HUMAN ECCRINE SWEAT GLAND EPITHELIAL CULTURES EXPRESS DUCTAL CHARACTERISTICS

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SUMMARY

1. Isolated human eccrine sweat glands were cultured in vitro. Cells were harvested and plated onto permeable supports to form confluent cell sheets, area 0.2 cm². These were used to study the electrogenic transepithelial transport of ions by measurement of short-circuit current (SCC). Epithelial sheets had a basal SCC of 5.89 ± 0.62 μA cm⁻² (n = 33) and a transepithelial resistance of 74.1 ± 5.6 Ω cm² (n = 33). The transepithelial potential difference varied between -0.2 and -1.8 mV with a mean value of -0.71 ± 0.09 mV (n = 33).

2. The basal current was abolished by addition of 10 μM-amiloride to the apical bathing solution. The concentration of amiloride which inhibited basal SCC by 50% (EC₅₀) was 0.4 μM. Cultures prepared from the secretory coil of sweat glands, rather than from whole glands, were similarly sensitive to amiloride (EC₅₀ = 0.8 μM).

3. Lysylbradykinin (LBK), carbachol, isoprenaline, prostaglandin E₂ (PGE₂) and A23187 all increased SCC in cultures from whole glands. LBK responses were obtained with basolateral and not with apical application. Furthermore LBK actions were not substantially altered by cyclo-oxygenase inhibition but showed marked desensitization upon repeated application. Sheet cultures prepared from sweat gland coils also showed SCC responses to both carbachol and LBK. Forskolin, an activator of adenylate cyclase, did not alter SCC in either type of preparation.

4. Replacement of chloride and of chloride and bicarbonate in the bathing solution did not cause attenuation of the responses to LBK or carbachol in whole-gland sheet cultures. Furthermore responses were unaffected by piretanide or acetazolamide. These results were taken to indicate that anion secretion was not the basis for the SCC responses.

5. Responses to LBK and carbachol were significantly reduced by amiloride (10 μM), this effect being reversible. No responses to LBK or carbachol were seen when N-methyl-D-glucamine (NMDG) was used to replace sodium, whereas reintroduction of sodium ions restored responsiveness to these agents.

6. The SCC responses to the muscarinic agonist carbachol and to LBK appear to be due to stimulation of amiloride-sensitive, electrogenic sodium absorption in whole-gland sheet cultures. Further it would appear that, in culture, the

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pleuripotential capacity of the cells is revealed since both whole-gland and secretory coil cultures exhibit some properties usually associated in vivo with duct cells. Many mammalian epithelia show electrogenic chloride secretion both in response to carbachol and LBK but also in response to activation of adenylate cyclase with forskolin. The behaviour of sweat gland cultures compared to other epithelia is discussed.

INTRODUCTION

The human eccrine sweat gland secretes an ultra-filtrate of plasma-like precursor fluid from the secretory coil and then actively resorbs sodium and chloride as the primary secretion passes along the duct. In this way a hypotonic surface sweat is produced (Schultz, 1969). Recently, several reports of in vitro culture of sweat gland ducts have been reported, (Pedersen, 1984; Collie, Buchwald, Harper & Riordan, 1985; Lee, Carpenter, Coaker & Kealey, 1986). In vitro culture offers the advantages of access to the apical surface and experimental manipulation in ways reserved for sheet-like epithelia.

Our aim here was to subculture cells from explants of sweat glands onto permeable supports for continuous short-circuit current recording. This technique would allow protocols for examining cellular physiological mechanisms of ion transport to be developed. Furthermore the relative simplicity of the technique allows precise quantitative data to be collected, in contrast to the more difficult procedures necessary with perfused glands.

We show that lysylbradykinin, a decapeptide produced by kallikrein from kininogen, stimulates electrogenic sodium absorption. We are unaware of another mammalian epithelium in which electrogenic sodium absorption is stimulated by kinin. Indeed most mammalian epithelia secrete chloride in response to this peptide (Cuthbert & Margolius, 1982; Manning, Snyder, Kachur, Miller & Field, 1982).

We also show that reabsorptive duct-like properties are expressed irrespective of whether the origin of the explant is from whole gland or secretory coil. The culture of cells from whole glands therefore represents the simplest system available to study reabsorption in human sweat gland epithelia. Importantly, the new information given here forms a background against which the behaviour of the cystic fibrosis epithelia can be compared, since sweat glands display the primary defect of the disease (Quinton, 1983; Sato & Sato, 1984; Welsh, McCann & Dearborn, 1987).

METHODS

Isolation of human sweat glands

Non-cauterized normal human skin samples (0.5 × 0.5 cm) without scar tissue were obtained from patients undergoing surgery. The skin was collected in sterile buffer containing (mm): NaCl, 117; KCl, 4.7; CaCl₂, 2.5; KH₂PO₄, 1.2; MgSO₄, 1.2; glucose, 11.1; HEPES-Tris, 10; sucrose, 15; penicillin, 100 U ml⁻¹; streptomycin, 100 μg ml⁻¹; and Phenol Red, 0.01% at a pH of 7.3. Under sterile conditions the skin was washed clean of blood and the excess fat removed with sharp scissors. The skin was transferred to a beaker containing 3–4 ml of sterile buffer without Phenol Red where it was chopped, very fine, with sterile sharp scissors. Chopping caused the glands to ‘pop out’ intact from surrounding tissue (Lee et al. 1986). The suspension was then further diluted 3-fold with buffer before being aliquoted into 25 cm² Petri dishes, each containing 2–3 ml.
To pick out the glands from the resulting suspension, ten drops of 0.05% Neutral Red were added to each dish. Within 2 min each gland became highlighted from the background of fat and collagen by the appearance of a diffusely red color in the tubules of the secretory coil and a thin dark red line in the reabsorptive duct (Quinton & Tormey, 1976). The glands were visible at 20 times magnification and could be picked out from other debris with fine forceps and transferred to buffer without Neutral Red. Fifty to sixty glands were collected into a sterile 25 cm² Petri dish containing buffer.

**Primary culture of whole glands**

Glands were transferred to William’s E medium containing collagenase type II (2 mg ml⁻¹) and fetal bovine serum (5%) and incubated with 5% CO₂ in air at 37 °C for 30 min. Afterwards glands were transferred to William’s E medium containing only bovine serum and incubated for 60 min. Tissue culture flasks, 25 cm², with 1 ml of William’s E medium containing L-glutamine (1 mM), penicillin–streptomycin (100 U ml⁻¹, 100 μg ml⁻¹), bovine insulin (10 μg ml⁻¹), hydrocortisone (10 ng ml⁻¹), transferrin (10 μg ml⁻¹), epidermal growth factor (EGF, 20 ng ml⁻¹) and trace element mix (0.5%) were equilibrated with 5% CO₂ in air at 37 °C. Glands were explanted into these flasks using A5 insect pins glued to the end of glass handles. It was essential to keep the glands moist in the first 24 h during which time attachment occurred. If more than 1 ml medium was used the tissues floated off the plastic surface. After 24 h a further 3–4 ml of medium were added to each flask and incubation continued. The medium was changed twice weekly. The methods used for isolation and culture of the glands was modified from that described by Lee et al. (1986).

Epithelial cells began to grow out from the explant after 2 days. After 2–3 weeks the outgrowth reached 1–2 cm diameter. Cells were removed from the flasks by dissociation with versene and trypsin (Cuthbert, Egiele, Greenwood, Hickman, Kirkland & MacVinish, 1987) and placed on Millipore filters (HAWP, 0.45 μm). The filters were coated either with collagen (0.25% in 0.2% acetic acid) or basement membrane Matrigel (diluted 1:1 with William’s E medium) and allowed to dry. A silicone washer with a 0.2 cm² hole was attached to the centre of each filter using Silastic 734 RTV adhesive creating a small well into which cells could be seeded. These units were sterilized by UV irradiation.

Aliquots of 100 μl of cell suspension containing 3 × 10⁶ cells at a viability of 95% (Trypan Blue exclusion test) were pipetted into each well. Fetal calf serum (4%) was added to the medium at this stage to promote cell attachment to the filters. Serum-containing medium was withdrawn after 2 days, especially to reduce the risk of fibroblast outgrowth. Cultures were ready for use after 5–8 days. In some instances primary cultures were grown from only the secretory coil part of the gland. To do this glands were microdissected as described in Lee et al. (1986), and cultured as described above with the omission of the 2 day exposure to fetal calf serum.

**Short-circuit current recording in epithelial monolayers**

The Millipore washer cell monolayer complexes were clamped between the two halves of a Ussing chamber. The epithelial area (0.2 cm²) was held in the centre of a window (area 0.6 cm²) of the double chamber. Edge damage was avoided as the chamber halves abutted the silicone washer. Monolayers were short circuited using a W–P dual-voltage clamp (model DVC 100) with the capacity to compensate for the fluid resistance between the tips of the potential electrodes. Short-circuit current (SCC) records were displayed on a pen recorder. Measurements of transepithelial potential difference and calculation of transepithelial resistance were made according to previous descriptions (Cuthbert, George & MacVinish, 1985). Each side of the tissue was bathed in 20 ml of Krebs–Henseleit (KH) solution maintained at 37 °C by a heat exchanger. Both sides of the chamber were continuously bubbled with 95% O₂–5% CO₂ which maintained pH at 7.4.

**Histology**

Monolayers were stained with Haemotoxylin–Eosin (Baker & Silverton, 1976). Electron microscopy was carried out with a Phillip’s 300 transmission electron microscope using standard fixation and sectioning techniques.

**Chemicals and solutions**

KH solution consisted of (mM): NaCl, 117; KCl, 4.7; CaCl₂, 2.5; MgSO₄, 1.2; NaHCO₃, 25; and glucose, 11.1. Chloride-free KH solution contained sodium gluconate (117 mM), potassium
gluconate (4-7 mM), and CaSO₄ (2-5 mM) as substitutes for NaCl, KCl and CaCl₂ respectively. Chloride–bicarbonate-free KH solution contained these replacements and in addition HEPES–Tris (10 mM), in place of NaHCO₃. This solution was gassed with 100% O₂. For sodium-free KH solution the replacement was either with choline chloride (117 mM) or N-methyl-D-glucamine (NMDG, 117 mM) neutralized to pH 7.4 with HCl and with HEPES–Tris (10 mM), as replacement for NaHCO₃. Substituted KH solutions were checked for tonicity using a freezing-point osmometer and tonicity corrected by adding sucrose where necessary. When preparations were not to be mounted in KH solution the compensation for fluid resistance was made in the modified solution. Substituted KH solutions were always used to bath both sides of the preparations.

William’s E medium, transferrin, EGF and trace element mix were obtained from Gibco (Paisley). Fetal calf serum was obtained from Flow Labs (Irvine). Collagenase, collagen, isoprenaline, amiloride, penicillin–streptomycin, glutamine, insulin, hydrocortisone, prostaglandin E₂ (PGE₂), lysylbradykinin (LBK), carbachol, atropine, piroxicam and A23187 were obtained from Sigma (Poole, Dorset). Piretanide was a gift from Hoescht (F.R.G.). Acetazolamide was obtained from Lederle Labs (Gosport) and forskolin was supplied by Calbiochem (Hoescht, U.S.A.). Matrigel TM was supplied by Universal Biologicals Ltd.

RESULTS

Patterns of growth

Epithelial cells migrated from whole-gland explants after about 2 days in culture. They continued to divide for up to 3 weeks forming a confluent circular monolayer around the explant. Some cell division continued after this time but multilayered structures were then formed. At around 25 days in culture cells became senescent, vacuoles developed and division ceased. Figure 1A and B shows the appearance of cultures at 4 and 18 days respectively. Only 40% of the explanted glands grew in culture; the reason for the failure of the others is unknown. Previous studies with cytokeratin antibodies have shown primary cells grown in this system are of epithelial lineage (Lee et al. 1986).

Sheet culture formation on permeable supports

Epithelial sheet cultures were grown on permeable supports as described in the Methods. It was essential to prepare cultures from explants grown for 18 days or less, that is when the cells were actively dividing. Cells obtained from cultures older than this gave structures with zero transepithelial potential and low transepithelial resistance; these were unsuitable for electrophysiological investigation. Satisfactory cultures required seeding with cells at a high density (1-3 × 10⁵ cells per well); sparsely seeded wells failed to attain confluence. It was not possible to visualize the cells growing on Millipore filters in the living state; consequently the cultures were

Fig. 1. A, 4-day-old whole-sweat-gland primary culture. Epithelial cells are migrating outward from the explant (S). Calibration 10 μm. B, confluent monolayer of epithelial cells produced in primary culture after 18 days. Dark area (S) is the remains of the primary explant. Calibration 10 μm. C, transverse section through an epithelial sheet grown on a Matrigel-coated filter 6 days after seeding. The apical surface (A) with microvilli is uppermost. M indicates the Matrigel substratum. Only the four upper layers appear to be composed of living cells. Note the profusion of desmosome structures between the living layers. Calibration 10 μm. D, high-power transverse section showing the apical junction between two cells (J) and a single microvillus (V). Calibration 0.1 μm. E, transverse section showing desmosomes (D) under high magnification. Calibration 0.1 μm.
Fig. 1. For legend see opposite.
stained after they had been used experimentally. In cultures showing no transepithelial potential subsequent histological examination showed that they were not confluent. In cultures which demonstrated good electrophysiological characteristics staining with Haemotoxylin and Eosin showed a confluent sheet of cells inside the well.

In other instances experimental sheet cultures were examined by electron microscopy (Fig. 1C, D and E). This revealed that the cultures were multilayered. Not all the layers were composed of living cells, senescent or dead layers forming the outermost or innermost structures. Apical microvilli were apparent in the outermost living cell layer, but no evidence of tight junctions was seen. Desmosomes were a prominent feature of the cultures.

**Table 1.** Comparison of the properties of cultures grown upon collagen or Matrigel-coated Millipore filters

|            | \( R \) (\( \Omega \) cm\(^2\)) | PD (mV) | LBK SCC (\( \mu A \) cm\(^2\)) |
|------------|---------------------------------|---------|---------------------------------|
| Matrigel   | 85·2 ± 8·2 (15)                 | -1·00 ± 0·77 (15) | 6·00 ± 0·77 (14)               |
| Collagen   | 34·1 ± 3·6 (9)*                 | -0·37 ± 0·11 (9)*  | 2·62 ± 0·36 (8)*              |

Measurements were carried out on three different batches of cultures, each batch containing both collagen and Matrigel-coated Millipore filters. The LBK values were the peak increases in SCC in response to basolateral addition of 0·1 \( \mu M \)-LBK. *\( P < 0·01 \), unpaired Student’s \( t \) test. \( R \), resistance; PD, potential difference. The number of separate cultures examined is given in parentheses.

In early experiments we investigated whether collagen or Matrigel was the better substrate for promoting sheet formation. The same epithelial cell suspensions were used to form sheets on the two substrates. Measurements were made of potential difference, resistance and the SCC responses to LBK, which we had shown in preliminary experiments caused a rapid, but often transient, increase in SCC. The results, given in Table 1, show that Matrigel gives cultures in which all three parameters were increased. Therefore Matrigel was used as the substrate for all in the experiments given in the remainder of this report.

Basal values for all epithelial sheet cultures grown on Matrigel and derived from whole glands between 5 and 8 days following seeding were 5·89 ± 0·62 \( \mu A \) cm\(^{-2}\) \((n = 33)\) for SCC, 74·1 ± 5·6 \( \Omega \) cm\(^2\) \((n = 33)\) for transepithelial resistance and 0·71 ± 0·09 mV \((n = 33)\), range -0·2 to -1·8 mV, for transepithelial potential difference.

**Responses to hormones and autacoids**

From preliminary results it was clear that the cultures prepared from human sweat glands did not have a high resistance and, in addition, they proved to be extremely fragile. Changing the bathing solution sometimes dislodged the monolayer from the supporting membrane. With these restrictions measurements of ion flux with isotopes to determine the nature of the transported species was not possible. Reliance was placed on ion substitution and designing experiments in ways to avoid, or at least to minimize, the number of solution changes. As the basal SCC in these preparations was relatively small, a number of agents with either known or postulated actions on sweat formation were used to probe the effects of ion substitution upon SCC responses.

Several substances were found to cause increases in SCC and typical responses are
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Fig. 2. Examples of SCC responses from a culture prepared from whole gland, 7 days after seeding (resistance, $R = 80 \ \Omega \ \text{cm}^2$, potential difference, $PD = -1.2$ mV). Compounds were added basolaterally, except forskolin which was added to both sides. Concentrations were: LBK, 0.1 $\mu$M; carbachol (CCh), 10 $\mu$M; isoprenaline (Iso), 10 $\mu$M; and forskolin (For), 10 $\mu$M. Horizontal line indicates zero SCC.

Fig. 3. Examples of SCC responses from a 5-day-old culture ($R = 100 \ \Omega \ \text{cm}^2$, $PD = -1.5$ mV). Agents were added basolaterally except A23187 and amiloride which were added to the apical side only. LBK was added at different times to the apical (A) and basolateral (B) side. Drug concentrations were: LBK, 0.1 $\mu$M; carbachol (CCh), 10 $\mu$M; atropine (Atr), 10 nM; A23187, 1 $\mu$M; acetazolamide (Acet), 0.45 mM; piretanide (Piret), 200 $\mu$M; and amiloride (Amil), 10 $\mu$M. Horizontal line indicates zero SCC.

Illustrated in Figs 2 and 3. LBK, carbachol, isoprenaline and A23187 all caused SCC increases. In some 60% of preparations the rapid increase in current to LBK was followed by oscillations (Fig. 2), which declined to baseline within 10–15 min. In the other preparations the response was a smooth increase and decrease in current (Fig. 3). No responses to LBK were seen when the peptide was added to the apical face of the monolayers. In twenty-four separate experiments the response to LBK (0.1 $\mu$M) was $6.65 \pm 0.58 \ \mu A \ \text{cm}^{-2}$.

In a given preparation which showed oscillatory responses to LBK, other agents also caused oscillatory-type responses after the initial response to LBK had subsided. Similarly preparations showing a smooth response to LBK also responded smoothly to other agents (Fig. 3). In twenty separate preparations carbachol (10 $\mu$M) caused responses of $7.54 \pm 1.03 \ \mu A \ \text{cm}^{-2}$ of which fourteen (60%) showed oscillations during the plateau phase. Only minute responses were obtained when carbachol was added to the apical bathing solution. It was not possible to discover if these responses were...
due to the presence of cholinoreceptors on the apical face or due to penetration of the agent to the basolateral side. Low concentrations of atropine (10 nM) caused an immediate return of carbachol-stimulated SCC to the basal value (Fig. 3). In three preparations pre-incubated with atropine (10 nM), responses were obtained to LBK but not to carbachol. In twenty-five preparations in the absence of atropine, responses were always obtained to both carbachol and LBK.

Isoprenaline caused an increase of 5.40 ± 1.03 μA cm⁻² in nine separate experiments (Fig. 2). Oscillations were seen in four of these preparations. However in some batches of preparations formed from a single-cell suspension no responses were seen to isoprenaline, while those to carbachol or LBK were normal. We must conclude that the response to isoprenaline is variable in a way which makes investigations problematical. Responses to apical addition of isoprenaline were small, as with carbachol. A23187 (1 μM) increased SCC more slowly than the other agents (Fig. 3). The peak increase in SCC was 5.70 ± 0.71 μA cm⁻² in eight experiments and in half of these preparations oscillations were seen in the plateau phase.

In four preparations forskolin (10 μM), an activator of adenylate cyclase, was applied to both sides of preparations but was without effect (Fig. 2). Neither did this agent affect the response to LBK or carbachol.

In six preparations application of PGE₂ (10 μM) to the basolateral face caused SCC to increase by 5.91 ± 0.5 μA cm⁻². Using lower concentrations (1 μM) it was shown that these responses were maximal. To examine whether eicosanoids were mediating the effect of LBK, responses to LBK were measured in the presence and absence of the prostaglandin synthesis inhibitor piroxicam (10 μM). In three pairs of tissues piroxicam had no effect on basal SCC per se and also failed to influence the subsequent LBK response (Fig. 4). This indicates that endogenous prostaglandin production is unlikely to be of more than minimal importance to either the maintenance of SCC or in the genesis of the LBK response. On different occasions the effect of piretanide (a Na⁺-K⁺-Cl⁻ co-transport inhibitor) and acetazolamide (a carbonic anhydrase inhibitor) on either basal or stimulated SCC was tried (e.g. Fig. 3). No effects of these agents were found except a minor stimulation of SCC following addition of piretanide.

**Ionic basis of the short-circuit current responses**

The pattern of responses of sweat gland cell sheet cultures resembles anion secretory mechanisms in other epithelial systems such as primary cultures of canine tracheal epithelium (Welsh, 1985), MDCK epithelial monolayers (Brown & Simmons, 1981), and rat colon (Cuthbert & Margolius, 1982). Unexpected characteristics found here were the failure of sweat gland monolayers to respond to forskolin and the lack of effect of blockers of Na⁺-K⁺-Cl⁻ co-transport and of carbonic anhydrase (Fig. 3). Additionally, the basal current was sensitive to amiloride which suggested that electrogenic sodium absorption was, at least in part, responsible for basal SCC.

To discover the ionic basis for the SCC responses experiments were carried out in which individual ions were substituted and responses compared to those in normal KH solution. Throughout we used LBK (0.1 μM) and carbachol (10 μM) as stimulating agents.

Figure 5 shows two examples of responses in chloride-free KH solution in which
Fig. 4. Illustration of apparent lack of inhibitory action of piroxicam (Pirox, 10 μM), on the responses to LBK (0·1 μM). Piroxicam was added to the fluid bathing both sides of the tissue while LBK and PGE₂ (1 μM) were added only to the basolateral bathing fluid. Piroxicam was added only to the tissue illustrated in the upper tracing. Both cultures were from the same batch. Horizontal lines indicate zero SCC.

Fig. 5. Examples of SCC records obtained in chloride-free KH solution. Both cultures were 5 days old. Resistance and potential difference were 65 Ω cm² and −0·8 mV (upper) and 75 Ω cm² and −1·0 mV (lower). Drugs were added to the basolateral side except acetazolamide (both sides) and amiloride (apically). Drug concentrations were: LBK, 0·1 μM; carbachol (CCh), 10 μM; acetazolamide (Acet), 0·45 mM; and amiloride (Amil), 10 μM. Horizontal lines indicate zero SCC.
Table 2. Comparison of SSC response to LBK and carbachol in whole-gland sheet cultures using KH, chloride-free KH and chloride–bicarbonate-free KH bathing solutions

| bathing solutions       | LBK (µA cm⁻²) | Carbachol (µA cm⁻²) |
|-------------------------|---------------|---------------------|
| KH                      | 6.00 ± 0.77 (14) | 7.81 ± 1.65 (8)    |
| Cl⁻-free KH             | 4.50 ± 0.81 (5)  n.s. | 5.20 ± 0.87 (5)  n.s. |
| Cl⁻–HCO₃⁻-free KH       | 7.42 ± 1.62 (5)  n.s. | 7.12 ± 1.64 (5)  n.s. |

Values are peak current responses to both agents after basolateral additions. Concentrations used were: LBK, 0·1 µM; carbachol, 10 µM (unpaired Student’s t test; n.s., not significant). Number of separate measurements given in parentheses.

![Graph](image-url)

**Fig. 6.** Examples of SCC records obtained in chloride–bicarbonate-free KH solution. Both cultures were 5 days old and had identical resistances and potential differences (100 Ω cm² and −1.8 mV). Drug concentrations were: LBK, 0·1 µM; carbachol (CCh), 10 µM; and amiloride (Amil), 10 µM. The latter was added to the apical side only while the other two were added to the basolateral bath. Amiloride was added either before (upper) or after (lower) the agonists. Horizontal lines indicates zero SCC.

Gluconate was substituted for chloride. This manoeuvre did not prevent a response to either agent and Table 2 shows that the responses in this solution were not different from those obtained in normal KH. In some epithelia anion secretory mechanisms can employ bicarbonate when chloride is absent (Grasl & Turnheim, 1984; Cuthbert & Hickman, 1985). Although acetazolamide (0·45 mM) had no effect during the plateau response to carbachol in chloride-free KH solution, experiments were also made in which both chloride and bicarbonate were substituted in the bathing solution. Table 2 and Fig. 6 illustrate that responses to LBK and carbachol were not reduced by the virtually complete removal of permeant anions.

In a final series of substitution experiments sodium ions were replaced either with choline or NMDG. The likely importance of sodium was already a possibility since using chloride- and bicarbonate-free KH solution we found that amiloride pre-treatment significantly inhibited subsequent responses to LBK and carbachol (P
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Table 3. Effect of amiloride on SCC responses to LBK and carbachol in chloride–bicarbonate-free KH

|       | LBK (µA cm⁻²) | Carbachol (µA cm⁻²) |
|-------|---------------|---------------------|
| (A)   |               |                     |
| Control | 7·42 ± 1·62  (5) | 7·12 ± 1·64  (5)  |
| Amiloride pre-treatment | 1·98 ± 0·17  (5)* | 1·96 ± 0·18  (5)* |
| (B)   |               |                     |
| Control | 8·40 ± 1·35  (4) | 5·80 ± 0·80  (4)  |
| Amiloride pre-treatment | 1·97 ± 0·10  (3)* | 1·94 ± 0·07  (3)* |

In A, responses were pooled from two batches of cultures. In B, responses were obtained within a single batch. Values are peak height SCC responses to basolateral additions of LBK (0·1 µM) and carbachol (10 µM). *P < 0·01, unpaired Student’s t test. Number of separate observations given in parentheses.

Fig. 7. SCC recordings from 5-day-old cultures bathed in chloride–bicarbonate-free KH solution. Resistance and potential difference measurements were: 125 Ω cm² and −1·5 mV (upper record) and 100 Ω cm² and −1·3 mV (lower record). Both records illustrate the effect of LBK (0·1 µM) and carbachol (CCh, 10 µM) before and after amiloride with washing (W) in between. In one experiment amiloride was added first before LBK and CCh while in the other the order was reversed. Only amiloride was added to the apical bathing solution; all other agents were added to the basolateral side. Amiloride (Amil) and atropine (Atr) were used at 10 µM and 10 nm respectively. Horizontal lines indicate zero SCC.

< 0·01) (Table 3). A typical example is shown in Fig. 6. Further evidence that LBK or carbachol caused stimulation of electrogeneric sodium absorption could be obtained in single preparations which were robust enough to allow washing. Figure 7 shows two examples of this. Amiloride almost abolished the responses to LBK and carbachol, the response to the latter being restored after the blocker was removed. Responses to LBK were not restorable as exposure to the peptide causes long-duration desensitization, as found for other epithelia (Cuthbert et al. 1987).

Replacement of sodium by choline was unsatisfactory as preparations became
unstable in this medium. Additionally, choline caused problems in relation to assessing the actions of carbachol, because of the weak muscarinic activity of the former. NMDG proved to be a satisfactory sodium substitute and results of five experiments with cultures from a single batch of cells are shown in Fig. 8. All had SCCs close to zero and LBK and carbachol had no effect in the absence of sodium. Addition of isotonic saline to give a final sodium concentration of 25 mM caused an immediate increase in SCC which was sensitive to amiloride (A). If sodium was added first the epithelium responded to both agents, even though the sodium concentration was only 25 mM (B). A further variation was to add sodium after one and before the other of the stimulating agents (C and D). Again responses were obtained only in the presence of sodium. Finally, prior addition of amiloride prevented the increase or SCC following the addition of sodium (E).
All five experiments were conducted at day 6 after seeding with cells from the same batch and therefore they may be presumed to represent tissues with very similar properties. Of the twenty additions made in the five experiments illustrated on Fig. 8, the responses were, without exception, consistent with the hypothesis that LBK and carbachol promote electrogenic sodium absorption in this tissue.

In several other experiments the effect of addition of ouabain (1 \( \mu \text{M} \)), basolaterally, on SCC was observed. The SCC declined slowly to a low value over 15–20 min, at which time amiloride caused no substantial further change in current. This finding is consistent with a model for active sodium transport as discussed later.

**Properties of secretory coil sheets in culture**

Microdissected secretory coils have been used to produce epithelial cultures using the same protocols as for whole glands. These preparations too had a basal SCC sensitive to amiloride, indicative of electrogenic sodium absorption. Concentration–response relationships for amiloride have been established for preparations produced from secretory coil and from whole glands. Similar values were obtained for the concentrations producing 50% of the maximal inhibition (EC\(_{50}\)), namely 0.8 \( \mu \text{M} \) for secretory coil preparations and 0.4 \( \mu \text{M} \) for whole-gland-derived monolayers (Fig. 9). The secretory coil preparations also responded to LBK and carbachol.

**DISCUSSION**

Three other groups have recently developed methods for growing sweat gland epithelial cells in culture using different methods for the assessment of transport function. Collie *et al.* (1985) and Lee *et al.* (1986) used hemicyst formation as an indication of transport function, while Pedersen & Larsen (1986) cultured cells on dialysis membranes in order to measure transepithelial movement. More recently Pedersen, Larsen, Hainau & Brandt (1987) have reported SCC measurements from cultured sweat gland ducts. As far as we are aware no previous studies of
continuously short-circuited epithelia have been made with sheet cultures of whole glands or coils.

The choice of tissue area (0.2 cm²) was a compromise between obtaining reasonable currents while economizing with limited amounts of cell suspensions. While the changes in SCC in response to various agonists can be measured with great accuracy (10 nA) the measurement of basal SCC is more problematical. The DVC 100 voltage clamp indicates transepithelial potential difference to within 0.1 mV, representing the maximum possible error in this parameter. As the mean value for the resistance was 75 Ω cm² then the basal SCC may be in error by 0.25 μA. Throughout we have indicated on the records the position of zero SCC for reference purposes. However amiloride did not always reduce SCC to zero, there often being residual currents of the magnitude given above.

The values for transepithelial potential and resistance given here are both substantially smaller than those found by Pedersen, Brant & Hainau (1985), who obtained values of −20 to −30 mV. These are greater than the values of −7 to −10 mV found for perfused isolated absorptive ducts (Quinton, 1983; Bijman & Quinton, 1987). A possible reason for the difference is that Pedersen et al. (1985) cultured only the absorptive duct. With regard to the resistance values we were not able to record any change in transepithelial conductance following amiloride, suggesting that our measurements of resistance were dominated by the intercellular pathways, part of which must relate to the mating between the edge of the culture and the silicone washer; this has not been measured. From a structural study of the tight junctions in human sweat glands (Briggman, Bank, Bigelow, Graves & Spicer, 1981), together with the relation between strand number and epithelial resistance (Claude, 1978), a ductal resistance of 300 Ω cm² was predicted while Pedersen et al. (1985) measured values of 500–1000 Ω cm². From cable analysis measurements with perfused human sweat ducts a value of only 10 Ω cm² was obtained which increased after the luminal application of amiloride (Bijman & Fromter, 1986). Additionally, we were not able to observe any tight junctions, although the apical edges of the cells were closely opposed, with the intervening space filled with a light staining material (Fig. 1E). Finally, a major problem with cultured sheets is that small, non-confluent areas, which cannot be viewed microscopically on the supports, short circuit the transepithelial potential difference as well as increase conductance. Nevertheless, in spite of the uncertainties about resistance, sheet cultures do provide the simplest system yet available for quantitative analysis, having many advantages over perfused segments.

The multilayered structure of the cultures is reminiscent of the sodium-absorbing epithelia of amphibian skin, as well as of the human epidermis. In the former function is the responsibility of the first reactive layer (Ussing & Windhager, 1964) so that the syncytial structure behaves as a functional monolayer.

It is likely from a variety of evidence (see Sato, 1977) that sweat glands are surrounded by nerve endings releasing both acetylcholine and catecholamines; further it is well known that acetylcholine and catecholamines increase sweating. From the use of simple in vivo sweat collection and analysis techniques a view of sweat gland function has emerged. A primary fluid is generated in the secretory coil, the composition of which is then modified as the sweat passes along the absorptive
duct. Theoretical modelling of these processes has been attempted (Schwartz & Thayssen, 1955; Slegers, 1967), particularly to relate sweat rate to the sodium concentration of the sweat. Important parameters are the Michaelis constant \( K_m \) for sodium of the absorptive process and the maximal absorptive capacity. Whether or not sodium absorption in the duct is limited by apical entry of sodium ions or the availability of basolateral pumps is unknown; however, our data show that the absorptive process in cultured sweat gland epithelia can be stimulated by humoral agents. This does not imply that such control necessarily occurs in vivo.

In regard to adrenergic innervation of sweat glands there is evidence that catecholamines affect secretion (Sato, 1977) and that they can also modify the chloride permeability of sweat duct monolayers (Pedersen & Larsen, 1986). In contrast, Quinton (1987), using microperfused sweat ducts, suggested that isoprenaline may stimulate sodium absorption. In our system responses to the \( \beta \)-adrenoceptor agonist, isoprenaline, have been variable, including no response when other agonists were effective, as also found by Quinton (1987). It has not been possible, therefore, to investigate these responses systematically. Effects in SCC would not necessarily be seen if the effect of catecholamines were only on counter-ion permeability. However, as forskolin too was ineffective a possible explanation is that the responses to isoprenaline represent modest \( \alpha \)-adrenoceptor stimulation working through similar second-messenger systems as does carbachol.

Of the responses we have been able to investigate systematically, that is to carbachol and LBK, there can be little doubt that they represent stimulation of an electrogenic sodium-absorbing process. Briefly the evidence is failure to be modified by chloride and bicarbonate removal, inhibition by sodium substitution and inhibition by amiloride. Taken together with the sensitivity of the basal SCC to amiloride and the near-zero SCCs obtained in sodium-free medium we have no evidence to suggest that the SCC in these monolayers is due to anything other than an inwardly directed sodium absorbtion. Therefore, the monolayers developed with our conditions exemplify a classical sodium-absorbing epithelium (Ussing & Zerahn, 1951) using energy derived from a sodium–potassium ATPase located on the basolateral membranes. The location of ouabain binding sites on the basolateral membranes of intact sweat ducts (Quinton & Tormey, 1976) and the effect of ouabain on SCC reported here add further support to the classical model, as indeed suggested by others (Bijman & Quinton, 1984).

It is of particular interest that our tissues were derived from whole glands. Their properties have been compared, in a limited way, with those derived only from the secretory coil. Both types of preparation show some of the characteristics of ductal epithelium indicating the pleuripotential character of secretory cells in culture. Cultures made only from duct also are similar, showing a cholinoreceptor-mediated SCC which was amiloride sensitive (Pedersen et al. 1987).

The effects of carbachol were shown to be due to an action at muscarinic receptors from the high potency of atropine as a blocking agent. In other epithelia activation of muscarinic receptors leads to raised intracellular calcium concentrations \([Ca^{2+}]_i\) through activation of a G protein and hydrolysis of phosphatidylinositol (Streb, Irvine, Berridge & Schultz, 1983). Here we have shown that the calcium ionophore, A23187, can also increase amiloride-sensitive SCC. Sato & Sato (1981) using the
isolated monkey sweat gland found that secretion caused by methacholine and isoprenaline was calcium dependent.

However results presented here differ in an important way from the examples given above in that carbachol stimulates sodium absorption and not a secretory process. We know of no such other examples in mammalian epithelia, although muscarinic agonists increase sodium absorption in frog skin epithelium (Cuthbert & Wilson, 1981) probably by adenylate cyclase activation and eicosanoid formation. Although from a functional viewpoint it would appear appropriate to increase ductal reabsorption at the same time as secretory activity was increased as a means of reducing salt loss while promoting efficient sweating, there is as yet no information to suggest that the absorptive process is under cholinergic control in vivo.

As far as it is possible to compare the transepithelial transport of ions in cultured sweat glands, whether they be derived from whole gland, secretory coil or duct, they appear to behave very similarly, showing an amiloride-sensitive SCC which can be stimulated by cholinocceptor agonists. This means it is not possible to distinguish between cultures which are characteristically ductal or show ductal characteristics. For example, Bell, Jones & Quinton (1987) showed that some cells in coil cultures are sensitive both to amiloride and methacholine, while duct cultures are sensitive only to amiloride. In contrast, Pedersen et al. (1987) showed that duct cultures are sensitive to cholinocceptor agents. At this stage, therefore, it is not possible to know if in culture ductal cells take on the characteristics of secretory coil cells or vice versa or, indeed, if epithelial cells of whatever type revert to a more primitive form in culture. Nevertheless, cultured cells have been important for the investigation of cellular transport mechanisms in human sweat glands and have led to insightful observations in relation to cystic fibrosis.

This is the first report of a definitive effect of LBK on the sweating process. Transient effects of bradykinin on sweat formation in the horse, donkey and cow were attributed to an effect upon the contraction of myoepithelial cells (Johnson, 1975), an effect which cannot be relevant in this situation. Gordon & Schwarz (1971) found that the high sodium concentration in the first drops of sweat formed at low secretion rates, characteristic of stimulation by many sudorific agents, could be corrected by bradykinin and cyclic AMP. Arginine esterases which can generate kinin have been found in eccrine sweat, one of which is a typical kallikrein (Fraki, Jensen & Hopsu-Havu, 1970) and a deficiency in arginine esterases in cystic fibrosis is reported (Rao, Posner & Nadler, 1972).

Typically, kinins stimulate electrogenic anion secretion of either chloride (Cuthbert & Margolius, 1982; Cuthbert et al. 1987) or of bicarbonate (Baird & Margolius, 1987). These effects may be dependent (Cuthbert & Margolius, 1982) or independent (Cuthbert et al. 1987) of eicosanoid formation. In sweat gland cultures the effect of LBK was not modified by cyclo-oxygenase inhibition. As with carbachol the effect of LBK was to increase electrogenic sodium absorption. At this stage there is no information about the second messengers involved although in other cultured epithelial cells bradykinin induces increased phosphatidylinositol turnover, again suggesting that raised $[\text{Ca}^{2+}]_i$ is crucial for the effect (Shayman & Morrison, 1985). In cultured MDCK cells an increase in intracellular calcium has been directly demonstrated in response to bradykinin (Pidakiti, Gamero, Gamero & Hassid, 1985).
which leads to the opening of calcium-sensitive K⁺ channels in the basolateral membranes (Paulmichl, Friedrich & Lang, 1987).

Carbachol and LBK, agents which have been most intensively studied here, have their receptors on the basolateral faces only of the epithelial cells. This seems entirely appropriate for muscarinic receptors sensing neuronally released acetylcholine. It is unknown where kinins might be generated in relation to the sweat glands although it might be expected that kininogen is present in tissue fluids. In both cultures from whole glands and secretory coil the potency of amiloride is similar and comparable to its activity in other epithelial systems (Cuthbert & Spayne, 1983; Cuthbert & Fanelli, 1987). It is now necessary to repeat the experiments reported here using cultures prepared from cystic fibrosis tissue to examine for important differences. The simple cellular system we describe may become a useful tool for assessing agents in the search for a therapeutic strategy for cystic fibrosis.

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REFERENCES

BAIRD, A. W. & MARGOLIUS, H. S. (1987). Bradykinin stimulates electrogenic bicarbonate secretion by guinea-pig gallbladder. British Journal of Pharmacology 91, 369 P.

BAKER, B. J. & SILVERTON, R. E. (1976). Introduction to Medical Laboratory Technology, 5th edn, pp. 404–405. London and Boston: Butterworths.

BELL, C. L., JONES, C. J. & Quinton, P. M. (1987). Characterisation of human eccrine sweat gland secretory coil and duct cells in culture. Pediatric Pulmonology, suppl. 1, 112.

BIJMAN, J. & FROMTER, E. (1986). Direct demonstration of high transepithelial chloride-conductance in normal human sweat duct which is absent in cystic fibrosis. Pflügers Archiv 407, S123–127.

BIJMAN, J. & Quinton, P. (1984). Influence of abnormal Cl⁻ permeability on sweating in cystic fibrosis. American Journal of Physiology 247, C3–9.

BIJMAN, J. & Quinton, P. (1987). Permeability properties of cell membranes and tight junctions of normal and cystic fibrosis sweat ducts. Pflügers Archiv 408, 505–510.

BRIGMAN, J. V., BANK, H. L., BIGELOW, J. B., GRAVES, J. S. & SPICER, S. S. (1981). Structure of the tight junctions of the human eccrine sweat gland. American Journal of Physiology 240, 357–368.

BROWN, C. D. A. & SIMMONS, N. L. (1981). Catecholamine-stimulation of Cl⁻ secretion in MDCK cell epithelium. Biochimica et biophysica acta 649, 427–435.

CLAUDE, P. (1978). Morphological factors influencing transepithelial permeability: a model for the resistance of the zonula occludens. Journal of Membrane Biology 39, 219–232.

COLLIE, G., BUCHWALD, M., HARPER, P. & RIORDAN, J. R. (1985). Culture of sweat gland epithelial cells from normal individuals and patients with cystic fibrosis. In Vitro Cellular and Developmental Biology 21, 597–602.

CUTHBERT, A. W., EGLEME, C., GREENWOOD, H., HICKMAN, M. E., KIRKLAND, S. C. & MACVINISH, L. J. (1987). Calcium- and cyclic AMP-dependent chloride secretion in human colonic epithelia. British Journal of Pharmacology 91, 503–515.

CUTHBERT, A. W. & FANELLI, G. M. (1978). Effects of pyrazinecarboxamides on sodium transport in frog skin. British Journal of Pharmacology 63, 139–149.

CUTHBERT, A. W., GEORGE, A. M. & MACVINISH, L. J. (1985). Kinin effects on electrogenic ion transport in primary cultures of pig renal papillary collecting tubule cells. American Journal of Physiology 249, F439–447.

CUTHBERT, A. W. & HICKMAN, M. E. (1985). Indirect effects of adenosine triphosphate on chloride secretion in mammalian colon. Journal of Membrane Biology 86, 157–166.
Cuthbert, A. W. & Margoliuc, H. S. (1982). Kinins stimulate net chloride secretion by the rat colon. *British Journal of Pharmacology* 75, 587–597.

Cuthbert, A. W. & Spayne, J. A. (1983). Conversion of sodium channels to a form sensitive to cyclic AMP by component(s) from red cells. *British Journal of Pharmacology* 79, 783–797.

Cuthbert, A. W. & Wilson, S. A. (1981). Mechanisms for the effects of acetylcholine on sodium transport in frog skin. *Journal of Membrane Biology* 59, 65–75.

Fraki, J. E., Jensen, C. T. & Hopsu-Havu, V. K. (1970). Human sweat kallikrein. *Acta dermatovenereologica* 50, 321–326.

Gordon, C. & Schwartz, V. (1971). Cyclic AMP, bradykinin and sweat gland function. *Journal of Physiology* 213, 68–69 P.

Grasl, M. & Turnhein, K. (1984). Stimulation of electrolyte secretion in rabbit colon by adenosine. *Journal of Physiology* 346, 93–110.

Johnson, K. G. (1975). Sweat gland function in isolated perfused skin. *Journal of Physiology* 250, 633–649.

Lee, C. M., Carpenter, F., Coaker, T. & Kealey, T. (1986). The primary culture of epithelia from the secretory coil and collecting duct of normal human and cystic fibrotic eccrine sweat glands. *Journal of Cell Science* 83, 103–118.

Manning, D., Snyder, S. H., Kachur, J. F., Miller, R. J. & Field, M. (1982). Bradykinin receptor mediated Cl secretion in the intestine. *Nature* 299, 256–259.

Paulmichl, M., Friedrich, F. & Lang, F. (1987). Effects of bradykinin on electrical properties of Madin–Darby canine kidney epithelioid cells. *Pflügers Archiv* 408, 408–413.

Pedersen, P. S. (1984). Primary culture of epithelial cells derived from the reabsorptive coiled duct of human sweat glands. *International Research Communications System Medical Science* 12, 752–753.

Pedersen, P. S., Brandt, N. J. & Hainau, B. (1985). Differentiated function in primary epithelial culture derived from the coiled reabsorptive segment of human sweat glands. *International Research Communications System Medical Science* 12, 752–753.

Pedersen, P. S. & Larsen, E. H. (1986). Effect of isoproterenol on ion transport in cell culture epithelial membranes derived from human sweat gland ducts. *International Research Communications System Medical Science* 14, 108–109.

Pedersen, P. S., Larsen, E. H., Hainau, B. & Brandt, N. J. (1987). Transepithelial ion transport in sweat duct cell cultures derived from normals and patients with cystic fibrosis. *Medical Science Research: Biochemistry* 15, 1009–1016.

Pidikiti, N., Gamero, D., Gamero, J. & Hassid, A. (1985). Bradykinin-evoked modulation of cytosolic Ca2+ concentrations in cultured renal epithelial (MDCK) cells. *Biochemical and Biophysical Research Communications* 130, 807–813.

Quinton, P. M. (1983). Chloride impermeability in cystic fibrosis. *Nature* 301, 421–422.

Quinton, P. M. (1987). Physiology of sweat secretion. *Kidney International* 32, suppl. 21, S102–108.

Quinton, P. M. & Tormey, J. McD. (1976). Localization of Na/K-ATPase sites in the secretory and reabsorptive epithelia of perfused eccrine sweat glands: a question to the role of the enzyme in secretion. *Journal of Membrane Biology* 29, 383–399.

Rao, G. J. S., Posner, L. A. & Nadler, H. L. (1972). Deficiency of kallikrein activity in plasma of patients with cystic fibrosis. *Science* 177, 610–611.

Sato, K. (1977). The physiology, pharmacology and biochemistry of the eccrine sweat gland. *Review of Physiology, Biochemistry and Pharmacology* 79, 52–131.

Sato, K. & Sato, F. (1981). Role of calcium in cholinergic and adrenergic mechanisms of eccrine sweat secretion. *American Journal of Physiology* 241, C113–120.

Sato, K. & Sato, F. (1984). Defective beta-adrenergic response of cystic fibrosis sweat glands in vivo and in vitro. *Journal of Clinical Investigation* 73, 1763–1771.

Schultz, I. J. (1969). Micropuncture studies of the sweat formation in cystic fibrosis patients. *Journal of Clinical Investigation* 48, 1470–1477.

Schwartz, I. L. & Thaysen, J. H. (1955). Excretion of sodium and potassium in human sweat. *Journal of Clinical Investigation* 34, 114–120.

Shayman, J. A. & Morrison, A. R. (1985). Bradykinin-induced changes in phosphatidylinositol turnover in cultured rabbit papillary collecting tubule cells. *Journal of Clinical Investigation* 76, 975–984.
SLEGERS, J. F. (1967). A mathematical approach to the two step reabsorption hypothesis. *Bibliotheca paediatrica (Basel)* **86**, 74–88.

STREB, H., IRVINE, R. F., BERRIDGE, M. J. & SCHULTZ, I. (1983). Release of Ca$^{2+}$ from a non-mitochondrial intracellular store in pancreatic acinar cells by inositol-1,4,5-triphosphate. *Nature* **306**, 67–69.

USSING, H. H. & WINDHAGER, E. E. (1964). Nature of shunt path and active sodium transport path through frog skin epithelium. *Acta physiologica Scandinavica* **61**, 484–504.

USSING, H. H. & ZERAHN, K. (1951). Active transport of sodium as the source of electric current in the short-circuited isolated frog skin. *Acta physiologica Scandinavica* **23**, 110–127.

WELSH, M. J. (1985). Ion transport in primary cultures of canine tracheal epithelium: methodology, morphology and electrophysiology. *Journal of Membrane Biology* **88**, 149–163.

WELSH, M. J., MCCANN, J. D. & DEARBORN, D. G. (1987). Chloride and sodium channel currents in normal and cystic fibrosis sweat duct epithelium. *Federation Proceedings* **46**, 1271 (abstract 5567).