Thermal Thresholds Predict Painfulness of Diabetic Neuropathies

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OBJECTIVE — Pathophysiology explaining pain in diabetic neuropathy (DN) is still unknown.

RESEARCH DESIGN AND METHODS — Thirty patients with peripheral DN (17 men and 13 women; mean age 52.4 ± 2.5 years) were investigated. Fifteen patients had neuropathic pain, and 15 patients were free of pain. Patients were followed over 2 years and examined at the beginning and thereafter every 6 months. Clinical severity and painfulness of the DN were assessed by the neuropathy impairment score and visual analog scales (VASs). Cold and warm perception thresholds as well as heat pain thresholds were obtained for evaluation of Aδ- and C-fibers. Nerve conduction velocities (NCVs) and vibratory thresholds were recorded for analysis of thickly myelinated fibers. Moreover, for assessment of cardiac vagal function, heart rate variability (HRV) was evaluated. In order to reduce day-to-day variability of pain, mean values of the five time points over 2 years were calculated and used for further analysis. Data were compared with an age- and sex-matched control group of healthy volunteers.

RESULTS — There were significant differences regarding electrophysiological studies, HRV and quantitative sensory testing (QST) between patients and healthy control subjects ($P < 0.001$). Generally, patients with neuropathic pain were indistinguishable from pain-free patients. In the pain group, however, VAS pain ratings were correlated to the impairment of small-fiber function (cold detection thresholds, $P = 0.02$; warm detection thresholds, $P = 0.056$).

CONCLUSIONS — Intensity of pain in painful DN seems to depend on small nerve fiber damage and deafferentation.

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Diabetic neuropathy (DN) is the most frequent neuropathy in western countries and affects ~60% of all diabetic patients (1). About 13% of patients with DN report neuropathic pain (2), which includes spontaneous pain such as burning feet or dysesthesia (3). Unfortunately, there are no predictors for the development of pain as a symptom of DN. The intensity of pain may vary substantially within days or weeks. There are mood, seasonal, social, and daily activity influences that modify pain intensity or pain-coping strategies (4). This variability complicates the quantification of clinical neuropathic pain. The detailed mechanisms leading to neuropathic pain are not specific for DN and may even vary between patients. The most important mechanisms are the accumulation of sodium channels on injured axons (5), sympatho-afferent coupling (6), disinhibition of nociception (7), and peripheral or central sensitization (8). However, the predominant pathophysiology in painful DN is unknown (9).

Tests to analyze nerve function in DN include assessment of sensory and motor nerve conduction velocity (NCV), quantitative sensory testing (QST) for different afferent fiber classes (10), analysis of heart rate variability (HRV) for vagal function, and sudomotor axon reflexes for peripheral sympathetic fibers (11). Histological data can be obtained from nerve or skin biopsies (12). However, all of these variables are not able to reveal information about the mechanisms of pain in DN. In some cases the presence of pain seems to be related to the rate of nerve de- and regeneration in teased fiber preparations (13). Later studies (14), however, failed to confirm these findings. Moreover, there was never a parameter explaining the presence or absence of neuropathic pain in different patients. One reason might be the variability of neuropathic pain, as indicated above.

In the present study, we therefore examined DN patients and followed them over a period of 2 years to get more stable values for neuropathic pain. Different neurophysiological parameters of peripheral nerve function and neuropathic pain on visual analog scales (VASs) were analyzed in intervals of 6 months.

RESEARCH DESIGN AND METHODS

— Thirty consecutive patients with signs of peripheral DN (17 men and 13 women; mean age 52.4 ± 2.52 years) were recruited from cooperation with primary care providers. The only exclusion criterion was an estimated impossibility to adhere to the five investigations (see below).

The mean duration of diabetes was 204 ± 24 months at the entrance of the study. The patients were divided into two patient groups according to the presence or absence of neuropathic pain. The first group consisted of 15 patients suffering from neuropathic pain (7 men and 8 women, mean age 51.8 ± 3.3 years; 7

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Abbreviations: CAN, cardiac autonomic neuropathy; DN, diabetic neuropathy; dSFN, diabetic small-fiber sensory neuropathy; HRV, heart rate variability; NCV, nerve conduction velocity; QST, quantitative sensory testing; VAS, visual analog scale; VT, vibratory threshold.

A table elsewhere in this issue shows conventional and Système International (SI) units and conversion factors for many substances.

with type 1 diabetes and 8 with type 2 diabetes). The second group consisted of 15 patients with painless neuropathy (10 men and 5 women, mean age 53.0 ± 4.0 years; 5 with type 1 diabetes and 10 with type 2 diabetes). There was no significant difference concerning signs of DN, duration of diabetes, or HbA_{1c} between the two patient groups. DN was staged as advanced in all patients due to electrophysiological and clinical results.

Neuropathic pain was treated exclusively by the primary care providers. The primary pain medication used was α-lipoic acid ($n = 8$). In rare cases, morphines ($n = 2$), antidepressants ($n = 1$), nonsteroidal anti-inflammatory drugs ($n = 1$), neuroleptics ($n = 1$), or vitamins ($n = 2$) were prescribed. We assessed pain medication at each visit but refrained from changing the therapy regimens. Since none of the patients became pain free, the pain treatment in general had to be regarded as mostly ineffective.

The five consecutive investigations were performed at the beginning of the study and every 6 months thereafter over a period of 24 months. Clinical severity of DN was analyzed by medical history and neuropathy impairment score (15). During each visit, the patients were thoroughly neurologically examined. Emphasis was laid to explore whether DN was painful. Patients were asked to fill in a pain diary for the purpose of recording severity of pain. This diary consisted of VASs ranging from 0 to 10 in which the value of 0 indicated "no pain" and 10 "maximum pain imaginable." Pain on the VAS was recorded five times a day for 14 days. Diabetes control was assessed at the end of the study and was found to be of suboptimal quality, with a mean HbA_{1c} of 8.1 ± 0.3%.

For normal values, 34 age- and sex-matched healthy control subjects were tested (19 men and 15 women, mean age 50.6 ± 2.2 years). Their state of health was determined by medical history and physical examination. Control subjects were investigated once at the beginning of our study.

Informed consent was obtained from all participants according to the Declaration of Helsinki, and the study was approved by the local ethics committee. All investigations were carried out in our temperature (23°C)- and humidity (50% relative humidity)-controlled laboratory. The time for acclimatization for all subjects was at least 1 h before starting the experiment.

Electrophysiological studies

Electrophysiological investigations were performed using standard surface recording techniques. We recorded motor NCV of the peroneal and tibial nerves. Sensory NCV was not analyzed systematically because only a minority of our patients ($n = 7$, assessed orthodromically) had measurable nerve potentials at the lower extremities.

HRV

HRV was evaluated for diagnosis of cardiac autonomic neuropathy (CAN). HRV was recorded using a ProsciCard analyzer (ProScience, MediSyst, Linden, Germany). Seven statistical values (four parameters during six per minute metronomic breathing: variation coefficient, root mean square of successive differences [RMSSD], difference of longest and shortest beat-to-beat interval, ratio of longest and shortest beat-to-beat interval; two parameters at rest: variation coefficient and RMSSD; and the Valsalva ratio) were obtained while all subjects were resting in a reclined position (16). CAN was diagnosed if the results of three or more of these statistical measures exceeded predetermined normative values (17).

QST

Vibratory thresholds were recorded at the internal medial ankle joint using an electromagnetic vibrameter (Somedic, Horby, Sweden) with a stimulus frequency of 100 Hz. The vibratory threshold (VT) was calculated as the statistical mean of three consecutive measurements (18).

Cold and warm perception thresholds as well as heat pain thresholds were determined with a thermal tester (Somedic). A 5-cm^2 peltier element (thermode) was placed on the dorsal side of the right foot. The baseline temperature of the thermode was 32°C for all measurements. The ramp rate of changing temperature for all threshold determinations was 1°C/s. The Marstock method of limits (19) was used to determine thermal thresholds in six consecutive measurements for warm and cold perception. The first two measurements were discarded, and the mean value from the remaining four was calculated.

Statistics

Statistics were calculated using a SPSS version 10.1 for Windows (SPSS, Chicago, IL) software package. Grand mean values were calculated out of the five consecutive measurements during follow-up to get more reliable parameters. For identification of significant influences of the different variables on the pain in DN, the Pearson correlation coefficient was calculated using the grand mean values. For comparison of the different patient groups, *t* tests were performed. All values are given as means ± SE. Statistical significance was considered at $P < 0.05$.

RESULTS

Pain and clinical status of patients over time

None of the patients switched permanently from painful to painless DN or vice versa. Individual pain ratings varied substantially between the five examinations. Unfortunately, some patients repeatedly refrained from filling in the pain diary. In these cases, available data were pooled and grand means calculated. For details see Table 1. Neither the neuropathy impairment score nor the results of the clinical neurological examination varied significantly within 2 years (P = NS).

NCV

Highly significant differences of tibial or peroneal NCV between patients and control subjects were recorded at the entrance of the study (*t* test, $P < 0.001$). No significant difference comparing the two patient groups was found (*t* test; P = NS). Subgroup analysis of patients with painful DN revealed no significant correlation of motor NCV and pain (Pearson, tibial NCV: $r = -0.46$, P = NS; peroneal NCV: $r = -0.35$, P = NS).

HRV

HRV was significantly impaired in patients (*t* test, $P < 0.001$). CAN was diagnosed in 10 patients (5 patients with pain and 5 patients without pain). This number remained unchanged during the follow-up. For follow-up statistics, the variation coefficient during metronomic inspiration was selected. It has been shown to be sensitive and hardly influenced by the heart rate itself. Statistical analysis did not detect any significant differences between the patient groups (P = NS) or any significant correlation be-

Table 1—*Incidence of pain and VAS values of patients with painful DN*

	Baseline		6 months		12 months		18 months		24 months		
Patient no.	Pain	VAS	Pain	VAS	Pain	VAS	Pain	VAS	Pain	VAS	VAS grand mean
1	1	7.7	1	8.6	1	9.3	1	*	1	8.9	8.6
2	1	3.8	1	2.4	1	2.4	1	5.5	1	*	3.5
3	1	3.3	1	4.4	1	4.3	1	6.0	1	5.2	4.6
4	1	4.2	1	3.4	1	3.6	1	3.6	1	3.7	3.7
5	1	4.9	1	4.2	1	6.9	1	5.4	1	5.8	5.5
6	1	4.2	1	4.4	1	4.9	1	2.8	1	4.5	4.2
7	1	1.2	1	1.8	1	3.7	1	2.0	1	1.8	2.1
8	1	3.6	1	1.4	1	3.3	1	2.0	1	1.0	2.3
9	1	5.1	1	4.7	1	*	1	*	1	*	4.9
10	1	1.3	1	0.6	1	1.8	1	0	1	0	0.8
11	1	4.4	1	6.2	1	3.5	1	*	1	0	3.5
12	1	*	1	3.8	1	*	1	*	1	3.1	3.5
13	1	5.8	1	4.0	1	5.4	1	5.3	1	4.4	5.0
14	1	3.7	1	5.2	1	*	1	*	0	0	2.9
15	1	*	1	5.3	1	*	1	*	0	0	2.6

*Missing pain diary completion.

tween CAN and the presence of neuropathic pain in DN (Pearson, $r = -0.45$, P = NS).

QST

VTs. VTs were significantly deteriorated in the patient groups (t test, $P < 0.001$). There were no significant differences between patients with or without pain (t test, P = NS) and no correlation between pain and VT in the painful DN group (Pearson, $r = 0.18$, P = NS).

Cold detection thresholds and warm detection thresholds. Thermal detection thresholds were significantly deteriorated in the patient groups (cold detection threshold, $P < 0.001$; warm detection threshold, $P < 0.001$). Differences between the two patient groups (painful and painless) were not significant (t test, P = NS). However, there was a significant positive correlation between deterioration of cold detection thresholds and intensity of pain in painful DN (Pearson: $r = 0.59$, $P = 0.02$) (Fig. 1*A*). In addition, correlation of warm perception thresholds and pain nearly reached significance ($r = 0.5$, $P = 0.056$) (Fig. 1*B*).

Heat pain thresholds. Heat pain thresholds were increased in both patient groups compared with control subjects ($P < 0.05$). Neither significant differences between the different patient groups (t test, P = NS) nor any significant correlation between heat pain thresholds and neuropathic pain (Pearson, $r = 0.41$, P = NS) could be detected.

CONCLUSIONS — The results of our investigation confirm the lack of a significant difference in nerve function in painful and painless DN. However, if pain was present, it was predominantly related to the impairment of fiber classes, which are involved in pain signaling. This is a new finding that emphasizes the importance of small-fiber loss and deafferentiation in painful neuropathies. However, it does not explain why DNs can be either painful or painless.

The perception of neuropathic pain may vary depending on internal and external factors (4). In order to get stable parameters, we repeatedly investigated the patients and found that pain ratings varied markedly. Therefore, all available values were pooled, and the means of all test results were calculated, thus minimizing the effect of fluctuations and occasional variations, which do not depend on changes of DN. Neither clinical data nor any neurophysiological results were able to predict the presence of pain in DN. This indicates that impairment of peripheral nerve function alone cannot explain neuropathic pain in DN. There are recent data showing that chronic pain may be influenced by genetic factors in rats (20,21) and in men (22). Differences in pain control systems in the spinal cord or brain, rather than diabetes-related peripheral nerve pathology, may further determine whether DN is painful. Accordingly, no patient in our study switched permanently between the painful and painless DN group. This unchanged clinical picture might be related to the stable pathology in advanced DN.

On the other hand, pain in patients with painful DN obviously depends mainly on the impairment of thinly myelinated and unmyelinated afferent fibers (diabetic small-fiber sensory neuropathy [dSFN]). These fibers are not only nociceptive afferents, but also mediate thermal sensations. This result confirms a previous study of our group (23), showing that the C-nociceptor–mediated neurogenic flare is diminished in painful DN. However, the reverse is not possible. The absence of pain does not necessarily predict well-preserved small afferent fiber function because the nonpainful DN patients had increased thermal thresholds as well. Function of large myelinated efferent motor nerve fibers or autonomic efferent fibers failed to correlate significantly to pain. This confirms that pain in DN may be related in particular to small-fiber damage. However, all parameters of nerve function were negatively related to pain in our study. If there was a random distribution, at least some positive correlations would be expected. This further emphasizes the importance of nerve degeneration regarding neuropathic pain in DN and once again indicates that DN is a systemic disease with a predominant pattern of fiber loss.

If nerve fibers degenerate, they may become spontaneously active and accumulate sodium channels, thus causing os-

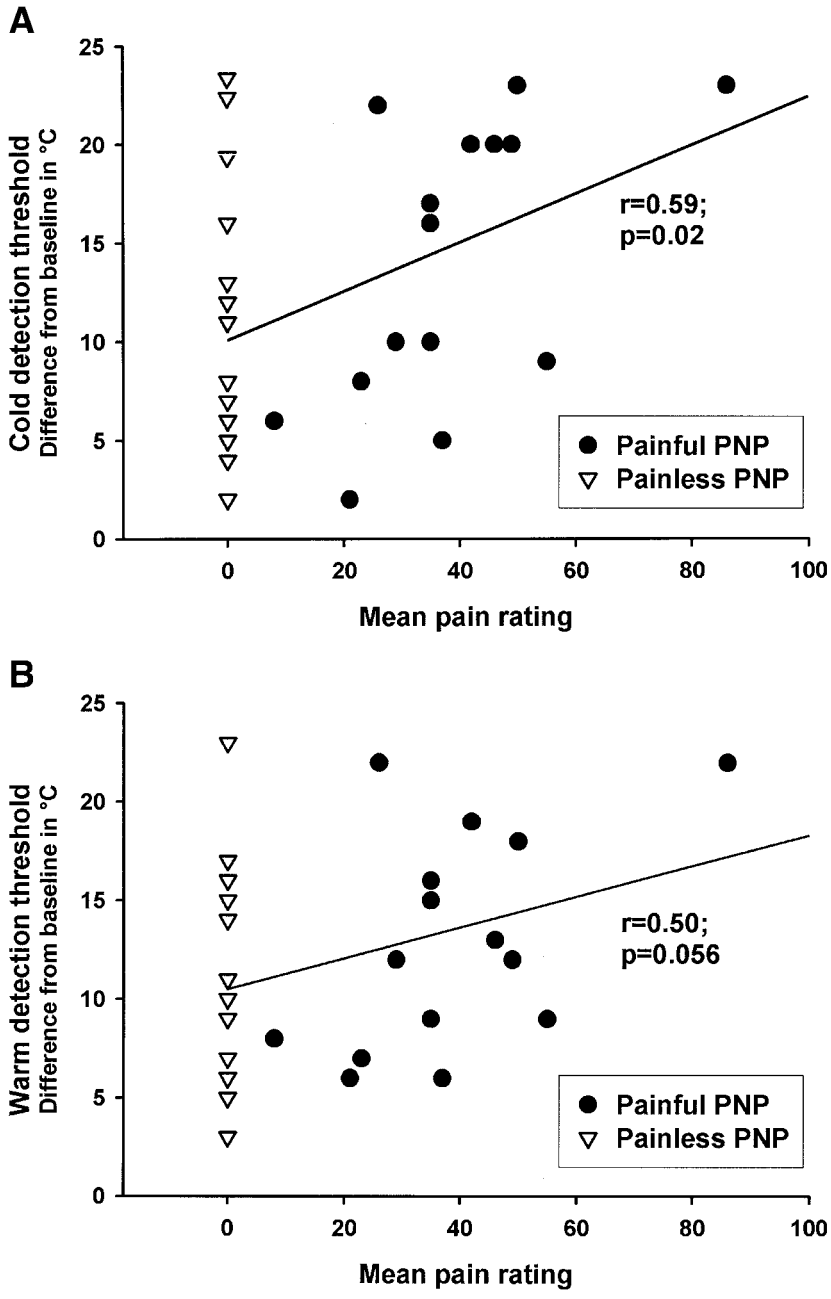

Figure 1—*Plotted are correlations between mean ratings on the VASs and thermal perception thresholds in all 30 patients. Within the painful DN patient group, a significant correlation between cold detection thresholds and mean VAS ratings was found* (A)*. The correlation of warm detection thresholds and mean VAS ratings nearly reached significance* (r = *0.5*, P = *0.056*) (B).

cillation of the membrane potential and bursts of spontaneous activity may occur (5). In accordance, spontaneous activity of C-fibers has been shown by microneurography in erythromelalgia (24), a disease that clinically resembles early dSFN with burning feet. Our results indicate that the ongoing damage of small afferent fibers correlates with an increase of pain intensity. This might be explained by the higher percentage of spontaneously active nerve fibers. Blocking sodium channels usually reduces the pain in ~50% of the patients. However, the remaining ~50% of the patients often do not satisfyingly respond to pain medication. In these cases, gradual deafferentiation, which is known to cause phantom limb pain and pain after cervical root avulsions (25), may also contribute to pain in DN. After peripheral nerve damage, spino-thalamic neurons in the spinal cord or brain stem may become spontaneously active (26,27). This has been shown in post-stroke pain patients, if the infarction involves primary afferent trigeminal neurons in the medulla (28). Furthermore, axonal damage causes an increase of Met-enkephalin and a decrease of β-endorphin in animal models for neuropathic pain (29), resulting in spinal disinhibition of the nociceptive system. Axotomy can also lead to an increase of cholecystokinin in dorsal root ganglia cells, where it antagonizes morphine receptors as well as upstream in the anterior cingulate cortex of the brain (30). As a result, the imbalance of the antinociceptive system will be further enhanced.

In neuropathies, damaged axons can be found in touch with intact axons. Due to Wallerian degeneration and related inflammatory changes, sensitization of intact axons within the whole nerve bundle might occur (31). Peripheral sensitization of intact axons is indicated by a decrease of heat pain thresholds. We found no indication for peripheral sensitization in our patients with advanced DN. This indicates that sensitization of peripheral nociceptors is of minor importance in this late stage of DN. However, it does not exclude an important contribution to pain in early and acute painful DNs (13). Furthermore, axon damage as indicated above leads to an increase of heat pain thresholds while peripheral sensitization decreases them. These confounding mechanisms make heat pain thresholds a less reliable parameter for the evaluation of DN (32).

A certain contribution of the autonomic nervous system to the development of neuropathic pain is under discussion. The information "pain" represents a stressor causing an arousal of the sympathetic nervous system measurable by HRV (33). A previous study (34) showed that the relief of chronic pain can be followed by an increase in HRV. However, this change in HRV was not correlated to the severity or type of pain (35). Vagal damage is well described in DN, but vagus nerve function does not necessarily contribute to pain (36). Moreover, HRV is an indirect measure of nerve function and shows a significant variation, influenced by pain itself but also by other arousals (37). This critical view corroborates our finding that impairment of HRV does not show differences between painless and painful DN. Unfortunately, we did not measure adrenergic sympathetic function in our patients due to the lack of a reliable method to quantify it. Sympathetic vasoconstrictor reflexes often have a huge variability, and there are many confounding factors such as diabetic vessel disease that prevent reliable interpretations. Cholinergic sudomotor function, assessable via quantitative sudomotor axon reflex testing, has no influence on nociceptive C-fibers (38). Therefore, our results finally do not exclude a contribution of the sympathetic catecholaminergic system to pain in dSFN. However, any theory about sympathetic maintained pain in DN is weakened by the fact that diabetes induces peripheral sympathetic damage (39).

In conclusion, our results provide evidence for a particular mechanism leading to neuropathic pain in DN, which is nerve fiber damage and deafferentiation predominantly of small afferent nerve fibers. If our findings can be confirmed in future experimental studies, the origin of neuropathic pain in DN may be better explained. However, the question of why some DNs are painful and others not is still far from being answered.

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