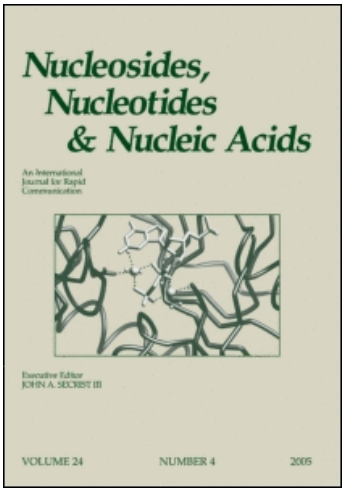

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APICALLY SORTED P2X7 RECEPTORS MEDIATE PURINERGIC-INDUCED PORE FORMATION PREFERENTIALLY IN APICAL DOMAINS OF THE PLASMA MEMBRANE

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□ *Treatment of human epithelial cervical cells CaSki attached on filters with the P2X7-receptor (P2X7-R) agonist BzATP induced acute transient influx of calcium, most likely the result of P2X7-R channel activation, followed by slower sustained calcium influx. In cultures incubated in the presence of ethidium bromide (EB), BzATP induced slow and sustained influx of the dye with a time-course similar to the late slow calcium influx, suggesting P2X7-R pore formation. The acute and late calcium effects of BzATP were greater if the agonist was added to the luminal solution, facing the apical membrane of the cells. The EB effect of BzATP initially occurred in the apical membrane, while effects in the basolateral membrane were delayed and smaller. These results suggest that in polarized epithelial cells under steady-state conditions the P2X7-R is located in the apical membrane, and activation of the receptor induces formation of P2X7-R pores preferentially in the apical membrane.*

Keywords Cervix; Epithelium; P2X7; Receptor; Apical; Basolateral

INTRODUCTION

The P2X7-receptor (P2X7-R) plays an important role in regulation of apoptosis of human uterine cervical epithelial cells.[1] The P2X7-R belongs to the P2X subfamily of P2 nucleotide receptors[2] which are membrane-bound, ligand-operated channels.[3] ATP is the naturally occurring ligand for the P2X7-R, and activation of the receptor by brief exposure to extracellular ATP opens cation channels that allow Ca^{2+}, Na^{+},

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and K^+ influx. Longer exposure to ATP allows passage of cations with progressively larger diameters, up to 900 Da, through formation of pores.[4] The mechanism of P2X7-R pore formation is unclear, and opinions vary between decreased filter selectivity of existing channels[5] to rearrangement of receptor molecules.[6] To test the latter hypothesis, the present study utilized the polarized cultured epithelium CaSki to determine to what degree baseline distribution of the P2X7-R changes after treatment with a ligand.

MATERIALS AND METHODS

The experiments utilized human epithelial cervical cells CaSki attached on filters. The cell characteristics, culture conditions and method of plating on filters were previously described.[1] Dynamic Confocal Laser Scanning Microscopy was used to determine changes in intracellular (cytosolic) calcium and ethidium bromide (EB) fluorescence. Cells were seeded at $2\text{-}3\cdot10^5$ on filters and were allowed to reach confluence. For cytosolic calcium determinations cells were loaded with 5 μM Fluo-4/AM and imaged with a Zeiss LSM 510 inverted real-time confocal microscope equipped at 488 nm/505 nm (exc/emm). For ethidium bromide influx experiments, filters with cells were loaded onto the microscope and imaged at the same wavelengths (488 nm/505 nm exc/emm) after adding 5 μM of ethidium bromide to either the luminal and subluminal perfusates. Average fluorescence intensity was quantified from collated images using MetaVue software (Fryer Company Inc., Huntley, IL, USA) by subtracting the basal intensity values.

RESULTS AND DISCUSSION

Treatment of CaSki cells with the P2X7-R specific agonist 2',3'-0-(4-benzoylbenzoyl)-adenosine 5′-triphosphate (BzATP) induced a biphasic change in cytosolic calcium: an acute transient increase, and a slow sustained increase in cytosolic calcium (Figure 1). The BzATP effects could be blocked by pre-treatment with 1.2 mM EGTA (to chelate extracellular calcium) (not shown), indicating that both effects are the result of calcium influx. The likely explanation for the acute transient increase in cytosolic calcium is P2X7-R channel activation,[3] resulting in acute calcium influx via the open channel. The explanation for the slow secondary calcium influx is P2X7-R pore formation[3] (see also Figure 2).

Both calcium effects of BzATP depended on the sidedness of treatment. Thus, a greater effect was observed when BzATP was added in the luminal solution (facing the apical membranes), while a smaller effect was observed

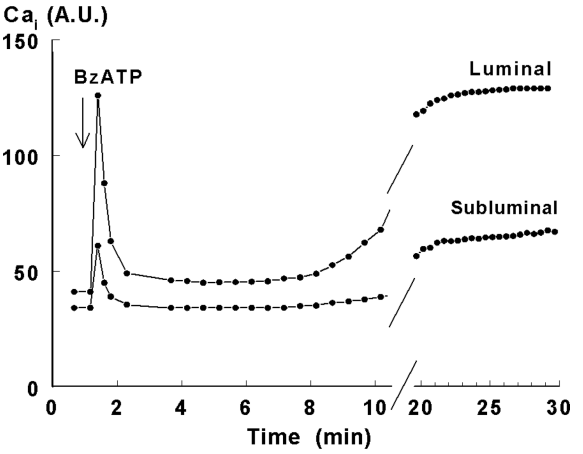

FIGURE 1 Effects of BzATP on cytosolic calcium. CaSki cells attached on filters were loaded with the calcium-sensitive dye Fluo-4 and treated with 100 μM BzATP (arrow) added either to the luminal or subluminal solutions (A.U. = arbitrary units).

when BzATP was added in the subluminal solution (facing the basolateral membrane) (Figure 1).

These data suggest that at baseline the P2X7-R is expressed predominantly at the apical plasma membrane, and ligation of the receptor induces channel activation in apical domains of the plasma membrane.

To determine the site of pore formation, cells were treated with BzATP, and at different time-intervals thereafter aliquots of the intracellular dye ethidium bromide (EB) were added either to the luminal or subluminal

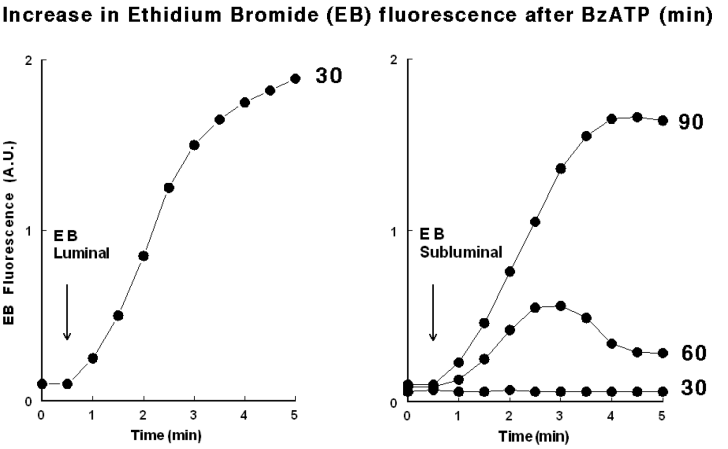

FIGURE 2 Effects of BzATP on ethidium bromide (EB) fluorescence. CaSki cells attached on filters were treated with 100 μM BzATP added to both the luminal and subluminal solutions. After 30–90 minutes, Aliquots of EB (5 μM, arrows) were added either to the luminal or subluminal solutions (A.U. = arbitrary units).

solution. EB (MW 394 Da) is a nuclear stain that does not enter cells having intact membranes; however, upon formation of pores in the plasma membrane (e.g., activation of P2X7-R pores),[3] EB can influx cells, bind to nuclear chromatin and elicit specific fluorescence.

In CaSki cells, under baseline conditions, addition of EB did not induce fluorescence (Figure 2). In contrast, in CaSki cells treated with BzATP addition of the EB to the solutions induced an increase in EB fluorescence, indicating pore formation (Figure 2). The effect was asymmetrical relative to the apical versus basolateral membranes. EB fluorescence was observed already 30 minutes after BzATP if EB was added to the luminal solution. In contrast, a smaller and delayed effect was observed when EB was added in the subluminal solution: the effect was observed only 30–60 min after BzATP (in contrast to $\leq$30 minutes if EB was added in the luminal solution); the increase in EB fluorescence did not reach maximal levels even 90 minutes after BzATP; and it was transient in contrast to a saturable effect when added in the luminal solution (Figure 2).

Collectively, the data in Figures 1 and 2 indicate that functional P2X7-Rs are expressed predominantly in apical domains of the plasma membrane, and upon activation P2X7-R pores are induced preferentially in the apical membrane. The delayed response of BzATP-induced EB influx suggests that activation of the P2X7-R induces recruitment of P2X7 receptors to the apical membrane, as well as to the basolateral membrane, and therefore supports the hypothesis that P2X7-R pore formation involves rearrangement of receptor molecules in the plasma membrane.

The biological significance of the present finding is at present unclear. In vivo, the natural agonist of the P2X7-R is ATP, which is continuously secreted by cells into their surrounding extracellular milieu, thereby activating the P2X7-R mechanism in a paracrine-autocrine manner.[1] In the cervix, the P2X7-R is expressed mainly in cells of the basal-parabasal germinative layer of the cervical epithelium (not shown). These cells determine the regeneration and growth of the cervical epithelium, and are under control of mitogenic and apoptotic stimuli. In the cervix the P2X7-R mechanism plays an important role in regulation of cell apoptosis.[1,7] In vivo, the basolateral surface of epithelial cells is exposed to the blood compartment, and ATP most likely would be either diluted, washed, or degraded by plasma ecto-ATPases and not accumulate in appreciable amounts to exert an efficient effect on the P2X7-R. On the other hand, the apical surface of the basal-parabasal cells faces the differentiating suprabasal layers of cells, and ATP secreted from those cells effectively could activate the apically-located P2X7-R of the basal-parabasal cells. This speculation provides basis to a novel hypothesis of regulation of cell growth whereby differentiating suprabasal cells of the cervical epithelium control the growth of germinative basal-parabasal cells through the P2X7-R mechanism.

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