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Calcium-Dependent and -Independent Mechanisms of P2Y Receptor Regulated Anion Secretion in Polarized Epithelia

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Extracellular nucleotides such as adenosine 5'-triphosphate (ATP) or uridine 5'-triphosphate (UTP) have been shown to regulate ion transport processes in a variety of epithelia (1). In some secretory epithelia, this effect is mediated by plasma membrane P2Y receptors, which belong to the G protein-coupled receptor family. Occupancy of P2Y receptors by various agonists activates the inositol 1,4,5-triphosphate (IP_3) – mediated intracellular Ca^{2+} signalling cascade. Empty of intracellular Ca^{2+} stores triggers the opening of plasmalemmal Ca^{2+} entry pathway, a phenomenon known as "capacitative calcium entry" or "store-operated Ca^{2+} influx" (2). Ion transport processes (e.g. activation of apical Cl^- channels) are then stimulated via the increase in intracellular free calcium concentration ($[Ca^{2+}]_i$).

There is, however, a paucity of information on the mechanisms of stimulus-secretion coupling in polarized epithelia. For example, the relationship between intracellular Ca^{2+} responses (i.e. Ca^{2+} release and influx via different membrane domains) and anion secretion remains a partially unresolved issue. Moreover, it is now emerging that certain types of P2Y receptor (e.g. P2Y2) are coupled to multiple, signal transduction pathways in which changes in $[Ca^{2+}]_i$ is not an obligatory component (3-6). More importantly, the functional expression of at least some P2Y receptor subtypes appears to be a feature of the polarized phenotype. These receptors may not be detected in isolated, non-polarized cells that have been grown on glass coverslips (7, 8). Apical Cl^- channel regulation in cells grown in monolayer culture therefore cannot be compared with that in isolated epithelial cells. Simply using the methods traditionally applied to single cells (e.g. microspectrofluorimetry, patch clamping) may fail to reveal how extracellular nucleotides regulate anion secretion in polarized epithelia.

In this study, a technique which allows us to monitor nucleotide-evoked short-circuit current (I_{SC}) and $[Ca^{2+}]_i$ concurrently in a polarized epithelium was employed (8, 9). The effect of various nucleotides on $[Ca^{2+}]_i$ and I_{SC} were explored in an equine sweat gland epithelial cell line (10). Previous studies have shown that it will form polarized monolayer on permeable support (11) and express both apical purine and pyrimidine receptors (12). The relationships between nucleotide-evoked $[Ca^{2+}]_i$ signalling and anion secretion was also investigated.

Cells were grown on Transwell-Col membranes for 4 days using standard culture techniques. Confluent monolayers were clamped in modified Ussing chamber and perfused bilaterally in normal Krebs solution containing 2.5 mM Ca^{2+}. ATP applied to the apical membrane increased both $[Ca^{2+}]_i$ and I_{SC} dose dependently. In another series of experiment, the internal Ca^{2+} stores were depleted by exposing the luminal membrane to 100 μM ATP under Ca^{2+}-free condition. Releasing of Ca^{2+} from internal stores, as measured by a change in Fura-2 fluorescence ratio, evoked a transient increase in I_{SC}. Re-introduction of Ca^{2+} in the basolateral bathing solution led to the increases in both $[Ca^{2+}]_i$ and I_{SC}. After the I_{SC} and $[Ca^{2+}]_i$ had returned to basal level, re-introduction of Ca^{2+} in the apical bathing solution induced a smaller increases in $[Ca^{2+}]_i$ and I_{SC}. Reversing the sequence of perfusing the epithelium with Ca^{2+}-containing solution (i.e. apical followed by basolateral) or emptying the internal stores by the sacro/endoplasmic reticulum Ca^{2+}-ATPase (SERCA) pump inhibitor thapsigargin revealed a similar pattern. These data suggest that the capacitative Ca^{2+} influx induced either by luminal P2Y receptors or thapsigargin was predominately occurred via basolateral membrane, which subsequently evoked anion secretion. This is in close agreement with our previous finding using conventional I_{SC} measurement (13). Similar phenomenon is also observed in MDCK-C7 cells (14), pancreatic acinar cells (15), and more recently in human colonic T84 and bronchial epithelial cells (16 HBE14o$^-$) (16).

Previous study using conventional I_{SC} measurement also demonstrates that basolateral application of ATP could only elicit a small and variable I_{SC} and therefore it was concluded that the P2Y receptors are essentially

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confined to the luminal membrane (12). When both $[Ca^{2+}]_i$ and I_{SC} were monitored simultaneously, basolateral application of 100 μM ATP evoked an increase in $[Ca^{2+}]_i$ but the increase in I_{SC} was only minimal (<1 μAcm^{-2}). Further experiments showed that other nucleotides such as UTP and UDP evoked dose-dependent increase in $[Ca^{2+}]_i$ when applied to either apical or basolateral membrane. Basolateral application of these nucleotides, however, did not evoke discernible changes in I_{SC}. Using the "Ca^{2+} store depletion and Ca^{2+} re-introduction" protocol as described earlier, basolateral ATP-activated Ca^{2+} influx was also predominately located at the basolateral membrane. Moreover, results indicated that apical ATP and basolateral ATP discharged Ca^{2+} from separate internal stores. It appears that although P2Y receptors are present in both luminal and basolateral membranes and initiate the same signalling cascade, there can be differences in responsiveness to the rise in $[Ca^{2+}]_i$.

One of the possibilities to explain the discrepancy between $[Ca^{2+}]_i$ and I_{SC} responses is that there may be direct coupling between the apical P2Y receptors and anion channels. Therefore, activation of apical anion channels can occur without the involvement of $[Ca^{2+}]_i$ increase. To explore this "calcium-independent" pathway, the epithelia were pre-stimulated with 1 μM thapsigargin in order to elevate the $[Ca^{2+}]_i$ to a level that could not be further raised by the Ca^{2+}-mobilising action of extracellular nucleotides. Under such condition, application of 100 μM ATP elicited an increase in I_{SC} but not $[Ca^{2+}]_i$. Therefore, ATP could evoke anion secretion without accompanied by a rise in $[Ca^{2+}]_i$ suggesting that the nucleotide-activated I_{SC} can be partly attributed to the $[Ca^{2+}]_i$-independent regulation by P2Y receptors.

In summary, both apical and basolateral aspects of the equine sweat gland epithelia express P2Y receptors. Although activation of these receptors evoked an increase in $[Ca^{2+}]_i$, only apical P2Y receptors could stimulate a substantial increase in anion secretion. Taken together, these data suggest that apical P2Y receptors may i) activate additional signalling pathways, ii) directly regulate apical anion channels to evoke secretion, and iii) elicit a localized $[Ca^{2+}]_i$ that are required to activate the opening of apical anion channels.

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