PERSPECTIVES

More cross-talk between purinergic receptors

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How does a simple molecule found in every cell exert a variety of specific actions throughout the body? The answer of course is that its actions are mediated via different receptors on different cells. A striking example is provided by ATP, which provides the energy source for all cells but also acts as an extracellular signalling molecule. It is a neurotransmitter in the central and peripheral nervous systems, and a regulator of afferent signalling, blood flow, inflammatory responses, cell proliferation and cell death (Surprenant & North, 2009). It acts upon a large number of different receptors, which can be classified into the metabotropic P2Y and ionotropic P2X receptors. The P2X receptors are multimeric proteins which can coassemble to make homotrimeric and heterotrimeric complexes with different functions. Given that the different P2X subtypes are frequently coexpressed in the same cell, the potential for mixing and matching of subunits is a physiologically important mechanism for increasing the diversity of responses mediated via this class of receptors.

The paper by Casas-Pruneda *et al.* (2009) in this issue of *The Journal of Physiology* provides evidence for a functional interaction between $P2X_4$ and $P2X_7$ receptors, both when heterologously coexpressed and for the endogenous receptors in mouse salivary acinar cells. $P2X_4$ receptors have a high affinity for ATP and $P2X_7$ receptors, a low affinity, and they are coexpressed in many different cells including immune cells, epithelia and endothelia. As homomeric receptors they differ in their signalling properties as well as their pharmacology, and there has been much speculation about whether or not they interact with each other. There is also considerable interest in these receptors as potential drug targets for a wide variety of disorders including chronic inflammatory disease, neurodegenerative disease and cancer. An understanding of the subunit composition of the native $P2X_4$ and $P2X_7$ receptors is therefore required.

Casas-Pruneda *et al.* (2009) examined the relative contributions of $P2X_4$ and $P2X_7$ receptors to the acinar cell currents evoked by a high concentration of ATP (5 mM). With Na^+ as the charge-carrying ion, the acinar cell currents had a time course resembling recombinant $mP2X_7$ receptor currents in HEK293 cells with no obvious $P2X_4$ receptor-mediated component. This is not altogether unexpected because endogenous $P2X_4$ receptors in other cells have been shown to have a predominantly intracellular distribution (Qureshi *et al.* 2007). When Na^+ was replaced with TEA^+ in the recording solutions, however, the time course of the acinar cell currents changed; they decayed much faster in the continued presence of ATP and no longer resembled the kinetics of the recombinant $mP2X_7$ receptor currents recorded under similar conditions. Neither did the current resemble $mP2X_4$ receptor currents, which, surprisingly, were abolished in TEA^+ solutions. This is surprising in so far as the rat isoform of $P2X_4$ is reported to undergo pore dilatation in the presence of ATP (Khakh *et al.* 1999) and the dilated I_2 state is permeable to $NMDG^+$ and therefore would be expected to also be permeable to TEA^+. Coexpression of $P2X_4$ with $P2X_7$ in HEK293 cells altered the time course of the ATP-evoked TEA^+ currents; they decayed more rapidly than $mP2X_7$ receptor currents, they could not be described by the sum of currents carried by two independent populations of $P2X_4$ and $P2X_7$ receptors, and they were similar in time course to the acinar cell currents. In further experiments, Casas-Pruneda *et al.* (2009) showed that when $mP2X_4$ and $mP2X_7$ were coexpressed in HEK293 cells, Na^+ currents evoked by a high concentration of ATP (5 mM) were insensitive to the $P2X_4$-selective positive modulator, ivermectin (IVM, 3 μM). In contrast, when $mP2X_4$ was expressed alone the currents were enhanced 3-fold by IVM and there was also a small potentiation of the $mP2X_7$ receptor currents. The lack of IVM sensitivity seen at high agonist concentration for the coexpressed recombinant receptors was reproduced in acinar cells, when currents were measured under similar conditions.

This demonstration of a functional interaction between $P2X_4$ and $P2X_7$ for both heterologously expressed and native receptors is in agreement with a recent study by Guo *et al.* (2007). Neither study elucidated the nature of the interaction between $P2X_4$ and $P2X_7$, which could occur within a heterotrimeric complex or between homotrimers. Coassembly of subunits to form heterotrimers is subtype specific but the molecular determinants that govern this have not yet been established. One argument against $P2X_4$ and $P2X_7$ preferentially assembling as heterotrimers is that where the distribution of these receptors has been examined, they differ in their subcellular localization; $P2X_7$ is predominantly at the plasma membrane whereas $P2X_4$ is predominantly intracellular (Qureshi *et al.* 2007; Boumechache *et al.* 2009). There is, however, some overlap in their distribution; $P2X_4$ receptors have been shown to traffic to and from the surface of microglia (Boumechache *et al.* 2009), and both receptors are found in phagosomes (Qureshi *et al.* 2007; Kuehnel *et al.* 2009).

Two recent studies investigated the subunit composition of native $P2X_4$ and $P2X_7$ receptor complexes by taking advantage of differences in their molecular mass to discriminate between homo- and heterotrimers (Nicke, 2008; Boumechache *et al.* 2009). Both studies conclude that the preferred assembly pathway for $P2X_4$ and $P2X_7$ is formation of homotrimers. It therefore seems likely that currents with novel characteristics are produced because of an interaction between the $P2X_4$ and $P2X_7$ homotrimers rather than because of the generation of heterotrimers. With the recent report of an ATP-signalling mechanism within phagosomes (Kuehnel *et al.* 2009) and the demonstration of $P2X_4$ and $P2X_7$ existing here (Qureshi *et al.* 2007; Kuehnel *et al.* 2009), it will be important to determine how these two receptors co-ordinate responses to luminal as well as extracellular ATP.

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