A PRIMITIVE ATP RECEPTOR FROM THE LITTLE SKATE *RAJA ERINACEA*

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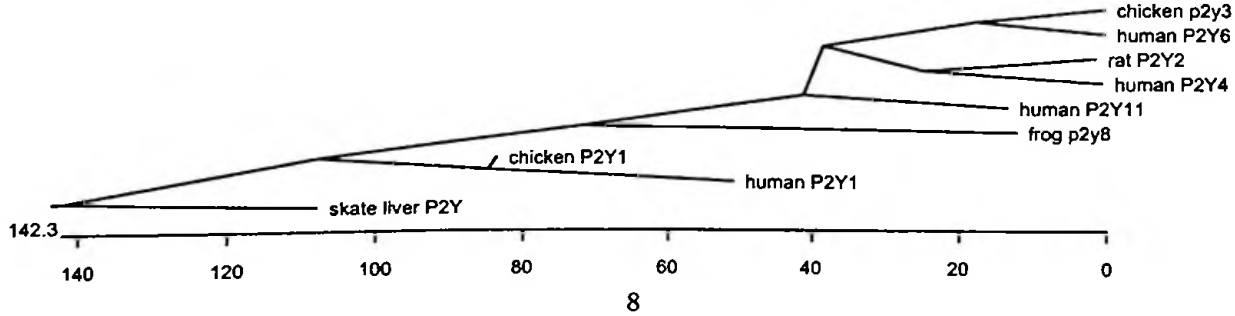
P2Y ATP receptors are G protein-coupled receptors linked to generation of $Ca^{2+}{}_i$. These receptors regulate a variety of processes in cells, including epithelial secretion, platelet aggregation, vasomotor responses, and volume homeostasis. Although P2Y receptors of higher organisms share a common seven transmembrane domain structure, P2Y subtypes vary widely in primary structure. These differences in primary structure are associated with widely varied nucleotide specificity.

In contrast to the specific pharmacologic profiles of P2Y receptor subtypes of higher organisms, P2Y receptors in hepatocytes from the little skate *Raja erinacea* are activated equally by a variety of P2Y receptor ligands (Am. J. Physiol. 270:R561-570, 1996). One possible explanation for this observation is that these cells express a primitive P2Y ATP receptor lacking pharmacologic selectivity. To test this hypothesis, we cloned and characterized the skate liver P2Y receptor.

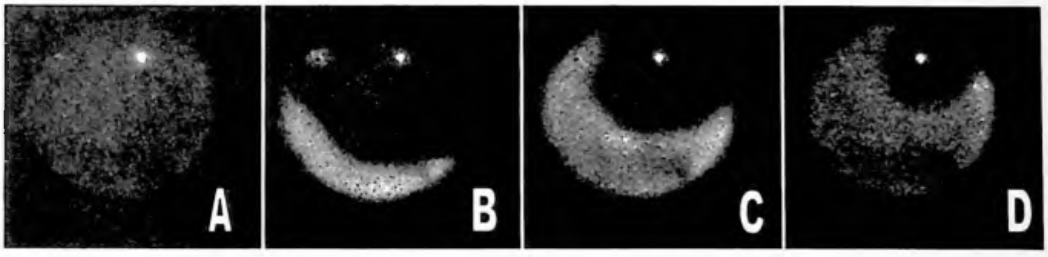
Skate liver RNA was screened with RT-PCR using degenerate oligonucleotide probes designed to amplify sequences conserved in avian and mammalian P2Y receptors. This generated a single 600 bp PCR product. Sequencing of this product revealed similarity to cloned P2Y receptors of higher organisms. The PCR product was used to screen a skate liver λ phage cDNA library; this produced a single 2700 bp cDNA clone. This clone contained a 972-base pair open reading frame, encoding a predicted protein sequence of 324 amino acids. Sequence analysis of this clone revealed approximate 60% similarity to avian and mammalian $P2Y_1$ receptors, and approximate 30% similarity to avian and mammalian $P2Y_2$, $P2Y_4$, and $P2Y_6$ receptors. A computer-generated (Meg-Align, DNASTAR, Inc., Madison, WI) phylogenetic analysis is seen in Figure 1.

Figure 1. Computer-generated phylogenetic analysis of cloned P2Y receptors. The skate ATP receptor is related to the common ancestor of P2Y receptors of higher organisms. Scale is in arbitrary divergence units and represents amino acid substitutions relative to the hypothetical ancestor.



To test whether the putative skate liver P2Y receptor functions as a P2Y receptor lacking pharmacologic selectivity, *Xenopus laevis* oocytes were injected with skate P2Y receptor cRNA, and subsequently loaded with the Ca^{2+}-sensitive dye fluo-3. Increases in Ca^{2+}_i in response to a variety of nucleotides were monitored using confocal video microscopy (Figure 2). Ca^{2+}_i increases were seen in response to ATP, ADP, UTP, and UDP at similar concentrations (10 μM-1 mM). No such increases were seen in water-injected oocytes. This suggests that the skate liver P2Y receptor functions as a P2Y receptor, and that this receptor lacks pharmacologic selectivity.

Figure 2. Serial Ca^{2+}_i increases monitored in a *Xenopus* oocyte expressing the skate liver P2Y receptor. Cells were loaded with the Ca^{2+}-sensitive dye fluo-3 and serially monitored after stimulation with ATP (100 μM). Images shown are at 0 sec (A), 15 sec (B), 30 sec (C), and 45 sec (D). A Ca^{2+} wave is seen crossing the cell.



To examine which skate tissues express the skate liver P2Y receptor, multiple skate tissues were examined with RT-PCR. Expression of the skate liver P2Y receptor was seen in liver, common bile duct, duodenum, rectal gland, gill, heart, brain, spleen, and testis. This suggests that the distribution of this P2Y receptor is widespread in the skate.

To examine which organisms express genes similar to the skate liver P2Y receptor, a phylogenetic range of organisms were examined with genomic DNA dot blots. Expression of the skate liver P2Y receptor was seen strongly in blue crab, skate, dogfish shark, and flounder, and weakly in sea urchin, frog, and human. This suggests that a variety of marine species express P2Y ATP receptors.

These results demonstrate the presence of a primitive, nonselective, ATP receptor in the skate. This primitive signaling mechanism may mediate a variety of cellular processes in skate tissues, presumably through increases in Ca^{2+}_i. Comparison of the gene for this receptor to P2Y receptors in higher organisms may provide insight into the molecular pharmacology of those receptors.

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