Quantitative Pilomotor Axon Reflex Test

A Novel Test of Pilomotor Function

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Background: Cutaneous autonomic function can be quantified by the assessment of sudomotor and vasomotor responses. Although piloerector muscles are innervated by the sympathetic nervous system, there are at present no methods to quantify pilomotor function.

Objective: To quantify piloerection using phenylephrine hydrochloride in humans.

Design: Pilot study.

Setting: Hospital-based study.

Participants: Twenty-two healthy volunteers (18 males, 4 females) aged 24 to 48 years participated in 6 studies.

Interventions: Piloerection was stimulated by iontophoresis of 1% phenylephrine. Silicone impressions of piloerection were quantified by number and area. The direct and indirect responses to phenylephrine iontophoresis were compared on both forearms after pretreatment to topical and subcutaneous lidocaine and iontophoresis of normal saline.

Results: Iontophoresis of phenylephrine induced piloerection in both the direct and axon reflex–mediated regions, with similar responses in both arms. Topical lidocaine blocked axon reflex–mediated piloerection post-iontophoresis (mean [SD], 66.6 [19.2] for control impressions vs 7.2 [4.3] for lidocaine impressions; P < .001). Subcutaneous lidocaine completely blocked piloerection. The area of axon reflex–mediated piloerection was also attenuated in the lidocaine-treated region postiontophoresis (mean [SD], 46.2 [16.1] cm² vs 7.2 [3.9] cm²; P < .001). Piloerection was delayed in the axon reflex region compared with the direct region. Normal saline did not cause piloerection.

Conclusions: Phenylephrine provoked piloerection directly and indirectly through an axon reflex–mediated response that is attenuated by lidocaine. Piloerection is not stimulated by iontophoresis of normal saline alone. The quantitative pilomotor axon reflex test (QPART) may complement other measures of cutaneous autonomic nerve fiber function.


Piloerector muscles are present throughout hairy skin and are activated centrally by cold exposure, fever, and strong emotions. In the periphery, piloerection is evoked directly by mechanical, thermal, electrical, or pharmacological stimuli or, as previously reported, indirectly via an axon reflex. The sudomotor axon reflex test is widely used clinically to assess autonomic function and may be the most sensitive physiological test in the assessment of small-fiber peripheral neuropathies. Vasomotor or nociceptor axon reflex tests are also widely used, particularly in research studies, as measures of neuropathic inflammation. Despite rigorous physiological studies dating back more than 80 years, to our knowledge, there is no widely used test of pilomotor function. We sought to evoke axon reflex–mediated piloerection to complement the sudomotor and vasomotor axon reflex–mediated cutaneous tests.

METHODS

SUBJECTS

Twenty-two healthy volunteers (18 males, 4 females) aged 24 to 48 years were recruited. Approval from the Beth Israel Deaconess Medical Center institutional review board was obtained, and full written informed consent was given by each subject. None of the subjects had evidence by history or examination of neuropathy, tobacco use, current use of medications, or medical disease.
TESTING PROTOCOL
All measurements were performed in a semirecumbent position in a temperature-controlled room (mean [SD], 20 [1]°C).

Iontophoresis
A drug delivery capsule electrode (LI-611; Perimed) was affixed on the testing area on the dorsal forearm. The inner chamber of this capsule, open to the skin surface, was filled with 0.4 mL of 1% phenylephrine solution (Sandoz International Corp). The drug delivery electrode was then connected to the iontophoresis stimulation box (Phoresor-PM850; IOMED). Iontophoresis was performed over a 1-cm diameter skin region with 0.5 mA for 5 minutes.

Silicone Impressions of Piloerection
Silicone impressions were used to create a local topographic map of piloerection. A silicone-based material (Silasoft; Microsonic) was placed over the skin for 10 seconds. The short placement time of 10 seconds was selected to minimize congealed sweat droplet impressions. The silicone cured for 5 minutes and had toner applied to mark the pilomotor impressions; excess toner was wiped free and the silicone was scanned to capture the image digitally. Blinded observers analyzed silicone scans using image analyzing software (Image Pro Plus 6.0; Media Cybernetics). Silicone impressions of erect hair follicles were quantified by number and area. The outline of the total area of piloerection was defined as a line connecting the outer edges of the most peripheral erect hair follicle impressions. The indirect, presumed axon reflex–mediated region was calculated by subtracting the area of phenylephrine application from the total area of piloerection.

Studies
Twenty-two subjects participated in 6 different studies to define the pilomotor axon reflex (8 subjects completed more than 1 study). In study 1, 1% phenylephrine was iontophoresed on the right and left dorsal forearms on 2 different days in 3 subjects to evaluate side-to-side pilomotor response differences. The pilomotor response was measured with silicone impressions. The order (right or left) was randomized. There were at least 3 days between test days.

In study 2, 1% phenylephrine was iontophoresed on both dorsal forearms on a single day. Lidocaine gel (2%) was applied to a skin region surrounding the region of phenylephrine iontophoresis (ie, the axon reflex region) to measure the ability of topical lidocaine to block piloerection. Lidocaine was applied 10 minutes prior to and during iontophoresis in 13 subjects. The opposite arm was tested without lidocaine. The side of lidocaine application was randomized. Measurements of piloerection were performed 20 minutes after iontophoresis and 2 minutes after removal of lidocaine.

In study 3, iontophoresis was performed on both dorsal forearms in 3 subjects (current intensity of 0.5 mA for 5 minutes) with 0.4 mL of 0.9% sodium chloride solution without phenylephrine to measure the effects of electrical stimulation without phenylephrine on the pilomotor response.

In study 4, injections of 1% lidocaine were administered over a 1 × 3-cm skin area lateral to the iontophoretic site on the right dorsal forearm in 3 subjects. Piloerection was measured beyond the injection site to measure the ability of the subcutaneous injection of lidocaine to block axon reflex–mediated piloerection.

In study 5, 1% phenylephrine was iontophoresed, followed by sequential imaging of piloerection using high-resolution photographs (Cyber-shot DSC-T90 with Carl Zeiss Vario-Tessar lens, Sony Electronics). Photographs were taken every 20 seconds for 15 minutes in 3 subjects to determine the latency of response in the direct and axon reflex region.

In study 6, the vasodilating agent nitroprusside (0.4 mL of 1% sodium nitroprusside) was iontophoresed on a dorsal forearm, while the contralateral forearm was tested without nitroprusside in 3 subjects. Phenylephrine (1%) was then iontophoresed on both forearms to address the role played by vasoconstriction in piloerection. Laser Doppler imaging (Periscan PIM 3; Perimed) measured changes in a 36-cm² area over the iontophoresis site to document cutaneous blood flow during testing.

Statistics
Variables are presented as means and standard deviations. Paired t tests were used to compare pilomotor function parameters with and without lidocaine application within the same individual. All testing was 2-tailed, and P < .05 was considered statistically significant. All calculations were performed using Stata version 9.2 (Stata Corp).

RESULTS

STUDY 1: SIDE-TO-SIDE COMPARISON
Iontophoresis of phenylephrine induced local piloerection in the area of phenylephrine application (direct region) and the axon reflex–response region (indirect region) in all subjects (Figure 1). There was no side-to-side difference in the number of erect hair follicles (mean [SD], direct region: 13.4 [2.7] for impressions on the right and 13.0 [2.0] for impressions on the left; indirect region: 66.0 [13.9] for impressions on the right and 62.0 [11.3] for impressions on the left) and the area of the axon reflex–mediated response (mean [SD], 51.0 [3.2] cm² for the right and 51.5 [3.2] cm² for the left).
STUDY 2: RESPONSE TO TOPICAL LIDOCAINE APPLICATION

Twenty minutes after phenylephrine iontophoresis, there was no difference in the number of impressions in the direct region with and without lidocaine pretreatment. In contrast, the number of impressions was reduced in the indirect region after pretreatment with lidocaine (mean [SD], 66.6 [19.2] for the control vs 7.2 [4.3] with lidocaine; \( P < .001 \)). The area of piloerection spread was also reduced in the lidocaine-treated region (mean [SD], 46.2 [16.1] cm² vs 7.2 [3.9] cm²; \( P < .001 \)) (Figure 2).

STUDY 3: RESPONSE TO ELECTRICAL STIMULATION

Iontophoresis of a 0.9% saline solution did not induce piloerection in the direct or indirect regions.

STUDY 4: EFFECTS OF SUBCUTANEOUS LIDOCAINE INJECTION ON AXON REFLEX PILOERECTION

Axon reflex spread of piloerection was abolished beyond the lidocaine anesthetic barrier (mean [SD], 58.3 [6.5] for hair follicle impressions in the control indirect region vs 0 [0] impressions beyond the lidocaine injection barrier; \( P < .001 \)) (Figure 3).

STUDY 5: RESPONSE LATENCY OF PILOERECTION

Maximal piloerection in the direct region occurred for a mean [SD] of 13.3 [5.8] seconds postiontophoresis, whereas maximal piloerection in the indirect region occurred for a mean [SD] of 600 [90] seconds postiontophoresis (\( P < .001 \). Figure 4).
STUDY 6: EFFECT OF NITROPRUSSIDE ON AXON REFLEX VASODILATION

Nitroprusside pretreatment did not alter axon reflex–mediated piloerection (mean [SD], 51 [9.0] erect hair follicle impressions with nitroprusside vs 48.7 [9.6] without nitroprusside; the \( P \) value was not significant). Nitroprusside pretreatment did not alter the number of hair follicle impressions in the direct region (mean [SD], 12.5 [4.2] impressions with nitroprusside vs 11.9 [4.6] impressions without nitroprusside). Laser Doppler imaging confirmed vasoconstriction with iontophoresis of phenylephrine in the direct region that was blocked by nitroprusside (blood flow decreased a mean [SD] of 58 [19]% with phenylephrine vs 8 [12]% with nitroprusside and phenylephrine). Vasodilation (as previously reported)\(^6\) was observed in the indirect region with phenylephrine iontophoresis and was not blocked by iontophoresis of nitroprusside.

COMMENT

The major findings in this study were that (1) piloerection can be evoked by phenylephrine iontophoresis directly beneath the stimulation site and in the surrounding region; (2) piloerection is not evoked by iontophoretic current alone of the same magnitude; (3) there is no side-to-side difference in the response; (4) there is a difference in response latency between the direct and axon reflex regions; and (5) piloerection in the surrounding region is significantly reduced after pretreatment with lidocaine and was abolished by lidocaine injection. Taken together, these data suggest that phenylephrine evokes piloerection directly and via an axon reflex.

Piloerection may be evoked centrally or locally. Strong emotions and lowering of ambient temperature are common central provocative stimuli for piloerection, although intersubject variability, vigilance, and habituation limit the use of this approach as a clinical test. Locally, piloerection may be evoked by direct stimulation of the arrector pili muscles or via an axon reflex.

Lewis and Marvin\(^1\) first characterized axon reflex–mediated piloerection evoked locally by external current. This response, consistent with an axon reflex, extended beyond the site of stimulation and was attenuated by local anesthesia and nerve degeneration.\(^1\) Similarly, in a series of experiments in humans and cats, Coon and Rothman\(^3\) showed that acetylcholine chloride evokes piloerection that is attenuated by local anesthesia, deep skin incision, and nerve degeneration. Piloerection also was evoked by intradermal injections of nicotine sulfate and picrate, and it was not inhibited by nerve block or intravenous atropine, consistent with a nicotinic receptor–mediated axon reflex.\(^3\)

Previous studies have demonstrated that phenylephrine iontophoresis,\(^4\) but not microdialysis,\(^7\) can evoke axon reflex–mediated responses. Phenylephrine iontophoresis evoked axon reflex–mediated vasodilation that was abolished by pretreatment with topical anesthesia. The response also was reduced by pretreatment with topical ibuprofen, suggesting that prostanoids are implicated in the response.\(^8\) Similarly, phenylephrine evoked axon reflex–mediated sweating in subjects with complex regional pain syndrome type 1.\(^8\) The mechanism whereby phenylephrine evokes axon reflex–mediated piloerection is not fully elucidated.

In prior reports, piloerection was evoked with electric current in vitro using an isolated cat piloarrector muscle\(^9\) as well as in vivo in humans directly and via an axon reflex.\(^1\) We have evoked axon reflex–mediated piloerection with higher current intensities than those used in our study (2 mA for 5 minutes over a 1-cm diameter region); however, the lower current intensities used to evoke axon reflex–mediated piloerection with phenylephrine in this study, when used with saline alone, did not evoke piloerection.\(^2\) Similarly, Low et al\(^5\) showed that iontophoresis of saline with a 1-mA current for 5 minutes did not induce an axon reflex–mediated sudomotor response. We also observed increased latency in the pilomotor axon reflex response compared with the direct response.\(^10\) Taken together, these data suggest that in our study, axon reflex–mediated piloerection was not evoked by current alone or vasoconstriction, and that the response was elicited by phenylephrine.

In recent studies, expression of \(\alpha_{1c}\)-adrenoreceptors was observed in unmyelinated and myelinated sensory
nerves in the skin of adult male Wistar rats. In contrast, there was no evidence of expression of α1-adrenoceptors in sympathetic efferent nerves.11 While these studies did not exclude the possibility of presynaptic α1-adrenoceptors in human sympathetic nerves, they support the possibility that axon reflex–mediated piloerction evoked by phenylephrine is due to the activation of sensory afferents.

Structural studies have shown that pilomotor nerve fiber density is decreased in skin biopsies of diabetic subjects12 and subjects treated with topical capsaicin.2 Viewed in conjunction with these structural studies, our data suggest that the quantitative pilomotor axon reflex test (QPART) may complement vasomotor and sudomotor axon reflex–mediated tests of small-fiber function.

Studies in larger populations and patients with peripheral neuropathy are necessary to confirm these findings. Further development of the QPART technique will need to include piloerction measurement with temporal resolution. In addition, structural nerve fiber assessment is needed to define the adrenergic receptor subtypes present on pilomotor nerves and piloarrector muscles to elucidate the physiology of pilomotor responses.

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