P2Y<sub>1</sub> receptor modulation of endogenous ion channel function in *Xenopus* oocytes: Involvement of transmembrane domains

So Yeong Lee<sup>1,4</sup>, Robert A. Nicholas<sup>2</sup> & Scott M. O’Grady<sup>3</sup>

<sup>1</sup>Department of Physiology and Molecular Veterinary Biosciences Graduate Program, University of Minnesota, St. Paul, Minnesota, USA; <sup>2</sup>Department of Pharmacology, University of North Carolina School of Medicine, Chapel Hill, North Carolina, USA; <sup>3</sup>Department of Physiology and Animal Science, University of Minnesota, St. Paul, Minnesota, USA; <sup>4</sup>Department of Pharmacology, College of Veterinary Science, Seoul National University, Seoul, Korea

Received 31 August 2004; accepted in revised form 14 October 2004

**Key words:** activation gating, divalent ion block, G protein-coupled receptors, P2Y receptors, T<sub>in</sub> channels, voltage-dependent ion channels

**Abstract**

Agonist activation of the hP2Y<sub>1</sub> receptor expressed in *Xenopus* oocytes stimulated an endogenous voltage-gated ion channel, previously identified as the transient inward (T<sub>in</sub>) channel. When human P2Y<sub>1</sub> (hP2Y<sub>1</sub>) and skate P2Y (sP2Y) receptors were expressed in *Xenopus* oocytes, time-to-peak values (a measure of the response to membrane hyperpolarization) of the T<sub>in</sub> channel were significantly reduced compared to oocytes expressing the hB<sub>1</sub>-bradykinin receptor or the rat M<sub>1</sub>-muscarinic (rM<sub>1</sub>) receptor. Differences in activation were also observed in the T<sub>in</sub> currents elicited by various P2Y receptor subtypes. The time-to-peak values of the T<sub>in</sub> channel in oocytes expressing the hP2Y<sub>4</sub>, hP2Y<sub>11</sub>, or hB<sub>1</sub>-bradykinin receptors were similar, whereas the channel had significantly shorter time-to-peak values in oocytes expressing either the hP2Y<sub>1</sub> or sP2Y receptor. Amino acid substitutions at His-132, located in the third transmembrane domain (TM3) of the hP2Y<sub>1</sub> receptor, delayed the onset of channel opening, but not the kinetics of the activation process. In addition, Zn<sup>2+</sup> sensitivity was also dependent on the subtype of P2Y receptor expressed. Replacement of His-132 in the hP2Y<sub>1</sub> receptor with either Ala or Phe increased Zn<sup>2+</sup> sensitivity of the T<sub>in</sub> current. In contrast, truncation of the C-terminal region of the hP2Y<sub>1</sub> receptor had no effect on activation or Zn<sup>2+</sup> sensitivity of the T<sub>in</sub> channel. These results suggested that TM3 in the hP2Y<sub>1</sub> receptor was involved in modulating ion channel function and blocker pharmacology of the T<sub>in</sub> channel.

**Introduction**

Nucleotides, including ATP and UTP, are released from most cells and function as extracellular signaling molecules in a variety of cell types. Their biological effects are mediated through P2 receptors, which are divided into two classes: Ionotropic P2X receptors and metabotropic P2Y receptors [1–4]. Eight mammalian P2Y receptors (P2Y<sub>1</sub>, P2Y<sub>2</sub>, P2Y<sub>4</sub>, P2Y<sub>6</sub>, P2Y<sub>11</sub>, P2Y<sub>12</sub>, P2Y<sub>13</sub>, and P2Y<sub>14</sub>) have been cloned and characterized functionally [4–9]. P2Y<sub>1</sub>, P2Y<sub>2</sub>, P2Y<sub>4</sub>, and P2Y<sub>6</sub> receptors are known to couple solely to activation of phospholipase C through G<sub>q</sub> [10–13]. The P2Y<sub>1</sub> receptor, which has 61%–64% sequence similarity to the hP2Y<sub>1</sub> receptor, activates G<sub>q</sub>/phospholipase C with similar rank nucleotide selectivity as that of the hP2Y<sub>1</sub> receptor [14]. The hP2Y<sub>11</sub> receptor activates both phospholipase C and adenylyl cyclase [15], whereas P2Y<sub>12</sub>, P2Y<sub>13</sub>, and P2Y<sub>14</sub> receptors are coupled exclusively to G<sub>i</sub> and inhibition of adenylyl cyclase [5, 7, 16].

Agonist activation of the hP2Y<sub>1</sub> receptor expressed in *Xenopus* oocytes stimulated a previously characterized transient inward (T<sub>in</sub>) channel. The channel responsible for the T<sub>in</sub> current has not been cloned, but appears to represent a new family of ion channels that has not been previously characterized at the molecular level. The T<sub>in</sub> current was initially identified following injection of mRNA from rat brain [19] and subsequently observed when cloned 5-HT<sub>1A</sub> and 5-HT<sub>2C</sub> receptors were expressed in oocytes [20]. T<sub>in</sub> activation required both membrane hyperpolarization and an increase in intracellular calcium in addition to agonist activation of these receptors [19, 20]. The T<sub>in</sub> channel can be distinguished from endogenous Ca<sup>2+</sup> and hyperpolarization-activated Cl<sup>−</sup> channels in *Xenopus* oocytes by its inactivation gating which occurs within 4 s following hyperpolarization [21]. Moreover, expression of Go<sub>q</sub> in stage V and VI oocytes was found to
be sufficient for activation of the T$_m$ current, thus demonstrating a role for G$_q$ in regulation of this channel [22].

We showed recently that P2Y receptors are capable of activating and modulating the voltage dependence and inactivation of the T$_m$ channel through interactions involving the C-terminal domains of these receptors that was independent of PDZ-binding motifs [18, 23]. In the present study, we investigated the role of TM3 of the hP2Y$_1$ receptor in modulation of the T$_m$ channel. Our results indicated that activation and Zn$^{2+}$ sensitivity were dependent on the subtype of P2Y receptor expressed and that mutations within TM3 of the hP2Y$_1$ receptor significantly altered these properties of the channel.

Materials and methods

Materials

_Xenopus laevis_ frogs were purchased from _Xenopus_ I (Ann Arbor, Michigan, USA) and maintained in aquaria as suggested by the supplier. Collagenase and gentamicin were obtained from Invitrogen (Carlsbad, California, USA). 2Methylthio-ADP (2MeS-ADP) and 2Methylthio-ATP (2MeS-ATP) were obtained from Research Biochemicals (Natick, Massachusetts, USA). Carbachol and bradykinin were obtained from Sigma-Aldrich (St. Louis, Missouri, USA).

Site-directed mutagenesis

Site-directed mutagenesis was done with the Altered Sites mutagenesis system kit from Promega (Madison, Wisconsin, USA). Site-directed mutagenesis was done with the Altered Sites mutagenesis system kit from Promega (Madison, Wisconsin, USA). Carbachol and bradykinin were obtained from Sigma-Aldrich (St. Louis, Missouri, USA).

Oocyte isolation and cRNA injections

Ovarian lobes from adult _X. laevis_ frogs were removed from _Xenopus_ I (Ann Arbor, Michigan, USA) following the manufacturer’s instructions.

Electrophysiological measurements

Electrophysiological measurements were made using the two-electrode voltage clamp technique at 20 °C. Recordings were performed in Cl$^-$-free MBS solution (in mM): 90 NaMeSO$_4$, 2 KMeSO$_4$, 0.82 MgSO$_4$, 0.74 CaGluconate, 10 HEPES (pH 7.4). Electrodes were placed in a separate Cl$^-$ containing MBS solution and connected to the oocyte bathing solution with an agar bridge. Current and voltage measuring electrodes were pulled from borosilicate filament glass to resistances between 2 and 5 MΩ when filled with 0.5 M KCl. Data acquisition and analysis was performed using pCLAMP 8 software (Axon Instruments, Union City, California, USA).

Agonist stimulation of expressed receptors

Agonist concentrations used for these experiments were selected to produce maximum receptor activation, based on previous concentration–response studies on P2Y receptors expressed in _Xenopus_ oocytes [17]. Agonists were first added to the bathing solution to activate the receptors and then the membrane potential was stepped from 0 to −140 mV to activate the T$_m$ channel. Maximum current responses to the voltage step protocol were achieved within 5 min after agonist addition to the bathing solution. 2MeS-ADP (20 μM) was used to stimulate hP2Y$_1$, mutants of hP2Y$_1$, and sP2Y receptors. 2MeS-ATP (40 μM) was used to stimulate hP2Y$_{11}$ receptors. Bradykinin (2 μM) and carbachol (10 μM) were used to stimulate hB$_1$-bradykinin and rM$_1$-muscarinic receptors, respectively.

Analysis and statistics

Statistical significance was determined using Student’s t-test. Statistical significance was accepted at P-values <0.05. Conductance–voltage relationships were analyzed using a Boltzmann function $Y = 1/1 + \exp(V_{50} - X/slope)$, where $V_{50}$ represents the voltage at which the conductance is half-maximal, slope factor represents the degree of voltage dependence (steepness of the curve), $Y$ represents the normalized conductance, $G/G_{\text{max}}$ (mV), and $X$ represents a specific voltage). IC$_{50}$ values were calculated using four-parameter logistic function ($Y = 100/(1 + \exp(\log IC_{50} - X) \times slope)$), where $X$ is the logarithm of concentration and $Y$ is the response.

Results and discussion

$T_m$ channel activation following stimulation of G$_q$ coupled receptors expressed in _Xenopus_ oocytes

Time-to-peak measurements were used as an indicator of the activation time course and were defined as the time between the capacitance current and the peak inward current (Figure 1A). It is important to note that the time-to-peak measurement does not reflect the time course of agonist binding or receptor activation, but instead is a measure of the time course of channel opening following the voltage step. When the hP2Y$_1$ receptor and sP2Y receptor were expressed in _Xenopus_ oocytes, the time-to-peak activation of the T$_m$ channel at all voltages tested was...
significantly reduced compared to oocytes expressing the hB1-bradykinin receptor or G\(_q\alpha\) subunit (Figure 1B). In contrast, when either rM1-muscarinic, hP2Y\(_b\), or hP2Y\(_{11}\) receptors were expressed in oocytes, time-to-peak values of the T\(_{in}\) channel were similar to those of hB1-bradykinin receptor or G\(_q\alpha\) subunit but significantly different from hP2Y\(_1\) or sP2Y receptors (Figure 1C). These results indicated that hP2Y\(_1\) and sP2Y receptors were capable of decreasing the time interval between the initiation of membrane hyperpolarization and the beginning of T\(_{in}\) channel opening compared to other G\(_q\) coupled receptors, including other subtypes of P2Y receptors and the G\(_q\alpha\) subunit. Expression of hP2Y\(_1\) or sP2Y receptors did not affect the rate of channel opening, as reflected by the slope of the current trace between the point of initiation and the peak inward current, compared to the hB1-bradykinin receptor (data not shown). These findings suggest that, unlike hB1-bradykinin or rM1-muscarinic receptors, an interaction occurs between the hP2Y\(_1\) (or sP2Y) receptor and the T\(_{in}\) channel that influences the ability of the channel to respond to membrane hyperpolarization.

Effect of C-terminal domain truncation

We showed previously that the C-terminal domain of the P2Y\(_1\) receptor was involved in modulating the voltage dependence of activation of the T\(_{in}\) channel in Xenopus oocytes [18]. To determine whether the C-terminal domain of the hP2Y\(_1\) receptor was also involved in modulating activation, we compared the time-to-peak currents of the T\(_{in}\) channel in oocytes expressing either the wild-type hP2Y\(_1\) receptor or one of several C-terminal truncation mutants of the hP2Y\(_1\) receptor (Figure 2A). As shown in Figure 2B, the time-to-peak values of the T\(_{in}\) channel in oocytes expressing any of the truncation mutants were not

---

**Figure 1.** Effects of expressed G\(_q\) coupled receptor stimulation on hyperpolarization-induced activation of the endogenous T\(_{in}\) channel in Xenopus oocytes. A) Representative trace of the T\(_{in}\) channel current at -140 mV following stimulation of the expressed hP2Y\(_1\) receptor. Time-to-peak current measurements were used to assess hyperpolarization-induced activation of the channel and are defined as the elapsed time following the capacitance current to the peak inward current. B) Comparison of time-to-peak measurements for hB1-bradykinin (n = 13), G\(_q\alpha\) alpha subunit (n = 13), hP2Y1 receptor (n = 14), and sP2Y (n = 13). C) Comparison of time-to-peak measurements for hB1-bradykinin (n = 13), rM1-muscarinic (n = 12), hP2Y\(_4\) (n = 12), and hP2Y\(_{11}\) (n = 13) receptors.

---

**Figure 2.** Effect of C-terminal domain truncation on channel activation. A) The relative locations of TM3 mutations (hP2Y\(_1\)-H132A, hP2Y\(_1\)-H132D, and hP2Y\(_1\)-H132F) and C-terminal truncation sites within the hP2Y\(_1\) receptor are indicated. B) Comparison of time-to-peak measurements for hB1-bradykinin (n = 13), hP2Y\(_1\) (n = 14), hP2Y\(_{1334}\)tr (n = 9), hP2Y\(_{1342}\)tr (n = 15), hP2Y\(_{1349}\)tr (n = 11), hP2Y\(_{1360}\)tr (n = 15), and hP2Y\(_{1369}\)tr (n = 13) receptors as the function of voltage.
significantly different from the values derived from oocytes expressing the wild-type hP2Y1 receptor, suggesting that the C-terminal domain was not involved in modulating activation of the channel.

**Effect of the TM3 mutations on activation and conductance–voltage relationships**

The P2Y1 receptor contains a unique histidine residue at position 132 that is not present in other P2Y receptor subtypes. To determine if TM3 of the hP2Y1 receptor is important in modulating activation, we mutated His-132 to alanine (hP2Y1-H132A), aspartic acid (hP2Y1-H132D), and phenylalanine (hP2Y1-H132F) (Figure 2A), expressed the mutants in *Xenopus* oocytes, and determined the time-to-peak value of the Tm current following receptor activation. Time-to-peak current measurements of all TM3 point mutations were significantly increased compared to the wild-type hP2Y1 receptor (Figure 3A). Mutation of His-132 to alanine, aspartic acid, or phenylalanine increased time-to-peak current values compared to the wild-type hP2Y1 receptor, suggesting that the primary structure of TM3 plays a role in modulating channel activation. It is worth noting that TM3 of the hP2Y1 receptor is known to be important in agonist recognition [24–26]. The H132A mutation was previously shown to produce a shift in the 2-MeS-ADP EC50 for phospholipase C activation from 1.94 (Table 1).  

**Table 1.** V50 and slope factors of Tm currents elicited by agonist-activated receptors.

| Receptors      | V50 (mV) | Slope factor |
|----------------|----------|--------------|
| hB1 (n = 13)   | −43.0    | −13.4        |
| hP2Y4 (n = 9)  | −53.8    | −18.8        |
| rM1 (n = 16)   | −51.8    | −16.2        |
| hP2Y1 (n = 13) | −72.5*   | −18.5        |
| hP2Y1-H132A (n = 5) | −73.7* | −18.2        |
| hP2Y1-H132D (n = 6) | −80.3* | −17.3        |
| hP2Y1-H132F (n = 5) | −69.5* | −12.3        |

*V50 is defined as the voltage at which the currents fell to one-half of the current at −140 mV (see Materials and methods for details).

* The value was significantly different (P < 0.05) from the hB1-bradykinin receptor value.

Figure 3. Effect of third transmembrane domain mutations on activation and conductance–voltage relationships. A) Time-to-peak measurements for hB1-bradykinin (n = 13), wild-type hP2Y1 (n = 14), hP2Y1-H132A (n = 5), hP2Y1-H132D (n = 7), hP2Y1-H132F (n = 10), and hP2Y1-Q307K (n = 7) receptors as a function of voltage. B) Normalized conductance–voltage relationships for hB1-bradykinin (n = 13), wild-type hP2Y1 (n = 13), hP2Y1-H132A (n = 5), hP2Y1-H132D (n = 6), hP2Y1-H132F (n = 6). The V50 values and slope factors for each conductance are listed in Table 1.

Figure 4. Effects of Zn2+ on the inward currents elicited by step hyperpolarization to −140 mV. A) Representative current traces showing Tm currents activated by hP2Y1 receptor in the presence of 20 μM 2MeS-ADP. Agonist-stimulated inward current was inhibited by ZnCl2 in a concentration-dependent manner. B) Concentration–response relationship for Gq (n = 7) and hB1-bradykinin (n = 5). C) Inhibition of hP2Y1 (n = 6), sP2Y (n = 5), and hP2Y11 (n = 5) receptor-elicited Tm currents by [Zn2+]. The IC50 values are listed in Table 2.
$T_0$ to 17.7 ± 4.7 nM when expressed in COS-7 cells [26].

To ensure full activation of the H132 mutant receptors, the 2MeS-ADP concentration used in these experiments was set at 20 µM.

We also analyzed the conductance–voltage properties of the $T_0$ channel currents activated by the mutant receptors. As shown in Figure 3B, the conductance–voltage relationships for these mutants were not significantly different from the wild-type hP2Y$_1$ receptor. The $V_{50}$ values, which are defined as the voltages at which the current is one-half of that at −140 mV (see Materials and methods for more details), for each of the hP2Y$_1$ receptor mutants were similar to the $V_{50}$ value of wild-type hP2Y$_1$ receptor, but were significantly more negative than the $V_{50}$ value measured for the hB$_1$-bradykinin receptor (Figure 3B, Table 1). These data demonstrated that the point mutations in TM3 of the hP2Y$_1$ receptor did not modulate the voltage sensitivity of the $T_0$ channel, as was previously shown for the C-terminal domain of the receptor [18, 23].

**Effects of Zn$^{2+}$ on the inward current elicited by step hyperpolarization to −140 mV**

Divalent cations have been shown previously to block $T_0$ currents, with Zn$^{2+}$ being the most potent of these blockers [17, 19]. Therefore, ZnCl$_2$ was used to compare divalent ion sensitivity of the $T_0$ current activated by hB$_1$-bradykinin and P2Y receptors. Figure 4A shows the current response at −140 mV of the $T_0$ channel elicited by agonist-activated hP2Y$_1$ receptors in the presence of increasing concentrations of ZnCl$_2$. The amplitudes of the $T_0$ currents were reduced in a concentration-dependent manner in the range of 1 to 500 µM ZnCl$_2$. Curves were analyzed by using a four-parameter logistic function as described in Methods. The IC$_{50}$ value of ZnCl$_2$ at the $T_0$ channel activated by the hB$_1$-bradykinin receptor was 65 µM, which was not significantly different from IC$_{50}$ values obtained following activation by the G$_\alpha$$_\text{q}$ subunit (98 µM; Figure 4B, Table 2) or hP2Y$_1$ receptor (83 µM; Figure 4C, Table 2). It is worth noting that the inhibition of $T_0$ channel current by ZnCl$_2$ in oocytes expressing the G$_\alpha$$_\text{q}$ subunit indicated that the effect of Zn$^{2+}$ was not on receptor-mediated regulation of the $T_0$ channel. In contrast, the IC$_{50}$ of ZnCl$_2$ for inhibition of $T_0$ currents activated by the hP2Y$_1$ receptor was significantly higher (Figure 4C, Table 2). The Zn$^{2+}$ IC$_{50}$ for inhibition of the currents elicited by the sP2Y receptor, which also has a His residue at position 132, was essentially identical to that of hP2Y$_1$ receptor.

To investigate whether His-132 is important in modulating the Zn$^{2+}$ sensitivity of the $T_0$ current, we examined the effect of hP2Y$_1$-H132A and hP2Y$_1$-H132F mutations in the hP2Y$_1$ receptor on Zn$^{2+}$ sensitivity of the channel (Figure 5A). The Zn$^{2+}$ IC$_{50}$ values for currents elicited by either the hP2Y$_1$-H132A or the hB$_1$-bradykinin receptor were similar, whereas the IC$_{50}$ value of Zn$^{2+}$ for currents elicited by wild-type hP2Y$_1$ receptor was significantly higher. In contrast, the hP2Y$_1$-H132F receptor-activated current was considerably more sensitive to Zn$^{2+}$ compared to wild-type hP2Y$_1$ and the hP2Y$_1$-H132A receptors (Figure 5A, Table 2), suggesting that mutations in the TM3 alter both Zn$^{2+}$ sensitivity of the $T_0$ channel and activation. A significant change in the slope of the concentration–response relationship was also observed following stimulation of the hP2Y$_1$-H132F receptor.

---

**Table 2** IC$_{50}$ values from Zn$^{2+}$ concentration–response relationship in Figure 4.

| Receptors        | IC$_{50}$ (µM) |
|------------------|---------------|
| hP2Y$_1$ (n = 6) | 187           |
| hP2Y$_{13}$ (n = 4) | 83*        |
| sP2Y (n = 5)     | 170           |
| hP2Y$_1$-H132A (n = 3) | 87*        |
| hP2Y$_1$-H132F (n = 5) | 13*        |
| hB$_1$ (n = 5)   | 65*           |
| G$_\alpha$ (n = 7) | 98*        |

* The value was significantly different ($P < 0.05$) from the hP2Y$_1$ receptor value.
change in slope reflected an increase in Zn$^{2+}$ sensitivity of the channel and a dramatic shift in the threshold for Zn$^{2+}$ inhibition of the channel. In contrast, C-terminal truncation nor the hP2Y$_1$-Q307K mutation (an amino acid substitution in TM7) had no effect on Zn$^{2+}$ sensitivity of the $T_{in}$ channel (Figure 5B).

Taken together, the results of this study and our previous work [18] suggest the hypothesis that hP2Y$_1$ and sP2Y receptors interact with the $T_{in}$ channel at multiple sites, including their C-terminal tails and TM3 regions. Whereas the C-terminal tails appeared to be involved in voltage dependence and inactivation kinetics of the $T_{in}$ channel, the TM3 region appeared to affect the response of the channel to membrane hyperpolarization and ZnCl$_2$ sensitivity. The point mutations in TM3 might alter a direct interaction of the receptor with the channel or indirectly affect coupling between the receptor and the channel at a site other than the TM3 domain through changes in receptor tertiary structure. It is clear from these studies, however, that the C-terminal domain of the receptor does not influence hyperpolarization-induced activation or Zn$^{2+}$ sensitivity. Moreover, activation and Zn$^{2+}$ sensitivity were not affected by expression of the hP2Y$_{11}$ receptor, even though this receptor subtype was made for hyperpolarization-induced activation, in that the point mutations in TM3 might alter a direct interaction of the receptor with the channel or indirectly affect coupling between the receptor and the channel.

The results reported in this study and our previous work suggests that certain P2Y receptors are capable of modulating ion channel function through membrane-delimited interactions that do not appear to be associated with classical G-protein signaling mechanisms. The observation that mutations in TM3 produce significant changes in $T_{in}$ channel function suggests that receptor TM domains may participate in channel regulation. Although a specific molecular interaction between the expressed P2Y receptor and the $T_{in}$ channel has yet to be identified, one possible interpretation of our results might be a physical coupling between the two proteins. This would explain how relatively subtle changes in TM3 structure could produce changes within the $T_{in}$ channel that lead to altered activation and Zn$^{2+}$ sensitivity. Future experiments will be necessary to determine the molecular basis of $T_{in}$ channel modulation by P2Y$_1$ receptors.

Acknowledgement

This study was supported by a grant from the NIH (AI50494) to SMO.

References

1. Dubayak GR, El-Moattassim C. Signal transduction via P2-purinergic receptors for extracellular ATP and other nucleotides. Am J Physiol 1993; 265: C577–606.
2. Boarder MR, Weisman GA, Turner JT, Wilkinson GF. G protein-coupled P2 purinoceptors: From molecular biology to functional responses. Trends Pharmacol Sci 1995; 16: 133–9.
3. Boarder MR, Hourani SM. The regulation of vascular function by P2 receptors: Multiple sites and multiple receptors. Trends Pharmacol Sci 1998; 19: 99–107.
4. Ralevic V, Burnstock G. Receptors for purines and pyrimidines. Pharmacol Rev 1998; 50: 413–92.
5. Chambers JK, Macdonald LE, Sarau HM et al. A G protein-coupled receptor for UDP-glucose. J Biol Chem 2000; 275: 10767–71.
6. Von Kugelgen I, Wetter A. Molecular pharmacology of P2Y-receptors. Naunyn-Schmiedeberg’s Arch Pharmacol 2000; 362: 310–23.
7. Communi D, Gonzalez NS, Deheuex M et al. Identification of a novel human ADP receptor coupled to G, J Biol Chem 2001; 276: 41479–85.
8. Nicholas RA. Identification of the P2Y$_{12}$ receptor: A novel member of the P2Y family of receptors activated by extracellular nucleotides. Mol Pharmacol 2001; 60: 416–20.
9. Sak K, Webb TE. A retrospective of recombinant P2Y receptor subtypes and their pharmacology. Arch Biochem Biophys 2002; 397: 131–6.
10. Parr CE, Sullivan DM, Paradiso AM et al. Cloning and expression of a human P2U nucleotide receptor, a target for cystic fibrosis pharmacotherapy. Proc Natl Acad Sci USA 1994; 91: 13067.
11. Communi D, Pirotton S, Parmentier M, Boeynaems JM. Cloning and functional expression of a human uridine nucleotide receptor. J Biol Chem 1995; 270: 30849–52.
12. Schachtner JB, Li Q, Boyer JL et al. Second messenger cascade specificity and pharmacological selectivity of the human P2Y$_{1}$-purinoceptor. Br J Pharmacol 1996; 118: 167–73.
13. Lazarowski ER, Rochelle LG, O’Neal WK et al. Cloning and functional characterization of two murine uridine nucleotide receptors reveal a potential target for correcting ion transport deficiency in cystic fibrosis gallbladder. J Biol Chem Exp Ther 2001; 297: 43–9.
14. Dronaff JA, O’Neill AF, Franco AM et al. A primitive ATP receptor from the little skate Raja erinaceus. J Biol Chem 2000; 275: 30701–6.
15. Communi D, Govaerts C, Parmentier M, Boeynaems JM. Cloning of a human purinergic P2Y$_{1}$ receptor coupled to phospholipase C and adenylyl cyclase. J Biol Chem 1997; 272(51): 31969–73.
16. Hollopo G, Jantzen HM, Vincent D et al. Identification of the platelet ADP receptor targeted by antiplatelet drugs. Nature 2001; 409: 202–7.
17. O’Grady SM, Elmezquist E, Filtz TM et al. A guanine nucleotide-independent inwardly rectifying cation permeability is associated with P2Y$_{1}$ receptor expression in Xenopus oocytes. J Biol Chem 1996; 271: 29080–7.
18. Lee SY, Wolff SC, Nicholas RA, O’Grady SM. P2Y$_{2}$ receptors modulate ion channel function through interactions involving their C-terminal domain. Mol Pharmacol 2003; 63(4): 878–85.
19. Parker I, Gundersen CB, Miledi R. A transient inward current elicited by hyperpolarization during serotonin activation in Xenopus oocytes. Proc R Soc London Ser B Biol Sci 1985; 223: 279–92.
20. Ni YG, Panicker MM, Miledi R. Efficient coupling of 5-HT$_{2A}$ receptors to the phospholipase C pathway in Xenopus oocytes. Brain Res Mol Brain Res 1997; 51: 115–22.
21. Kowdley GC, Ackerman SJ, John JE et al. Hyperpolarization-
activated Chloride currents in *Xenopus* oocytes. J Gen Physiol 1994; 103: 217–30.

22. Guttridge KL, Smith LD, Miledi R. *Xenopus* Gα subunit activates the phosphatidylinositol pathway in *Xenopus* oocytes but does not consistently induce oocyte maturation. Proc Natl Acad Sci USA 1995; 92: 1297–301.

23. Lee SY, Nicholas RA, O’Grady SM. P2Y2 and P2Y6 receptors expression in *Xenopus* oocytes modulates activation and inactivation gating of an endogenous ion channel. FASEB J 2002; 16(4): A191.

24. Van Rhee AM, Fischer B, Van Galen PJ, Jacobson KA. Modelling the P2Y purinoceptor using rhodopsin as template. Drug Des Discov 1995; 13: 133–54.

25. Jiang Q, Guo D, Lee BX et al. A mutational analysis of residues essential for ligand recognition at the human P2Y1 receptor. Mol Pharmacol 1997; 52: 499–507.

26. Moro S, Guo D, Camaioni E et al. Human P2Y1 receptor: Molecular modeling and site-directed mutagenesis as tools to identify agonist and antagonist recognition sites. J Med Chem 1998; 41: 1456–66.