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QDIRT: Quantitative Direct and Indirect Testing of Sudomotor Function

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Abstract

Objective—To develop a novel assessment of sudomotor function.

Background—Post-ganglionic sudomotor function is currently evaluated using quantitative sudomotor axon reflex testing (QSART) or silicone impressions. We hypothesize that high-resolution digital photography has advanced sufficiently to allow quantitative direct and indirect testing of sudomotor function (QDIRT) with spatial and temporal resolution comparable to these techniques.

Methods—Sweating in 10 humans was stimulated on both forearms by iontophoresis of 10% acetylcholine. Silicone impressions were made and topical indicator dyes were digitally photographed every 15 seconds for 7 minutes after iontophoresis. Sweat droplets were quantified by size, location and percent surface area. Each test was repeated 8 times in each subject on alternating arms over 2 months. Another 10 subjects had silicone impressions, QDIRT and QSART performed on the dorsum of the right foot.

Results—The percent area of sweat photographically imaged correlated with silicone impressions at 5 minutes on the forearm ($r = 0.92$, $p < 0.01$) and dorsal foot ($r = 0.85$, $p < 0.01$). The number of sweat droplets assessed with QDIRT correlated with the silicone impression although the droplet number was lower (162 ± 28 vs. 341 ± 56 , $p < 0.01$; $r = 0.83$, $p < 0.01$). QDIRT and QSART sudomotor assessments measured at the dorsum of the foot correlated (sweat response ($r = 0.63$, $p < 0.05$) and sweat onset latency ($r = 0.52$, $p < 0.05$).

Conclusions—QDIRT measured both the direct and indirect sudomotor response with spatial resolution similar to silicone impressions, and with temporal resolution that is similar to QSART. QDIRT provides a novel tool for the evaluation of post-ganglionic sudomotor function.

Introduction

Assessment of the sudomotor system is used to provide a measure of cholinergic sympathetic function. This assessment has taken on increasing importance in the evaluation of autonomic function in peripheral nerve disease; sudomotor dysfunction is the most prevalent neurophysiologic abnormality in patients with distal small fiber neuropathy.¹ Sudomotor function can be quantified using the thermoregulatory sweat test,² the quantitative sudomotor axon reflex test and the silicone imprint method.^{3,4}

The thermoregulatory sweat test provides a semi-quantitative, topographic measure of pre- and postganglionic sudomotor function over the entire body. This technique assesses the

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function of sudomotor pathways from the hypothalamus to the eccrine sweat gland. The quantitative sudomotor axon reflex test (QSART) and the silicone imprint method measure postganglionic sudomotor function over a restricted area. While QSART and silicone impressions both assess postganglionic sudomotor function in response to a pharmacological provocation, there are several technical differences between them. The QSART -- by measuring provoked changes in relative humidity within a sweat capsule -- assesses the sudomotor response with temporal resolution; the latency, magnitude and duration of the response are all measured in real time. In contrast, the silicone imprint method provides an assessment of individual sweat droplet size and number but does not provide temporal resolution - the latency and duration of the response cannot be determined.

We hypothesized that digital photography has advanced sufficiently to enable dynamic, real-time quantification of sudomotor function using indicator dyes in combination with iontophoresis of acetylcholine. We theorized that the technique would provide a measure of sudomotor function with temporal resolution similar to the QSART, and spatial resolution - droplet size and number - similar to the silicone imprint technique. We describe a novel approach, the quantitative direct and indirect reflex test of sudomotor function (QDIRT), to simply quantify direct and axon reflex mediated sudomotor function.

Methods

The protocol was approved by the Institutional Review Board of the Beth Israel Deaconess Medical Center.

Subjects

Twenty healthy subjects (12 male, 8 female), aged 24–42 participated in the study over 1 year. Two male subjects with distal small fiber neuropathy (normal strength, reflexes, proprioception, and vibration; mild reduction to pinprick and temperature sensation) ages 27 and 31 also underwent testing.

Study design

Ten healthy subjects (7 male, 3 female) participated in 8 test visits. Experiments were performed at a room temperature of 24–26°C. Sweat responses were quantified on alternating ventral forearms by silicone impression and QDIRT at each visit. Thus, each control subject had silicone impressions and QDIRT performed 8 times. QDIRT was performed 4 times using the indicator dye povidone-iodine and 4 times using alizarin red. Only results from the ipsilateral forearms were compared.

In order to identify the sensitivity of QDIRT to variables that could affect the sudomotor response, four of the above healthy subjects underwent additional testing on the anterior thighs to determine the effects of hydration and caffeine. Subjects were tested after a 12 hour overnight fluid restriction; the test was then repeated after hydration with 40 ounces of fluid. Subjects were also tested before and after ingestion of 12 ounces of coffee to determine the effects of caffeine on the sweat response assessed by QDIRT.

Ten additional subjects (5 male, 5 female) also underwent a test of QDIRT (using alizarin red), silicone impression and QSART on the dorsal aspect of the distal right foot. These tests were performed on 3 different test days, under the same testing conditions.

Iontophoresis

Sweating was stimulated by iontophoresis of 10% acetylcholine (Penta International Corporation, Fairfield, NJ; 10% in sterile water) for 5 minutes at 2mA using a custom

polycarbonate capsule. The capsule is 20 mm in diameter and has a 12mm opening in the center with an implanted platinum wire stimulator and ports for acetylcholine filling, similar to the design of a QSART probe (see E-Appendix 1).² The capsule holds 0.4 cc of acetylcholine and adheres to the skin through custom fitted medical grade adhesive (3M, St. Paul, MN) that prevents extrusion of acetylcholine into the surrounding region. The polycarbonate capsule was placed within an imaging template to enable photographic analysis of the direct and axon reflex response (Described in detail in E-Appendix 1).

Silicone impressions

Silicone impressions (Silasoft, Detax GmbH & Co. KG, Ettlingen, Germany) were performed using standard protocols.⁵ After iontophoresis of acetylcholine, silicone material was applied for 5 minutes, the resulting sweat droplet impressions were imaged and counted by computer analysis (Image Pro Plus, Media Cybernetics, Bethesda MD).⁶ The sweat droplets were quantified by number, size and percent area over a 4.5 cm² region centered over the stimulation site.

QDIRT

Sweating on the contralateral forearm was measured after iontophoresis by drying the stimulated region and dusting with indicator dye (povidone-iodine and cornstarch, or alizarin red, cornstarch, sodium pyruvate) followed by digital photographs taken every 15 seconds for 7 minutes (Canon 8.2 Megapixel EOS 30D with 100mm Macro Lens). Images of QDIRT were directly uploaded as a sequence and analyzed (Image Pro Plus, Media Cybernetics, Bethesda, MD) with automated image alignment, normalization and graphical thresholding display of individual droplets for QDIRT images. Sweat droplets were quantified by number, size and percent (of the total) area over a region centered over the stimulation site for each image. The direct sweat response was the region directly in contact with the acetylcholine (a circle of 12mm in diameter), while the indirect (axon reflex) response was a circle 24 mm in diameter that excluded the inner direct region (described in detail in E-Appendix 1). Changes in droplet number, size and area are quantified at 15 second intervals and results expressed as area of sweat production for total, direct and indirect responses. The change in sweat area over change in time (Δ sweat area/ Δ time) is also calculated for direct, indirect and total areas. To compare results to QSART, change in sweat area over change in time in the indirect testing region was compared to the QSART area under the curve.

QSART

Quantitative sudomotor axon reflex testing was performed with iontophoresis of 10% acetylcholine using standard protocols.² Area under the curve was measured for 15 minutes post stimulation.

Statistics

Statistical analysis of results was performed using Systat 11 (Systat Software Inc, San Jose, CA). Data are presented as mean $\pm$ standard deviation. Pearson correlation coefficients (r) were calculated to assess simple relationships between variables, with Bonferroni adjustments for multiple comparisons. A *p* value <0.05 was used to define statistical significance for all data sets. Photoshop CS3 Extended (Adobe, San Jose, CA) and Image Pro Plus (Media Cybernetics, Bethesda, MD) were used for data acquisition. Detailed specific methods for iontophoresis, QDIRT technique, and sweat droplet analysis are provided in E-Appendix 1.

Results

The total, direct, and indirect areas that were analyzed were 4.5 cm², 1.13 cm² and 3.4cm² accordingly for both silicone impressions and QDIRT (See E-Appendix 1). The total area of sweat photographically imaged with povidone-iodine correlated with silicone mold impressions: at 1 minute ($r = 0.55$, $p = \text{NS}$), at 4 minutes ($r = 0.81$, $p = 0.05$) and at 5 minutes ($r = 0.88$, $p < 0.01$, figure 1). The total area of sweat photographically imaged with alizarin red also correlated with silicone mold impressions: at 1 minute ($r = 0.36$, $p = \text{NS}$), at 4 minutes ($r = 0.82$, $p < 0.01$), and at 5 minutes ($r = 0.92$, $p < 0.01$, figure 1). The povidone-iodine and alizarin red QDIRT techniques correlated ($r = 0.93$, $p < 0.01$).

Silicone impressions showed more sweat droplets than QDIRT at 5 minutes (341 ± 56 vs. 162 ± 28 ; $p < 0.01$), despite the percent areas of sweat coverage being equal ($34 \pm 5\%$ silicone vs. $38 \pm 6\%$ QDIRT). Although the absolute number of sweat droplets was less using QDIRT at 5 minutes, the relative number of sweat droplets correlated with the silicone impression technique ($r = 0.77$, $p < 0.05$ povidone-iodine; $r = 0.83$, $p < 0.05$ alizarin red). Many small sweat droplets were seen using silicone impressions that were not seen by QDIRT, but these sweat droplets did not substantially contribute to the total sweat area. The total number of sweat droplets visible by QDIRT began to decrease after 5 minutes despite an increase in the total area of sweat production. This was largely an effect of increasing sweat droplet confluence (figure 2).

Tests performed on the dorsum of the feet showed similar responses. There was high correlation between percent area of QDIRT (alizarin red) and silicone impressions at 5 minutes ($r = 0.85$, $p < 0.01$). The total number of sweat droplets using QDIRT was less than silicone impressions (143 ± 51 vs. 297 ± 75 , $p < 0.01$), but still showed a high correlation across individuals ($r = 0.76$, $p < 0.01$).

A comparison of QDIRT to QSART revealed that the area under the curve analysis of QSART (Stimulation + 15 minutes) correlated with the indirect sweat response at 7 minutes ($r = 0.63$, $p < 0.05$). The mean latency for sweat onset with QSART in our subjects was 136 ± 37 seconds (with time 0 at the start of iontophoresis). The mean indirect sweat latency for QDIRT was 29 ± 16 seconds (time 0 begins immediately after completion of iontophoresis, i.e. 5 minutes later than QSART). Those subjects with longer latencies during QSART also tended to have longer latencies with QDIRT ($r = 0.52$, $p < 0.05$).

Sweat production, measured with QDIRT, differed between males and females ($p < 0.01$) (figure 1), and was consistent with results seen by silicone impressions and QSART. In the 4 control subjects with additional testing, sweat production measured by QDIRT was reduced by $30 \pm 12\%$ if subjects were not adequately hydrated, and was reduced by $22 \pm 9\%$ if subjects ingested a caffeinated beverage within 2 hours of testing. Subjects that were not adequately hydrated and ingested a caffeinated beverage showed large reductions in sweat production with QDIRT ($58 \pm 19\%$).

In the two subjects with small fiber neuropathy, the onset of sweating was delayed compared to control subjects (47 ± 14 vs 10 ± 9 seconds direct sweat area; 48 ± 12 seconds vs 29 ± 16 seconds indirect sweat area), and the overall sweat production area was lower in both the direct and indirect regions. A sample analysis of the time course of sweat production was determined for the direct, indirect, and total sweat areas of the image seen in figure 2; results are shown in figure 3. The indirect sweat production in the healthy control subject peaked rapidly at 30 seconds, rapidly decreased, then stabilized for several minutes before stopping; the direct sweat response rapidly increased then stabilized for several minutes before decreasing. The small fiber neuropathy subject had decreased total, direct and indirect sweat response (measured in percent area) compared to the control subject. The small fiber

neuropathy subject also had reduced indirect peak sweat production rate and a delay in the latency to indirect peak sweat production. The direct sweat production rate was also reduced, but showed a normal latency (figure 3).

Discussion

The major findings of this study are: 1) QDIRT test results correlate well with silicone impressions in the assessment of sudomotor function, 2) QDIRT allows analysis of both direct and indirect sweat response, 3) QDIRT measures the sudomotor response with temporal resolution that is not obtained by silicone impressions, and 4) QDIRT digital images are rapidly and easily analyzed using automated software.

We describe a new approach to analysis of sweat production utilizing many readily available tools for simple and inexpensive investigation of post-ganglionic sudomotor function. This technique, QDIRT, combines some of the advantages of silicone impressions and QSART by providing data on droplet number, droplet topographical distribution and temporal resolution in direct and axon reflex mediated regions.

QDIRT provides the ability to measure both sweat droplet number and area and can analyze both direct and axon reflex mediated responses. The total areas of sweat produced correlated well with silicone impressions, although the total number of sweat droplets was lower. The differences in number of sweat droplets may be due to increased sweat droplet confluence seen during later QDIRT images, particularly in subjects with large sweat responses. Alternately, some smaller droplets seen with silicone impressions may be due to debris, hair follicles and other cutaneous artifacts and may have no impact on the results. Nevertheless, despite the smaller number of sweat droplets, the overall area of sweat produced was similar using the two techniques in the ventral forearms and the dorsum of the foot.

Our results indicate a good correlation between silicone impressions and QDIRT when quantifying the surface area of sweat distribution. We also compared 2 indicator dyes using QDIRT: povidone-iodine and alizarin red. While both methods were good indicators of sweat droplet production, alizarin red was easier to use, provided better photographic image quality (particularly in individuals with darker skin where contrast was enhanced) and gave slightly higher correlations with sweat droplet production using silicone impressions.

Silicone impressions measure the size and number of sweat droplets; this information is used to estimate the volume of sweat production.⁷ This technique provides the unique ability to study the quantity of sweat produced from individual sweat glands. This may be clinically important in assessment of neuropathic disease states where sweat volume production may remain stable, while sweat droplet number is reduced. This is likely a consequence of denervation supersensitivity, reinnervation, or sweat gland hypertrophy.⁶ Although the silicone impression technique is inexpensive and easy to use, there are limitations. The quantification of sweat droplets is labor intensive and time consuming. Furthermore, this technique does not measure the time course of the sudomotor response; the silicone impression is applied for 5 minutes, and is then removed.

QSART is also used to measure post-ganglionic sudomotor function. This technique measures the relative change in humidity over time and provides an assessment of the overall duration of sweat production, but does not provide data on individual sweat unit production as seen by silicone impression.⁸ Despite the relative expense, QSART has attained widespread clinical use, primarily due to the simplicity with which results are obtained and analyzed.

QDIRT, similar to the QSART, measures the changes in sweat over time, providing the response latency and duration for both direct and indirect sudomotor function (figure 3). Our results suggest that the duration of sweat production is longer in QSART than in QDIRT. This observation has several possible explanations. First, the sealed capsule in QSART may extend the duration of time that relative changes in humidity are detected. Second, additional sweat production using QDIRT is not recorded in areas where indicator dyes have already changed.

QDIRT also measures the sudomotor response with temporal resolution that is similar to the QSART. There were modest correlations between QSART and QDIRT sweat latency ($r=0.52$) despite several methodological differences. QSART measures the sweat response during and after stimulation, while QDIRT measures the sweat response shortly after stimulation. In addition, the QDIRT indirect test results in the dorsum of the foot correlated with QSART measurements in the same region, suggesting that although the techniques and measurement tools differ, there is internal consistency between these various tests of sudomotor function.

There are many factors that may alter autonomic function. Known variables include body temperature, humidity, room temperature, nicotine, and hydration status.^{9,10} These variables all appear to impact the use of QDIRT. Of particular concern is the reduction in sweat with caffeine and an overnight fluid restriction. Such large variations in sweat production have not been reported with QSART, although we found similar results while using silicone impressions (data not shown), suggesting this is not an isolated artifact with the use of QDIRT. Female subjects had much lower sweat responses using QDIRT, a finding consistently seen with both our QSART and silicone impression tests as well. Although there are many variables that appear to alter sudomotor function, QDIRT appears to respond similarly to other well established techniques.

There are limitations to QDIRT. Ambient room temperature and humidity need to be controlled to prevent cool dry air from causing evaporation of sweat production. We did not control temperature at the testing site; we only controlled ambient temperature in this study. QSART and silicone impressions are tested within an enclosed environment and are less subject to alterations in humidity. It will be important to establish normative values for this technique to avoid over diagnosis of sudomotor dysfunction. We have also described an alternate method in E-Appendix 1 using a flexible clear cover slip to reduce evaporation (data not shown).

Additional investigation is necessary to determine the utility of QDIRT in disease states that alter sudomotor structure or function. Nevertheless, these results suggest that QDIRT may aid in the evaluation of post-ganglionic sudomotor function. This technique may be suitable for clinicians who do not have access to specialized autonomic testing centers.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.

Correlation scatter plots of QDIRT vs. silicone impression techniques using both povidone-iodine and alizarin red at 1 minute and 5 minutes in male and female subjects.

The total sweat production in a 4.5 cm² region of the forearm comparing silicone impressions to QDIRT. QDIRT results are shown at 1 minute (A&B), and 5 minutes (C&D), for both povidone-iodine (A&C) and alizarin red (B&D). All silicone impressions are only measured at 5 minutes following standard protocols. Distribution of male and female subjects can be seen in each figure; male subjects exhibited greater sweat production at all time points using all testing methods ($P<0.01$).

Figure 2.

QDIRT images and analysis: example in healthy control and small fiber neuropathy subject. QDIRT images of a healthy control (A) and (SFN) small fiber neuropathy subject (B) tested on the lateral thigh. The large circle indicates the total area of sweat analyzed (4.5 cm^2), the inner circle is the direct sweat response (1.13 cm^2). Indirect sweat response is derived by subtracting the inner from the outer circle. Total sweat response over time is shown for each subject (C) and is separated into direct and indirect response (D).

Figure 3.

Change in sweat area over time in the healthy control and small fiber neuropathy subject shown in Figure 2.

The change in sweat area over time (Δ sweat area/ Δ time) between consecutive 15 second images identifying the total (A), direct (B) and indirect (C) sweat response for both the healthy control and small fiber neuropathy (SFN) subject. The maximal rate of sweat production is reduced in the SFN subject in the total, direct and indirect regions. There is also a delay in latency to peak sweat production in the indirect and total regions (the delay in the indirect latency appears to be the major contributor to the total response).