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Short communication

Nucleotide-evoked ion transport and $[Ca^{2+}]_i$ changes in normal and hyperhidrotic human sweat gland cells

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Abstract

Apical and basolateral application of ATP and UTP evoked $[Ca^{2+}]_i$ and short circuit current (Isc) increases in normal and hyperhidrotic human eccrine sweat gland cells grown into functionally polarised epithelia on permeable supports. Basolateral application to hyperhidrotic cells exhibited a markedly greater increase in Isc than in normal cells. Hyperhidrotic cells also demonstrated differences from the normal in $[Ca^{2+}]_i$ and Isc responses to ATP when pre-treated with thapsigargin. The data demonstrate the presence of apical and basolateral receptors that allow nucleotides to increase $[Ca^{2+}]_i$ and Isc. The results suggest that changes from the normal in transepithelial ion transport contribute to the characteristic excessive fluid production of hyperhidrotic sweat glands.

Keywords: P2Y receptor; Sweat gland; Anionic secretion; Hyperhidrosis; Ca^{2+}, intracellular; Ussing chamber

1. Introduction

P2Y receptors are found in the apical membranes of polarised epithelia (Dubyak, 1999) where they allow nucleotides to increase intracellular calcium $[Ca^{2+}]_i$ and exert control over transepithelial ion transport. P2Y receptors have been identified on cells from a transformed human eccrine sweat gland cell line (Wilson et al., 1994) and human secretory coil cells grown on glass cover slips (Clunes et al., 1999). However, expression of receptors in transformed human cells and equine sweat gland cells on glass cover slips was limited, compared to those on permeable supports (Ring and Mork, 1997; Ko et al., 1997).

The aim was to confirm the presence of P2Y receptors in the apical membranes of polarised human sweat gland cells, using simultaneous measurement of $[Ca^{2+}]_i$ signaling and transepithelial ion transport, to create a model for the study of cells from hyperhidrotic patients. Hyperhidrosis is characterised by the production of an inappropriately large volume of sweat and can be a primary condition or a manifestation of a more serious illness (Sato et al., 1989); the cause of the primary syndrome is unknown. There is no simple, non-invasive cure and no specific corrective therapy; remedial options are generally severe, hazardous and not always effective. Anatomical evidence indicates that the syndrome is not due to a morphological defect (Bovell et al., unpub.). The causal defect is most likely physiological in one or all of four areas: (1) Excessive levels of exogenous neuro-hormonal secretagogues. (2) Abnormal secretory cells with an increased number of membrane receptors or increased receptor sensitivity. (3) Altered ionic membrane conductance and hence, increased salt and water movement. (4) A defective second messenger system within the secretory cells. Initial findings on possibilities 3 and 4 are reported on samples from two individuals, the number obtained over a year. Although hyperhidrosis is a relatively common condition, skin samples are scarce.

2. Materials and methods

Eccrine sweat glands were isolated from skin samples (Lee et al., 1984) obtained from control patients undergo-

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ing general surgery and by biopsy from hyperhidrotic individuals. These procedures had the approval of the local medical ethics committee and the informed consent of the patients.

Primary cultures were established from freshly isolated glands by standard culture techniques using mammary epithelial growth medium (Bio-Whittaker, UK). The reabsorptive duct was removed prior to growth for 16–18 days. Cells were removed from the culture flasks using standard techniques (i.e. Trypsin/EDTA), re-suspended in fresh medium and 2.2×10^5 cells cm^{-2} were plated onto permeable supports (Transwell-Col membranes, 0.4 μm pore diameter, Costar, UK). Culture area was 0.1 cm^2 and membranes bearing cells were floated on medium in Petri dishes and incubated for 6 days until the cells formed a confluent monolayer. Transepithelial resistance and short circuit current (Isc) were measured by mounting the membranes in miniature Ussing Chambers attached to the stage of an inverted microscope (Ko et al., 1999). Transepithelial potential difference was expressed with respect to the basolateral solutions. Positive currents (displayed as upward deflections) were defined as those carried by anions moving from the basolateral to apical solution. Changes in $[Ca^{2+}]_i$ were monitored by FURA-2 loading and the fluorescence ratio (340:380 nm) recorded from 30–40 cells, while Isc was being monitored (Ko et al., 1999).

Nucleotides (100 μM) were added to a bicarbonate-buffered saline (containing in mM: NaCl, 100; KCl, 5; KH_2PO_4, 1.2; $MgCl_2$, 1; $CaCl_2$, 1; D-Glucose, 10; $NaHCO_3$, 20; adjusted to 7.4 pH with NaOH) superfusing either the apical or basolateral membrane. Thapsigargin (3 μM) was added to the apical bath, while calcium-free conditions were achieved by adding 1 mM EGTA to a nominally calcium-free solution. Experimentally induced changes in $[Ca^{2+}]_i$ and Isc were quantified at the peak response by subtraction of the equivalent values obtained immediately before stimulation. Pooled data for normal cells are presented as means ± SE, n = number of experiments from five normal donors and means ± SD, $n = 6$ experiments from two hyperhidrotic patients. Statistics were performed using Students t-test.

3. Results

After 4–6 days in culture, cells had formed confluent layers on the Transwell-Col membranes. Under open-circuit conditions, transepithelial resistance was 123 ± 15 (normal cells) and 137.9 ± 0.4 Ω cm^{-2}. The cultured epithelia generated a potential difference of -0.6 ± 0.1 (normal) and -1.3 ± 0.3 mV (hyperhidrotic). Basal Isc was 0.6 ± 0.1 (normal) and 0.13 ± 0.02 μA cm^{-2} (hyperhidrotic).

Changes in $[Ca^{2+}]_i$ and Isc in normal and hyperhidrotic cells in response to ATP and UTP are presented in Table 1. Apical and basolateral application of ATP and UTP to normal cells elicited small, sustained rises in Isc (Fig. 1(A)). $[Ca^{2+}]_i$ consistently increased in response to the agonists, with apical responses significantly larger than basolateral responses ($P < 0.05$). In hyperhidrotic cells, apical ATP and UTP both caused increases in Isc, which were not markedly different from normal cells. Basolateral application, however, induced considerably larger increases in Isc than normal (Fig. 1(B)). In the hyperhidrotic cells, the addition of the agonists to both the apical and basolateral sides evoked increases in $[Ca^{2+}]_i$ which were not significantly different from one another. Statistical comparisons between normal and hyperhidrotic cells will be made when further samples become available.

Changes in normal and hyperhidrotic cells to thapsigargin are also presented in Table 1. Thapsigargin elicited a slowly developing but sustained increase in $[Ca^{2+}]_i$ and in

Table 1
Simultaneous measurements of Isc (μA cm^{-2}) and $[Ca^{2+}]_i$ (fluorescent ratio units) in response to ATP, UTP, Thapsigargin (Tg), Tg + ATP, Tg followed by removal of Ca^{2+} (Tg − Ca^{2+}), and Tg − Ca^{2+} with subsequent addition of ATP (Tg − Ca^{2+} + ATP)

		ATP	UTP	Tg	Tg + ATP	Tg − Ca^{2+}	Tg − Ca^{2+} + ATP
Normal							
Isc	Apical	1.81 ± 0.61	4.16 ± 1.4[a]		0.04 ± 0.02		0.6 ± 0.02
	Basolateral	1.7 ± 0.23	1.99 ± 0.75	1.52 ± 0.6		0.03 ± 0.01	
Ca^{2+}	Apical	0.4 ± 0.05[b]	0.33 ± 0.06[b]		0.09 ± 0.06		0.06 ± 0.01
	Basolateral	0.21 ± 0.02	0.21 ± 0.02	0.34 ± 0.03		−0.23 ± 0.16	
Hyperhidrotic							
Isc	Apical	1.16 ± 0.28	1.79 ± 0.35		2.17 ± 0.4		2.32 ± 1.44
	Basolateral	19.12 ± 1.12[c]	19.28 ± 0.95[c]	3.29 ± 0.6		8.29 ± 2.46	
Ca^{2+}	Apical	0.07 ± 0.02	0.06 ± 0.02		0.06 ± 0.01		0.04 ± 0.01
	Basolateral	0.11 ± 0.02	0.11 ± 0.02	0.34 ± 0.3		−0.26 ± 0.1	

Statistical comparisons have not been performed between normal (experiments from five subjects) and hyperhidrotic (experiments from two subjects) results.

[a] Significantly greater than the response to apical ATP ($P < 0.05$).

[b] Significantly greater increases in $[Ca^{2+}]$ as compared to basolateral application ($P < 0.001$).

[c] ATP and UTP induced substantial increases in Isc when applied basolaterally to the hyperhidrotic cells.

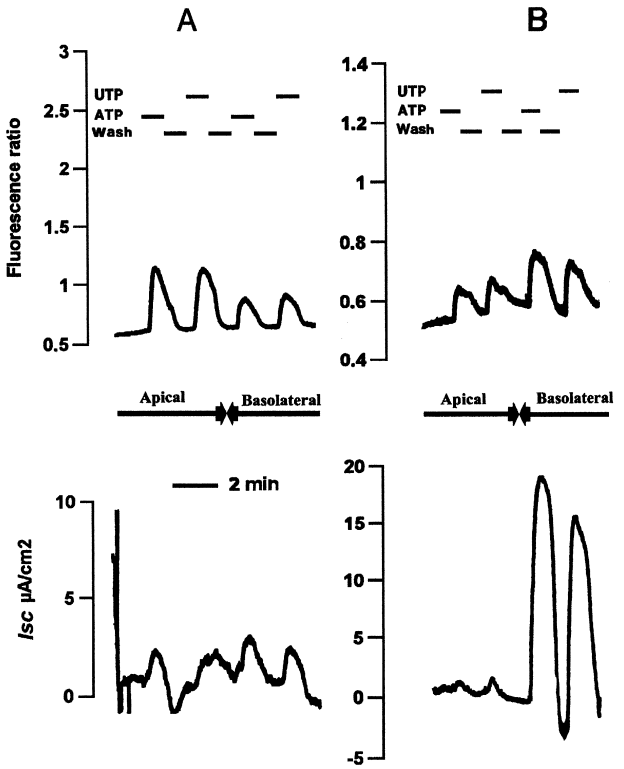

Fig. 1. The effects of apical and basolateral application of ATP and UTP (100 µM) upon fluorescent ratio and Isc were recorded from Fura-2 loaded, (A) normal and (B) hyperhidrotic epithelia.

Isc ($n = 3$). Subsequent apical application of ATP ($n = 3$) caused a small increase in $[Ca^{2+}]_i$ with no apparent change in Isc. Thapsigargin application to hyperhidrotic cells evoked a rise in $[Ca^{2+}]_i$ and a slowly increasing level of Isc to a sustained level ($n = 3$). The subsequent apical addition of ATP further increased $[Ca^{2+}]_i$ and Isc.

Following resultant changes in $[Ca^{2+}]_i$ in response to thapsigargin under control conditions, Ca^{2+} was removed from the bathing solution. This caused a reduction in $[Ca^{2+}]_i$ with no apparent affect on Isc. Subsequent apical addition of ATP caused no further increase in Isc, however, a small increase in $[Ca^{2+}]_i$, was observed ($n = 3$).

In hyperhidrotic cells, the removal of external Ca^{2+} during thapsigargin treatment, evoked a sustained increase in Isc ($n = 3$) with a transient increase and subsequent reduction in $[Ca^{2+}]_i$ back to control levels. The application of ATP apically further increased Isc with an accompanying transient rise in $[Ca^{2+}]_i$, suggesting that activation of the P2Y receptors in these cells, like normal cells, enhances Ca^{2+} release.

Repeated stimulation with either ATP or UTP at apical or basolateral membranes resulted in rapid desensitization. Cross-desensitization experiments with the normal cells demonstrated that apical addition of ATP increased Isc (3.47 ± 1.26 µA cm^{-2}; $n = 4$), with subsequent apical UTP application inducing a comparable response (4.6 ± 1.4 µA cm^{-2}; $n = 4$). Similarly, ATP added basolaterally increased Isc (4.7 ± 0.6 µA cm^{-2}; $n = 4$), and subsequent UTP application elicited a similar increase (4.1 ± 0.7 µA cm^{-2}, $n = 4$).

UTP added apically increased Isc as before (3.5 ± 0.9 µA cm^{-2}, $n = 4$), while subsequent apical addition of ATP produced a reduced response (0.2 ± 0.1 µA cm^{-2}, $n = 4$). A similar reduced response to ATP (0.9 ± 0.2 µA cm^{-2}, $n = 4$) was obtained following basolateral application of UTP. In this instance, the basolateral UTP evoked a rise in Isc (7.9 ± 1.2 µA cm^{-2}, $n = 4$).

4. Discussion

The data confirm the presence of apical and basolateral receptors on human eccrine sweat gland cells that allow nucleotides to increase $[Ca^{2+}]_i$ and Isc. Purinoceptors have been shown to be almost exclusively on the luminal membranes of equine cells (Wilson et al., 1996) implying an autocrine source for the nucleotides. In contrast, our findings suggest a neural source of the nucleotides influencing the basolateral receptors.

Desensitization experiments on the normal cells reveal the presence of at least two $[Ca^{2+}]_i$-mobilising P2Y receptor subtypes, a population stimulated by both ATP and UTP, and a second population stimulated by UTP alone, which cannot be desensitized by ATP. These anion channels are not refractory to repeated stimulation even after prolonged exposure to ATP.

The slow increase in Isc after exposure to thapsigargin, a substance that blocks the sarcoplasmic Ca^{2+} uptake pump, differs from a significant rise seen in equine secretory epithelial cells (Ko et al., 1999) and from an absence of response reported in human sweat gland cells (Pickles and Cuthbert, 1992). The first may be due to species differences. The second may well be a consequence of the use of different culture mediums. MEG medium (a derivative of MCDB 170) facilitates the growth of two distinct cell types from human eccrine secretory coil (Reddy et al., 1992), while the medium used by Pickles and Cuthbert (1992), facilitates the growth of cells with ductal characteristics.

The small increase in $[Ca^{2+}]_i$ and Isc upon exposing thapsigargin-treated normal cells to ATP agrees with the findings of Pickles and Cuthbert (1992). This however, also differs from results in equine cells under similar conditions, where ATP caused an increase in Isc but no further increase in $[Ca^{2+}]_i$ (Ko et al., 1999). In human cells, when external Ca^{2+} was removed during thapsigargin treatment, the addition of ATP elicited an increase in $[Ca^{2+}]_i$ with no apparent effect on Isc. This again contrasts with the equine cell (Ko et al., 1999). Thapsigargin thus increases $[Ca^{2+}]_i$ in normal human sweat gland epithelial cells but has no effect upon transepithelial ion transport, whereas Ca^{2+}-mobilising agonists such as ATP and UTP increase both parameters. This suggests that Isc is uncou-

pled from $[Ca^{2+}]_i$ in at least some types of sweat gland secretory epithelia.

In hyperhidrotic cells, the markedly greater increase in Isc to basolateral application of agonists, compared to normal cells, while still based on small numbers, was consistent and is intriguing. It suggests that differences in the transepithelial transport mechanisms could contribute to the inappropriately large volume of sweat produced by this condition. Furthermore, the set of experiments involving thapsigargin treatment and ATP stimulation of hyperhidrotic cells, demonstrated increases in Isc which were absent in normal cells, suggesting thapsigargin-insensitive sub-compartments of an ATP releasable Ca^{2+} store, which can induce ion movements. The rise in $[Ca^{2+}]_i$ and Isc in the hyperhidrotic cells upon removal of external Ca^{2+} during thapsigargin treatment, suggests that there is a transient release of Ca^{2+} from intracellular stores in response to Ca^{2+} removal, which is different from normal cells. The sustained increase in Isc under these conditions is surprising but implies that external Ca^{2+} removal alters membrane permeability to ions. This clearly warrants further investigation. The increase in $[Ca^{2+}]_i$ and further transient increase in Isc upon the addition of ATP to these cells, suggests that intracellular sub-compartments containing releasable amounts of Ca^{2+}, which can alter transepithelial ion transport, might exist in the hyperhidrotic cells. These further differences from the normal cells suggest that hyperhidrotic cells express different characteristics and that these differences could further contribute to the excessive sweat secretion. The cellular basis of hyperhidrosis will be further investigated as and when scarce material is obtained.

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