Large pore formation uniquely associated with P2X
7 purinergic receptor channels. Focus on “Are second messengers crucial for opening the pore associated with P2X7 receptor?”

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P2X RECEPTOR CHANNELS ARE RECEPTORS for extracellular nucleotides (8, 9). The same P2X receptor membrane protein also forms a Ca2+-permeable, nonselective cation channel (8, 9). Seven subtypes have been cloned (9). They share the same overall topology of intracellular NH2 and COOH termini, two transmembrane α-helices, and a very large extracellular domain that accounts for 70% of the molecular mass in all P2X receptor channel subtypes (8, 9). This topology is also shared with the epithelial Na+ channel (ENaC) and ENaC relatives in mammals and in lower organisms such as the acid-sensing ion channels, brain ENaCs, degenerins of the nematode, and ripped pocket and pickpocket genes of Drosophila (3, 9). Within the large extracellular domain in each subfamily, there is an even number of conserved cysteines that are thought to participate in intrachain disulfide bridging (8). P2X receptors are fascinating membrane proteins to study in epithelial and other cells. Their large extracellular domains are exposed to diverse extracellular microenvironments (9, 15, 16). A schema of a P2X receptor channel is shown in Fig. 1. It is hypothesized that P2X plasma membrane proteins serve at least three functional roles for a cell. First, they are clearly receptors for extracellular nucleotides and for biomolecules such as zinc. Second, they are cation channels that can serve as Ca2+-entry channels to elicit a sustained increase in cellular Ca2+. Third, by virtue of their large extracellular domain, they are extracellular sensors for ATP, zinc, protons, and cations (9). Emerging evidence suggests that there are binding sites for all of these substances within this elaborate extracellular domain (8, 9).

The study by Faria et al. (Ref. 5, see p. C194 in this issue) tackles a problem that has confounded P2X receptor biologists for some time. It has been known for many years that millimolar concentrations of extracellular ATP can promote membrane permeabilization (9). Even as the new class of P2X purinergic receptors was emerging through molecular cloning, experts thought that the ATP-induced permeabilization receptor would fall into a third molecular class of purinergic receptors (1). Before its cloning, the ATP-induced permeabilization receptor was referred to as the P2Z receptor to distinguish it from P2X receptor channels and P2Y G protein-coupled purinergic receptors (1). However, Suprenant et al. (13) cloned a seventh P2X receptor channel gene that had significant homology with P2X1–6. It was classified as P2X7 and was shown to confer two phenotypes on a cell in which it was expressed. First, a Ca2+-permeable, nonselective cation channel of a defined single-channel conductance was observed, a phenotype consistent with P2X1–6 (13). P2X6 was thought to be a silent channel, although a recent study suggested that this may not be true (6). However, the P2X7-expressing cell also expressed a larger membrane pore that was permeable to large compounds and dyes that were ≈1,000 Da (13). Uptake of such dyes is used to measure P2X7 function as well as the state of apoptosis. P2X7 and apoptosis are closely linked; however, it is not clear whether apoptosis induces P2X7 expression (i.e., P2X7 is a marker of apoptosis) or whether P2X7 is essential to drive a cell into programmed cell death (8, 13).

North (8), in a recent comprehensive review of the molecular physiology of P2X receptors, summarized P2X7-mediated pore formation, dye uptake, and apoptosis. Other recent papers (4, 7, 11, 12, 14), in addition to the article in focus by Faria et al. (5), have addressed these issues. Figure 1 shows a molecular feature of P2X7 that sets it apart from P2X1–6. It has a substantially longer and larger COOH terminus that resides in the cytosol. Specific regions of the COOH-terminal tail have been examined and show the possible existence of a domain for binding of bacterial lipopolysaccharide, an SH3 domain, and a possible domain for binding α-actinin (8). Deletion of the entire COOH terminus reduces dye uptake through large pores but not cation channel function. A glutamate (E496) also has been implicated in pore formation and dye uptake (8). It lies within a stretch of amino acids rich in cysteines, arginines, and lysines (8). In another study, a proline (P582) was also found to be critical for dye uptake (11). Removal of this residue within a truncation or mutation to a glycine residue abolished or diminished pore formation and dye uptake (11). Wilson et al. (14) have begun to use the P2X7 COOH-terminal tail as bait to pull down proteins that interact with P2X7 receptors to mediate pore formation, dye uptake, membrane blebbing, and ultimately apoptosis. They have found some interesting candidate proteins in a thymic epithelial cell expression system that may tie P2X7 receptors to membrane blebbing (14).

The issue of large pore formation associated with P2X7 is addressed in the study by Faria et al. (5). The essence of their argument is that signal transduction initiated by P2X7 receptor is essential to observe large pore formation and dye uptake. In particular, a sustained increase in cell Ca2+ and activation of MAP kinases are required to observe pore formation but not P2X7 receptor cation channel activity. Evidence for a requirement for both p38 MAPK and MEK kinases was found with selective pharmacological inhibitors. The data with regard to MAP kinases are in agreement with a recent study showing p38 MAP kinase involvement in activation of caspases and Rho-kinase triggered via P2X7 engagement; these enzymes are intimately involved in apoptotic events (5, 7). In fact, data...
published in 2003 by Amstrup and Novak (2) showed that P2X7 also activates ERK1 and ERK2 MAP kinases. However, they showed that the COOH terminus is important for Ca2+ entry, while the NH2 terminus as well as proximal parts of the COOH terminus are likely involved in ERK activation. Taken together, these data clearly demonstrate that P2X7 may be closely coupled to MAP kinase-dependent signal transduction pathways.

Ca2+ entry was also essential to observe P2Xγ-associated pore formation and dye uptake in the study by Faria et al. (5). Of particular interest, 10 μM ionomycin induced pore formation independently of P2X7 receptor activation with millimolar concentrations of ATP or ATP analog. Pore formation was abolished in reduced or absent extracellular Ca2+ solutions and by chelation of intracellular free Ca2+. Dye exclusion from the large pores also was carefully analyzed, confirming a cutoff of 900 Da. These data are consistent with a maitotoxin-sensitive, large, dye-permeable pore characterized by Schilling et al. (10) in some of the same cell models. Using pharmacology and dye exclusion, Faria et al. (5) have ruled out the involvement of connexin hemichannels (i.e., gap junctional channel subunits that are not juxtaposed between cells but are expressed on the cell surface as a complex).

Faria et al. (5) have clearly answered the question posed in their article’s title. Second messengers are indeed crucial for opening the large dye-permeable pore associated with P2X7 expression. However, larger puzzles remain. One relates to the precise role of P2X7 in apoptosis. Is it just one of the many apoptotic markers upregulated in a cell undergoing programmed cell death? Or is P2X7 a critical initiator in the immediate early phase of apoptosis? Moreover, does the same P2X7 receptor channel protein also dilate or undergo conformational change to form a larger pore upon prolonged exposure to millimolar concentrations of ATP? Or does the P2X7 receptor channel open a closely associated but separate, larger membrane pore through initiation of multiple signal transduction events? These interrelated questions remain unanswered; however, Faria et al. have helped turn the tide toward the latter explanation.

In the review by North (8), arguments for and against the dilation of the channel pore into the dye-permeable pore of the P2X7 receptor were presented. North initially stated that the simplest explanation is that P2X7 itself performs both functions. Arguments in favor of this idea include 1) the change in permeability from channel-like to pore-like is gradual, 2) this change is observed in virtually every heterologous cell expression system, 3) several maneuvers and reagents block both the pore and the channel [Faria et al. (5) have found that MAP kinase antagonists blocked both functions], and 4) the two properties also have been shown upon expression of other P2X receptor subtypes.

However, the above evidence is not ironclad. This view is underscored by the findings in another study (12) showing that P2Xγ-mediated pore formation and induction of apoptosis in retinal microvasculature depends on signal transduction and other Ca2+ entry channels. In that study, the authors have shown that extracellular ATP and P2X7 mediated pore formation and apoptosis in retinal microvascular cells. Intracellular Ca2+ was essential for the effect. However, they found that pore formation was minimized by P2Y4 receptor activation, which somehow prevented P2X7 pore formation. Moreover, they found that P2X7 opening caused profound depolarization in this cell model, causing voltage-dependent Ca2+ channels (VDCC) to allow lethal Ca2+ influx. They concluded that retinal vasotoxicity by purinergic agonists was normally prevented under physiological circumstances by nitric oxide inhibition of VDCC and by P2Y4-mediated inhibition of P2Xγ-associated pore formation. That study, too, implicated signal transduction pathways as vital to coupling P2X7 activation with pore formation and apoptosis.

In his recent review (8), North argued that P2X7 and its COOH terminus are critical to initiating signal transduction that couples P2X7 with a separate but closely associated pore-forming membrane protein. First, antagonists of P2Xγ
channel function such as calmidazolium do not affect large pore permeability. Second, maitotoxin opens a large pore with properties very similar to those of the P2X7-associated pore, as shown by Dubyak et al. (10). Maitotoxin does not affect or bind to P2X7. Third, in some Xenopus oocyte preparations expressing P2X7, only cation channel activity is observed and not large pore formation. Fourth, YO-PRO-1 dye permeability varies in different transfections within a single cell expression system [e.g., human embryonic kidney (HEK)-293 cells], while cation currents show equivalent and reliable expression.

Taken together, Faria et al. (5) and Sugiyama et al. (12) speak directly and elegantly to the key issues surrounding P2X7 function. While open questions remain, these authors and researchers at other laboratories are clearly searching for the closely associated membrane protein that is associated with apoptosis, opened by P2X7-mediated signal transduction, and confers millimolar ATP induction of membrane permeabilization. We expect that this pore-forming protein will be identified in the near future.

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REFERENCES