# PROPERTIES OF A VOLTAGE-DEPENDENT INWARD CURRENT ASSOCIATED WITH EXPRESSION OF THE SKATE P2Y RECEPTOR IN *XENOPUS* OOCYTES

Scott M. O'Grady, Peter J. Maniak, So Yeong Lee and Jonathan A. Dranoff*
Department of Physiology and Animal Science, University of Minnesota
St. Paul, MN 55108
*Department of Medicine, Yale University School of Medicine
New Haven, CT 06510

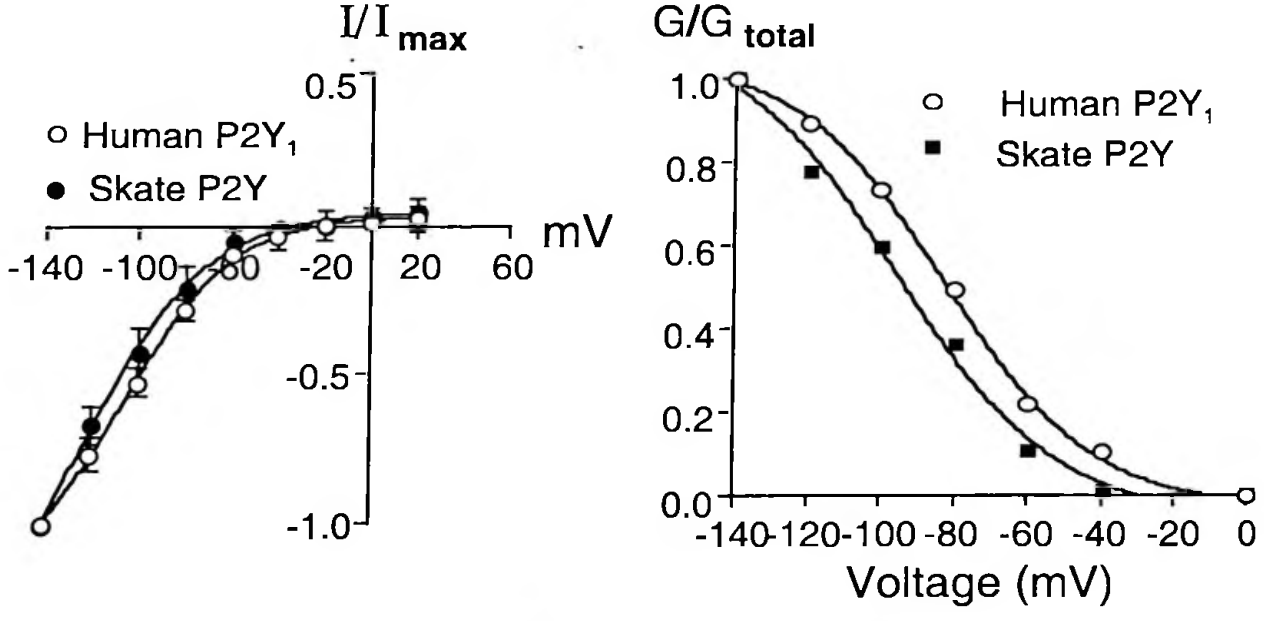
P2Y receptors are G-protein coupled receptors that are specifically activated by extracellular purine (ADP, ATP) and pyrimidine nucleotides (UDP, UTP). These receptors consist of seven membrane spanning domains, an extracellular n-terminal sequence and an intracellular c-terminal sequence. Several mammalian subtypes of P2Y receptors have been cloned including the $P2Y_1$, $P2Y_2$, $P2Y_4$, $P2Y_6$ and $P2Y_{11}$ receptors. Two of these subtypes, $P2Y_4$ and $P2Y_6$, are selective for UTP and UDP respectively and are not activated by purines. The $P2Y_2$ receptor is the only P2Y receptor that is activated by ATP and UTP with equal potency. The $P2Y_1$ receptor is most selective for ADP and the $P2Y_{11}$ receptor is the only P2Y receptor that is selective for ATP (Ralevic V. and G. Burnstock, Pharmacol. Rev. 50(3):413-492, 1998). The first cloned P2Y receptor was isolated from chick brain and was shown to be activated by agonists with a potency order of 2Me-S-ATP > ATP with α,β Me-ATP, β,γ Me-ATP and UTP being inactive (Webb T.E., et al., FEBS Lett 324:219–225, 1993). Homologs of the chick brain $P2Y_1$ receptor have been cloned from species and cell types that have different agonist activity sequences. In addition, the relative potency of ADP and ATP appears to differ widely between cloned $P2Y_1$ receptors and endogenous $P2Y_1$ receptors suggesting variations in receptor structure associated with agonist selectivity. Recently, a primitive P2Y receptor has been cloned from the liver of the skate, *Raja erinacea* (Dranoff, J.A., et al., J. Biol. Chem. 275(39):30,701-30,706, 2000). This receptor shows significant overall sequence homology (approximately 60%) to the mammalian $P2Y_1$ receptor and is thought to be an evolutionary ancestor to $P2Y_1$ receptors found in birds and mammals. Unlike mammals however, there appears to be only one subtype of P2Y receptor present in tissues from the skate. The skate receptor is activated by a variety of purine and pyrimidine nucleotides and this lack of specificity is a unique property of the skate receptor.

Most P2Y receptors couple to G-proteins that activate PLC leading to $IP_3$ formation and $Ca^{2+}$ mobilization from intracellular stores. Studies of the $P2Y_1$ receptor in turkey erythrocytes identified the G-protein that couples to the receptor as a $G_q$ protein, $G_{11}$, which is insensitive to both cholera and pertussis toxins (Waldo G.L. et al., Mol. Pharmacol., 40:480–489, 1991). The α subunit of $G_{11}$ activates PLCβ in turkey erythrocytes producing increases in $IP_3$ formation. There is also some evidence to suggest that activation of endogenous $P2Y_1$-like receptors in rat brain endothelial cells leads to inhibition of adenylyl cyclase presumably mediated by activation of $G_i$ (Boyer J. L., et al., Br J Pharmacol. 116:2611–2616, 1995). Nucleotide activation of the native P2Y receptor in isolated skate hepatocytes leads to an increase in intracellular calcium and a transient increase in bile secretion in skate liver (Nathanson, M.H. and K. Mariwalla, Am. J. Physiol. 270:R561-R570, 1996). When the cloned skate receptor was expressed in *Xenopus* oocytes, an increase in calcium release from intracellular stores was also observed (Dranoff, J.A., et al., J. Biol. Chem. 275(39):30,701-30,706, 2000).

Our previous studies of human and turkey $P2Y_1$ receptors expressed in *Xenopus* oocytes showed that treatment with ATP or ADP analogs stimulated a slowly activating inward current

that exhibited strong rectification and steady-state inactivation at membrane potentials below -40 mV (O'Grady S.M., et al., J. Biol. Chem. 271:29080-29087, 1996). This current was blocked by divalent cations including $Zn^{2+}$, $Cu^{2+}$, $Mg^{2+}$ and $Ba^{2+}$. Remarkably, blocking G-protein activation with GDP-β-S did not affect activation of this current.

In the present study we expressed the skate P2Y receptor in *Xenopus* oocytes and investigated the effects of 2Me-S ADP stimulation on whole cell currents using the two electrode voltage clamp technique. Experiments were conducted in Cl free saline solution (in mM, 96 $NaMeSO_4$, 2 $KMeSO_4$, 1 $MgSO_4$, 1 $CaSO_4$, 5 HEPES, pH 7.5) Bath electrodes were placed in a separate solution with Cl containing saline solution and connected to the oocyte bathing solution with an agar bridge. Oocytes were injected with skate receptor cRNA (20 ng) and maintained in Cl-free saline solution. Addition of 20 μM 2Me-S-ADP and hyperpolarization from a holding potential of zero mV to –140 mV resulted in the activation of a slow inward current that required approximately 500 msec to reach full activation and then inactivated over a time course of 3-4 seconds. The activation and inactivation kinetics of this current was nearly identical to that of the human $P2Y_1$ receptor. **Figure 1a** shows the current voltage relationships for the agonist-activated skate P2Y and human $P2Y_1$ receptors normalized to the maximum inward current measured at –140 mV. The reversal potentials for the human $P2Y_1$ receptor and skate receptor were –23 mV and –36 mV respectively and were not significantly different from each other. Both current voltage relationships exhibited similar degrees of inward rectification. Comparison of slope values obtained from the conductance-voltage relationship shown in **figure 1b** revealed that no significant differences in voltage dependence exists between human and skate receptors.




These results extend our earlier findings showing that purinergic receptors related to the $P2Y_1$ subtype exhibit a unique agonist and hyperpolarization dependent ion channel activity when expressed in *Xenopus* oocytes. Activation of this channel does not appear to be dependent upon conventional mechanisms of G-protein coupling to ion channels in oocytes and may represent a novel ionotropic property associated with $P2Y_1$ receptors in mammalian tissues.

This study was supported by a New Investigator Award from MBIBL to SMO.