Differential Innervation of Secretory Coils and Ducts in Human Eccrine Sweat Glands

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Abstract

Background: Previous studies demonstrate that eccrine sweat glands are innervated by both cholinergic and adrenergic nerves. However, it is still unknown whether the secretory coils and ducts of eccrine sweat glands are equally innervated by the sympathetic nerve fibers. To well understand the mechanisms on sweat secretion and reabsorption, the differential innervation of secretory coils and ducts in human eccrine sweat glands was investigated in the study.

Methods: From June 2016 to June 2017, six human skins were fixed, paraffin-embedded, and cut into 5 µm-thick sections, followed by co-staining for nerve fiber markers protein gene product 9.5 (PGP 9.5), tyrosine hydroxylase (TH) and vasoactive intestinal peptide (VIP), and eccrine sweat gland markers K7, S100P, and K14 by combining standard immunofluorescence with tyramide signal amplification (IF-TSA). Stained sections were observed under the microscope, photographed, and analyzed.

Results: The fluorescent signals of PGP 9.5, TH, and VIP were easily visualized, by IF-TSA, as circular patterns surrounding eccrine sweat glands, but only PGP 9.5 could be observed by standard IF. The IF-TSA method is more sensitivity than standard IF in detecting antigens expressed at low levels. PGP 9.5, TH, and VIP appeared primarily surrounding the secretory coils and sparsely surrounding the sweat ducts.

Conclusion: Sweat secretion is mainly controlled by autonomic nerves whereas sweat reabsorption is less affected by nerve activity.

Key words: Duct; Eccrine Sweat Glands; Innervation; Nerve Fibers; Secretory Coil; Tyramide Signal Amplification

Introduction

In humans, eccrine sweat glands play important roles in regulating body temperature. Secretory coils and ducts are two components of eccrine sweat glands and have different structures and functions. Eccrine sweat glands are innervated mainly by terminal nerve fibers, which are expressed at low levels. Therefore, it is difficult to detect these peripheral nerve fibers by standard histochemical techniques, and signal amplification is required. The tyramide signal amplification (TSA) system is a technique used to amplify weak signals and can be easily integrated into standard immunofluorescence (IF) protocols. In this study, we combine standard IF with TSA to characterize weakly expressed nerve fiber antigens.

Previously, studies have demonstrated that eccrine sweat glands are innervated by both cholinergic and adrenergic nerves, with cholinergic nerves playing the primary roles. However, it is unknown whether the secretory coils and ducts of eccrine sweat glands are equally innervated by the sympathetic nerve fibers. To investigate the differential innervation of secretory coils and ducts, full-thickness skins were co-stained for nerve fiber markers protein gene product 9.5 (PGP 9.5), tyrosine hydroxylase (TH) and vasoactive intestinal peptide (VIP), and eccrine sweat gland markers by combining standard IF with tyramide signal amplification (IF-TSA).

Methods

Ethical approval

The study was conducted in accordance with the Declaration of Helsinki and was approved by the Ethics Committee of

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Received: 26-03-2018 Edited by: Peng Lyu
How to cite this article: Ouyang Z, Li HH, Zhang MJ, Xie ST, Cheng LH. Differential Innervation of Secretory Coils and Ducts in Human Eccrine Sweat Glands. Chin Med J 2018;131:1964-8.
Skin samples
Six noncauterized and full-thickness skin specimens were obtained from individuals undergoing plastic surgery in the Plastic Surgery Department of our hospital. The patients’ ages varied between 6 and 18 years, and the regions of specimens included fingers, abdomens, arms, and legs. The full-thickness skins were fixed in 4% paraformaldehyde, paraffin-embedded, and cut into 5 µm-thick sections.

Standard immunofluorescence staining for innervation of eccrine sweat glands
Standard IF staining was performed according to the schematic shown in Figure 1a. First, sections were dewaxed in xylene and rehydrated in descending ethanol concentrations. Second, antigen retrieval was done by heating the sections to 95°C in 0.01 mol/L citric acid buffer (pH 6.0) for 15 min. Third, nonspecific sites were blocked by incubating the sections with normal goat serum in PBS for 30 min at 37°C. Fourth, the sections were incubated, respectively, with rabbit anti-PGP 9.5 (1:100 dilution, Abcam, ab8189, MA, USA), rabbit anti-TH (1:1000 dilution, Sigma, T8700, NJ, USA), or rabbit anti-VIP (1:1000 dilution, Immunostar, 20077, WI, USA), at 4°C overnight, followed by incubation with Alexa Fluor 488-labeled goat anti-rabbit secondary antibody (1:500, Beyotime, A0423, Jiangsu, China) for 1 h in the dark at room temperature. Finally, sections were counterstained with 5 µg/ml 4’, 6-diamidino-2-phenylindole (Beyotime, Jiangsu, China) for 10 min in the dark at room temperature and mounted with antifade mounting medium (Beyotime, Jiangsu, China). Sections were washed three times with PBS between steps. The results were observed with a fluorescence microscope (Olympus BX51, Tokyo, Japan).

Standard immunofluorescence with tyramide signal amplification staining for innervation of eccrine sweat glands
The protocols for IF-TSA staining [Figure 1b] were the same as for standard IF staining [Figure 1a] except for the following steps. Horseradish peroxidase-labeled goat anti-rabbit secondary antibody (Beyotime, A0208, Jiangsu, China) was used to substitute for Alexa Fluor 488-labeled goat anti-rabbit secondary antibody, and then, the sections were incubated with fluorescein isothiocyanate (FITC)-labeled tyramide (1:50) in a ×1 amplification solution (Perkin Elmer, Wellesley, MA, USA) for 10 min at room temperature.

Double immunofluorescence staining of nerve fiber and eccrine sweat gland markers
Protocols for double IF staining were similar to IF-TSA staining except for the following steps. After sections were stained with FITC-labeled tyramide for PGP9.5, TH or VIP, sections were incubated, respectively, with mouse anti-K7 (ZSGB-BIO, ZM0071, Beijing, China), rabbit anti-S100P (Novus, NB51-95671, MO, USA), or rat anti-K14 (ZSGB-BIO, ZA0540, Beijing, China) for 2 h at room temperature in the dark, followed by incubation with Cy3-labeled goat anti-rabbit (Beyotime, A0516, Jiangsu, China) or Cy3-labeled goat anti-mouse (Beyotime, A0521, Jiangsu, China) secondary antibodies for 10 min at room temperature in the dark.

Analysis of the study
Staining was observed under the microscope, photographed, and analyzed by two experienced pathologists in our hospital.

Results
Skin tissues reacted with the primary antibody to PGP 9.5 [Figure 2a], but not to TH [Figure 2c] or VIP [Figure 2e] when stained by standard IF methods. PGP 9.5 was easily visualized on nerve fibers surrounding the eccrine sweat glands [Figure 2a]. As for IF-TSA staining, all the three primary antibodies reacted with nerve fibers surrounding sweat glands [Figure 2b, 2d and 2f]. The immunoreactivity of PGP 9.5 was comparable between standard IF and IF-TSA staining [Figure 2a and 2b].

The fluorescence of PGP 9.5 [Figures 2b and 3a1-3a3], TH [Figures 2d and 3b1-3b3], and VIP [Figures 2f and 3c1-3c3] appeared on nerve fibers surrounding the eccrine sweat glands, indicating that human eccrine sweat glands were innervated by both adrenergic and cholinergic fibers. To further identify the sympathetic types innervating different parts of eccrine sweat glands, we doubly stained for different sympathetic nerve fiber markers and eccrine sweat gland markers including secretory coil marker K7 [Figure 3a1, 3b1 and 3c1], duct marker S100P [Figure 3a2, 3b2 and 3c2], and sweat gland marker K14 [Figure 3a3, 3b3 and 3c3]. Positive staining for PGP 9.5 [Figure 3a1-3a3], TH [Figure 3b1-3b3] and PGP 9.5 [Figure 3a1-3a3] stainings vary among different parts of eccrine sweat glands.
and VIP [Figure 3c1-3c3] predominantly surrounded the secretory coils of eccrine sweat glands in a dotted circular pattern [Figure 3], and only sparsely surrounded sweat ducts [Figure 3], suggesting that sweat secretion is mainly regulated by autonomic nerves and sweat absorption is less affected by nerve activity.

**Discussion**

In the present study, we compared the sensitivity of standard IF and IF-TSA methods in detecting peripheral nerve fibers. The results showed that the fluorescent signals of PGP 9.5, TH, and VIP were easily visualized by the IF-TSA method, but only PGP 9.5 signal can be visualized by standard IF, which indicates that standard IF alone is insufficiently sensitive for detecting antigens expressed at low levels, but that such antigens can be detected by the IF-TSA method. The expression of PGP 9.5 can be detected by both IF and IF-TSA methods, and there was no apparent difference in the sensitivity of PGP 9.5 detected by the two methods, with the IF-TSA method only slightly elevating the background.

All the three never fiber markers, namely PGP 9.5, TH, and VIP, were found to surround the eccrine sweat glands, indicating human eccrine sweat glands are innervated by both sympathetic adrenergic and sympathetic cholinergic nerves, which are consistent with previous studies. However, until now, it was unknown whether the two components of eccrine sweat glands are equally innervated by sympathetic nerve fibers. To address the issue, we investigated the innervation of different components of eccrine sweat glands by double IF staining for nerve fiber markers and eccrine sweat gland markers. In eccrine sweat glands, K7 is selectively expressed in secretory cells, S100P is specifically expressed in inner layer cells of ducts, and K14 is expressed in myoepithelial cells of secretory coils and ducts, so we can differentiate secretory coils from ducts according to the differential localization of K7, S100P, and K14. IF double staining shows that PGP 9.5, TH, and VIP predominantly surround secretory coils and sparsely surround sweat ducts, suggesting that the innervation of secretory coils and ducts are not equal.

Previous studies have demonstrated that the maturation and maintenance of secretory responsiveness is regulated by innervations. Eccrine sweat glands cannot produce sweat in the absence of innervation, indicating secretory responsiveness is innervation dependent. Further studies show that the secretory response of eccrine sweat glands is reduced by the cholinergic antagonist atropine, alpha-adrenergic antagonist phentolamine, or beta-adrenergic antagonist propranolol, but is totally blocked only by a combination of the above three antagonists, which also indicates that sweat secretion is innervation-dependent, and both cholinergic and adrenergic innervations are involved in sweat secretion. In the clinic, approximately 1–3% of the population exhibits primary hyperhidrosis, which interferes with their daily activities and causes social embarrassment and psychological distress. After selective sympathectomy to block the secretory responsiveness of sweat glands, the life quality of these patient improved, which further indicates that sweat secretion is innervation dependent. The secretory coils of eccrine sweat glands produce isotonic sweat that is then reabsorbed by sweat ducts, leading to hypotonic sweat and conserving electrolytes. When the rate of sweat production is low, most of the sodium and chlorine are reabsorbed by the sweat duct; when the sweat rate is high, less sodium and chlorine are reabsorbed and more water is evaporated on the skin. These studies indicate that sweat reabsorption primarily depends on the rate of sweat production and less on innervation. The differential innervation of secretory coils and ducts in human eccrine sweat glands indicates that the mechanisms of sweat secretion and absorption are different. Sweat secretion is mainly controlled by autonomic nerves, whereas sweat reabsorption is less affected by nerve activity.

There is a limitation to this study. Co-immunohistochemistry is used to detect the differential innervation of secretory coils and ducts. Western blotting and transmission electron microscope are other methods which may make the results more accurate.
There are no conflicts of interest.

Financial support and sponsorship
This study was supported by grants from National Natural Science Foundation of China (No. 81772102, and No. 81471882).

Conflicts of interest
There are no conflicts of interest.

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摘要

背景: 先前的研究表明人汗腺受胆碱能和肾上腺素能神经双重支配，但并不太清楚是否分泌部和导管部等同受到交感神经纤维支配。本研究的目的是调查分泌部和导管部的差异神经纤维支配。

方法: 从2016年6月-2017年6月，六个人皮肤标本常规固定、石蜡包埋、切成5 μm 厚的切片。采用共免疫荧光组织化学-酪氨酸信号放大系统(IF-TSA)方法检测神经标志物: 蛋白基因产物9.5 (PGP 9.5), 酪氨酸羟化酶 (TH)和血管活性肠肽(VIP)，和汗腺标志物: K7, S100P 和K14，的表达。显微镜下观察、拍照和分析染色结果。

结果: IF-TSA方法能够很容易的检测到PGP 9.5, TH和VIP荧光信号呈环状分布于汗腺周围，而传统的IF法仅检测到PGP 9.5在汗腺的表达。IF-TSA方法比传统的IF法在检测低表达抗原方面更敏感。PGP 9.5, TH和VIP主要分布于分泌部周围，较少分布于导管部周围。

结论: 汗液分泌主要受神经支配，而汗液重吸收较少受神经影响。