PROCEEDING

PAR-2 receptor-induced effects on human eccrine sweat gland cells

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Abstract : Serine proteases can induce cell signaling by stimulating G-protein-coupled receptors, called proteinase-activated receptors (PAR's) on a variety of epithelial cells. While PAR-2, one such receptor, activates cell signaling in a secretory cell line derived from human sweat glands, there was no information on their presence and effects on intact sweat glands. PAR-2 presence and activation of eccrine sweat glands isolated from human skin samples was investigated using Western blot analysis, immunohistochemistry, electron microscopy (EM) and Ca^{2+} imaging. Anti-human PAR-2 antibody demonstrated the presence of these receptors in eccrine sweat glands. EM showed that PAR-2 activation resulted in degranulation of secretory cells. Ca^{2+} imaging using PAR-2 activators demonstrated a two phase increase in $[Ca^{2+}]_i$ which was dependent on extracellular Ca^{2+} for the second phase, and that the response could be blocked by prior incubation with xestospongin, the IP_3 receptor blocker. The results demonstrated that PAR-2 receptors are present in human sweat gland secretory cells and that these receptors are functionally active and can induce changes associated with secretory events in eccrine glands. J. Med. Invest. 56 Suppl. : 371-374, December, 2009

Keywords **:** *proteinase activated receptors, eccrine sweat glands, ussing chamber, immunofluorescence*

INTRODUCTION

Serine proteases can regulate cell signaling by stimulating G-protein-coupled receptors, called proteinase-activated receptors (PAR's) that are activated by the proteolytic cleavage of their N-terminal domain. This cleavage exposes a receptor-activating peptide sequence that binds to the receptor and initiates intracellular signaling (1). PAR-2, one such receptor, is activated by trypsin, mast cell tryptase, endogenous and exogenous proteinases and synthetic activating peptides (AP) (see review (2))

PAR-2 receptors have been located in various organs and evoke ion secretion in a variety of epithelial cells [2] through a rise in intracellular calcium ($[Ca^{2+}]_i$) (3, 4). PAR-2 agonists can also increase $[Ca^{2+}]_i$ in eccrine sweat glands and induce anion secretion in a sweat gland cell line-NCL-SG3 (5). There are two types of sweat gland in the human body, namely the eccrine type, which produces the NaCl-rich solution that is found in response to heat or exercise, and the apocrine, which releases a lipid-rich secretion. Eccrine gland sweat is produced by a secretory coil and modified by a reabsorptive duct before exiting on to the skin surface. The secretory epithelia consist of light and dark cells, with light cells thought to produce the primary NaCl-rich solution and the dark cells releasing secretory granules, thought to be the source of proteins and antimicrobial peptides in sweat (6, 7).

The pathway(s) associated with increased $[Ca^{2+}]_i$ and the subsequent anion secretion in the eccrine

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secretory cells have not been fully elucidated, nor is it understood what effect PAR-2 activation has on the release of dark cells granules. In this study, the effects of PAR-2 activation were investigated in sweat glands isolated from human skin samples.

METHODS

Human skin samples were obtained with ethical approval and patient consent. Intact skin samples were used for electron microscopy (EM) and immunohistochemistry using anti-human PAR-2 antibody. Eccrine sweat glands were isolated by repeated shearing (8), and either attached to Cell Tak™ coated glass coverslips and incubated with FURA-2 AM and used for Ca^{2+} imaging, or used for Western blotting. FURA-2 loaded glands were superfused with physiological salt solutions containing PAR-2 activators (trypsin and synthetic activating peptide-(SLIGKV)) and the reverse peptide (VKGILS) and changes in intracellular calcium monitored by recording changes in fluorescent ratio.

RESULTS

Western blot analysis demonstrated the presence of these receptors in eccrine sweat glands, while immunohistochemistry located receptors to the secretory coil and reabsorptive duct (Fig 1). EM showed that PAR-2 activation by trypsin or AP resulted in degranulation of the dark cells, when compared to unstimulated cells. Calcium imaging experiments demonstrated that perfusing the glands with 10 µM trypsin could evoke an increase in $[Ca^{2+}]_i$ that could not be readily repeated by the re-addition of trypsin (Fig 2). Perfusing the glands

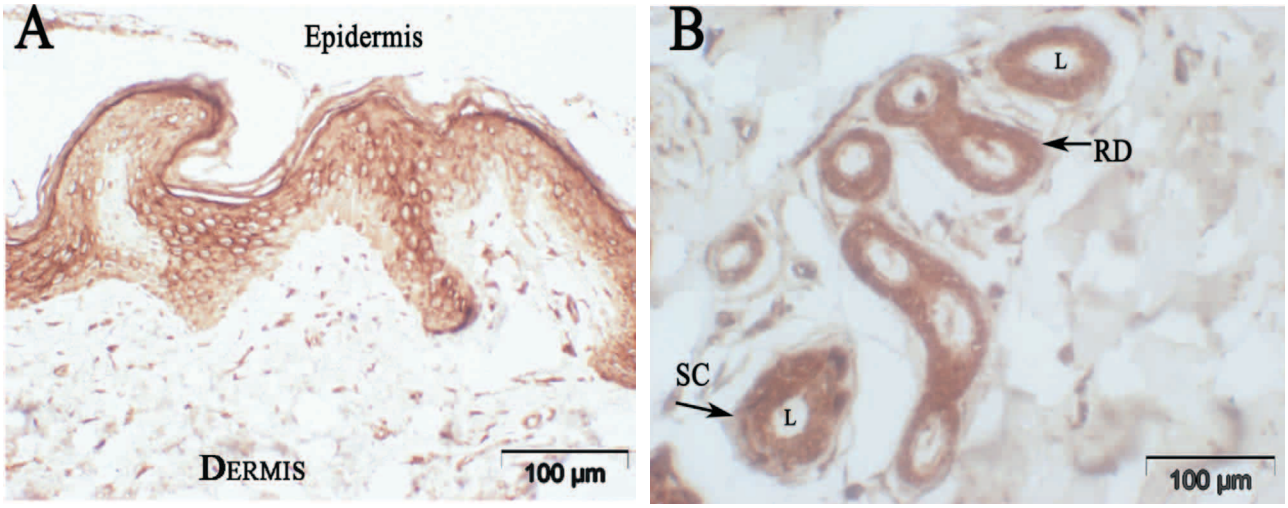

Figure 1. A). Immunohistochemistry of PAR 2 receptors on epidermal keratinocytes (control). B). PAR 2 immunoreactivity in an eccrine gland.

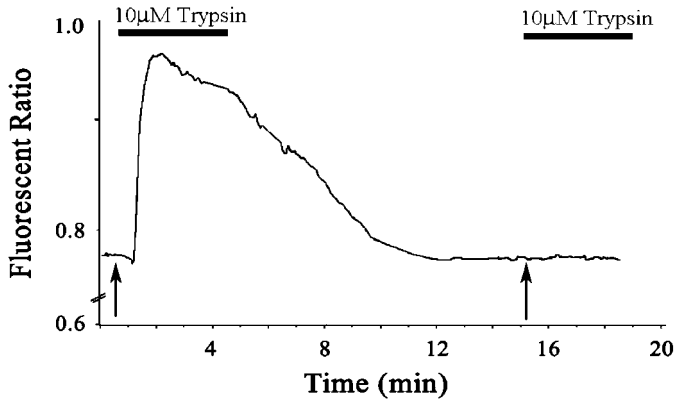

Figure 2. The trace shows the increase in fluorescent representing an increase in $[Ca^{2+}]_i$, in response to 10 µM trypsin. The figure shows that a second addition of trypsin after washout failed to cause an increase in $[Ca^{2+}]_i$.

with 100 μM PAR-AP (SLIGKV) demonstrated an increase in $[Ca^{2+}]_i$ (Fig 3), which was not significantly different from the changes seen in response to trypsin. Perfusing the glands with the reverse peptide did not bring about an apparent change in $[Ca^{2+}]_i$. The increase in $[Ca^{2+}]_i$, in response to 100 μM PAR-AP, unlike that of trypsin, was readily repeated after washout of the peptide (Fig 3). Perfusing the glands with PAR2-AP in nominally Ca^{2+}-free saline brought about an increase in $[Ca^{2+}]_i$, which would increase further on the re-addition of Ca^{2+} to the perfusing solution in the continued presence of the PAR2-AP. The increase in $[Ca^{2+}]_i$ in intact glands could be blocked by prior incubation with xestospongin, the IP_3 receptor blocker.

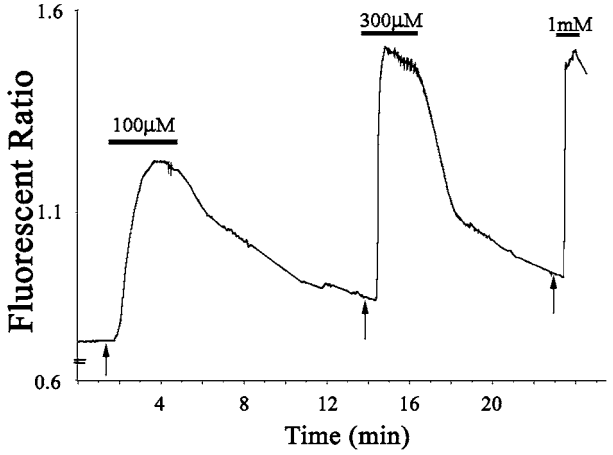

Figure 3. A representative trace showing repeatable increases in $[Ca^{2+}]_i$ in response to 100, 300 μM and 1 mM PAR2-AP.

DISCUSSION

This study demonstrates that PAR-2 proteinase-activated G protein-coupled receptors are present in intact isolated human eccrine sweat glands. The results shows that trypsin-induced increases in $[Ca^{2+}]_i$ in isolated glands cannot be readily repeated, whereas the changes induced by PAR-2-AP could be repeated after washing out the peptide, suggesting that the trypsin effects are longer lasting. The results of exposing the glands to PAR2-AP in the absence of extracellular Ca^{2+} and then re-adding Ca^{2+} back to the solution demonstrates that the changes in $[Ca^{2+}]_i$ are a result of the release of Ca^{2+} from intracellular stores and an influx of Ca^{2+} from extracellular fluid. The blocking of the PAR-2-induced calcium increases by Xestospongin, indicates that these receptors act via the inositol-trisphosphate pathway.

The results of this study indicate a possible physiological function of PAR-2 receptors on eccrine sweat glands, which are in addition to the well-documented effects of cholinergic, adrenergic and purinergic receptors in regulating transepithelial ion transport and fluid secretion from the eccrine secretory coil. It is as yet unclear whether trypsin activation of PAR-2 receptors reduces the effectiveness of the gland to continue to secrete or to release secretory granules. This requires further investigation, as the effects of trypsin on sweat glands may have consequences in health and disease. *e.g.* in atopic dermatitis patients where increased rates of skin bacterial infections occur.

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