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Rapid activation of basolateral potassium transport in human colon by oestradiol

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1 We investigated the effect of oestradiol on basolateral potassium channels in human colonic epithelium.

2 Ion transport was quantified using short circuit current (I_{sc}) measurements of samples mounted in Ussing chambers. Serosal K transport was studied using nystatin permeabilization of the apical membrane. Intracellular pH changes were quantified using spectroflouresence techniques.

3 Experiments were performed with either 10 nM or 1 μM Ca^{2+} in the apical bathing solution. With 10 nM Ca^{2+} in the apical bathing solution addition of oestradiol (1 nM) to the basolateral bath produced a rapid increase in current ($\Delta I_K = 11.2 \pm 1.2$ $\mu A.cm^{-2}$, $n = 6$). This response was prevented by treatment of the serosal membrane with tolbutamide (1 μM). With 1 μM Ca^{2+} in the apical bathing solution addition of oestradiol produced a rapid fall in current ($\Delta I_K = -12.8 \pm 1.4$ $\mu A.cm^{-2}$), this response was prevented by treatment of the basolateral membrane with tetra-pentyl-ammonium (TPeA). These responses were rapid and occurred independently of protein synthesis.

4 Inhibition of basolateral Na^+/H^+ exchange with either amiloride or a low sodium bathing solution prevented this response. These responses were prevented by inhibition of protein kinase C (PKC) with bis-indolyl-maleimide.

5 Oestradiol (1 nM) produced a rapid intracellular alkanization (mean increase = 0.11 pH units; $n = 6$; $P < 0.01$).

6 These results suggest that oestradiol rapidly modulates serosal K transport in human colon. These effects depend upon intact Na^+/H^+ exchange and protein kinase C. We propose a non-classical, possibly membrane linked, mechanism for oestradiol action in human colonic epithelium. *British Journal of Pharmacology* (2000) **131,** 1373–1378

Keywords: Oestradiol; non-genomic; potassium transport; Na^+/H^+ exchange; human colon

Abbreviations: BCECF-AM, 2′,7′-bis-(2-carboxyethyl)-5,6-carboxyfluoroscein-acetoxymethyl ester; BIM, bis-inolyl-maleimide; EC_{50}, half maximal concentration; EIPA, 5-(*N*-ethyl-*N*-isopropyl) amiloride; Gt, transepithelial conductance; ΔI_K, change in potassium dependent current; I_K, potassium dependent current; I_{max}, maximal current response; I_{SC}, short circuit current; I_t, transepithelial current; K_{ATP}, ATP regulated potassium channel; K_{Ca}, calcium activated potassium channel; PKC, protein kinase C; TPeA, tetra-pentyl-ammonium; V_t, transepithelial potential difference

Introduction

High oestrogen states such as pregnancy and treatment with the oral contraceptive pill are associated with hypertension and sodium and water retention (Chasen Taber *et al*., 1996; Hall, 1997). However very little is known about the direct effects of oestradiol on salt absorbing epithelia. The distal human colon is an important site for salt and water reabsorbtion and a target for the natriferic hormone, aldosterone (Binder & Sandle, 1994). It shares many physiological properties with other salt absorbing epithelia such as distal renal tubule and sweat duct (Binder & Sandle, 1994). The distal human colon is therefore an accessible human model for investigating the effects of oestrogens on salt absorbing epithelia. In this study we investigated the effects of 17β-oestradiol on basolateral potassium transport in human colon.

The mechanism of salt absorption in human colon is well understood. Sodium ions enter the cell through Na^+ channels or a Na^+/H^+ exchanger located on the luminal membrane of the cell. These sodium ions are extruded across the serosal membrane by basolateral Na^+/K^+ exchange pumps (Binder *et al*., 1987; Binder & Rawlins, 1973; Frizzell *et al*., 1976; Schultz, 1984). To maintain a favourable electrochemical gradient for continued sodium absorption the K^+ ions are recycled through basolateral ion channels. There are two potassium conductive pathways on the basolateral membrane of human colonic epithelial cells (Maguire *et al*., 1994; McNamara *et al*., 1999). The first pathway has properties consistent with transport through a K_{ATP} channels. It is inhibited by increasing intracellular ATP and by the sulphonylurea tolbutamide (Maguire *et al*., 1994; McNamara *et al*., 1999). The second pathway is consistent with potassium transport through a calcium activated potassium channel, K_{Ca}. It is inhibited by quarternary ammonium cations such as tetra-pentyl-ammonium (TPeA) and is activated by increasing intracellular calcium, (Maguire *et al*., 1994; McNamara *et al*., 1999). K_{Ca} channels maintain a favourable electrochemical gradient for chloride secretion by the colon (McNamara *et al*., 1999; Winter *et al*., 1996). These potassium conductive pathways are pH sensitive. K_{ATP}

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dependent transport is activated by increasing intracellular pH, in contrast, K_{Ca} channels are inhibited by increasing intracellular pH (Maguire *et al*., 1995).

In contrast to oestrogens the effects of the natriferic hormone aldosterone on salt absorbing epithelia are well understood (Frizzell & Schultz, 1978; Rajendran *et al*., 1989; Turniman & Binder 1990). Aldosterone promotes salt absorption by a number of mechanisms. It enhances the permeability of the apical membrane to Na^+ by increasing the number of active sodium channels on the luminal membrane. It also promotes Na^+ extrusion through the serosal membrane by activating basolateral Na^+/K^+ ATPase pumps (Barbry & Hofman, 1997; Fuller & Verity, 1996; Horsberger & Rossier, 1992). Aldosterone also activates K_{ATP} channels in human colon. This involves two mechanisms (Maguire *et al*. 1995; 1999). Aldosterone has a delayed effect that occurs approximately 1 h after treatment of colon with the hormone. This involves the genomic mechanism of aldosterone action, i.e., binding to type Ia intracellular aldosterone receptors followed by alterations in DNA transcription and RNA translation (Maguire *et al*., 1995; 1999). Aldosterone also mediates rapid (<1 min) effects through a cell signalling pathway that is independent of protein synthesis and involves PKC dependent activation of basolateral Na^+/H^+ exchange thus modulating pH dependent K transport (Maguire *et al*., 1995). In this study we demonstrate that oestradiol also activates K_{ATP} dependent K^+ transport. We found that this response is non-genomic and independent of DNA transcription and RNA translation. We also investigated the role of Na^+/H^+ exchange and PKC in producing these responses.

Methods

Source and preparation of colonic mucosa

Non-diseased samples of human distal colon from patients undergoing resection for carcinoma were used. The use of human tissue in these experiments was approved by the Cork University Teaching Hospitals' Ethics Committee and were performed with full consent and in accordance with the Declaration of Helsinki. The sample was transported to the laboratory in 0.9% saline within 30 min of surgery.

The epithelial layer was separated from underlying smooth muscle and connective tissue by blunt dissection. 0.5 cm^2 sheets of this tissue were mounted in modified Perspex Ussing chambers (A.D.I. Instruments U.K. Ltd.). The initial bathing solution was a Kreb's solution of the following ionic composition in mM NaCl 118, $NaHCO_3$ 25, glucose 11, KCl 4.7, $CaCl_2$ 2.5, $MgSO_4$ 1.2, and KH_2PO_4 1.2. The solution was equilibrated in 5% CO_2 in oxygen, pH 7.4. The bath temperature was maintained at 37°C using a heated water jacket.

Electrophysiological techniques

The spontaneous transepithelial potential difference (V_t) was measured using an EVC 4000 Amplifier (WPI., U.K.), the potential was clamped to 0 mV by the application of a short circuit current (I_{sc}) which is a measure of electrogenic ion transport (Koefoed-Johnsen & Ussing, 1958). Transepithelial resistance was measured by measuring the current response to a 5 mV pulse. The current signal was digitized using an MP100 analogue/digital converter (Biopac Systems Inc., U.S.A.) and analysed using the 'Acqknowledge' 3.0 software (Biopac Systems Inc., U.S.A.) on a Macintosh Quadra 650 personal computer (Macintosh Ireland Ltd.). All drugs and chemicals were obtained from Sigma Inc., U.S.A.

Nystatin permeabilization

In nystatin permeabilization experiments the basolateral membrane was bathed in a low chloride Kreb's solution of the following ionic composition in mM: NaGluconate 100, NaCl 20, $NaHCO_3$ 25, glucose 11, KCl 4.7, $CaCl_2$ 2.5, $MgSO_4$ 1.2, and KH_2PO_4 1.2. pH was maintained at 7.4 by gassing with 95% O_2/5% CO_2. The apical membrane was bathed in potassium rich solution of the following ionic composition in mM: KGluconate 120, NaCl 20, $MgSO_4$ 3, KH_2PO_4 1.2, Glucose 11, ethylenediaminetetraacetic acid (EDTA) 5, and N-[2-hydroxyethyl] piperazine-N-[2-ethane-sulphonic acid] (HEPES), and $CaCl_2$ was 260 μM or 2 mM to give final free calcium concentrations of 10 nM or 1 μM, respectively (at 37°C and pH 7.2), pH was adjusted to 7.2 by the addition of KOH. Electrogenic Na^+ transport was abolished by treatment of the apical membrane with amiloride (100 μM). The use of symmetrical low chloride solutions was found to be necessary to avoid cell volume changes.

The apical membrane was treated with the polyene antibiotic nystatin (500 IU.ml^{-1} in methanol $<0.01\%$) and the transepithelial current allowed to reach a steady state. Nystatin forms cation permeable pores in the apical membrane, which allows the electrical properties of the serosal membrane to be studied more closely (Lewis *et al*., 1977). Under the conditions of a mucosa to serosa K^+ gradient changes in the K^+ conductance of the basolateral membrane will produce changes in the transepithelial current.

Intracellular pH measurements

Intact human crypts were isolated by exposing mucosal segments, microdissected from resected segments, to a calcium chelation solution (composition in mM: NaCl 96, KCl 1.5, HEPES/Tris 10, NaEDTA 27, Sorbitol 45, Sucrose 28) for 30 min at room temperature. A pellet of isolated crypts was formed by centrifugation at 200 r.p.m. for 1 min and was resuspended in Kreb's solution. Freshly isolated crypts were exposed to 3 μmol/l 2′,7′-bis-(2-carboxyethyl)-5,6-caboxyfluoroscein-acetoxymethyl ester (BCECF-AM) at room temperature for 30 min. Crypts were then rinsed twice, transferred to glass cover slips treated with poly-L-lysine, and were mounted on an inverted epi-fluorescence microscope (Diaphot 200, Nikon). The light from a Xenon lamp (Nikon) was filtered through alternating 440 and 480 nm interference filters (10 nm bandwidth, Nikon). The emitted fluorescence was passed through a 400 nm dichroic mirror, filtered at 510 nm and then collected using an intensified CCD camera system (Darkstar, Photonic Science). Images were digitized and analysed using the Starwise Fluo system (Imstar, Paris, France). Six regions of interest (containing 3–4 cells) were analysed in each of six crypts from six separate distal colonic specimens, such that $n=6$ represents recordings from a total of >144 cells. Results represent mean $\pm$ s.e.mean.

Statistical methods

All values are expressed as the mean $\pm$ standard error of the mean. Students *t*-test was used to determine statistical significance. We used half maximal inhibitory concentration (EC_{50}) as a summary statistic to compare concentration/

inhibition characteristics. EC_{50} was calculated by fitting concentration and response values to the following equation which is analogous to the Michaelis-Menten equation. This was done using the method of least squares. All statistical analysis and curve fitting was performed using a commercially available statistical package (SPSS for windows, SPSS inc. U.S.A.).

Equation 1 $\Delta I = \Delta Imax.\ [Oestradiol]/(EC_{50} + [Oestradiol])$
Where ΔI = Change in I_{sc} or I_K for a given concentration of 17-β oestradiol.
$\Delta Imax$ = maximum change in I_K or I_{sc} produced by 17-β oestradiol.

Results

Serosal K transport

We investigated basolateral K^+ transport using nystatin perforation of the apical membrane. Treatment of the apical membrane of mammalian colonic epithelium with nystatin has been previously shown to remove the electromotive force and resistance generated by the apical membrane. This allows investigation of the basolateral membrane in isolation. In the presence of high mucosal [K^+] and low [Ca^{2+}] (10 nM), the addition of nystatin produced an immediate increase in transepithelial current (Table 1) and increase in transepithelial conductance (control $G_t = 3.0 \pm 0.2$ mS.cm^{-2} post nystatin $G_t = 6.3 \pm 2.9$ mS.cm^{-2}, $n = 20$), at V_t clamped to zero mV. The K^+-dependence of this current was tested by graded substitution of all potassium salts with sodium salts in the apical bath (Figure 1, $n = 6$). The nystatin-induced current was abolished following total substitution of apical K^+ by Na^+. There was a curvilinear relationship with a zero intercept between the reversal potential for potassium, E_k and the transepithelial current (Figure 1). The slope of the line the portion of the curve over which our experiments were performed gives an estimate of transmembrane K^+ conductance of 1.6 ± 0.2 mS.cm^{-2}. The conductance not accounted for by K^+ transport at the basolateral membrane may reflect the conductance of the non-selective paracellular pathway. It may also reflect the conductance of other non K^+ dependent pathways such as basolateral Cl^- channels. However it is unlikely that these channels contribute to the current as the current falls to zero when there is no K^+ gradient. The nystatin-induced transepithelial current therefore predominantly reflects K^+ flow across the basolateral membrane and is hereafter referred to as transbasolateral K^+ current (I_K). Further proof for this conclusion was obtained from the effects of K^+ channel inhibitors on the nystatin-induced current. There was a transepithelial HCO_3^- gradient generated by the solutions used for our nystatin method. This did not result in a significant diffusion potential (the HCO_3^- gradient was maintained during the sodium ion substitution protocol; when the K^+ gradient was zero there was no measurable I_{SC} despite the maintained HCO_3^- gradient).

Table 1 Sensitivity of the transepithelial current to TPeA 1 μM, tolbutamide (100 nM) and ouabain following addition of nystatin

	10 nM Ca^{2+}	*1 μM Ca^{2+}*
Basal I_t (μA.cm^{-2})	22.3 ± 0.8	17.6 ± 2.8
Nystatin (μA.cm^{-2})	51.7 ± 1.2	48.6 ± 5.3
+ Tolb. (μA.cm^{-2})	16.1 ± 1.2	47.2 ± 4.9
+ TPeA (μA.cm^{-2})	6.9 ± 0.8	5.2 ± 0.9
+ Ouabain (μA.cm^{-2})	6.8 ± 0.8	5.1 ± 0.9

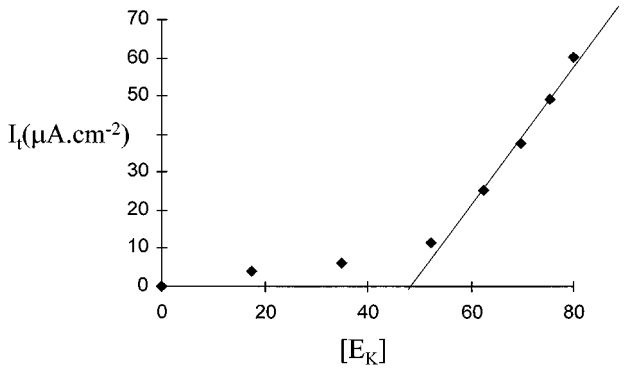

Figure 1 Effect of varying the transepithelial *K* gradient (Nagluconate for K-gluconate substitution) on transmembrane current (I_t). The best linear fit, for the range included in our experiments, estimated between the reversal potential for potassium, E_K for each transepithelial *K* gradient and I_t is shown.

Under conditions designed to preserve the intracellular ionic environment (apical bath containing high [K^+] 120 mM, low [Cl^-] 20 mM and low [Ca^{2+}] 10 nM), the current present under nystatin-permeabilized conditions was almost completely inhibited by basolateral tolbutamide (100 nM) and, to a much lesser extent, by tetra-pentyl-ammonium (Table 1). This sensitivity profile of I_K to these K^+ channel inhibitors was independent of the order of addition of drugs (data not shown). This current was insensitive to inhibition of Na^+/K^+ ATPase with ouabain (50 μM). The current remaining after combined tolbutamide and TPeA treatment was insensitive to Ba^{2+} and may be generated by paracellular K^+ or Na^+ leak from mucosa to serosa or Ba^{2+} insensitive K channels, since it was abolished by elimination of the transepithelial K^+ and Na^+ gradient (mucosal Na^+ substitution for K^+). These results imply that under low intracellular [Ca^{2+}], basolateral K^+ transport occurs through a sulphonylurea-sensitive K^+ conductance pathway.

Under experimental conditions with approximately 1 μM-free calcium, the addition of nystatin produced an immediate increase in K^+-dependent transepithelial current (Table 1) and an increase in transepithelial conductance (control $G_t = 3.1 \pm 0.2$ mS.cm^{-2}, post nystatin $G_t = 6.9 \pm 0.4$ mS.cm^{-2}, $n = 20$). Under these conditions of high $[Ca^{2+}]_i$, the I_K was almost completely abolished by TPeA (10 μM) with relatively low sensitivity to tolbutamide (Table 1). Again there was curvilinear correlation between the reversal potential for potassium, E_K and I_t (correlation co-efficient $r = 0.89$, $P < 0.01$). The slope of the line in the steepest portion of the curve gives an estimate of transmembrane K^+ conductance of 1.8 ± 0.4 mS.cm^{-2}. Again the current remaining after treatment with tolbutamide and TPeA was insensitive to ouabain (Table 1). Ouabain had no effect when added before tolbutamide and TPeA.

Effect of oestradiol on serosal K transport

Under conditions where I_K was tolbutamide sensitive (10 nM Ca^{2+} in the apical bath) addition of 17β-oestradiol (1 nM) to the basolateral membrane of this preparation produced rapid and concentration dependent increase in *K* dependent current (Figure 2, Table 2) that was associated with an increase in transepithelial conductance ($\Delta Gt = 0.2 \pm 0.04$ mS.cm^{-2} $n = 6$). This effect occurred within 1–2 min and reached a maximum within 30 min.

The concentration response characteristics of this effect are shown in Figure 3, maximal response occurred at a concentration of 1 nM with a EC_{50} of 0.09 ± 0.03 nM ($n=6$). To confirm that the oestradiol effect was due to activation of K_{ATP} dependent current the basolateral membrane was first treated with tolbutamide (1 μM). Under these conditions oestradiol did not produce an increase in current (Table 3, $P<0.01$, $n=6$).

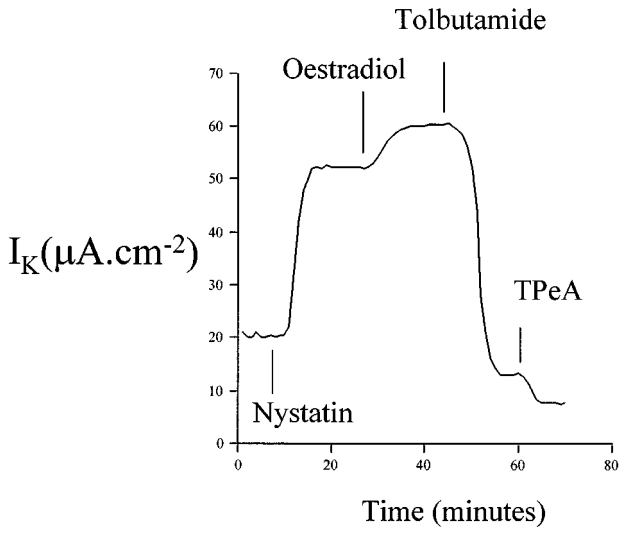

Figure 2 Effect of oestradiol on basolateral K^+ dependent current (I_K) after nystatin perforation. These experiments were performed with 10 nM Ca^{2+} in the apical bathing solution. Addition of oestradiol to the serosal membrane produces a rapid and sustained increase in I_K, most of the subsequent current was inhibited with tolbutamide suggesting that this was K_{ATP} dependent current.

Table 2 Effect of oestradiol on I_K under conditions of high (10 μM) apical Ca^{2+} and low (10 nM) apical Ca^{2+} conditions

	1 μM Ca^{2+}	*10 nM Ca^{2+}*
+ Nystatin ($\mu A.cm^{-2}$)	57.4 ± 5.3	53.3 ± 4.9
+ Oestradiol ($\mu A.cm^{-2}$)	44.6 ± 3.6	64.4 ± 5.7
ΔI_K ($\mu A.cm^{-2}$)	-12.8 ± 1.4	11.2 ± 1.2

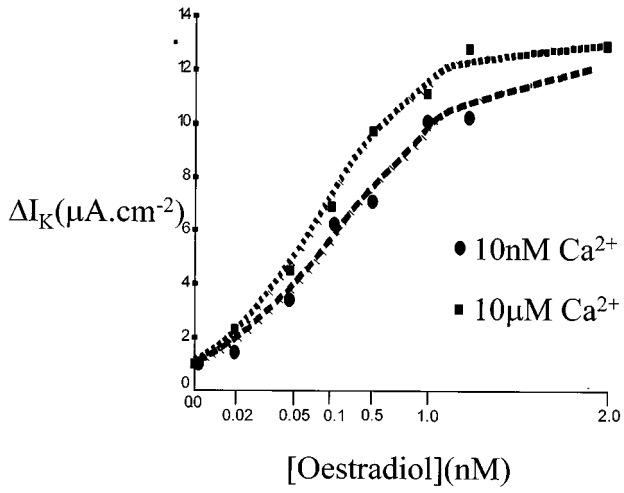

Figure 3 Dose response characteristics for the rapid activation of basolateral K_{ATP} dependent current by oestradiol and for the rapid inhibition of the K_{Ca} dependent. Maximal effect was seen at approximately 1 nM. Responses are shown as means ($n=6$).

Under conditions where I_K was TPeA sensitive (1 μM Ca^{2+} in the apical bathing solution), addition of 17 β-oestradiol (1 nM) to the basolateral bath produced a rapid and concentration dependent decrease in I_K (Table 2, Figure 4), that was associated with a fall in transepithelial conductance ($\Delta Gt = 0.3 \pm 0.05$ mS/cm^2, $n=6$). Maximum effect occurred at 1 nM with an EC_{50} of 0.1 ± 0.02 nM ($n=6$) (Figure 3). This fall in current was prevented by pre-treatment with TPeA (1 μM) (Table 3).

Role of Na^+/H^+ exchange

Na^+/H^+ exchange was inhibited by pre-treatment of the basolateral membrane with amiloride (500 μM). Subsequent addition of oestradiol did not alter the current when the current was TPeA sensitive or when the current was tolbutamide sensitive (Table 3). Na^+/H^+ exchange was also inhibited by performing experiments in a low sodium solution (N-methyl-D-glutamine substitution for sodium at the serosal membrane). Subsequent addition of oestradiol did not alter the current when current was predominantly tolbutamide sensitive and when the current was predominantly TPeA sensitive (Table 3).

Role of protein synthesis

To investigate the effect of protein synthesis on the oestradiol response, samples of colon were pre-treated with cyclo-heximide (100 μM) an inhibitor of RNA translation. This cyclo-heximide dose has been previously shown to inhibit the genomic effect of aldosterone on potassium transport in colonic epithelium (Maguire *et al*., 1999). Subsequent addition of oestradiol produced an effect equivalent to that in normal controls when the current was both tolbutamide sensitive and TPeA sensitive (Table 4).

Table 3 Effect of pretreatment of the basolateral membrane with either tolbutamide (100 nM) or TPeA under low (10 nM) and high apical Ca^{2+} (10 μM) conditions

	1 μM Ca^{2+}	*10 nM Ca^{2+}*
+ TPeA ($\mu A.cm^2$)$^+$	2.1 ± 0.5	13.2 ± 2.0
+ Tolbutamide ($\mu A.cm^2$)	-12.3 ± 1.4	-1.9 ± 0.6

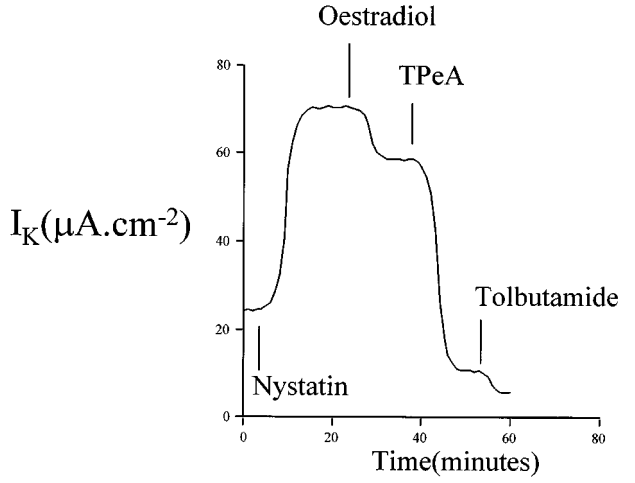

Figure 4 Effect of oestradiol on K_{Ca} dependent current, these experiments were performed with 1 μM Ca^{2+} in the apical bathing solution. Treatment of the serosal membrane with oestradiol causes a rapid and sustained fall in K dependent current. Most of the subsequent current is inhibited by TPeA.

To investigate the role of PKC samples of colon were treated with the specific protein kinase C inhibitor 1, 25 bis-indolyl-maleimide (BIM, 25 nM). Subsequent addition of oestradiol had no effect on I_K (Table 4).

Effect of tamoxifen

To study the effect of tamoxifen on this response, tamoxifen (10 μM) was added 10 min before oestradiol. Subsequent addition of oestradiol had no effect on ion transport when the current was predominantly tolbutamide sensitive and when the current was predominantly TPeA sensitive (Table 4).

Intracellular pH measurements

The basal cytosolic pH recorded from the human colonic crypts was 7.29 ± 0.02 ($n=6$). Perfusion of the crypts with 17β-oestradiol (10 nM) induced a rapid increase in pH within 1 min, although this did not achieve statistical significance (pH of 7.33 ± 0.01 at 1 min; $n=6$; $P=0.28$). This alkalinization was significant at 5 min (7.40 ± 0.03; $n=6$; $P<0.02$) and reached a plateau (Figure 5). This represents a maximal pH change from 7.29 ± 0.02 to 7.40 ± 0.02 (mean increase = 0.11 pH units; $n=6$; $P<0.01$). The alkalinization was blocked with 50 μM 5-(*N*-ethyl-*N*-isopropyl) amiloride (EIPA), a specific sodium-hydrogen exchange inhibitor (Ginstein *et al*., 1989). Mean decrease was 0.24 pH units ($n=6$; $P<0.01$). This indicates that an increase in Na^+/H^+ activity mediates the rapid onset, oestrogen-induced alkalinisation, similar to that which is observed in response to aldosterone (Winter *et al*., 1996).

Table 4 Response to oestradiol treatment following pre-incubation of the basolateral membrane with cyclo-heximide, 1.25-BIM, tamoxifen amiloride, $0Na^+$ or ouabain

Drug (concentration)	*10 nM Ca^{2+}* ($\mu A.cm^{-2}$)	*10 μM Ca^{2+}* ($\mu A.cm^{-2}$)
Cycloheximide (100 μM)	11.0 ± 1.3	-9.5 ± 1.0
1.25 BIM (25 nM)	-0.2 ± 0.05**	0.0 ± 0.0**
Amiloride (500 μM)	-0.5 ± 0.1**	0.1 ± 0.0**
Tamoxifen (10 μM)	0.1 ± 0.0**	0.0 ± 0.0**
$0Na^+$	0.4 ± 0.1**	-0.5 ± 0.2**
Ouabain	13.3 ± 1.6	-10.2 ± 1.5

*$P<0.05$, **$P<0.01$.

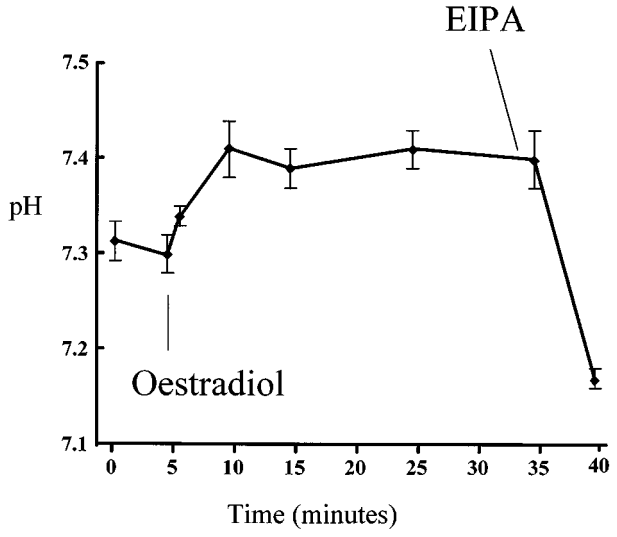

Figure 5 Intracellular pH measurements. These experiments were performed on isolated colonic crypts. Addition of oestradiol to the basolateral membrane produced a rapid intracellular alkanisation. Values are shown as mean $\pm$ s.e.mean.

Discussion

K^+ channels maintain the electrochemical gradient that allows sodium absorption to occur. We found that under certain conditions oestradiol produces a small but significant activation of serosal K^+ transport. The rapid activation of K transport would increase the potassium conductance of Na^+ absorbing cells allowing increased Na^+ absorption. We also found that oestradiol rapidly inhibits K_{Ca} dependent transport. K_{Ca} channels play an important role in maintaining chloride secretion in human colon-oestradiol. Therefore creates an unfavourable electrochemical gradient for chloride secretion in human colon (McNamara *et al*., 1999). Similar mechanisms may occur in other salt absorbing and secreting epithelia. These effects may therefore contribute to the salt and water retention associated with high oestrogen states.

The classical mechanism of steroid action involves hormone entry to the cell, binding to intracellular receptors resulting in changes in the rate of DNA transcription and RNA translation. There is also evidence for a non-classical mechanism for steroid action. Aldosterone activates K_{ATP} channels in human colonic epithelium. This effect occurs within minutes and is independent of DNA transcription or RNA translation (Maguire *et al*., 1995). This non-genomic mechanism of aldosterone action would appear to involve activation of Na^+/H^+ exchange. Similar non-genomic effects of aldosterone have been described in other cell types. In mono-nuclear lymphocytes aldosterone also activates Na^+/H^+ exchange, this effect is rapid and is independent of DNA transcription and RNA translation (Wehling *et al*., 1993). A similar response has been identified in single K_{ATP} channels on the basolateral membrane of frog skin epithelium (Urbach *et al*., 1996). The oestradiol effect on potassium transport in human colonic epithelium would appear to involve a similar non-genomic mechanism. Firstly, the oestradiol effect is rapid and independent of protein synthesis. Secondly, the oestradiol effect is prevented by inhibition of basolateral Na^+/H^+ exchange with either basolateral amiloride or a Na^+ free Kreb's and oestradiol causes rapid intracellular alkanization at similar concentrations. The oestradiol effect on Na^+/H^+ occurs more rapidly, maximal intracellular alkanization occurred at around 5 min. This discrepancy may be due to compartmentalization of the cell, introducing diffusion barriers to H^+, slowing the K^+ channel response to intracellular alkanization. These findings strongly support the hypothesis that the oestradiol effects on K transport are due to activation of Na^+/H^+ exchange. There is compelling evidence to suggest that PKC also plays an important role in the cellular transduction of this response. The phorbol ester PMA, a known activator of protein kinase C, produces a similar rapid activation of K_{ATP} channels in human colonic epithelium (Maguire *et al*., 1999). We have also found rapid activation of PKC by aldosterone and oestradiol in colon and the colonic cell line T-84 (Doolan & Harvey, 1996). In this study we show that inhibition of PKC prevents the rapid modulation of K transport by oestradiol. These results suggest that oestradiol alters basolateral K^+ transport in human colonic epithelium by a rapid mechanism that involves activation of PKC resulting in upregulation of Na^+/H^+ exchange.

The characteristics of the receptor involved in these non-genomic effects of steroids are unknown. A 50 K_d membrane receptor for aldosterone has been identified in human mononuclear lymphocytes (Wehling *et al*., 1993). This receptor appears to be structurally and functionally distinct from the classical mineralocorticoid receptor. Our results would suggest that the rapid effect of oestradiol on human epithelial cells does not involve the classical human oestrogen receptor (hER). Our effect had lower EC_{50} than would be expected given published values for the dissociation constant of the hER ($\approx$0.3 nM) (Neff *et al*., 1994). These responses were inhibited by the oestrogen receptor antagonist, tamoxifen. However this result may be explained by the fact that tamoxifen is a known inhibitor PKC (Cheng *et al*., 1998). Membrane linked binding sites for oestradiol have been identified in endometrial cells (Pietras & Szego, 1976) and rapid effects of oestradiol have also been identified in endothelial cells, hypothalamic neurones and cortical neurones (Rusko *et al*., 1995; Lagrange *et al*., 1994; 1995; Joels, 1997). This would suggest that there is second, non-genomic, signal transduction pathway for oestradiol. Our results support a non-genomic mechanism of action for oestrogens in human epithelial cells. Studies with neuroactive steroids suggest that there may be multiple non-genomic signal transduction pathways for steroids (Revelli *et al*., 1998). We propose that rapid activation of PKC with subsequent modulation of intracellular pH and membrane conductance is an important non-genomic signalling pathway for oestrogens.

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