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Regulation of Na^+ Absorption and Cl^- Secretion in the Endometrium: Switching Mechanisms

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It is believed that the endometrial epithelium lining the lumen of the uterus has electrolyte transport activities which provide a suitable fluid environment for sperm movement, embryo implantation and development. The uterine fluid composition is largely determined by the absorptive and secretory activities of the endometrial epithelium. We have recently established a primary culture of mouse endometrial epithelium grown on permeable supports on which the electrogenic ion transport across the endometrial epithelium could be studied using the short-circuit current (I_{SC}) technique (1). The cultured monolayers exhibited a substantial basal I_{SC} which was attributed to Na^+ absorption. However, the cultured endometrial epithelium responded to a number of neurohormonal agents, e.g., adrenaline, prostaglandins and ATP, with predominant anion secretion (2-6). The switching mechanisms for the two opposing processes remain largely unknown.

Inhibition of ENaC by CFTR

Previous studies using the short-circuit current and whole-cell patch-clamp techniques have demonstrated the involvement of CFTR in mediating the secretory responses to adrenaline, prostaglandins in mouse endometrial epithelial cells (6). In the present study, using RT-PCR, CFTR and epithelial Na^+ channel (ENaC) were found to be co-expressed in mouse endometrial cells. The endometrial cells, isolated using the method described previously (1, 2), formed functional monolayers which exhibited a basal current of 4.6 ± 0.3 mA/cm^2. Of this basal current, $61.7\pm2.4\%$ was inhibited by apically applied Na^+ channel blocker, amiloride (10 mM). However, after stimulation with adenylate cyclase activator, forskolin, the amiloride-sensitive current, expressed as percentage of the basal current, was decreased to $21.8\pm4.2\%$. Similar reduction in amiloride-sensitive current, $26.6\pm2.5\%$, was also observed after stimulation with adrenaline which had been shown to activate β-adrenoceptor and a cAMP-dependent pathway leading to the activation of CFTR in the mouse endometrial cells (6). Pretreatment of the monolayers with amiloride did not alter the agonist-induced currents, however, pretreatment with Cl^- channel blocker, DPC (1 mM), completely abolished the agonist-induced currents, indicating that the agonist-induced current was entirely due to activation of Cl^- secretion but not Na^+ absorption. The observed decrease in the amiloride-sensitive current after stimulation with cAMP-evoking agonists may be due to down- regulation of Na^+ channel via activation of CFTR.

Inhibition of Na^+ absorption by extracellular ATP

Previous studies (2) have demonstrated activation of endometrial Cl^- secretion by extracellular ATP via P_{2U} receptor and Ca^{2+}-dependent pathway involved the Ca^{2+}-activated Cl^- Channel (CaCC). The present study investigated the signaling pathway involved in the ATP-induced inhibition of Na^+ absorption by the cultured endometrial epithelium using the short-circuit current (I_{SC}) technique. Basal current (I_b) of cultures appeared predominant Na^+ absorption. Apical addition of ATP resulted in an averaged decrease in the I_{SC}, and subsequent Forskolin resulted in an additional decrease, indicating different inhibitory mechanisms involved. Both ATP-induced inhibition of Na^+ absorption and Cl^- secretion, but not those induced by forskolin, could be abolished by P_2 receptor antagonist, reactive blue, indicating the involvement of P_2 receptor in mediating the ATP response. However, the ATP-induced inhibition of Na^+ absorption could be mimicked by ionomycin, suggesting that the inhibitory effect was mediated by Ca^{2+} and could surpass the receptor level. Chelating extracellular Ca^{2+} with EGTA reduced the ATP- but not the

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forskolin-induced inhibition, further indicated the involvement of Ca^{2+} in mediating the inhibitory effect of ATP. The ATP-induced inhibition of Na^+ absorption could also be abolished by DIDS, indicating the involvement of CaCC. The present results suggest that in addition to its role in stimulating Cl^- secretion, extracellular ATP may inhibit Na^+ absorption by a Ca^{2+}-dependent ENaC-inhibiting mechanisms involving CaCC, which is independent of CFTR, in mouse endometrial epithelial cells.

In summary, the mouse endometrial epithelium is capable of switching from predominant Na^+ absorption to Cl^- secretion by inhibiting ENaC upon stimulation or activation of anion channels. There appear to be different mechanisms involved, which are important for the regulation of uterine fluid enviornment.

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