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Short communication

T_{84} epithelial cells respond to 5-hydroxytryptamine when grown in serum-free media

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Abstract

The aim of this study was to establish the cause of insensitivity of T_{84} human colonic epithelial cells to 5-hydroxytryptamine (5-HT). Monolayers of T_{84} cells were placed in modified Ussing chambers for measurement of short-circuit current, an index of secretion. When grown in serum-supplemented media, T_{84} cells gave secretory responses to acetylcholine and forskolin but not to 5-HT. When grown in AIM V serum-free media, T_{84} cells responded to 5-HT. Chromatographic analysis with fluorimetric detection showed high levels of 5-HT (1.8 μM) in the serum. This contamination is probably responsible for subsequent desensitization of T_{84} cells to 5-HT.

Keywords: 5-Hydroxytryptamine (5-HT, serotonin); Desensitization; Serum; T_{84} cell

1. Introduction

The T_{84} cell line was originally derived from lung metastases in a patient with a colonic adenocarcinoma. The cells grow as confluent monolayers, retain cellular polarity, form tight junctions, and exhibit directional ion transport when grown in serum containing media. The cells can be grown in serum-free media, but instead of monolayers, gland-like structures were formed which closely resembled the original tumour morphology (Murakami and Masui, 1980). The T_{84} cell shows a close structural resemblance to the human colonic crypt cell for which it serves as a model for ion transport studies (Dharmsathaphorn and Madara, 1990; Dharmsathaphorn et al., 1984). T_{84} cells have subsequently been shown to respond to a wide variety of neurotransmitters and hormones with a chloride secretory response, and as such, have gained wide acceptance as a general model of intestinal transepithelial chloride transport (Barrett, 1993). However, T_{84} cells do not respond to 5-hydroxytryptamine (5-HT) (Dharmsathaphorn et al., 1984) despite the fact that 5-HT is an effective intestinal secretagogue (Cooke and Reddix, 1994) which causes non-neural secretory responses in human isolated colonic mucosa (Borman and Burleigh, 1996). A possible explanation may have been that the cells were tested for their sensitivity to 5-HT only 40–56 h after seeding onto the semi-permeable membranes used for the ion transport studies. Alternatively, it was suggested that foetal bovine serum might contain sufficient 5-HT to desensitize T_{84} cells to the indolealkylamine (Hamilton, personal communication). The purpose of this investigation was to determine whether T_{84} cells can be made to respond to 5-HT by manipulating the conditions under which they are grown.

2. Materials and methods

T_{84} cells were obtained from the European Collection of Cell Cultures (ECACC) and used between passages 70 and 85. They were cultured in Dulbecco's modified Eagle's medium/Ham's F12 (1:1) supplemented with: *N*-2-hydroxyethylpiperazine-*N*′-2-ethane sulfonic acid (HEPES) (15 mM), $NaHCO_3$ (1.2 g l^{-1}), L-glutamine (2 mM), penicillin (100 units ml^{-1}), streptomycin (0.1 mg.ml^{-1}), and 10% foetal bovine serum at 37°C in a humidified atmosphere of 5% CO_2 in 95% room air. Cells were initially grown in 75 cm^2 flasks and media was changed

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every 2–3 days. On reaching 95–100% confluence, cells were washed twice with Ca^{2+}/Mg^{2+}-free phosphate buffered saline and detached using 0.25% trypsin plus 0.9 mM ethylenediaminetetra-acetic acid (EDTA). They were then subcultured in a 1:3 ratio. The following protocol was used for growing cells in serum-free media; recommended concentrations of AIM V (registered trade name), Episerf and Defined Keratinocyte media were used and supplemented with L-glutamine and antibiotics as above. Observation of the 75-cm^2 flasks revealed that all three types of media supported cell growth. To assess the adaptation from serum-containing to serum-free media, cells were initially grown in all three types of media, each of which was made up to contain 5%, 2% or 0% foetal bovine serum. Cells appeared to grow as readily in serum-free media as in media containing 2% or 5% foetal bovine serum.

For Ussing chamber experiments, cells (10^6) were seeded onto collagenised (100 $\mu g\ ml^{-1}$) semi-permeable membranes (Costar Snapwell Culture Inserts, 0.4 μM pore size, 12 mm internal diameter) and cultured immediately in serum-free media. Monolayers were used 9 or 11 days after seeding cells into inserts; the last media change contained no antibiotics and media was not changed the day before an experiment. Inserts were placed into specially designed Ussing chambers (Snap Chamber, World Precision Instruments) with 10 ml of Krebs buffer, bathing both sides of the monolayer. Drugs were added basolaterally and no monolayer received more than one concentration of a given compound. Monolayers were clamped at zero potential by a high impedance voltage clamp (DVC-1000, World Precision Instruments) and transmural short-circuit current was measured and continuously recorded. Transepithelial resistance was calculated from the change in short-circuit current when monolayers were intermittently clamped for 20 s every 5 min at 2 mV. Both current passing and voltage detecting electrodes utilised a system of silver–silver chloride half-cells connected to large diameter agar bridges (4% agar in modified Krebs buffer, i.e., minus calcium and glucose). Determination of 5-HT levels in foetal bovine serum used reversed phase high-pressure liquid chromatography (HPLC) with fluorimetric detection (Bearcroft et al., 1995). All data are expressed as arithmetic mean $\pm$ S.E.M., n = number of monolayers. Statistical comparisons used the Mann–Whitney U-test with $P < 0.05$ being taken to represent a significant difference.

2.1. Drugs and reagents

AIM V (registered trade name), Episerf and Defined Keratinocyte media were all obtained from Life Technologies (Gibco BRL); all other culture consumables obtained from Sigma Type 1 rat trail collagen, 5-HT creatinine sulphate, acetylcholine chloride, forskolin, and dimethyl sulfoxide (DMSO) were also obtained from Sigma.

3. Results

When grown in serum-supplemented media, basal short-circuit current was 1.0 ± 0.3 and conductance was 2.3 ± 0.1 mS cm^{-2} (equivalent to a resistance of 441 ± 17 Ω) after an equilibration period of 30 min ($n = 54$). Basal short-circuit current was not affected by duration of culture whereas a maximum resistance of 538 ± 35 Ω was attained after 7 days ($n = 12$). When cells were grown in the presence of serum, 5-HT (1–100 μM, $n = 6$) had no significant effect on basal short-circuit current ($P > 0.05$, Fig. 1). Forskolin (25 μM) tested on the same monolayer produced a rise in short-circuit current of 65 ± 9 $\mu A\ cm^{-2}$ ($n = 9$) while forskolin vehicle (DMSO) was without effect ($n = 4$). In contrast to 5-HT, acetylcholine (1–100 μM, $n = 6$) produced significant, concentration-dependent increases in short-circuit current ranging from 4.0 ± 0.9 to 11.8 ± 1.6 $\mu A\ cm^{-2}$ ($P < 0.05$ compared to aqueous vehicle, Fig. 1).

Analysis of serum for 5-HT content revealed levels of 1.8 μM which may explain the insensitivity of T_{84} cells to the indolealkylamine. For this reason an attempt was made to culture T_{84} cells in three types of serum-free media: AIM V, Episerf and Defined Keratinocyte. Cells grew to confluence and appeared normal when grown in plastic flasks in all three types of media. However, only with AIM V media, was a monolayer transepithelial resistance recorded that approached that was attained when cells were grown in serum supplemented media. Transepithelial resistances for monolayers grown in both Episerf and Defined Keratinocyte medium were much lower than values obtained in serum supplemented media (15 ± 3 $n = 7$ and 58 ± 12 $n = 8$, respectively, $P < 0.05$). Ussing experiments were therefore carried out on cell monolayers grown in AIM V media. Following incubation in AIM V media basal short-circuit current was 2.0 ± 0.6 $\mu A\ cm^{-2}$ and

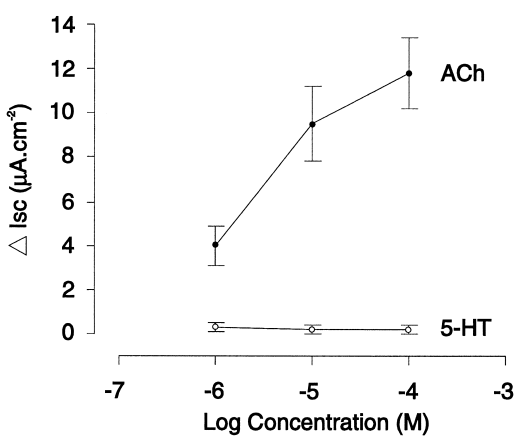

Fig. 1. Comparison of the sensitivity of T_{84} cell monolayers, grown in foetal bovin serum, to 5-hydroxytryptamine (5-HT, ○) and acetylcholine (ACh, ●). Secretory responses were measured as increases in short-circuit current (I_{sc}), and expressed as mean $\pm$ S.E.M. from $n = 6$ monolayers.

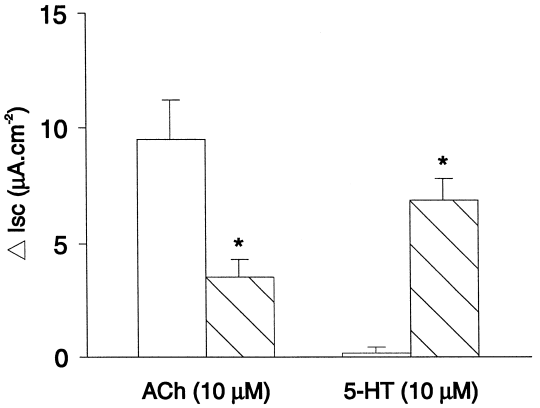

Fig. 2. Effect of 5-hydroxytryptamine (5-HT, 10 μM) and acetylcholine (ACh, 10 μM) on T_{84} cell monolayers grown in serum supplemented (□, $n = 6$) and serum-free media (▨, $n = 10$). Secretory responses measured as increases in short-circuit current (I_{sc}) and expressed as mean ± S.E.M. * Indicates significant difference between serum-supplemented and serum-free media, $P < 0.05$.

monolayer resistance was 378 ± 26 Ω after an equilibration period of 30 min ($n = 19$). 5-HT (10 μM, $n = 10$) and acetylcholine (10 μM, $n = 10$) produced significant increases in short-circuit current of 6.9 ± 0.9 and 3.5 ± 0.8 μA cm^{-2}, respectively, when compared to aqueous vehicle ($P < 0.05$, Fig. 2). Forskolin (25 μM) increased short-circuit current by 78 ± 5 μA cm^{-2} ($n = 13$).

4. Discussion

Although T_{84} cells serve as a model for ion transport by human colonic mucosa, there are differences in their electrical properties. Firstly, T_{84} cells have the functional characteristics of intestinal crypt cells, which are mainly secretory in function. The lack of differentiation of T_{84} cells into absorptive cells is reflected in the virtual absence of a basal short-circuit current. Secondly, in contrast to short-circuit current, the transepithelial resistance developed by monolayers of T_{84} cells is significantly higher than the resistance across the multi-cell thickness preparations of colonic mucosa (Borman and Burleigh, 1996). Despite their significance, these differences in basal electrical properties are unlikely to result in insensitivity to 5-HT as T_{84} cells respond to a wide range of secretagogues which activate basolaterally located receptors to produce transepithelial transport of chloride ions and resultant increases in short-circuit current (Binder and Sandle, 1994).

T_{84} monolayers comprise a homogenous population of epithelial cells, there are no neural or endocrine elements and no possibility of regional differences in function which may well exist between human proximal and distal colon. This latter point is relevant as the location of the primary tumour, from which the T_{84} cells originated, is unknown and therefore it is not possible to say whether T_{84} cells reflect the function of proximal or distal human colonic crypt cells. However, as both ascending and sigmoid colon give quantitatively similar concentration-dependent increases in short-circuit current when exposed to 5-HT, the absence of responses of T_{84} cells to 5-HT cannot be ascribed to T_{84} cells originating from a region of the colon which is relatively insensitive to 5-HT. Similarly, as tetrodotoxin did not reduce secretory responses of human colonic mucosa to 5-HT (Borman and Burleigh, 1996), then the lack of responsiveness of T_{84} cells to 5-HT cannot be ascribed to the compound acting on neural elements in intact mucosal sheets. In their original characterisation of the electrolyte transporting properties of T_{84} cells, Dharmsathaphorn et al. (1984) almost certainly did not allow sufficient time for cells to reach maturity (40–56 h); for instance, it was shown in a later publication that maximum transepithelial resistance required almost three times as long to develop (Dharmsathaphorn and Madara, 1990). However, we have shown that sensitivity to 5-HT cannot be increased by allowing longer incubation times for monolayers. 5-HT stimulates intact colonic mucosa by an action on 5-HT_{2A} receptors (Borman and Burleigh, 1996). As T_{84} cells were sensitive to acetylcholine, which like 5-HT_{2A} receptor stimulation, acts via the phosphoinositide system, it is possible that 5-HT receptors may not be expressed in this undifferentiated cell line or if they are present, are not functionally linked to the second messenger. At this point in the investigation, it was pointed out that foetal bovine serum can contain high levels of 5-HT despite being heat-treated (Hamilton, personal communication; Wood et al., 1997). We confirmed this was the case with the batch of serum used in the present investigation. Even allowing for dilution in making up the media, cells would have been continuously exposed to 0.2 μM 5-HT. The problem could possibly be resolved by growing the cells in serum-free media. This had been previously attempted using a serum-free medium supplemented with growth factors. Unfortunately, cells formed gland-like structures rather than monolayers (Murakami and Masui, 1980). It was therefore decided to empirically try three commercially available serum-free media without all the growth supplements recommended by Murakami and Masui (1980). For one of these media, AIM V, T_{84} cells grew to confluence and developed transepithelial resistances comparable with values obtained when cells were grown in serum-supplemented media. Moreover, such cells responded to 5-HT with increases in short-circuit current greater than those obtained using equimolar concentrations of acetylcholine.

Desensitization is a widespread phenomenon in which exposure of a receptor to an agonist results in decreased responsiveness on subsequent exposure. 5-HT receptors are recognised as being susceptible to agonist-induced desensitization. Desensitization of the 5-HT_{2A} receptor has been previously encountered, and investigated in detail, in rat cortical neurons. It was shown that 5-HT was able to induce acute desensitization upon first contact with the 5-HT_{2A} receptor, and long term desensitization upon sec-

ond contact (Rahman and Newman, 1993). As with other receptors which activate protein kinase C, desensitization of 5-HT_{2A} receptors results from feedback inhibition mediated by protein kinase C (Roth et al., 1986; Aghajanian, 1990). In addition, it is thought likely that the receptors undergo concentration-dependent internalization or sequestration, a phenomenon which has already been reported for the 5-HT_4 receptor (Ansanay et al., 1992).

We conclude from our investigations that the most likely explanation of T_{84} cell monolayer insensitivity to 5-HT, when grown in serum-supplemented culture medium, is the presence of 5-HT in the serum which causes desensitization of the 5-HT receptors.

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