Sudomotor function in familial dysautonomia

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Background: Patients with familial dysautonomia (FD) manifest episodic hyperhidrosis despite the reduction of sudomotor fibres and sweat glands associated with this autonomic neuropathy. We assessed peripheral sudomotor nerve fibre and sweat gland function to determine if this symptom was due to peripheral denervation hypersensitivity.

Methods: In 14 FD patients and 11 healthy controls, direct and axon reflex mediated sweat responses were determined by measuring transepidermal water loss (TEWL) after application of acetylcholine via a microdialysis membrane, a novel method to evaluate sudomotor function in neuropathy patients. Results were compared with data from conventional quantitative sudomotor axon reflex testing (QSART). Using microdialysis, interstitial fluid was analysed for plasma proteins to evaluate protein extravasation induced by acetylcholine as an additional parameter of C-fibre function.

Results: Although reduced axon reflex sweating was expected in FD patients, neither direct or axon reflex mediated sweat responses, nor acetylcholine induced protein extravasation differed between control and patient groups. However, the baseline resting sweat rate was higher in FD patients than controls (p < 0.05). TEWL and QSART test results correlated (r = 0.64, p = 0.01), proving the reliability of TEWL methodology in evaluating sudomotor function.

Conclusion: The finding of normal direct and axon reflex mediated sweat output in FD patients supports our hypothesis that, in a disorder with severe sympathetic nerve fibre reduction, sudomotor fibres, but not the sweat gland itself, exhibit chemical hypersensitivity. This might explain excessive episodic hyperhidrosis in situations with increased central sympathetic outflow.

Familial dysautonomia (FD) is a rare autosomal recessive disorder with extensive central and peripheral autonomic dysfunction resulting in protean manifestations, including abnormal sweat production. At rest, sweating is normal or diminished, but with emotional excitement, profuse sweating is noted over the head and trunk but sparing the hands and feet. Excessive sweating also occurs during the initial phases of sleep in many patients. A few patients will exhibit gustatory sweating but it tends to be limited to the bridge of the nose and forehead.

The pathophysiology of this clinical paradox is still unclear. FD is one member of the group of disorders known as hereditary sensory and autonomic neuropathy (HSAN). Diminished populations of small and unmyelinated fibres characterise all HSANs, but sweating characteristics between the disorders vary. Unlike the disorders associated with anhidrosis, sweating characteristics in FD have not been extensively studied. Theoretically, a dysfunction of either central or peripheral sympathetic pathways, or abnormal reactivity of sweat glands might contribute to abnormal sweating reactions. In FD, both afferent and efferent sympathetic fibres in the peripheral nervous system are largely reduced. Therefore, decreased sympathetic sudomotor innervation was postulated to explain the sweating abnormalities. Our previous finding of a reduced sympathetic skin response (SSR) in FD patients supports decreased peripheral innervation causing abnormal sweating, but SSR depends on afferent and efferent nervous pathways, which may both be altered in FD patients. SSR therefore cannot differentiate between disturbed afferent or efferent signal transmission.

Pathohistological studies have shown that the number and structure of sweat glands do not differ between FD patients and healthy controls. Green et al. found lowered thresholds when testing sweat gland responsiveness to local heat application and therefore postulated a central disturbance of thermoregulation in FD. Other authors favour the hypothesis of a peripheral hypersensitivity of sweat glands due to denervation.

To evaluate whether sweating is altered in response to the physiological sudomotor transmitter acetylcholine (ACh) and whether there is evidence for a peripheral sudomotor dysfunction, we studied sweat responses in FD patients using two systems that stimulate sweat glands and peripheral sudomotor fibres. The quantitative sudomotor axon reflex response (QSART) was tested by iontophoresis of ACh. In addition, ACh was directly delivered to sweat glands in the subcutaneous tissue using microdialysis. A device measuring transepidermal water loss (TEWL) was applied to test direct and axon reflex mediated sweating simultaneously.

MATERIALS AND METHODS

Patients

Fourteen patients with familial dysautonomia (6M, 8F; age range 18–43 years; mean (SEM) 22.8 (3.2) years and 34.5 (2.9) years respectively) were studied at the Dysautonomia Treatment and Evaluation Center at New York University Medical Center. All patients fulfilled the accepted clinical diagnostic criteria of FD, which include Ashkenazi Jewish ancestry, absent or diminished deep tendon reflexes, absence of overflow tears, absence of lingual fungiform papillae, and absent axon flare response following intradermal histamine injection. In addition, all patients were homozygous for the most common genetic haplotype, the intron 20 mutation on the IKBBP gene. All patients had given their written consent.

Abbreviations: ACh, acetylcholine; FD, familial dysautonomia; HSAN, hereditary sensory and autonomic neuropathy; QSART, quantitative sudomotor axon reflex test; SSR, sympathetic skin response; TEWL, transepidermal water loss; TPC, total protein content
informed consent to participate in the study, and the institutional review board of New York University Medical Center approved the protocol. All patients were asked to discontinue their medication, including fludrocortisone and midodrine, 18 hours prior to testing. Patients who were not able to comply with this requirement were not included in the study. The study participants were seated comfortably in a near supine position, with only the head lifted by 30° or less. Eleven age and sex matched healthy volunteers (6M, 5F; age range 22–37 years; mean (SEM) 26.5 (1.9) years and 29.4 (2.3) years, respectively) served as controls.

**Microdialysis for application of ACh and evaluation of protein extravasation**

For microdialysis, the skin at the right lower leg above the tibial anterior muscle was used as the test site. Hollow plasmapheresis fibres (0.4 mm in diameter, cut off 3000 kDa; Asahi, Japan) were inserted intracutaneously at a length of 1.5 cm in the skin by means of a 25 gauge cannula. All membranes were inserted transversely, orientated approximately 10 cm proximal to the ankle region. The applied technique ensured a strictly intracutaneous position of the membranes with an insertion depth of between 0.4 and 0.9 mm. Local anaesthesia was not necessary, as insertion of the membranes was only slightly painful. After insertion, each microdialysis fibre was connected via a Tygon tube (Novodirekt, Germany) to a syringe in a microdialysis pump (No. 540230; TSE GmbH, Bad Homburg, Germany) containing Ringer’s solution. Perfusion of the membranes with Ringer’s solution was started immediately after insertion of the membrane and continued throughout the whole experimental session of 120 minutes at a flow rate of 4 μl/min. Previous microdialysis experiments had established that a stable baseline level of plasma extravasation is reached approximately 45 minutes after membrane insertion. Therefore, our baseline values were obtained at 60 minutes, and then the microdialysis membrane was connected to a second syringe containing acetylcholine (ACh) in Ringer’s solution at a concentration of 10⁻⁴ mol/L. Membranes were perfused with ACh for 30 minutes, then perfusion was switched back to Ringer’s solution without ACh. After skin passage the dialysate was collected in polyethylene cups attached to the skin. Samples were taken every 15 minutes for the total period of 120 minutes. Probe volume per sample was 60 μl, corresponding to the flow rate of 4 μl/min. All samples were frozen immediately after the testing session and stored at −20°C or lower until analysis was performed.

To determine the total protein content of the microdialysis samples, we used the method according to Bradford, where a test reagent containing Coomassie blue, a dye that reacts unspecifically with proteins, is added to the dialysate. This reaction was then detected photometrically using an MRX reader (Dynatech, Germany).

**Flare response**

ACh stimulation induces a neurogenic axon reflex flare that develops around the stimulation membrane during ACh instillation. To measure the size of the axon reflex flare, we transferred the maximum extent of the ACh induced axon reflex erythema (flare) to a transparency at the end of the stimulation period, and the maximum diameter of the flare reaction was compared between the patients and controls.

**Evaporimeter**

Direct and indirect sweat responses in the microdialysis area were monitored using an evaporimeter (Tewameter™; Courage and Khazaka, Cologne, Germany). The device consists of a cylindrical chamber with a diameter of 2 cm, open at both sides and containing two hygrometer sensors inside the cylinder at different fixed distances from the skin surface. The probe was placed manually on the skin surface. Evaporation from the skin in the area of the rounded opening of the evaporimeter probe causes a gradient of humidity inside the cylindrical chamber, which is measured by the two hygrometers. Total amount of water passing through the chamber (transdermal water loss, TEWL) is automatically calculated from the gradient of humidity and air temperature by a connected microcomputer and continuously displayed at the computer monitor. Stable baseline values of TEWL were reached after approximately 60–90 seconds. The computer software of the Tewameter™ allows recalculation of TEWL data to sweating rates, given in g H₂O/h m² skin surface. To measure the direct sweat output in response to ACh instillation, the evaporimeter probe was placed directly above the microdialysis membrane, and to measure axon reflex mediated sweat response, the probe was placed on the skin at a distance of 10 mm to the microdialysis membrane. The evaporimeter probes were always placed at exactly the same site on the skin, which had been marked by a pen. All Tewameter™ measurements were performed at 5 minute intervals 15 minutes before, during, and 30 minutes after ACh iontophoresis.

**Quantitative sudomotor axon reflex test**

Quantitative sudomotor axon reflex test (QSART) measurements were performed using a commercially available device (QSweat; WR Medical, Stillwater, MN, USA). The QSART chamber was placed on the skin of the left lower leg in the same location that on the right leg was used for microdialysis and evaporimeter measurements. Baseline sweat rate was assessed and expressed as μmol/min. Ringer’s solution containing 10% ACh was iontophoresed through the skin for 5 minutes by means of a 2 mA constant current. ACh induced sweat production showed a typical time course with a peak after about 2 minutes. Sweat responses were calculated as the area under the curve expressed as μmol (total sweat volume). Additionally, the time needed for onset of the indirect sweat response was measured as response latency (seconds). To monitor the return to baseline, QSART was recorded for an additional 5 minutes after the electrical stimulation was discontinued in healthy controls and up to 15–20 minutes in FD patients. Prolonged recordings in FD patients were performed in case the return of the sweat output to baseline sweat rates after the end of the ACh installation was delayed.

**Statistical analysis**

Standard one way analysis of variance tests were performed using Statistica software (version 7.0; StatSoft Inc., Tulsa, USA). When appropriate, nonparametric statistics were applied. Differences were regarded to be significant at p values below 0.05.

**RESULTS**

**Protein content of the dialysate**

Total protein content (TPC) of all dialysate probes, sampled at 15 minute intervals during the experimental session of 120 minutes, was evaluated in controls and FD patients. In both groups, TPC was elevated to 150–175% of baseline values in patients and controls. In healthy controls, TPC decreased quickly afterwards, but in FD patients, TPC was elevated in the probes sampled during the last 15–20 minutes after ACh iontophoresis. Prolonged recordings in FD patients were performed in case the return of the sweat output to baseline sweat rates after the end of the ACh installation was delayed.
increase after ACh stimulation and decrease after the end of the ACh application began sooner in FD patients than controls (fig 1). However, differences in protein extravasation were not significant between both groups, either when protein concentrations were compared at corresponding time intervals or when maximum and minimum protein concentrations were compared to correct for a possibly different time course of protein extravasation.

**Results of postganglionic sudomotor function tests**

Total sweat volume was calculated from the area under the sweat curve in the QSART experiment. Total sweat volume was not significantly different between patients with familial dysautonomia and healthy controls (Mann-Whitney U test; p>0.05) and response latency was slightly shorter in FD patients than in controls (48.8 v 72.9 seconds); however this difference was not quite significant (p = 0.06). In contrast, baseline sweat rates were significantly higher in patients with familial dysautonomia (4.3 (0.6) μmol/min) than in the controls (2.4 (0.9) μmol/min, p<0.05).

There was a close correlation between the QSART and Tewameter results. During ACh stimulation, total sweat response measured by the QSART device correlated significantly with the sweat response in the axon reflex area measured by TEWL (r = 0.64; p = 0.01). Although a reduced sweat response was postulated in FD patients due to their peripheral autonomic neuropathy, neither direct nor axon reflex mediated sweat responses differed between patients and controls at any time point (fig 2).

**Acetylcholine mediated flare response**

The neurogenic flare response to acetylcholine, an additional parameter related to the integrity of peripheral unmyelinated afferents, developed around the microdialysis membrane during ACh installation. In FD patients and controls, the extent of the flare reaction was maximal approximately 15 minutes after the onset of ACh stimulation. However, similar to the sweat responses, the ACh flare was not reduced in FD patients. The maximum flare diameter was even slightly, although not significantly, larger in the patients than in the controls (11.2 (3.2) cm v 9.9 (4.7) cm).

**DISCUSSION**

In contrast to the clinical observation that hyperhidrosis is only an episodic phenomenon in familial dysautonomia (FD), we noted that baseline sweat rates in our FD subjects, as measured by QSART, were significantly higher than in controls (4.3 (0.6) μmol/min v 2.4 μM/min (0.9) μmol/min, p<0.05). However, when provoked with ACh stimulation, direct and axon reflex mediated sweat responses did not differ between FD patients and controls whether assessed by evaporimeter or QSART (fig 2). Furthermore, the indirect parameters of vascular responses to ACh, the size of the ACh induced neurogenic axon reflex flare, and the ACh induced plasma extravasation did not differ between patients and controls.

In our experiments, we used different techniques that selectively tested the function of peripheral sudomotor nerves and of sweat glands. Microdialysis was used to apply neurotransmitters intradermally, because the procedure is painless after the initial insertion of the microdialysis fibres. Therefore, this technique is especially helpful in experiments that test the peripheral sympathetic nervous system, as any pain induced arousal of the tested person might activate central sympathetic pathways during the examination and thus bias the results. Recently, we demonstrated the advantages of this technique in another study evaluating peripheral noradrenergic pathways in FD patients. To differentiate between sweat gland and sudomotor C-fibre function, it is necessary to simultaneously assess direct...
Acetylcholine, applied intradermally by microdialysis, excites muscarinic ACh receptors, located at sweat glands, and nicotinergic ACh receptors (n-ACh-R), located at the cholinergic axons, simultaneously (1). Activation of n-ACh-R induces axon reflex sweating (2). Hypersensitive n-ACh-R might compensate for reduced C-fibre density in FD patients. Similar hypersensitivity of n-ACh-R located at the proximal terminal of the postganglionic sudomotor fibre (3) might contribute to excessive sweating during dysautonomic crises.

Figure 4

Evaporimeter and QSART results were closely correlated ($r = 0.64, p = 0.01$), suggesting that the evaporimeter results are as reliable as the QSART measures.

In summary, the parameters evaluating exclusively peripheral sudomotor and sweat gland function showed no significant differences between FD patients and controls. Normal sweat output in FD patients is unexpected, as patients with some other autonomic neuropathies commonly demonstrate diminished or even absent sweat responses in QSART tests. However, our findings are consistent with those of Low et al., who reported normal sweat output in a single FD patient.

Because sympathetic innervation in FD patients is severely impaired, “normal” sweat responses cannot be explained without postulating mechanisms that would compensate for the loss of sympathetic nerve fibres. Such mechanisms might include episodic increases of central sympathetic activation, differences in signal transmission in peripheral sympathetic neurones, or modified sweat gland responsiveness.

In our study, we focused on the evaluation of peripheral mechanisms of sweating. Tests evaluating central sweat responses (for example whole body thermoregulatory sweat testing, TST) cannot be easily conducted in FD patients. An increase of body temperature during TST might induce life threatening autonomic instability caused by profound hypertension resulting from vasodilatation. Moreover, the indicator powder used to visualise thermoregulatory sweating in larger body areas could cause corneal pathology in the FD patients due to their insensitive corneas and absence of lacrimation.

Consequently, central mechanisms of sweat disturbances in FD patients have not been studied, other than one report that attributed increased sweat response to thermal stimulation at the forearm to an increased excitatory state of reflex centres in the CNS. Central excitation, however, is consistent with hyperhidrosis during generalised enhanced sympathetic surges noted during dysautonomic crises. The increased sweating rate at baseline seen in our QSART results might also be explained by such a mechanism. Although we took great care to avoid any arousal reactions during the experimental session, the procedures might still have induced a stress reaction in some subjects and account for a central upregulation of sweat output at baseline.

The most unexpected result of our study, the normal axon reflex sweat response, is independent of central innervation; it only depends on the integrity and chemical responsiveness of postganglionic sudomotor fibres, thus a marked reduction of the axon reflex response would be expected. Reduced nociceptor axon reflexes are a major feature of familial dysautonomia. The reduced response of nociceptor C-fibres to intradermal instillation of histamine, which produces a local wheal reaction due to plasma extravasation, but no visible flare in FD patients, is one of the diagnostic criteria of this autonomic neuropathy.

Because there was a normal direct response of sweat glands to ACh in our study, it seems unlikely that there is a significant reduction or dysfunction of the sweat glands themselves in FD patients. Previous histological studies have not demonstrated a reduced number or histological abnormalities of sweat glands in FD patients, while the innervation of sweat glands was reduced in skin biopsies from calf and back of FD patients (Hilz et al. and personal communication), corresponding to the reduction of sympathetic ganglia at the spinal cord level. It is commonly held that sweat glands do not develop hypersensitivity to ACh after denervation, in contrast to other organs innervated by sympathetic or parasympathetic nerves, such as the pupil. In animal models of acute peripheral denervation, there was a reduction of sweat responses after application of pilocarpine to denervated...
sweat glands. Furthermore, receptor properties and the density of the muscarinic ACh receptors of sweat glands were found to be unchanged despite the unresponsiveness of the sweat gland to muscarinic stimulation 1 week after acute denervation. It is not known if these mechanisms also apply to the sympathetic nervous system outflow is increased such as with "dysautonomic crises".

In conclusion, our study suggests hypersensitivity of the peripheral cholinergic sudomotor nerves in FD patients. Peripheral hypersensitivity is likely to contribute to the excessive hyperhidrosis that occurs when sympathetic nervous system outflow is increased such as with "dysautonomic crises".

**REFERENCES**


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