Stimulation of anion secretion by β-adrenoceptors in the mouse endometrial epithelium

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1. Regulation of anion secretion by adrenoceptors in primary culture of mouse endometrial epithelium was investigated using the short circuit current (I_{SC}) technique.

2. Adrenaline stimulated a sustained increase in the I_{SC} in a concentration-dependent manner. The adrenaline-induced I_{SC} could be inhibited by pretreatment with diphenylamine 2,2′-dicarboxylic acid (DPC) or replacement of external Cl^- and HCO_3^-, but not by amiloride or replacement of Na^+ in apical solution.

3. The concentration-dependent responses of the adrenaline-induced I_{SC} to the Cl^- channel blockers glibenclamide and DPC were examined and exhibited IC_{50} values of 380 and 960 μM, respectively.

4. The effect of various adrenoceptor agonists on the I_{SC} was examined. The order of potency appeared to be isoprenaline > adrenaline > noradrenaline, while no response was elicited by the α-adrenoceptor agonist methoxamine, indicating a predominant involvement of β-adrenoceptors.

5. The β-adrenoceptor antagonist propranolol was found to be much more effective than the α-adrenoceptor antagonist phentolamine in inhibiting the I_{SC} responses induced by all adrenoceptor agonists examined.

6. The effect of adrenaline on the I_{SC} was mimicked by an adenylate cyclase activator, forskolin, but suppressed by the adenylate cyclase inhibitor MDL 12,330A, indicating the involvement of cAMP.

7. Our results demonstrate that anion secretion by the mouse endometrial epithelium is regulated by β-adrenoceptors and involves a cAMP-dependent mechanism.

It is believed that the endometrial epithelium, the mucosal lining of the uterus, has both absorptive and secretory activities which may be important for the formation of a uterine fluid environment suitable for sperm transport and embryo implantation. Measurements of luminal Na^+ and K^+ concentrations in a number of species, including humans (Casslen & Nilsson, 1984), rats (Nilsson & Ljung, 1985; Nordenvall, Ulmsten & Ungerstedt, 1989) and sows (Iritani, Sato & Nishikawa, 1974), have indicated that Na^+ may be absorbed and K^+ secreted by the endometrial epithelium during various reproductive events. Electrophysiological studies on primary cultures of human endometrial epithelial cells (Matthews, McEwan, Redfern, Thomas & Hirst, 1992, 1993*a*; Matthews, Thomas, Redfern & Hirst, 1993*b*) and the intact endometrial epithelium from immature pigs (Vetter & O'Grady, 1996) has provided direct evidence for regulated Na^+ absorption and K^+ secretion by the endometrial epithelium. However, less is known for the regulation of endometrial anion secretion, although short circuit current measurements on intact rat uteri have indicated that electrogenic transfer of Cl^- and HCO_3^- into the luminal fluid occurs (Kyriakides & Levin, 1973), and X-ray microanalyses of anions in uterine secretions in the rat have demonstrated an increase in Cl^- concentration during blastocyst implantation (Nilsson & Ljung, 1985).

Recently, a primary culture of mouse endometrial epithelial cells grown on permeable supports has been established and shown to have a basal short circuit current (I_{SC}) predominantly mediated by Na^+ absorption (Chan *et al.* 1997*a*). It has also been demonstrated that the cultured epithelium responds to a number of agonists with increases in the I_{SC} which can be predominantly attributable to Cl^- secretion (Chan *et al.* 1997*a*,*b*). In the present study the regulation of anion secretion by adrenoceptors in the mouse endometrial epithelium has been investigated further. The results suggest that anion secretion across the mouse endometrium epithelium can be regulated by β-adrenoceptors and involves a cAMP-dependent mechanism.

METHODS

Materials

Dulbecco's modified Eagle's medium (DMEM), Hank's balanced salt solution, Ham's F-12 nutrient mixture, penicillin, streptomycin, noradrenaline, phenylephrine, propranolol, glibenclamide and *N*-methyl-D-glucamine (NMDG) were purchased from Sigma, while phosphate-buffered saline (PBS), fetal bovine serum, non-essential amino acids and pancreatin were from Gibco. Diphenylamine-2,2′-dicarboxylic acid (DPC) was obtained from Riedel de Haen Chemicals (Hannover, Germany), and amiloride hydrochloride from Merck Sharp & Dohme Research Laboratories. Adrenaline was obtained from David Bull Laboratories (Victoria, Australia), isoprenaline from Pharmax Ltd (Dartford, UK) and phentolamine from Ciba Geigy. Methoxamine hydrochloride and MDL 12,330A hydrochloride (MDL) were purchased from Research Biochemicals International.

Cell isolation and culture

Endometrial epithelial cells were enzymatically isolated from the mouse uterus according to the method described by McCormack & Glasser (1980) with slight modifications (Chan *et al.* 1997*a*). Samples of uteri were obtained from 3·5- to 4-week-old immature ICR mice to avoid the complication of the endometrial cycle. Animals were killed by placing them in a CO_2-gassed chamber for 3 min. Uteri were removed and placed into a Petri dish containing sterile PBS (without Ca^{2+} and Mg^{2+}). After washing with PBS and trimming off the remaining fatty and connective tissues, the uteri were sliced longitudinally. The sliced uteri were incubated in PBS supplemented with 7·5 mg ml^{-1} trypsin, 37·5 mg ml^{-1} pancreatin, 100 U ml^{-1} penicillin and 100 μg ml^{-1} streptomycin at 0 °C for 60 min and then at room temperature for another 60 min. After the enzyme digestion, the test-tube containing PBS and the tissues was shaken gently for 30 s. Uterine tissue was carefully removed and the crude cell solution was passed through a 70 μm fluorocarbon mesh filter (Spectra Mesh; Spectrum, Houston, TX, USA). The filtrate was centrifuged at 1000 *g* for 5 min. The supernatant was discarded and the cell pellet was resuspended in 12 ml PBS. The cells were allowed to settle for 5 min, and then the top portion (about 2 ml) of the cell suspension was discarded. The cell suspension was centrifuged again at 1000 *g* for 5 min. The washing procedures were then repeated once more. After centrifugation, the cell pellet was resuspended in Ham's F-12–DMEM culture medium containing 10% fetal bovine serum, 1% non-essential amino acids, 100 U ml^{-1} penicillin and 100 μg ml^{-1} streptomycin. The isolated cells were then ready for subsequent culture. For the I_{SC} measurements, the isolated endometrial cells were plated at a density of about $1·4 \times 10^6$ cells ml^{-1} onto nitrocellulose Millipore filters (with a surface area of 0·45 cm^2 for cell growth) floating on culture medium. Cultures were incubated at 37 °C in 95% air–5% CO_2, and reached confluence in 3–4 days.

Short circuit current measurement

The measurement of I_{SC} has been described previously (Ussing & Zerahn, 1951; Wong, 1988). Monolayers grown on permeable supports were clamped vertically between two halves of the Ussing chamber. The monolayers were bathed on both sides with Krebs–Henseleit solution which was maintained at 37 °C by a water jacket enclosing the reservoir. The Krebs–Henseleit solution had the following composition (mM): NaCl, 117; KCl, 4·5; $CaCl_2$, 2·5; $MgSO_4$, 1·2; $NaHCO_3$, 24·8; KH_2PO_4, 1·2; glucose, 11·1. In some experiments, ambient Cl^- and/or HCO_3^- was replaced by gluconate, and Na^+ was replaced by $NMDG^+$. The solution was bubbled with 95% O_2–5% CO_2 to maintain the pH of the solution at 7·4. When HCO_3^- was removed, the solution was gassed with 100% O_2. Drugs could be added directly to the apical or basolateral side of the epithelium. The epithelium exhibited a basal transepithelial potential difference for every monolayer examined, which was measured by the Ag–AgCl reference electrodes (World Precision Instruments) connected to a preamplifier which was connected in turn to a voltage clamp amplifier (DVC 1000; World Precision Instruments). The change in I_{SC} was defined as the maximal rise in I_{SC} following agonist stimulation and it was normalized as current change per unit area of epithelial monolayer (in μA cm^{-2}). In each experiment, a transepithelial potential difference of 0·1 mV was applied. The change in current in response to the applied potential was used to calculate the transepithelial resistance of the monolayer using the ohmic relationship. Small variations in I_{SC} between cultures were observed and experiments were normally repeated in different batches of culture to ensure that data were reproducible.

Statistical analysis

Results are expressed as means ± S.E.M., and n indicates the number of experiments. Comparisons between groups of data were made by Student's unpaired t test. A P value of less than 0·05 was considered statistically significant.

RESULTS

I_{SC} response to adrenaline

The mouse endometrial culture exhibited a mean basal I_{SC} of 4·7 ± 0·2 μA ($n = 132$), a transepithelial resistance of 786 ± 66 Ω cm^2 ($n = 139$) and a mean transepithelial potential of 4·2 ± 0·3 mV ($n = 139$), with the apical side negative with respect to the basolateral side. The cultured mouse endometrial epithelium responded to basolateral addition of adrenaline with an increase in the I_{SC} (Fig. 1). The I_{SC} response to 1 μM adrenaline usually reached a peak value of 5·1 ± 0·2 μA cm^{-2} ($n = 39$) 3 min after adrenaline stimulation and remained above 90% of the peak response for 5 min. The adrenaline-stimulated current gradually declined to 50% of the peak response over 20 min. The value of the peak response was used for analysis throughout the study.

Anion dependence of the adrenaline-stimulated I_{SC}

Ion substitution experiments were conducted to study the ion species involved in mediating the adrenaline-stimulated I_{SC} response. When Cl^- in the bathing solutions was replaced, the adrenaline-stimulated I_{SC} response was reduced by 61 ± 5% ($P < 0·001$, $n = 11$). In Cl^-- and HCO_3^--free solution, the I_{SC} response was further reduced (total reduction of 83 ± 2%; $n = 6$; $P < 0·001$), indicating that the adrenaline-stimulated I_{SC} response was anion dependent. However, replacement of external anions produced an insignificant effect on the basal I_{SC}. Bilateral replacement of Na^+ reduced the adrenaline-induced I_{SC} by 97 ± 1% ($n = 4$; $P < 0·001$) and the basal I_{SC} by 75 ± 5% ($P < 0·005$). However, apical replacement of external Na^+ did not reduce the adrenaline-stimulated I_{SC} response but suppressed the basal I_{SC} by 85 ± 4% ($n = 5$), indicating that Na^+ was involved in mediating the basal I_{SC}, as previously reported

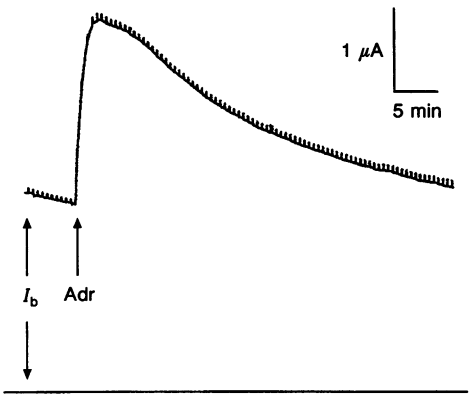


Figure 1. Effect of adrenaline on I_{SC}
I_{SC} recording ($n = 39$) with arrows marking the extent of the basal current (I_b) and the time at which basolateral adrenaline (Adr, 1 μM) was added. Experiments were performed in normal Krebs–Henseleit solution. The line below the trace represents zero I_{SC}. The transient current pulses resulted from an intermittently applied voltage of 0·1 mV, from which transepithelial resistance could be calculated. $I_b = 4{\cdot}7$ μA cm^{-2}.

(Chan *et al.* 1997*a*), but not the adrenaline-stimulated response.

Another set of experiments was performed to examine the sensitivity of the adrenaline-stimulated I_{SC} to various channel blockers. As shown in Fig. 2*A*, when amiloride was added at a concentration (10 μM, apical) known to block Na^+ channels prior to the addition of adrenaline, about 70% of the basal current was reduced, as previously reported (Chan *et al.* 1997*a*). Treatment with amiloride did not affect the adrenaline-elicited I_{SC} significantly ($6{\cdot}1 \pm 1{\cdot}2$ μA cm^{-2}, $n = 4$, with amiloride compared with the control value of $4{\cdot}6 \pm 0{\cdot}4$ μA cm^{-2}, $n = 3$; $P > 0{\cdot}05$), but the adrenaline-induced I_{SC} could be blocked substantially by subsequent addition of a Cl^- channel blocker, DPC (2 mM; Fig. 2*A*). Pretreatment of the endometrial epithelial cells with DPC (2 mM, apical), as shown in Fig. 2*B*, almost completely abolished the increase in the adrenaline-stimulated I_{SC} ($0{\cdot}3 \pm 0{\cdot}3$ μA cm^{-2}, $n = 3$, $P > 0{\cdot}005$), indicating that adrenaline stimulated anion secretion rather than Na^+ absorption.

The sensitivity of the adrenaline-stimulated I_{SC} to a number of different Cl^- channel blockers was also examined. Different blockers at various concentrations were added to the apical membrane after adrenaline (1 μM) stimulation. Figure 3*A* shows the concentration-dependent effects of DPC and glibenclamide on the adrenaline-stimulated I_{SC} responses (IC_{50} values were 380 and 960 μM for glibenclamide and DPC, respectively). 4,4′-Diisothiocyanatostilbene-2,2′-disulphonic acid (DIDS) also had a small effect on the adrenaline-stimulated I_{SC}, producing a maximal reduction of 20 ± 2% at 200 μM ($n = 7$; $P < 0{\cdot}05$). Basolateral addition of a Cl^- channel blocker, either glibenclamide ($n = 10$) or DPC ($n = 5$), produced an insignificant effect on the adrenaline-stimulated I_{SC} compared with apical addition, as shown in Fig. 3*B* and *C*.

Effects of adrenoceptor agonists

In order to study the involvement of adrenoceptors in the mouse endometrial epithelial cells, different adrenoceptor agonists were employed. The effect of noradrenaline, an agonist more potent for α-receptors but less potent for

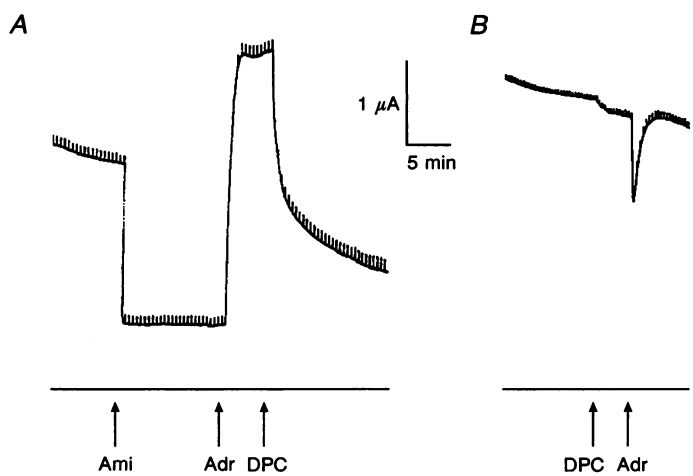


Figure 2. Effect of channel blockers on the adrenaline-stimulated I_{SC}
A, I_{SC} recording of the adrenaline (Adr)-stimulated response after treatment with the Na^+ channel blocker amiloride (Ami, 10 μM, apical). $I_b = 6{\cdot}0$ μA cm^{-2}. *B*, diminished adrenaline-stimulated response after treatment with the Cl^- channel blocker DPC (2 mM, apical). $I_b = 7{\cdot}6$ μA cm^{-2}.

β-receptors than adrenaline (Rang, Dale & Ritter, 1991), on the I_{SC} was examined and compared with that of adrenaline. As shown in Fig. 4, adrenaline stimulated I_{SC} in a concentration-dependent manner with an EC_{50} of about 45 nM. The kinetics of the noradrenaline- and adrenaline-induced I_{SC} responses were similar, but the concentration–response curve indicated that noradrenaline was less effective in activating the I_{SC}, with an EC_{50} of 5·3 μM, which was about two orders of magnitude higher than that of adrenaline (Fig. 4). The sensitivity of the noradrenaline-stimulated I_{SC} to various Cl^- channel blockers, e.g. DIDS, DPC and glibenclamide, was similar to that of the adrenaline-stimulated I_{SC} (data not shown). After stimulation by noradrenaline, the I_{SC} could be further increased, from 4·8 ± 0·3 to 6·4 ± 0·3 μA cm^{-2} ($n = 5$; $P < 0·01$), by addition of adrenaline, as shown in Fig. 5*A*. However, if stimulation with adrenaline preceded that with noradrenaline, no further increase in I_{SC} was elicited (Fig. 5*B*). The total current increase induced by noradrenaline followed by adrenaline did not exceed that induced by adrenaline alone (Fig. 5*C*).

The effects of potent α- and β-receptor agonists were also examined. Methoxamine, a specific α_1-receptor agonist, did not have any effect on the I_{SC}, even at a concentration of 10 μM ($n = 4$; Fig. 6*A*). Isoprenaline, a β-receptor agonist, induced a I_{SC} response similar to that stimulated by adrenaline in both concentration dependence (i.e. similar EC_{50}; Fig. 4) and sensitivity to Cl^- channel blockers (Fig. 6*B*). However, the maximal I_{SC} response to isoprenaline was greater than that to adrenaline (9·7 ± 0·6 μA cm^{-2} ($n = 3$) compared with 5·2 ± 0·3 μA cm^{-2}; $n = 6$; $P < 0·001$) at a concentration of 1 μM (Fig. 4). After stimulation with isoprenaline (0·05 μM), addition of adrenaline (1 μM) did not increase the current much further (Fig. 6*B*).

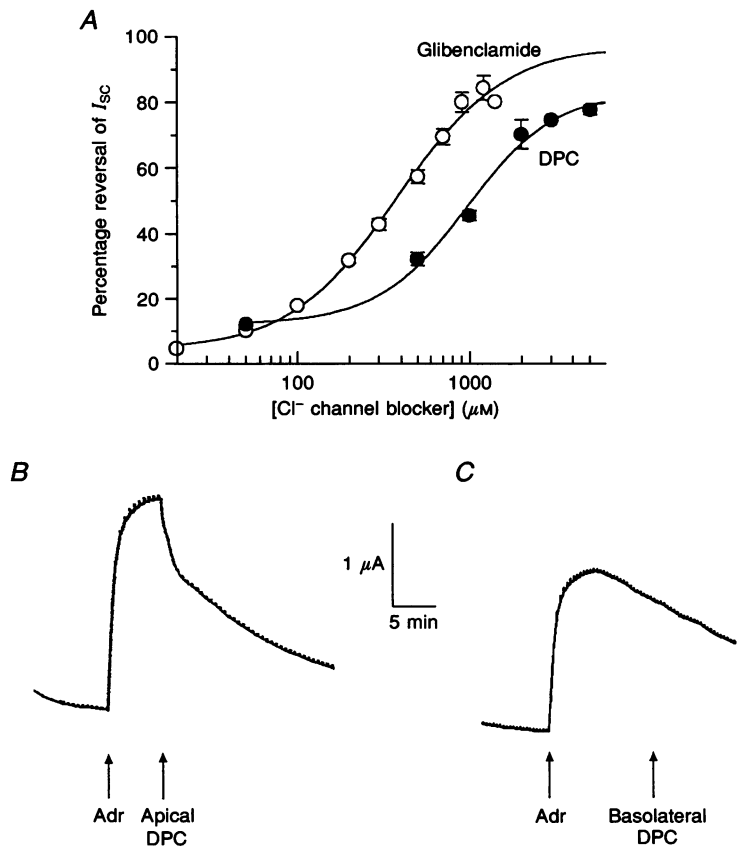


Figure 3. Effects of different Cl^- channel blockers on the adrenaline-stimulated I_{SC}

A, concentration-dependent effects of apical addition of DPC and glibenclamide on the I_{SC} induced by basolateral addition of 1 μM adrenaline. The percentage reversal values were derived from the formula $(I_{max} - I_{block})/I_{max}$, where I_{max} is the maximal current response and I_{block} is the current magnitude obtained in the presence of a blocker. *B*, I_{SC} recording ($n = 6$) with arrows marking the times at which basolateral adrenaline or apical DPC was added. $I_b = 3·9$ μA cm^{-2}. *C*, I_{SC} recording ($n = 5$) with arrows marking the times at which basolateral adrenaline or DPC was added. $I_b = 3·3$ μA cm^{-2}. Note that basolateral addition of DPC (*C*) produced an insignificant effect compared with that produced by apical DPC (*B*). Glibenclamide produced a similar effect.

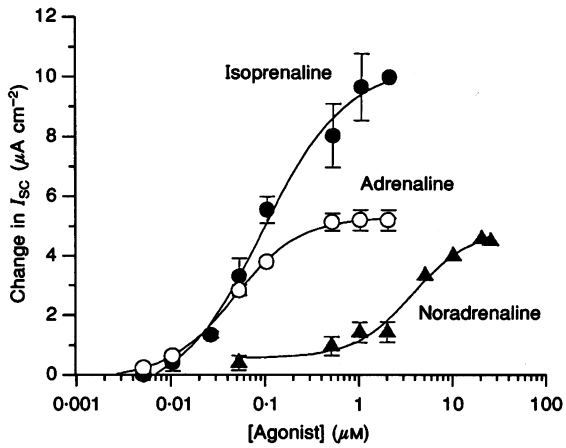
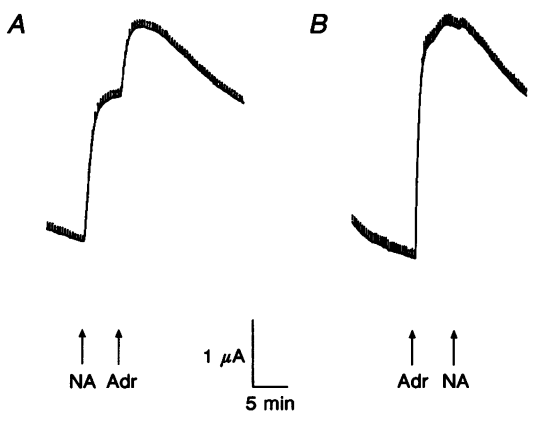


Figure 4. Concentration–response curves of adrenaline, noradrenaline and isoprenaline

Change in I_{SC} is plotted against hormone concentration. Drugs were applied on the basolateral side. Data for each point were obtained from at least 3 independent experiments. Symbols are means and bars where visible are S.E.M. values.



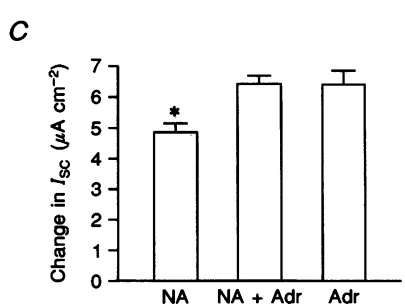


Figure 5. Combined effect of noradrenaline and adrenaline on I_{SC}

A, I_{SC} recording ($n = 5$) with arrows marking the times at which basolateral noradrenaline (NA, 10 μM) or adrenaline (Adr, 1 μM) were added. $I_b = 6{\cdot}7$ μA cm^{-2}. *B*, I_{SC} recording obtained when drugs were added in reverse order. $I_b = 6{\cdot}2$ μA cm^{-2}. *C*, summary of changes in I_{SC} stimulated by noradrenaline (10 μM) alone, by adrenaline (1 μM) alone, and by both adrenaline and noradrenaline. Columns and bars are means ± S.E.M.; $n = 5$ for all groups. * $P < 0{\cdot}01$ compared with the other groups individually.

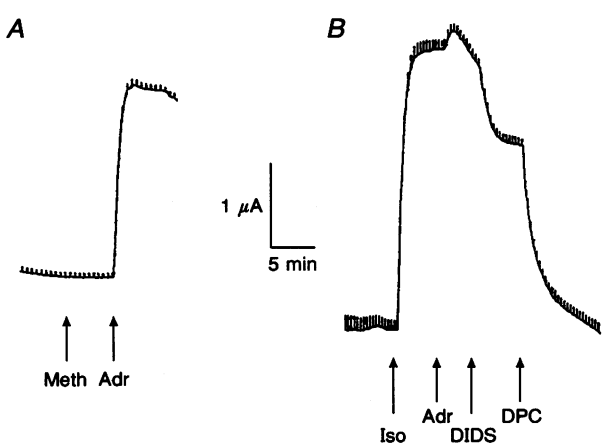


Figure 6. Effect of methoxamine and isoprenaline on I_{SC}

A, I_{SC} recording ($n = 4$) with arrows marking the times at which basolateral methoxamine (Meth, 10 μM) or adrenaline (Adr, 1 μM) was added. $I_b = 4{\cdot}3$ μA cm^{-2}. *B*, I_{SC} recording with arrows marking the times at which basolateral isoprenaline (Iso, 0·05 μM) and adrenaline (Adr, 1 μM), or apical DIDS (200 μM) and DPC (2 mM) were added. $I_b = 3{\cdot}3$ μA cm^{-2}.

Involvement of cAMP

The effect of adrenaline on the I_{SC} was mimicked by an adenylate cyclase activator, forskolin (10 μM; $n = 16$; Fig. 7*A*). The forskolin-induced I_{SC} was reduced by DIDS (200 μM) and DPC (2 mM) by 26 ± 1% ($n = 3$) and 100% ($n = 3$), respectively, reductions similar to those observed for the adrenaline-stimulated I_{SC}. As shown in Fig. 7*B*, the effects of both adrenaline (1 μM) and forskolin (10 μM) on the I_{SC} could be abolished by pretreatment with an adenylate cyclase inhibitor, MDL (20 μM; $n = 4$; $P < 0{\cdot}0001$), indicating that cAMP is involved in mediating the adrenaline-induced response.

Effects of adrenoceptor antagonists

Adrenoceptor antagonists were also used in conjunction with adrenoceptor agonists to investigate further the types of adrenoceptors involved. The β-adrenoceptor antagonist propranolol (1 μM) suppressed most of the responses to noradrenaline (10 μM) and adrenaline (1 μM) (Fig. 8*A*). The propranolol-treated cells responded to subsequent stimulation by ATP (Fig. 8*A*), indicating that the inhibitory effect of propranolol was specific and not due to other non-specific effects on the membrane. On the other hand, pre-treatment of endometrial epithelial cells with phentolamine (10 μM), an α-adrenoceptor antagonist, did not affect the I_{SC} induced by noradrenaline or adrenaline significantly. A comparison of the effects of α- and β-adrenoceptor antagonists is shown in Fig. 8*B* and *C*. The β-adrenoceptor antagonist propranolol produced a significant reduction in the agonist-stimulated I_{SC} response but the α-adrenoceptor antagonist phentolamine did not.

DISCUSSION

The present study has demonstrated stimulation of anion secretion across the mouse endometrial epithelium by adrenoceptor agonists. Previous studies on isolated human endometrial epithelial cells have shown that a number of neurohormonal agents, including adrenaline, stimulate electrogenic ion transport (Matthews *et al.* 1992, 1993*a*,*b*). However, the ion species mediating the response has not been fully investigated. The present study shows that the response of the mouse endometrial epithelium to adrenaline is mainly mediated by anion secretion. The supporting evidence comes from the experiments in which the I_{SC} was greatly reduced by replacement of Cl^- in the bathing solutions and the adrenaline-activated I_{SC} was blocked by various Cl^- channel blockers applied to the apical membrane. It should be noted that replacement of both HCO_3^- and Cl^- produced a further reduction in the adrenaline-stimulated I_{SC} response. One possible explanation is that HCO_3^- secretion could be stimulated concurrently, but clarification of this point requires further studies. The possibility that Na^+ absorption is involved in the adrenaline-stimulated I_{SC} was excluded by the observation that addition of amiloride at a concentration known to block apical Na^+ channels did not inhibit the adrenaline-stimulated I_{SC}. Replacement of apical Na^+ did not diminish the adrenaline-stimulated I_{SC} either, which also indicated that Na^+ absorption was not involved. The reduction in the adrenaline-stimulated I_{SC} following bilateral replacement of Na^+ observed in the present study may result from inhibition of certain basolaterally located Na^+-dependent transporters. A likely

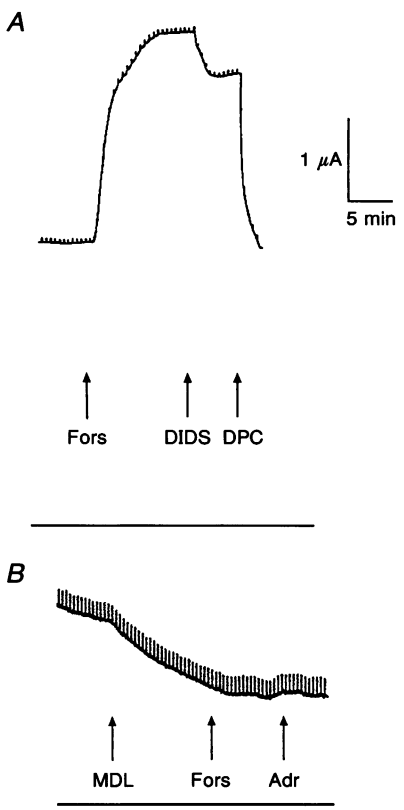


Figure 7. Effects of an adenylate cyclase activator (forskolin) and an inhibitor (MDL) on I_{SC}

A, I_{SC} recording ($n = 5$) with arrows marking the times at which basolateral forskolin (Fors, 10 μM), DIDS (200 μM) or DPC (2 mM) was added. $I_b = 4{\cdot}9$ μA cm^{-2}. *B*, I_{SC} recording ($n = 6$) with arrows marking the times at which basolateral MDL (20 μM), adrenaline (Adr, 1 μM) or forskolin (Fors, 10 μM) was added. $I_b = 4{\cdot}4$ μA cm^{-2}.

candidate would be the Na^+–K^+–$2Cl^-$ cotransporter which has been demonstrated to play an important role in active Cl^- secretion in many secreting epithelia, including the airways (review by Welsh, 1987). Another candidate could be the Na^+–HCO_3^- cotransporter, which has been demonstrated to be responsible for substantial HCO_3^- secretion across the pancreatic duct of the guinea-pig (Ishiguro, Steward, Wilson & Case, 1996). These possibilities are consistent with our contention that adrenaline stimulates anion secretion across the mouse endometrial epithelium.

The present study has also demonstrated that stimulation of anion secretion by adrenaline across the mouse endometrial epithelium is mainly mediated by β-adrenoceptors. Several lines of evidence support this contention. First, agonists with higher specificity for β-adrenoceptors are more effective in activating the I_{SC} (isoprenaline > adrenaline > noradrenaline), while the α-adrenoceptor agonist methoxamine is without effect. Adrenaline could produce further stimulation of I_{SC} if the initial stimulant was noradrenaline, but not if it was isoprenaline. The fact that the effects of adrenaline and isoprenaline on I_{SC} were not additive suggests that the action of adrenaline is similar to that of isoprenaline, which acts on β-adrenoceptors. While the effect of adrenaline following noradrenaline on the I_{SC} was additive, the combined effect was similar to that produced by adrenaline alone. In contrast, no additive effect could be seen if adrenaline was added prior to noradrenaline, suggesting that their effects are likely to be mediated by the same pathway, which involves β-adrenoceptors. This notion is further supported by the studies using adrenoceptor antagonists. Propranolol, a β-adrenoceptor antagonist, was found to be more potent than the α-adrenoceptor antagonist phentolamine in blocking the agonist-induced I_{SC}, demonstrating a predominant involvement of β-adrenoceptors.

As further support for a role for adrenoceptors in mediating the adrenaline response, the involvement of cAMP has also been demonstrated by mimicking the effect of adrenaline on the I_{SC} by an adenylate cyclase activator, forskolin. The effects of forskolin and adrenaline on the I_{SC} are similar in that they both induced a slow and sustained I_{SC} compared with the previously observed rapid and transient current elicited by Ca^{2+}-mobilizing agents such as ionomycin and ATP (Chan *et al.* 1997*b*). In addition, both forskolin- and adrenaline-induced I_{SC} could be inhibited by various Cl^- channel blockers to a similar extent. Together with the observed inhibition of the adrenaline-stimulated I_{SC} by pretreatment with the adenylate cyclase inhibitor MDL, these results suggest a role for cAMP in the action of adrenaline and add further support to the contention that β-adrenoceptors are involved in the regulation of anion secretion in the mouse endometrium. It is interesting to note that a mean reduction of 20% in these currents could be induced by DIDS, a blocker known to inhibit the Ca^{2+}-activated Cl^- channel but not the cAMP-activated Cl^- channel in various epithelia (Fuller & Benos, 1992). The adrenaline-induced response observed in the mouse

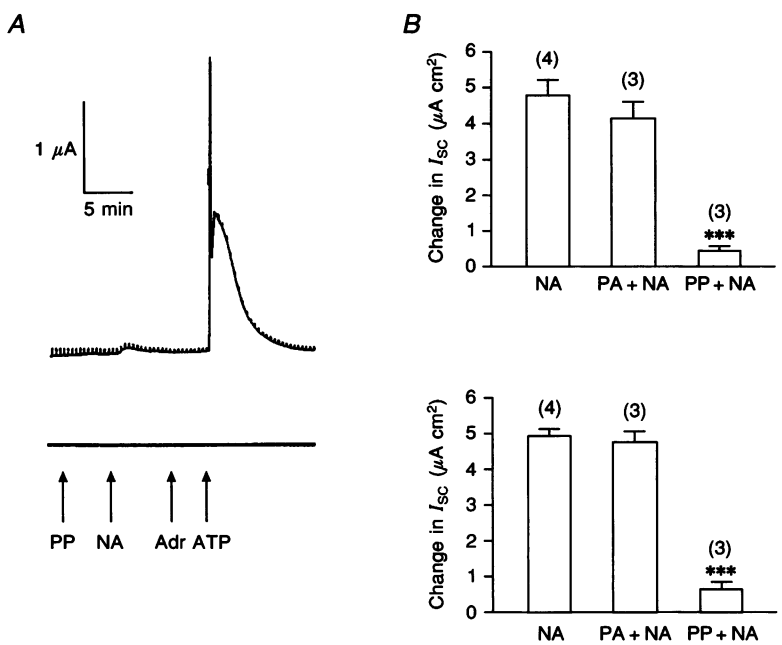


Figure 8. Effects of adrenoceptor antagonists

A, I_{SC} recording ($n = 4$) with arrows marking the times at which basolateral propranolol (PP, 10 μM), noradrenaline (NA, 10 μM), adrenaline (Adr, 1 μM), or apical ATP (10 μM) were added. $I_b = 2{\cdot}7$ μA cm^{-2}. *B*, comparison of the effects of α- and β-adrenoceptor antagonists on the adrenaline- and noradrenaline-stimulated I_{SC} responses. Columns and bars are means ± S.E.M.; numbers in parentheses are the number of experiments. *** $P < 0{\cdot}001$ compared with the control group.

endometrial epithelium may involve cross-talk between cAMP and Ca^{2+} signalling pathways.

Previous studies on intact rat uteri have yielded similar results, demonstrating that the effect of adrenaline on rat endometrial bioelectrical activity *in vivo* and *in vitro* was mediated by a β-adrenoceptor through cAMP and Ca^{2+}-sensitive pathways (Levin & Phillips, 1983; Levin & Sebkhi, 1989). In contrast to the present finding that adrenaline stimulates mainly Cl^- secretion in the mouse, the adrenaline-stimulated electrogenic ion secretion in the rat has been attributed to HCO_3^- secretion only (Levin & Scargill, 1987). Further investigation of the interaction between Cl^- and HCO_3^- ions in the adrenaline-stimulated I_{SC} response is required to understand fully the ionic mechanism(s) underlying the electrogenic ion transport process in the endometrium.

Regulation of ion transport across the endometrium by neurohormonal agents has also been observed in humans (Matthews *et al.* 1992, 1993*a*) and pigs (Vetter & O'Grady, 1996), but the responses appear to be different from those observed in the present study. The adrenaline-stimulated I_{SC} observed in primary cultures of human endometrial glandular epithelial cells, which was evident even in Na^+-free solution, was transient in nature in contrast to the sustained response observed in the present study. In addition, no I_{SC} response to forskolin was observed in the human cultures, which excludes the possibility that a cAMP-dependent mechanism mediates the effect of adrenaline in the human endometrial cells. Stimulation of Na^+ absorption and K^+ secretion rather than anion secretion by $PGF_{2\alpha}$ was observed in intact endometrium from pigs (Vetter & O'Grady, 1996), but activation of both anion secretion and Na^+ absorption by PGE_2 was observed in primary cultures of porcine glandular endometrium (Deachapunya & O'Grady, 1996). The different responses observed in the porcine endometrium have been attributed to the possibility that glandular cells in culture express a combination of luminal cell and glandular cell transport phenotypes and that receptors present in culture may not be present *in vivo*. The isolation and culture methods (McCormack & Glasser, 1980) that we have adapted for the mouse endometrium should yield predominantly luminal epithelial cells, although we cannot exclude possible contamination by glandular epithelial cells. At the present, it is difficult to determine whether the different responses of the endometrium to cAMP-evoking agents observed in different species was due to species specificity, or to the culture conditions, i.e. luminal *vs.* glandular epithelial cells, or intact *vs.* cultured cells. Clarification of this issue will require further investigation. It should also be noted that the present results were obtained from endometrial cells from reproductively immature mice. This may also contribute to the difference between the present results and those obtained from humans.

It would be of interest to investigate whether cAMP-dependent Cl^- secretion is mediated by the cystic fibrosis transmembrane conductance regulator (CFTR), which has itself been shown to be a cAMP-regulated Cl^- channel (Bear *et al.* 1992) and is expressed differently in the uterine epithelium of humans (Tizzano, Chitayat & Buchwald, 1993) and rodents (Trezise *et al.* 1993). Although the present study indicates that cAMP-dependent Cl^- secretion across the mouse endometrial epithelium is sensitive to glibenclamide and DPC, both of which have been shown to have potent effect on CFTR (Fuller & Benos, 1992; Sheppard & Welsh, 1992), further experiments using the patch clamp technique are required to identify the Cl^- channels involved. The role of the cAMP-stimulated endometrial Cl^- secretion in cystic fibrosis and infertility in CF women also remains to be elucidated.

In conclusion, the present study has demonstrated that anion secretion across the mouse endometrium could be regulated via a β-adrenoceptor and involve a cAMP-dependent mechanism. Regulated anion secretion may constitute the physiological basis for the observed high pH and HCO_3^- content in the rabbit uterus (Vishwakarma, 1962) and the increased Cl^- concentration during implantation in the rat (Nilsson & Ljung, 1985).

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