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$P2Y_1$ receptor modulation of endogenous ion channel function in *Xenopus* oocytes: Involvement of transmembrane domains

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Abstract

Agonist activation of the $hP2Y_1$ receptor expressed in *Xenopus* oocytes stimulated an endogenous voltage-gated ion channel, previously identified as the transient inward (T_{in}) channel. When human $P2Y_1$ ($hP2Y_1$) 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_{in} channel were significantly reduced compared to oocytes expressing the hB_1-bradykinin receptor or the rat M_1-muscarinic (rM_1) receptor. Differences in activation were also observed in the T_{in} currents elicited by various P2Y receptor subtypes. The time-to-peak values of the T_{in} channel in oocytes expressing the $hP2Y_4$, $hP2Y_{11}$, or hB_1-bradykinin receptors were similar, whereas the channel had significantly shorter time-to-peak values in oocytes expressing either the $hP2Y_1$ or sP2Y receptor. Amino acid substitutions at His-132, located in the third transmembrane domain (TM3) of the $hP2Y_1$ receptor, delayed the onset of channel opening, but not the kinetics of the activation process. In addition, Zn^{2+} sensitivity was also dependent on the subtype of P2Y receptor expressed. Replacement of His-132 in the $hP2Y_1$ receptor with either Ala or Phe increased Zn^{2+} sensitivity of the T_{in} current. In contrast, truncation of the C-terminal region of the $hP2Y_1$ receptor had no affect on activation or Zn^{2+} sensitivity of the T_{in} channel. These results suggested that TM3 in the $hP2Y_1$ receptor was involved in modulating ion channel function and blocker pharmacology of the T_{in} 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 ($P2Y_1$, $P2Y_2$, $P2Y_4$, $P2Y_6$, $P2Y_{11}$, $P2Y_{12}$, $P2Y_{13}$, and $P2Y_{14}$) have been cloned and characterized functionally [4–9]. $P2Y_1$, $P2Y_2$, $P2Y_4$, and $P2Y_6$ receptors are known to couple solely to activation of phospholipase C through $G_{q/11}$ (and possibly G_i) resulting in production of IP_3 and mobilization of intracellular Ca^{2+} [10–13]. The sP2Y receptor, which has 61%–64% sequence similarity to the $hP2Y_1$ receptor, activates G_q/phospholipase C with similar rank nucleotide selectivity as that of the $hP2Y_1$ receptor [14]. The $hP2Y_{11}$ receptor activates both phospholipase C and adenylyl cyclase [15], whereas $P2Y_{12}$, $P2Y_{13}$, and $P2Y_{14}$ receptors are coupled exclusively to G_i and inhibition of adenylyl cyclase [5, 7, 16].

Agonist activation of the $hP2Y_1$ receptor expressed in *Xenopus* oocytes stimulated a previously characterized transient inward (T_{in}) current [17, 18]. The channel responsible for the T_{in} 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_{in} current was initially identified following injection of mRNA from rat brain [19] and subsequently observed when cloned $5\text{-}HT_{1a}$ and $5\text{-}HT_{2c}$ receptors were expressed in oocytes [20]. T_{in} activation required both membrane hyperpolarization and an increase in intracellular calcium in addition to agonist activation of these receptors [19, 20]. The T_{in} channel can be distinguished from endogenous Ca^{2+} and hyperpolarization-activated Cl^- channels in Xenopus oocytes by its inactivation gating which occurs within 4 s following hyperpolarization [21]. Moreover, expression of $G\alpha_q$ in stage V and VI oocytes was found to

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be sufficient for activation of the T_{in} current, thus demonstrating a role for $G\alpha_q$ 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_{in} 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_{in} 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 *in vitro* mutagenesis system kit from Promega (Madison, Wisconsin, USA) following the manufacturer's instructions.

Oocyte isolation and cRNA injections

Ovarian lobes from adult *X. laevis* frogs were removed from anesthetized animals under sterile conditions and the tissue mass was dissociated with collagenase solution (in mM: 90 NaCl, 1 KCl, 0.82 $MgSO_4$, 10 HEPES (pH 7.4), 250 U/ml collagenase). Stage V and VI oocytes were sorted, defolliculated and maintained in modified Barth's saline solution (MBS solution; in mM: 90 NaCl, 2 KCl, 0.82 $MgSO_4$, 0.74 $CaCl_2$, 10 HEPES, pH 7.4, supplemented with 0.05 μg/μl gentamicin) at 19–20 °C. cRNA was synthesized using the Ambion Megascript Kit (Austin, Texas, USA) from linear cDNA encoding the hB_1-bradykinin receptor, wild-type P2Y receptors or mutants of the $hP2Y_1$ receptor. Oocytes were injected with cRNA transcripts (46 ng/oocyte) using a Drummond Nanoject oocyte injection system. Control oocytes were injected with 46 nl of sterile water. Oocytes were stored for 2–7 days in MBS solution.

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_{in} 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 $Y = 1/1 + \exp(V_{50} - X/\text{slope factor})$, 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, $G/G_{-140\ mV}$, and X represents a specific voltage). IC_{50} values were calculated using four-parameter logistic function ($Y = 100/(1 + \exp(\log IC_{50} - X) \times \text{slope})$), where X is the logarithm of concentration and Y is the response.

Results and discussion

T_{in} 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_{in} channel at all voltages tested was

significantly reduced compared to oocytes expressing the hB_1-bradykinin receptor or $G_q\alpha$ subunit (Figure 1B). In contrast, when either rM_1-muscarinic, $hP2Y_4$, or $hP2Y_{11}$ receptors were expressed in oocytes, time-to-peak values of the T_{in} channel were similar to those of hB_1-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 hB_1-bradykinin receptor (data not shown). These findings suggest that, unlike hB_1-bradykinin or rM_1-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.

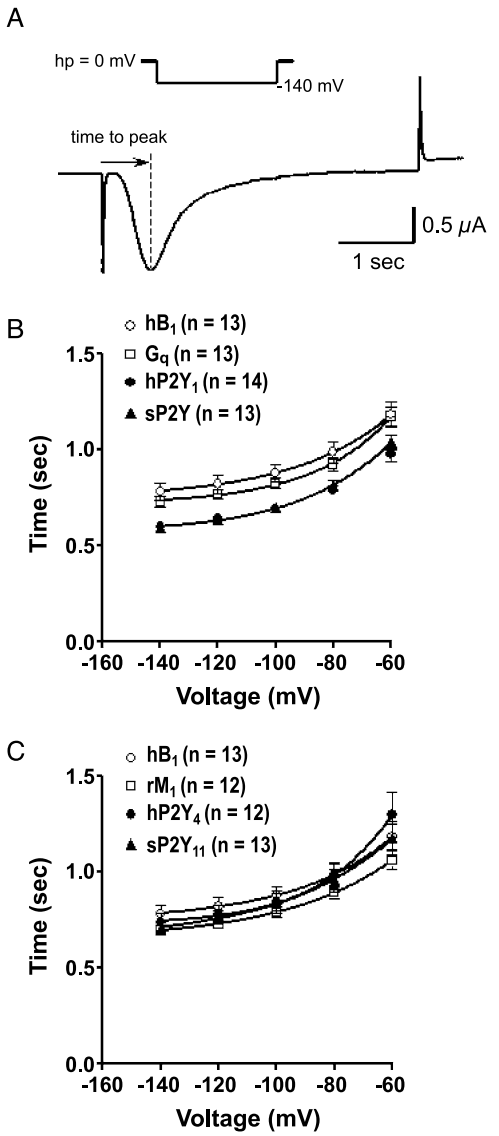

Figure 1. Effects of expressed G_q coupled receptor stimulation on hyperpolarization-induced activation of the endogenous T_{in} channel in *Xenopus* oocytes. A) Representative trace of the T_{in} channel current at −140 mV following stimulation of the expressed $hP2Y_1$ receptor. Time-to-peak current 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 hB_1-bradykinin ($n = 13$), G_q alpha subunit ($n = 13$), $hP2Y_1$ receptor ($n = 14$), and sP2Y ($n = 13$). C) Comparison of time-to-peak measurements for hB_1-bradykinin ($n = 13$), rM_1-muscarinic ($n = 12$), $hP2Y_4$ ($n = 12$), and $hP2Y_{11}$ ($n = 13$) receptors.

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

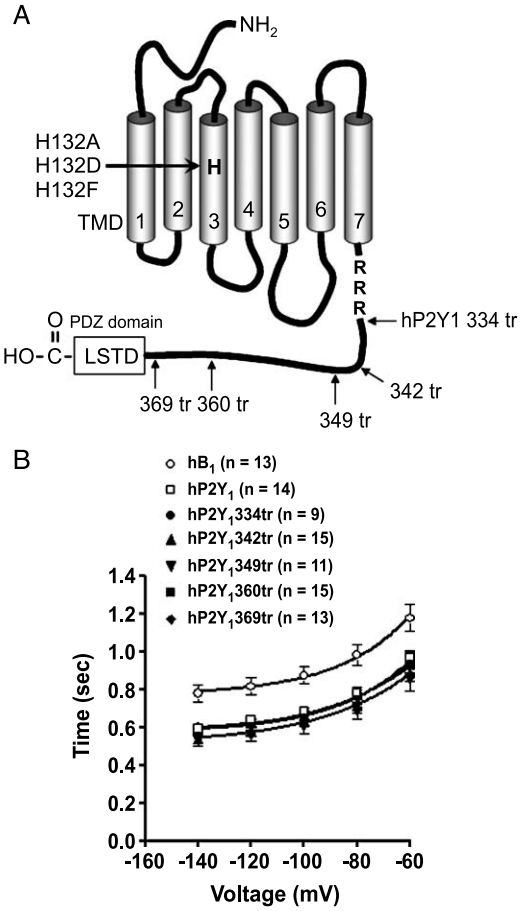

Figure 2. Effect of C-terminal domain truncation on channel activation. A) The relative locations of TM3 mutations ($hP2Y_1$-H132A, $hP2Y_1$-H132D, and $hP2Y_1$-H132F) and C-terminal truncation sites within the $hP2Y_1$ receptor are indicated. B) Comparison of time-to-peak measurements for hB_1-bradykinin ($n = 13$), $hP2Y_1$ ($n = 14$), $hP2Y_1$334tr ($n = 9$), $hP2Y_1$342tr ($n = 15$), $hP2Y_1$349tr ($n = 11$), $hP2Y_1$360tr ($n = 15$), and $hP2Y_1$369tr ($n = 13$) receptors as the function of voltage.

Table 1. V_{50} and slope factors of T_{in} currents elicited by agonist-activated receptors.

Receptors	V_{50} (mV)	Slope factor
hB_1 ($n=13$)	−43.0	−13.4
$hP2Y_4$ ($n=9$)	−53.8	−18.8
rM1 ($n=16$)	−51.8	−16.2
$hP2Y_1$ ($n=13$)	−72.5*	−18.5
$hP2Y_1$-H132A ($n=5$)	−73.7*	−18.2
$hP2Y_1$-H132D ($n=6$)	−80.3*	−17.3
$hP2Y_1$-H132F ($n=5$)	−69.5*	−12.3

V_{50} 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 hB_1-bradykinin receptor value.

significantly different from the values derived from oocytes expressing the wild-type $hP2Y_1$ 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 $P2Y_1$ receptor contains a unique histidine residue at position 132 that is not present in other P2Y receptor subtypes. To determine if TM3 of the $hP2Y_1$ receptor is important in modulating activation, we mutated His-132 to alanine ($hP2Y_1$-H132A), aspartic acid ($hP2Y_1$-H132D), and phenylalanine ($hP2Y_1$-H132F) (Figure 2A), expressed the mutants in *Xenopus* oocytes, and determined the time-to-peak value of the T_{in} current following receptor activation. Time-to-peak current measurements of all TM3 point mutations were significantly increased compared to the wild-type $hP2Y_1$ receptor (Figure 3A). Mutation of His-132 to alanine, aspartic acid, or phenylalanine increased time-to-peak current values compared to the wild-type $hP2Y_1$ receptor, suggesting that the primary structure of TM3 plays a role in modulating channel activation. It is worth noting that TM3 of the $hP2Y_1$ 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 EC_{50} for phospholipase C activation from 1.94

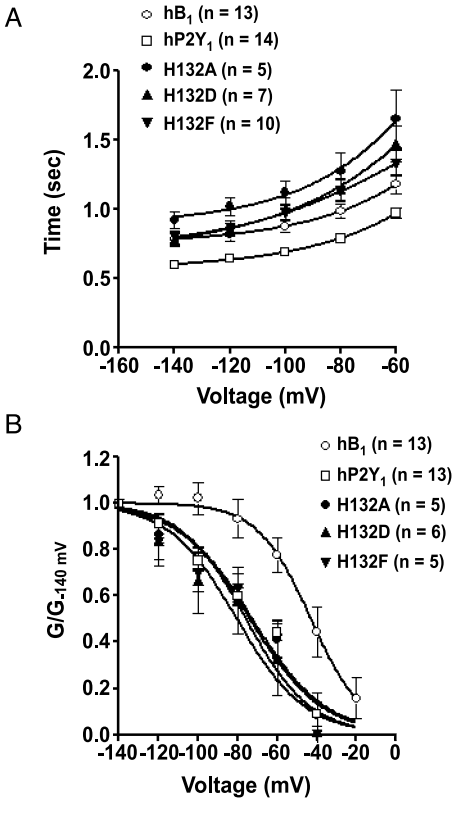

Figure 3. Effect of third transmembrane domain mutations on activation and conductance voltage relationships. A) Time-to-peak measurements for hB_1-bradykinin ($n=13$), wild-type $hP2Y_1$ ($n=14$), $hP2Y_1$-H132A ($n=5$), $hP2Y_1$-H132D ($n=7$), $hP2Y_1$-H132F ($n=10$), and $hP2Y_1$-Q307K ($n=7$) receptors as a function of voltage. B) Normalized conductance-voltage relationships for hB_1-bradykinin ($n=13$), wild-type $hP2Y_1$ ($n=13$), $hP2Y_1$-H132A ($n=15$), $hP2Y_1$-H132D ($n=6$), and $hP2Y_1$-H132F ($n=6$). The V_{50} values and slope factors for each conductance are listed in Table 1.

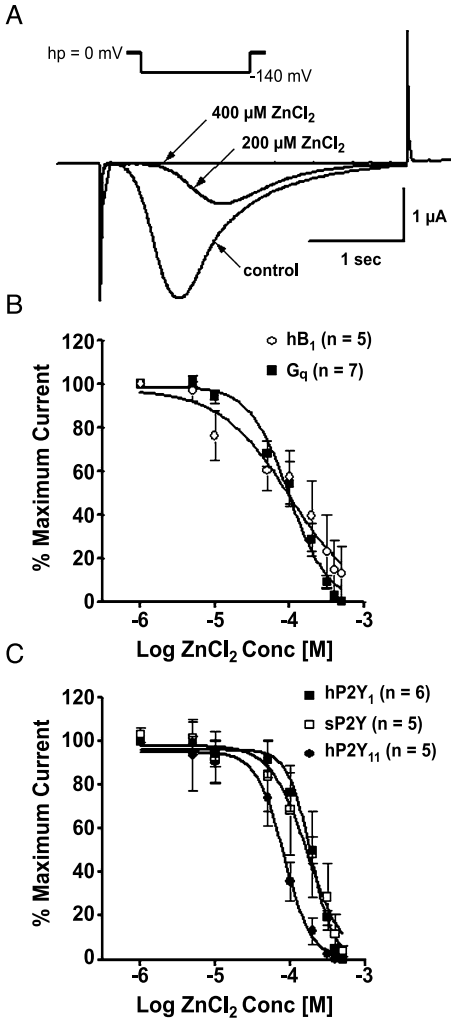

Figure 4. Effects of Zn^{2+} on the inward currents elicited by step hyperpolarization to −140 mV. A) Representative current traces showing T_{in} currents activated by $hP2Y_1$ receptor in the presence of 20 µM 2MeS-ADP. Agonist-stimulated inward current was inhibited by $ZnCl_2$ in a concentration-dependent manner. B) Concentration–response relationship for G_q ($n=7$) and hB_1-bradykinin ($n=5$). C) Inhibition of $hP2Y_1$ ($n=6$), sP2Y ($n=5$), and $hP2Y_{11}$ ($n=5$) receptor-elicited T_{in} currents by $[Zn^{2+}]$. The IC_{50} values are listed in Table 2.

± 0.8 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_{in} 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_{in} 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_{in} 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_{in} current activated by hB_1-bradykinin and P2Y receptors. Figure 4A shows the current response at −140 mV of the T_{in} channel elicited by agonist-activated $hP2Y_1$ receptors in the presence of increasing concentrations of $ZnCl_2$. The amplitudes of the T_{in} 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_{in} 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 $G_q\alpha$ subunit (98 μM; Figure 4B, Table 2) or $hP2Y_{11}$ receptor (83 μM; Figure 4C, Table 2). It is worth noting that the inhibition of T_{in} channel current by $ZnCl_2$ in oocytes expressing the $G_q\alpha$ subunit indicated that the effect of Zn^{2+} was not on receptor-mediated regulation of the T_{in} channel. In contrast, the IC_{50} of $ZnCl_2$ for inhibition of T_{in} 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.

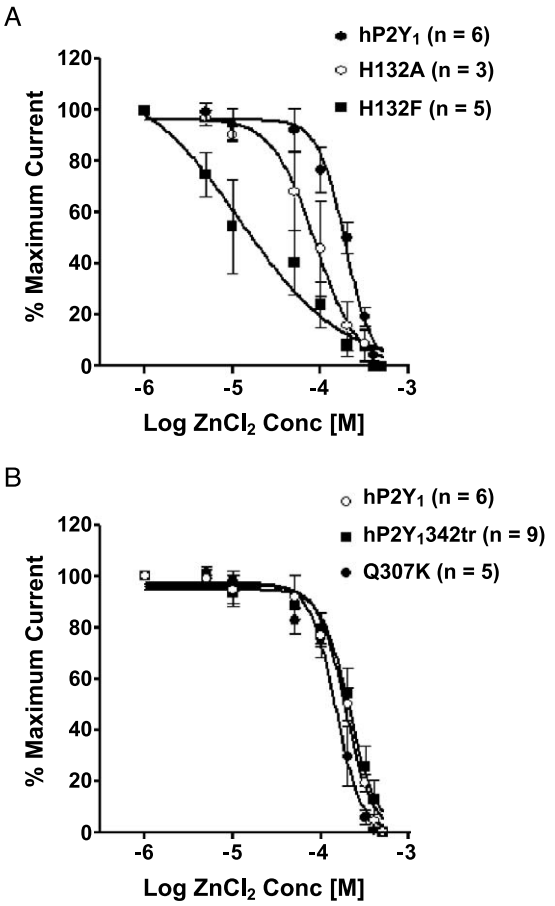

Figure 5. Effects of Zn^{2+} on the inward currents elicited after stimulation of mutant $P2Y_1$ receptors. A) Inhibition of $hP2Y_1$ ($n=6$), $hP2Y_1$-H132A ($n=3$) and $hP2Y_1$-H132F ($n=5$) receptor-elicited T_{in} currents by [Zn^{2+}]. B) Inhibition of $hP2Y_1$ ($n=6$), $hP2Y_1$342tr ($n=9$), and $hP2Y_1$-Q307K ($n=5$) receptor-elicited T_{in} currents by [Zn^{2+}]. The IC_{50} values are listed in Table 2.

To investigate whether His-132 is important in modulating the Zn^{2+} sensitivity of the T_{in} 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_{in} 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. This

Table 2. IC_{50} values from Zn^{2+} concentration–response relationship in Figure 4.

Receptors	IC_{50} (μM)
$hP2Y_1$ ($n=6$)	187
$hP2Y_{11}$ ($n=4$)	83*
sP2Y ($n=5$)	170
$HP2Y_1$-H132A ($n=3$)	87*
$HP2Y_1$-H132F ($n=5$)	13*
hB_1 ($n=5$)	65*
G_q ($n=7$)	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 shown previously to produce a shift in the voltage dependence of the channel similar to the $hP2Y_1$ and sP2Y receptors [18]. This observation is consistent with the idea that the C-terminal region and TM3 domains of the receptor independently affect distinct functional properties of the T_{in} channel.

Analysis of the $hP2Y_{11}$ receptor sequence showed that the corresponding residue to His-132 in TM3 is Thr-107. It is interesting to note that the Zn^{2+} IC_{50} values for the T_{in} currents elicited by $hP2Y_{11}$, $hP2Y_1$-H132A, and hB_1-bradykinin receptors were not significantly different, suggesting that the presence of His at this position is essential for producing the wild-type $hP2Y_1$ receptor effect on Zn^{2+} inhibition of the channel. A similar case can be made for hyperpolarization-induced activation, in that the $hP2Y_{11}$ receptor exhibited properties that were significantly different from the wild-type $hP2Y_1$ receptor but similar to the hB_1-bradykinin receptor.

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.

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References

1. Dubyak GR, El-Moatassim 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, Detheux M et al. Identification of a novel human ADP receptor coupled to G_i. 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. Schachter 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 Pharmacol Exp Ther 2001; 297: 43–9.
14. Dranoff JA, O'Neill AF, Franco AM et al. A primitive ATP receptor from the little skate *Raja erinacea*. J Biol Chem 2000; 275: 30701–6.
15. Communi D, Govaerts C, Parmentier M, Boeynaems JM. Cloning of a human purinergic P2Y receptor coupled to phospholipase C and adenylyl cyclase. J Biol Chem 1997; 272(51): 31969–73.
16. Hollopeter G, Jantzen HM, Vincent D et al. Identification of the platelet ADP receptor targeted by antithrombotic drugs. Nature 2001; 409: 202–7.
17. O'Grady SM, Elmquist 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 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 $5\text{-}HT_{1a}$ 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* G_q alpha 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. $P2Y_2$ and $P2Y_6$ 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 $P2Y_1$ receptor. Mol Pharmacol 1997; 52: 499–507.
26. Moro S, Guo D, Camaioni E et al. Human $P2Y_1$ receptor: Molecular modeling and site-directed mutagenesis as tools to identify agonist and antagonist recognition sites. J Med Chem 1998; 41: 1456–66.