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# AFM imaging reveals the assembly of a P2X receptor complex containing P2X2, P2X4 and P2X6 subunits

AP Stewart[1], LS Antonio[2], WA Varanda[2], RD Murrell-Lagnado[1], JM Edwardson[1]. [1]Department of Pharmacology, University of Cambridge, Cambridge, UK, [2]Department of Physiology, University of Sao Paulo, Ribeirao Preto, Brazil

Seven P2X purinergic receptor subunits have been identified: P2X1-P2X7. All except P2X6 assemble as homotrimers, and six heteromeric receptors (P2X1/2, P2X1/4, P2X1/5, P2X2/3, P2X2/6 and P2X4/6) have been described (Torres et al., 1999). In addition, P2X4 homomers associate with P2X2 or P2X7 homomers as dimers of trimers (Antonio et al., 2011). The various P2X receptors show individual functional properties, suggesting distinct physiological roles. The overlapping expression of P2X2, P2X4 and P2X6 subunits has been shown in different cell types, and functional analysis of P2X receptors in Leydig cells suggests that the three subunits interact (Antonio et al., 2009). In the present study, we investigated the potential assembly of P2X2, P2X4 and P2X6 subunits into heteromeric receptors.

tsA 201 cells were co-transfected with $His_6$-tagged P2X2, HA-tagged P2X4 and FLAG-tagged P2X6 subunits. After sequential co-immunoprecipitation using anti-HA and anti-FLAG resins, all three subunits were present, demonstrating their interaction. Proteins eluted from the resins were incubated with anti-$His_6$ antibodies and anti-HA Fab fragments, and analyzed by atomic force microscopy (AFM). In 466 AFM images, 32 central particles with volumes expected for P2X trimers were found to be doubly decorated by one antibody and one Fab fragment. In contrast, only one such complex was seen when the antibody/Fab incubation was omitted (223 images). Two complexes were seen after incubation with anti-Myc antibodies plus anti-V5 Fab fragments (control; 263 images). This result is consistent with the presence of a P2X2/4/6 heterotrimer.

We conclude that P2X2, P2X4 and P2X6 subunits interact, potentially forming a heterotrimeric receptor containing three different subunits.

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