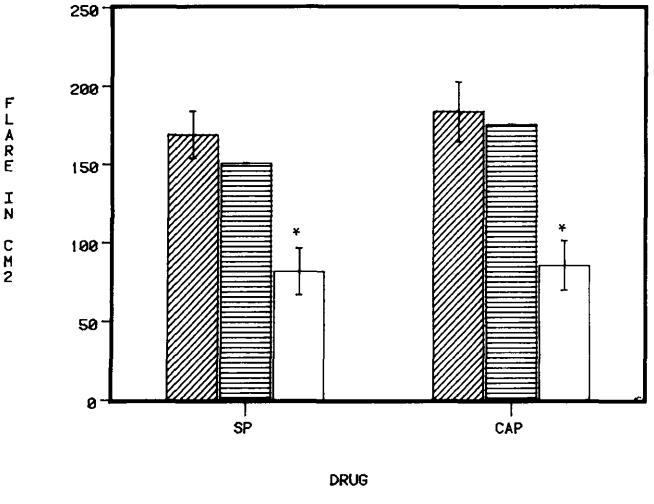

FIG. 2. Flare responses to intradermally injected substance P and capsaicin at University of Massachusetts Medical School. Substance P (SP) 3 ng in 20 μl and capsaicin (CAP) 100 ng/20 μl were injected into skin of controls (n = 9), nonneuropathic diabetic patients (n = 2), and neuropathic diabetic patients (n = 8). *Hatched bars*, control subjects; *horizontal-lined bars*, nonneuropathic diabetic patients; and *open bars*, neuropathic diabetic subjects. $^{*}P < .05$ vs. control.

which is generated from a precursor also containing substance P (24), and calcitonin-gene–related peptide contribute to the vasodilation and plasma protein extravasation of the axon response (23). Capsaicin causes release of these potential mediators of the axon response, and its use may reflect sensory fiber function more generally than substance P or histamine. In our study, injection of capsaicin caused similar flares in normal subjects and in diabetic patients without neuropathic changes. Thus, the smaller flare with capsaicin in neuropathic patients is consistent with a reduction in releasable substance P or other mediators from peripheral nerve endings.

Alternative explanations for the reduced flare need to be considered. The function of other components of the flare response, i.e., the mast cell and small blood vessels, also may be impaired. The diminished flare response to histamine may result not only from neural dysfunction with lower substance P release but also from an altered responsiveness of vasculature to substance P. Additionally, an impaired mast cell response to substance P administration would produce a smaller flare.

In diabetic patients, changes in function in small-caliber fibers may occur early in the course of the disease (22, 25,26). The neuropathy in our diabetic patients was most evident in the legs as decreased sensory perception, whereas sensory neuropathy in the forearm was clinically undetectable. One possible explanation for this finding is that the diminished flare response in the forearm reflects a small amount of dysfunction of the C-fiber, mast cell, or small blood vessel.

In the primate, there is evidence that the substance P–containing afferent C-fibers are unmyelinated and terminate in laminae I, II, and V, regions that give rise to spinothalamic and spinomedullary projecting pathways conveying nociceptive information (7). Impaired myelination has been implicated in severe, established-irreversible diabetic neuropathy (22,26). Because the C-fiber is unmyelinated, the reduced flare response, if based partly on changes in the cutaneous fibers, implicates mechanisms not dependent on proper myelination. Furthermore, substance P probably contributes to C-fiber function as a transmitter of nociception peripherally and centrally. Dale (27) proposed in 1935 that transmitter function at the peripheral ending of the pain fiber may "furnish a hint as to the nature of the transmission process at a central synapse." If an altered substance P release at the peripheral nerve ending is involved in the reduced flare, it is tempting to speculate that in the neuropathic diabetic subjects, there is an alteration of C-fiber function centrally, including substance P release.

In addition to the diminished perception of pain, the reduced flare response may explain another pathophysiological cause for injury to the skin, i.e., impaired vasodilation. It has been speculated that vasodilation promotes the removal of noxious agents from the skin (28). In denervated skin of diabetic patients with neuropathy, ordinarily minor noxious stimuli would remain, permitting further damage. In this regard, it is interesting that two of the neuropathic diabetic subjects at UAB reported hymenoptera "stings" on their upper extremities, both of which were totally painless.

In summary, our findings provide support that measurement of the flare response to substance P, histamine, or capsaicin injection is a quantifiable evaluation of sensory neuropathy in diabetic individuals. The reduction in the flare may reflect changes in C-fiber function primarily or dysfunction in mast cells or vascular reactivity. Studies are in progress to examine whether changes in the flare response to substance P, histamine, and capsaicin in neuropathic diabetic patients correspond to sensory and glycemic improvements.

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