FEBS Letters 436 (1998) 104–110 FEBS 20828

P2Y receptor subtypes differentially couple to inwardly-rectifying potassium channels

J. Mosbacher[a], R. Maier[a], B. Fakler[b], A. Glatz[a], J. Crespo[a], G. Bilbe[a,*]

[a] *Novartis Pharma A.G., CH-4002 Basel, Switzerland*
[b] *Dept. of Physiology II, University of Tübingen, 72074 Tübingen, Germany*

Received 15 June 1998; revised version received 3 August 1998

Abstract Subtypes of P2Y receptors are well characterized with respect to their agonist profile but little is known about differences in their intracellular signalling properties. When expressed in *Xenopus* oocytes, both $P2Y_2$ and $P2Y_6$ receptors effectively couple to endogenous Ca^{2+}-dependent Cl^--channels. However, only $P2Y_2$ receptors increased currents mediated by inward-rectifier K^+ channels of the Kir3.0 subfamily. This incrase in Kir-current was sensitive to pertussis toxin, while activation of Ca^{2+}-dependent Cl^--channels was not. In contrast, suramin, a P2 receptor antagonist, inhibited activation of both channels. These observations suggest that, in contrast to $P2Y_6$, $P2Y_2$ receptors couple to two different classes of G proteins.

Key words: P2Y; Purinoceptor; Pertussis toxin; G-protein activated Kir; *Xenopus* oocyte; G protein

1. Introduction

Receptors for extracellular nucleotides are widely expressed and involved in a variety of cellular functions. Based on pharmacological and structural properties, they have been subdivided into P1 or adenosine receptors and P2 receptors which are activated by nucleotide di- or triphosphates [1,2]. The P2 receptor family comprises both ionotropic receptors (P2X) and metabotropic receptors (P2Y) from the superfamily of G-protein-coupled receptors (GPCRs; [3,4]).

About 10 subtypes of P2Y receptors have been cloned from several species [2]. Each P2Y receptor subtype has a distinct pharmacological profile defined by its activation via ATP, UTP and respective analogs [1]. Stimulation of intracellular second messenger pathways by P2Y receptors is predominantly mediated by the $G\alpha_{q/11}$ subfamily of G-protein α-subunits [1] which activates phospholipase C (PLC) and subsequently stimulates inositol phosphates and 1,2-diacylglycerol (DAG) leading to a rise in intracellular free Ca^{2+} and activation of protein kinase C. All cloned P2Y receptor subtypes activate this pathway. Work on P2Y receptors in various types of cell and tissues, however, demonstrated quite a variability in effector types [5–10] as well as in G-protein α-subunits mediating this effector-coupling [8,10–12]. Moreover, extracellularly applied ATP activated an inwardly-rectifying potassium (K^+) current in atrial myocytes [12,13] in a pertussis toxin sensitive manner. These cells are known to express inward-rectifier K^+ (Kir) channels which are directly activated by G-protein β/γ subunits [14].

On the other side, only limited information is available from comparative studies that determine differences in signal transduction between recombinant P2Y receptor subtypes [15–17]. Part of the $P2Y_2$ and $P2Y_4$ receptor mediated mobilization of intracellular Ca^{2+} was pertussis toxin sensitive which indicates that these receptors may activate $G\alpha_{i/o}$ proteins [15,17]. However, only one report yet described activation of an $G\alpha_{i/o}$ protein mediated effect by a P2Y receptor subtype, namely the pertussis toxin sensitive inhibition of N-type Ca^{2+} channels by $P2Y_2$ receptors expressed in neurones [18].

To investigate the signal transduction properties of recombinant P2Y receptors, we expressed two mammalian receptor subtypes, $P2Y_2$ and $P2Y_6$, in *Xenopus* oocytes either alone or in combination with G-protein-activated Kir channels (GIRK channels or Kir channels of the Kir3.0 subfamily). This offered the advantage to monitor two different effectors of G protein activity, the endogenous Ca^{2+}-activated Cl^--channels [19,20] and the Kir channels directly sensitive to activated Gβ/γ.

2. Materials and methods

2.1. Materials

Inorganic chemicals were from Fluka and Sigma (Buchs, Switzerland); HPLC-purified ATP and UTP from Pharmacia (Heidelberg, Germany); UDP, suramin, the pertussis toxin holomer and hexokinase from Sigma; 2-methylthio-ATP (2MeATP) from RBI (Natick, MA, USA); the A protomer of pertussis toxin from Calbiochem (La Jolla, CA, USA); in vitro cRNA transcription kit from Ambion (Austin, TX, USA); 3-aminobenzoic acid ethyl ester (MS222) for frog anesthesia and collagenase from Sigma.

2.2. Expression of P2Y receptors and Kir3.0 potassium channels in Xenopus oocytes

Plasmids for mouse $P2Y_2$ (kind gift of D.J. Julius), human $P2Y_6$ receptors [14] and rat Kir3.0 channels were linearized and transcribed into capped cRNA using a cRNA transcription kit (Ambion, Houston, TX, USA). Stock solutions were prepared containing either all subtypes (Kir3.1/2/3/4) or Kir3.1/2 at the same concentration ratio. For oocyte preparation, female *Xenopus laevis* frogs (SNAKE, South Africa) were anesthetized in 0.15% 3-aminobenzoic acid ethyl ester and placed on ice during surgical removal of 4–5 lobes of oocytes. Oocytes were kept in modified Barth's solution (mBS; 88 mM NaCl, 1 mM KCl, 2.4 mM $NaHCO_3$, 0.82 mM $MgSO_4$, 0.34 mM $Ca(NO_3)_2$, 0.41 mM $CaCl_2$, 10 mM HEPES, pH 7.5) containing 100 U/ml penicillin and streptomycin and prepared for injection in the following way. Oocytes were mechanically isolated with forceps, incubated in 2 mg/ml collagenase (type IA) for 30 min at 37°C, washed twice with mBS, incubated in 4 mM EGTA (pH 8.5) for 4 min and slightly agitated until the follicular membranes were removed. Treated oocytes

*Corresponding author. Fax: (41) (61) 6964145.
E-mail: graeme.bilbe@pharma.novartis.com

Abbreviations: GPCR, G-protein-coupled receptor; Kir3.0, G-protein modulated inwardly rectifying potassium channels; PLC, phospholipase C; $I_{Cl(Ca)}$, Ca^{2+}-dependent Cl^--current; I_{Kir}, current mediated by Kir channels; Gβ/γ, G-protein β/γ subunit complex; Gα, G-protein α subunit; 2MeATP, 2-methylthioadenosine 5′-triphosphate; mBS, modified Barth's solution; NFR, normal frog Ringer; KFR, high-K′ frog Ringer; *I-V*, current-voltage relationship

PII: S0014-5793(98)01066-7

were washed and placed in mBS at 18°C for 6–20 h before injection. P2Y and Kir encoding mRNA were mixed prior to injection at a ratio of 1:1 (v/v) and a volume of about 50 nl was air-pressure injected into each defolliculated stage V–VI oocyte using thin-wall borosilicate capillaries with tip diameters of 15–30 μm. mRNA concentrations were adjusted to keep the stimulation of endogenous $I_{Cl(Ca)}$ at a submaximal level and to yield about the same current response for $P2Y_2$ and $P2Y_6$ receptors (see Fig. 3E,F). In some experiments, various amounts of either P2Y receptor or Kir3.0 channel encoding mRNA was injected to study coupling properties of P2Y receptors to Kir3.0 channels at various expression levels. After injection, oocytes were placed for 3–6 days at 17°C and mBS was changed every 72 h.

2.3. Electrophysiological measurement and analysis

For recording, oocytes were placed in a perfusion chamber (0.2 ml) with continuous gravity flow (2 ml/min) of normal frog Ringer (NFR) containing 115 mM NaCl, 2.5 mM KCl, 1.8 mM $CaCl_2$, 10 mM HEPES, pH 7.2), or high-potassium frog Ringer (KFR) where 87.5 mM NaCl was replaced by KCl. Recording electrodes for two-electrode voltage clamp contained 3 M KCl and had tip resistances of 0.7–2 MΩ. The following stock solutions were prepared: 100 mM HPLC-purified ATP and UTP, 100 mM UDP, 50 mM suramin and 10 mM 2MeATP, all stored at −20°C. Before use, UDP was treated for at least 1 h at 10 mM final concentration with 50 U/ml hexokinase and 100 mM glucose to remove contaminations of UTP. Pertussis toxin holomer was dissolved in dH_2O, added to the Barth's medium to give a final concentration of 1 μg/ml and preincubated with the oocytes for at least 40 h. The A-protomer of pertussis toxin was dissolved in high ionic strength buffer at a concentration of 100 μg/ml and stored at +4°C. For injection, the stock solution was dissolved 1:10 in H_2O, and 50 nl/oocyte were injected 10–20 h prior to measurement. A Geneclamp 500 amplifier was used together with a Digidata 1200 board and pClamp 6 (Axon Instruments, Foster City, CA, USA) to digitize the current and voltage signals. Voltage ramps from −100 to +50 mV in 2.5 s were used to estimate the current-voltage (*I-V*) relation in the absence and presence of test compound. Current signals were low-pass filtered (4-pole Bessel filter) at 50 Hz and digitized at 200 Hz for *I-V* and at 10 Hz for continuous sampling. Data were analyzed using Igor Pro (Wavemetrics, Lake Oswego, OR, USA). EC_{50} and IC_{50} values and their S.E.M. values were calculated from their logarithms giving unsymmetrically linear S.E.M. values. Student's *t*-test was used to test for statistically significant differences between two data sets.

3. Results

We recently cloned the human $P2Y_6$ receptor and studied its expression profile in a number of tissues and cell lines [21]. To elucidate possible differences between $P2Y_6$ and other P2 receptors, we first established the pharmacological profile of $P2Y_6$ and $P2Y_2$ heterologously expressed in *Xenopus* oocytes. As shown in Fig. 1, both receptor subtypes were about equally effective in activating endogenous Ca^{2+}-dependent Cl^--channels ($I_{Cl(Ca)}$; [19]) in defolliculated oocytes (Fig. 1A,C; current-voltage relation (*I-V*) in Fig. 1B,D). The agonist profile, however, was rather different. Whereas $P2Y_6$ receptors responded to UDP but not to 2MeATP, ATP or UTP, $P2Y_2$ receptors were stimulated by ATP and UTP, but not by UDP or 2MeATP (the preferred agonist for $P2Y_1$ receptors). In both cases the current response was blocked by 50 μM suramin, a non-specific inhibitor of P2Y receptor subtypes. The small Cl^--current observed upon wash-out of coapplied agonists and suramin most likely reflects residual nucleotides.

Since amplitude and time-course of the endogenous $I_{Cl(Ca)}$ varied significantly between individual oocytes, we coexpressed inward-rectifier K^+ channels of the Kir3.0 subfamily for a more detailed study of the pharmacological properties and the signalling pathways of our P2Y receptors. Kir3.0 channels, which deliver most robust currents when assembled

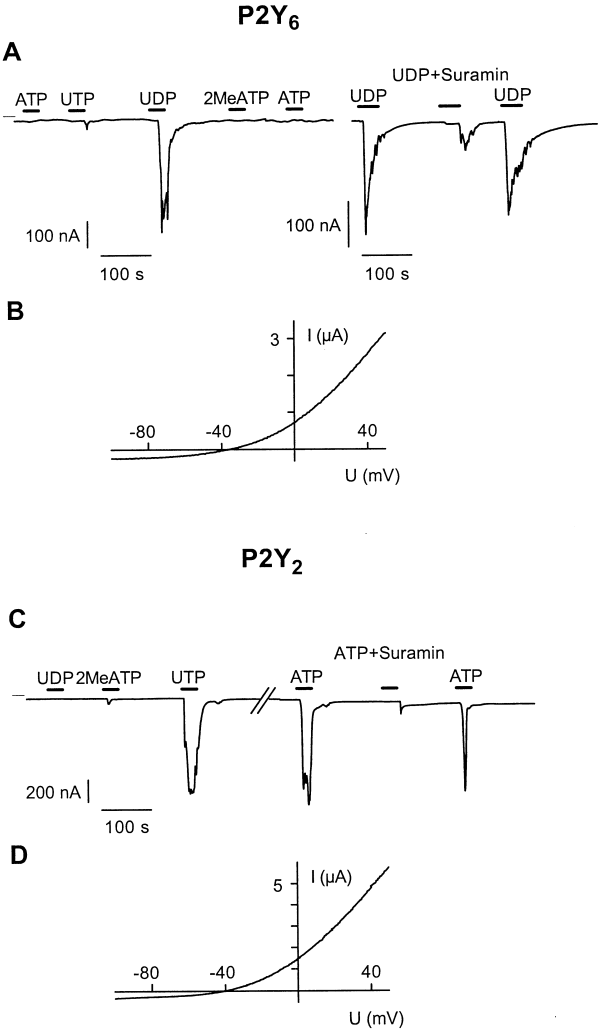

Fig. 1. Agonist profile of recombinant $P2Y_6$ and $P2Y_2$ receptors expressed in *Xenopus* oocytes. A: Whole-cell current measured in response to stimulation of human $P2Y_6$ receptors by various nucleotides at a concentration of 10 μM. Bath solution was NFR, holding potential was −70 mV. Fifty μM suramin partially blocked $I_{Cl(Ca)}$ elicited by 10 μM UDP. B: *I-V* of Ca^{2+}-dependent Cl^--channels measured in response to a voltage-ramp from −100 to +50 mV after activation of currents by 10 μM UDP. C: Experiment as in A but with oocytes expressing mouse $P2Y_2$ receptors. D: *I-V* as in B measured after stimulation of $P2Y_2$ receptors by 10 μM ATP.

as heteromultimers, are well known to couple to many GPCRs and to be activated by the β/γ complex of G proteins [14].

Surprisingly, receptor-stimulated activation of currents mediated by heteromultimeric Kir3.1/2 channels (I_{Kir}) was only observed for $P2Y_2$ receptors, while stimulation of $P2Y_6$ did not affect I_{Kir}. Fig. 2A and D show such experiments, where currents of whole oocytes were recorded at a constant membrane potential of −70 mV and agonist was applied in a solution containing 90 mM K^+. The initial increase in inward current (blocked by 1 mM $BaCl_2$, not shown) seen in both experiments results from the increase in extracellular K^+ concentration. While activation of $P2Y_2$ by ATP led to a stable and more than two-fold increase in inward I_{Kir} (Fig. 2D), stimulation of $P2Y_6$ by UDP just led to the transient increase in $I_{Cl(Ca)}$ (Figs. 1 and 2A). The differential effect of both receptors is more evidently illustrated in Fig. 2C and F which

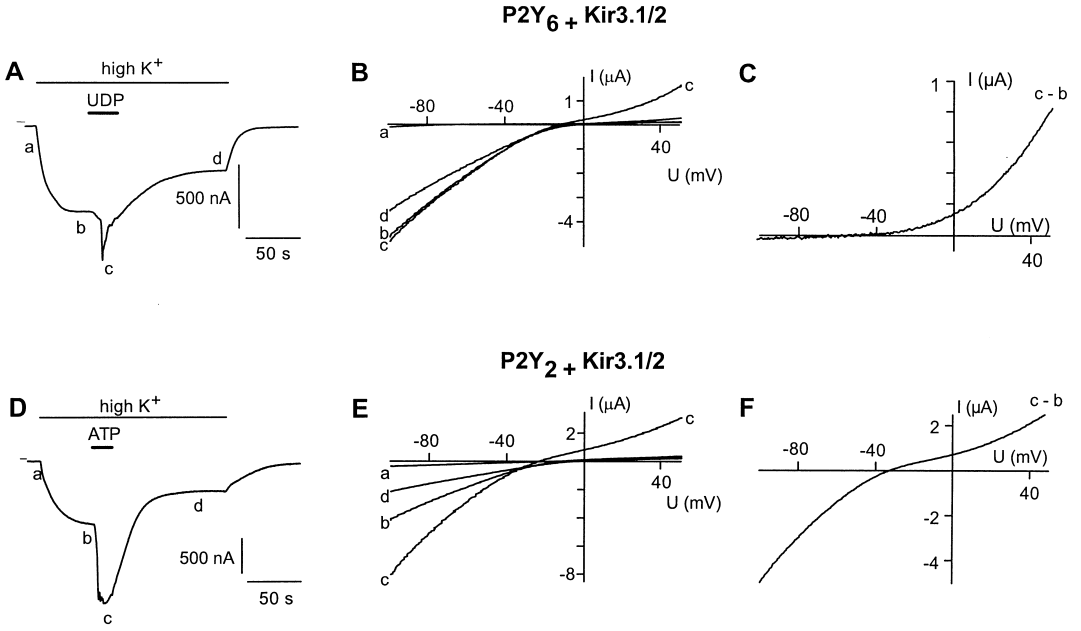

Fig. 2. $P2Y_2$ but not $P2Y_6$ activate coexpressed Kir3.1/2 channels. A: Currents recorded at −70 mV evoked by application of 10 μM UDP to oocytes coexpressing $P2Y_6$ receptors and Kir3.1/2 channels. Increase in I_{Kir} observed upon switch from NFR to KFR is due to the shift in K^+ reversal potential. B: *I-V*s measured as in Fig. 1 at the timepoints indicated in A. C: Agonist-induced current obtained by subtraction of the *I-V*s measured at c and b in B. D: Same experiment as in A but for $P2Y_2$ receptors activated by 10 μM ATP. E, F: *I-V*s and agonist-induced current as in B and C but for $P2Y_2$ receptors. Note in F that ATP activated both $I_{Cl(Ca)}$ and I_{Kir}.

represent *I-V*s obtained by subtracting the *I-V*s recorded before and during stimulation of either receptor (Fig. 2B,E). For $P2Y_6$ the result was very similar to that obtained without coexpressed Kir channels (Fig. 1BFig. 2C). The *I-V* resulting from stimulation of $P2Y_2$, however, clearly exhibited two components: the Kir-mediated inward current seen at potentials negative to about −30 mV and the outward-rectifying $I_{Cl(Ca)}$ (Fig. 2F). In addition, Fig. 2 shows that stimulation of either receptor resulted in a slowly developing decrease of the basal I_{Kir} (*I-V*s at timepoints b and d in Fig. 2A,B and D,E).

To determine whether the $P2Y_2$ mediated increase in I_{Kir} was agonist specific, 10 μM UTP was applied instead of ATP. UTP increased I_{Kir} to an extent similar to that observed for ATP (Fig. 3). The average ratio of current in the presence of agonist vs. baseline current ($I_{agonist}/I_{control}$) measured at −70 mV for $P2Y_2$ receptors was 3.6 ± 0.6 (mean ± S.E.M., $n = 9$; Fig. 3A) and 3.8 ± 0.4 ($n = 12$) for ATP and UTP, respectively. In contrast, little change in $I_{agonist}/I_{control}$ was seen for $P2Y_6$ receptors stimulated by 10 μM UDP under identical conditions (1.1 ± 0.04, $n = 11$; Fig. 3A). In the latter case, the $I_{Cl(Ca)}$ might account for the small upregulation seen with $P2Y_6$ receptors. At +50 mV, $I_{agonist}/I_{control}$ was not significantly different for both receptors. The respective values were 3.3 ± 0.3 ($n = 10$) for $P2Y_6$ receptors and 6.5 ± 0.8 ($n = 7$) and 3.8 ± 0.4 ($n = 8$) for $P2Y_2$ activated by 10 μM ATP and 10 μM UTP, respectively (Fig. 3B). As shown later, suramin was also able to block I_{Kir} activation by $P2Y_2$ receptors.

It might be that Kir3.0 subtypes are differentially sensitive to receptor stimulation. Therefore, all four Kir3.0 subunits cloned to date (Kir3.1, 3.2, 3.3, 3.4) were coinjected with $P2Y_6$ or $P2Y_2$ receptors. However, no differences to experiments with Kir3.1/2 heteromers were found. These results clearly indicate that while both $P2Y_2$ and $P2Y_6$ receptors effectively activate Ca^{2+}-dependent Cl^--channels, it is only $P2Y_2$ that couples to coexpressed Kir channels.

Two possible explanations may exist for this differential coupling of $P2Y_2$ receptors to $I_{Cl(Ca)}$ and I_{Kir}: $P2Y_2$ interacts with one type of G protein that activates both effectors, or $P2Y_2$ interacts with more types of G proteins that each activate a distinct effector. In order to address this question, oocytes expressing $P2Y_2$ and Kir3.1/2/3/4 channels were incubated for at least 48 h in 1 μg/ml pertussis toxin or injected with 50 nl of the A protomer of pertussis toxin (10 ng/μl) 20 h prior to electrophysiological recordings. As shown in Fig. 4, in pertussis-toxin treated cells Kir channels did not respond to receptor stimulation, whereas the increase in $I_{Cl(Ca)}$ was virtually left unchanged (Fig. 4A,B). Fig. 4C summarizes the results obtained from 6 oocytes tested and compares the result to untreated oocytes. This finding favors the hypothesis that $P2Y_2$ activates at least two G proteins, a pertussis toxin-sensitive G protein which couples the $P2Y_2$ receptor to I_{Kir} and a pertussis toxin-insensitive G protein which leads to stimulation of $I_{Cl(Ca)}$.

The fast inactivation of $I_{Cl(Ca)}$ and its desensitization upon repetitive receptor stimulation (Fig. 5A,B) can mask a heterologous desensitization of the receptor itself and makes it difficult to perform dose-response experiments. The upregulation of I_{Kir} did not show a significant desensitization (Fig. 5A,B) indicating that the $P2Y_2$ receptor does not desensitize within the time course of the experiment (20 min). Therefore, we used I_{Kir} to characterize the pharmacological properties of $P2Y_2$ receptors. The dose-response relationship for activation of $P2Y_2$ by ATP and UTP (Fig. 5C,E) showed that both agonists are equally potent: EC_{50} values were 1.2+0.4/−0.3 μM ($n = 8$) and 1.0 ± 0.1 μM ($n = 5$), with Hill coefficients of 1.1 ± 0.2 and 1.3 ± 0.2 for ATP and UTP, respectively. Inhibition of $P2Y_2$ by suramin measured in cumulative experiments in the presence of 10 μM ATP (Fig. 5D) resulted in a dose-

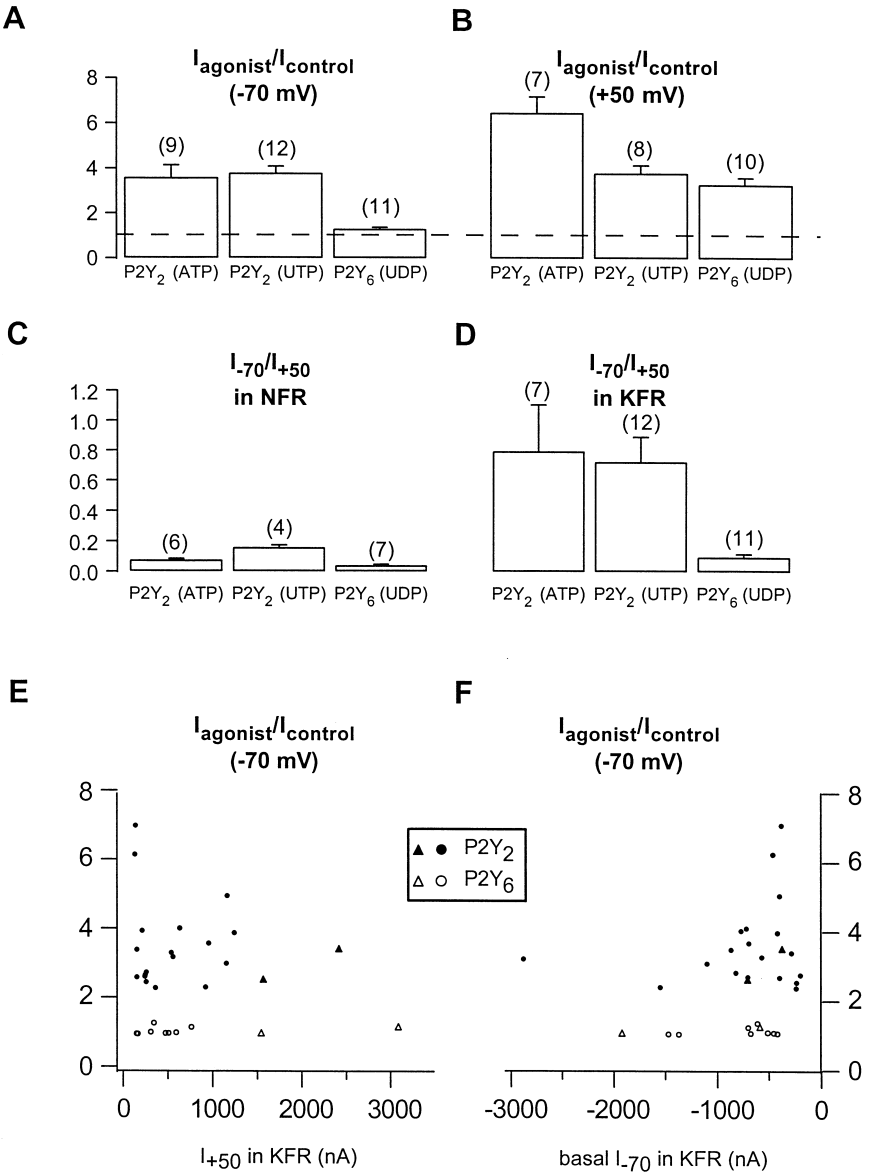

Fig. 3. Histograms illustrating the average effect of P2Y receptors on $I_{Cl(Ca)}$ and I_{Kir}. A: Average ratio of currents in the presence and absence of agonist (timepoint c and b in Fig. 2) measured at a potential of −70 mV. B: Ratio as in A for currents measured at +50 mV. Data are taken from *I-V* experiments as shown in Fig. 2B and E. C, D: Average rectification indices of agonist-induced currents (timepoints c–b in Fig. 2) in NFR (C) and KFR (D). A ratio of 1 indicates a linear *I-V*, values <1 indicate an outwardly-rectifying *I-V*. Error bars are S.E.M., number of cells tested is indicated. E, F: Upregulation of currents at −70 mV vs. current amplitudes in the presence of an agonist at +50 mV (E) and basal current at −70 mV (F) in KFR shown for single cells. Filled symbols show data for $P2Y_2$, open symbols for $P2Y_6$ receptors. Triangles represent data taken from oocytes injected with 10 times ($P2Y_2$) or 5 times ($P2Y_6$) higher amount of mRNA. Experiments shown in this figure are from at least 3 batches of oocytes, data from cells either coexpressing Kir3.1/2 or Kir3.1/2/3/4 channels are pooled.

response curve with an IC_{50} of 33+9/−7 μM and a Hill coefficient of 1.4 ± 0.4 ($n = 5$) (Fig. 5F).

4. Discussion

The initial goal of this study was a pharmacological and functional comparison of two P2Y receptors, $P2Y_2$ and $P2Y_6$. We used the *Xenopus* oocyte expression system with two ion channels as effector systems, the Ca^{2+}-activated Cl^--channel endogenous to the oocytes, and coexpressed Kir3.0 channels which are directly activated by GPCRs via the β/γ complex of G proteins.

The observation that both $P2Y_2$ and $P2Y_6$ receptors activate $I_{Cl(Ca)}$ in the oocyte indicates that $P2Y_2$ and $P2Y_6$ both activate PLC [4,22]. In support of this, stimulation of both receptor subtypes induced a slow downmodulation of I_{Kir} which was shown to be mediated by protein kinase C dependent phosphorylation of the Kir3.0 channels [23].

However, we found that only $P2Y_2$ receptors are able to activate I_{Kir} even though the stimulation of $I_{Cl(Ca)}$ was comparable between $P2Y_2$ and $P2Y_6$ expressing oocytes. This may imply that both subtypes couple to different intracellular signalling pathways. It was suggested that some P2Y receptors induce a rise in intracellular free Ca^{2+} via the β/γ complex of a $G\alpha_{i/o}$ protein [8,10,24]. Therefore, we tried to block the $P2Y_2$ receptor mediated responses by pertussis toxin. Surprisingly,

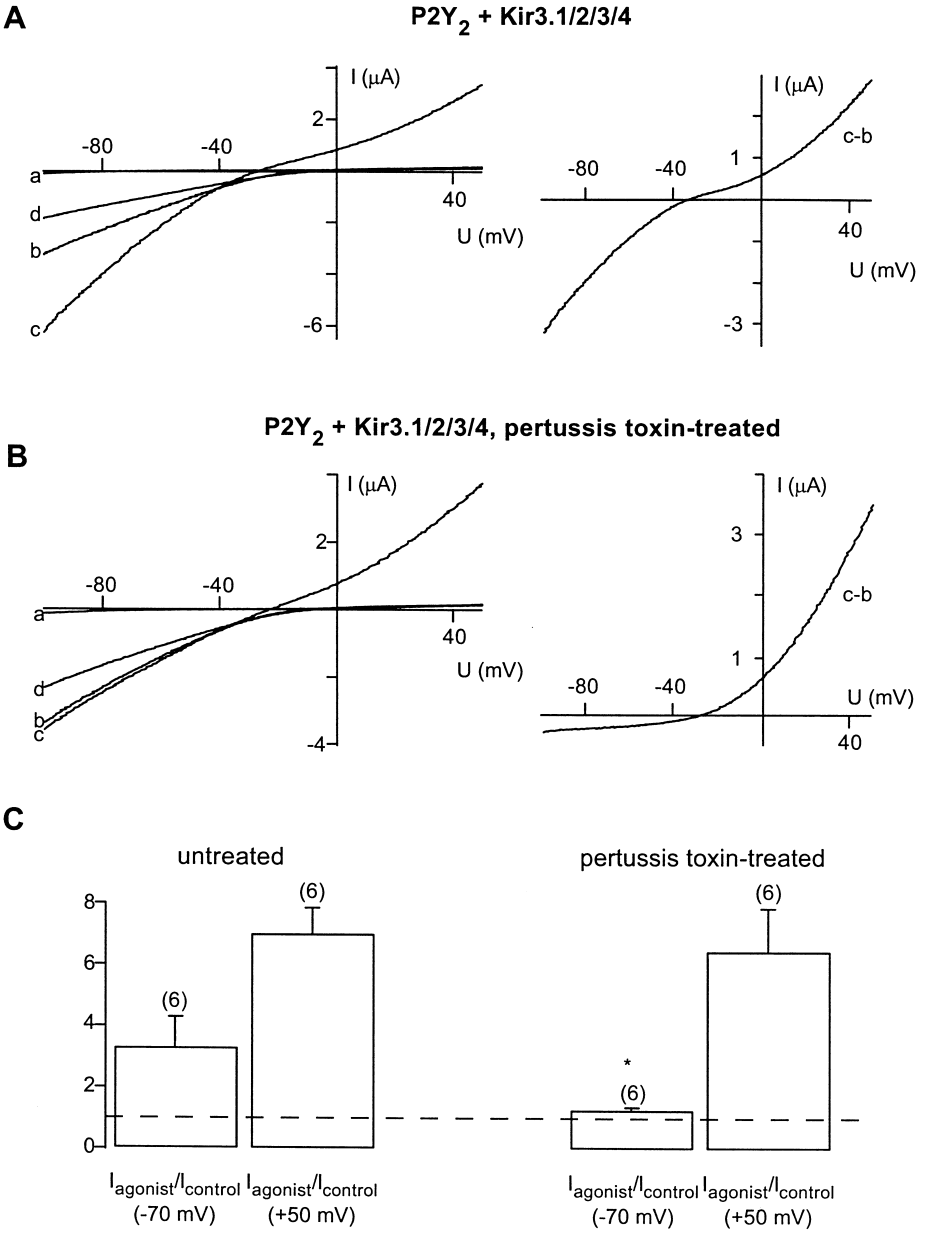

Fig. 4. Pertussis toxin selectively abolished coupling of $P2Y_2$ receptors to Kir3.0 channels. A: *I-V* recordings from oocytes coexpressing $P2Y_2$ receptors and Kir3.1/2/3/4 channels. Experimental conditions as in Fig. 2. B: *I-V*s from oocytes incubated in 10 ng/μl pertussis toxin for 48 h prior to recordings. Experimental conditions as in Fig. 2. C: Average effect of pertussis toxin treatment (n=6). Controls and pertussis toxin treated oocytes were from the same two batches of oocytes. Star indicates statistically significant reduction of the agonist-induced current at −70 mV in pertussis toxin-treated cells vs. controls ($^*P < 0.05$, Student's t-test).

only the coupling to the Kir3.0 channels was disrupted in pertussis toxin treated cells while the stimulation of $I_{Cl(Ca)}$ was almost unaffected. In parallel, the stimulation of $I_{Cl(Ca)}$ by $P2Y_6$ receptors remained unaltered upon treatment with pertussis toxin. These results strongly suggest that $P2Y_2$ receptors are multifunctional GPCRs activating two different G proteins from the $G\alpha_{i/o}$ and $G\alpha_{q/11}$ families whereas $P2Y_6$ receptors only activate $G\alpha_{q/11}$ proteins. This finding on recombinant P2Y receptors is in agreement with reports showing a pertussis toxin sensitive coupling of native P2Y receptors in atrial myocytes to inward potassium channels [12,13] and to Ca^{2+} channels in chromaffin cells [5], as well as with findings that aortic endothelial cells respond to application of UTP in a pertussis toxin sensitive manner [24].

It remains unclear why $P2Y_6$ receptors unlike many other GPCRs do not couple to Kir channels. Almost all of the coexpression studies with Kir3.0 channels were done with GPCRs coupling to $G\alpha_{i/o}$ and $G\alpha_s$ proteins. Thus, it is possible that the number of Gβ/γ complexes activated by a receptor coupling to $G\alpha_{q/11}$ is too low to allow a significant upregulation of the Kir3.0 channels. Differences in expression level of G proteins or in their GTPase activity might account for this effect. Alternatively, the β/γ complex of $G\alpha_{q/11}$ proteins may differ from the β/γ complex associated with $G\alpha_{i/o}$

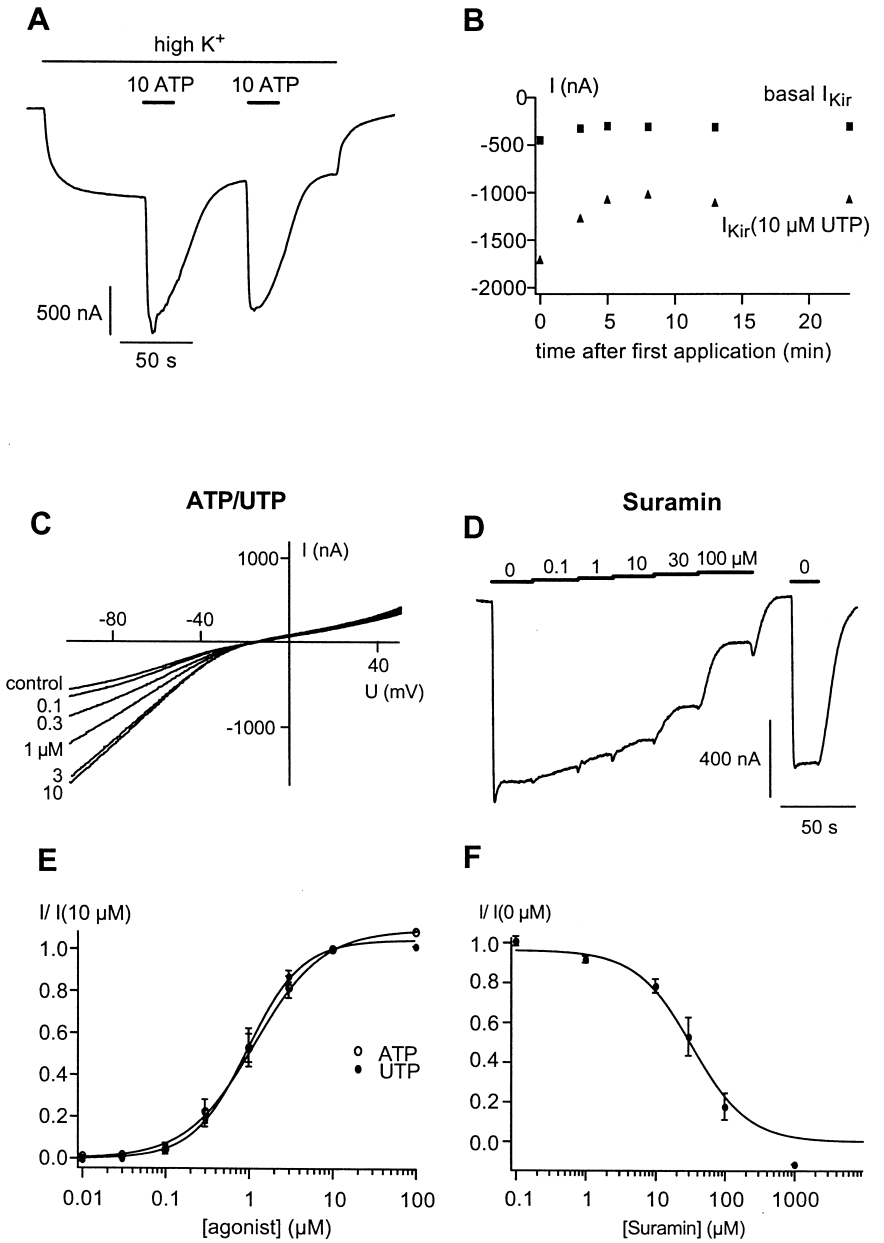

Fig. 5. Dose response relationship of $P2Y_2$ receptors for ATP, UTP and for suramin as obtained from activation and inhibition of I_{Kir}. A: Current response of oocytes coexpressing $P2Y_2$ receptors and Kir3.1/2 on repetitive application of ATP. While the peak amplitude of $I_{Cl(Ca)}$ desensitizes after the first application, upregulation of I_{Kir} remains almost unaltered upon the second application. B: Time course of current amplitudes measured at −70 mV from consecutive voltage ramps in the presence (I_{Kir}(10 µM ATP)) and absence (basal I_{Kir}) of agonist in high K^+. C: *I-V*s recorded at different concentrations of UTP; leak current was not subtracted. D: Concentration-response curves for ATP (open circles) and UTP (filled circles). Agonist-induced current amplitudes at −70 mV from different oocytes were normalized to the value measured at 10 µM agonist concentration. Error bars are S.E.M. from at least 8 independent experiments. Lines represent fits of a Hill equation to the data points. E: I_{Kir} in KFR activated by 10 µM ATP were inhibited by increasing concentrations of coapplied suramin. Bars show application of indicated concentrations of suramin in µM. F: Dose-response relation for suramin on $P2Y_2$ receptors obtained from 5 experiments as in E. Steady-state currents were normalized to their amplitude in 10 µM ATP and 0 µM suramin. Line represents fitted Hill equation.

proteins and only the latter types can activate the Kir3.0 channels. Third, the $P2Y_6$ receptor and the G protein might not form a functional complex with the Kir channels which seems to be necessary for the membrane-delimited Gβ/γ signalling [14]. There is at least one case where $G\alpha_{q/11}$ coupled receptors activate coexpressed Kir3.0 channels [25]. However, it was speculated that this coupling of an mGluR1 receptor arose from promiscuous coupling of overexpressed receptor proteins to $G\alpha_{i/o}$ or $G\alpha_s$ because the same experiment could not be repeated with lower expression levels in the same expression system [23]. A dependence of the coupling pathway on the expression level was also found for other receptors [22]. In our case, we do not assume that overexpression of the $P2Y_2$ receptor explains the differential behavior of the P2Y subtypes since the stimulated $I_{Cl(Ca)}$ is comparable and at a submaximal level for both P2Y receptors tested. Taking the

stimulated $I_{Cl(Ca)}$ as a measure of P2Y receptor expression, we did not find any correlation between expression level and I_{Kir} upregulation (Fig. 3E,F).

There are several examples of GPCRs which couple to two G proteins of different families [22,26]. D2 dopamine receptors, thrombin receptors and 5-HT 1a receptors couple both negatively to adenylyl cyclase and stimulatory to PLC [27–29]. The properties of the $P2Y_2$ receptor are similar to those GPCRs as one pathway is pertussis toxin sensitive and the other induces a rise in intracellular Ca^{2+}. In the case of the thrombin receptor, it could also be shown that the stimulation of inositol phosphates was not blocked by treatment with pertussis toxin [29] similar to our study.

It might be questioned whether the bifunctional behavior of $P2Y_2$ receptors that we have found is specific for our expression system. However, several studies have found activation of PLC and inhibition of adenylyl cyclase by native $P2Y_1$- and $P2Y_2$-like receptors in different cell types [4,10,24]. In addition, Communi et al. [16] recently cloned a P2Y receptor which stimulates adenylyl cyclase and PLC when expressed in CHO K1 and 1321N1 astrocytoma cells, respectively. This suggests that $P2Y_2$ receptors expressed in oocytes as in other systems are able to activate multiple second messenger pathways.

Acknowledgements: We like to thank Dr. D.J. Julius for providing us with the $P2Y_2$ cDNA, Drs. J.P. Ruppersberg, K. Lingenhöhl and R. Brom for kind support, Drs. G. Standke, S. Herlitze and I. Vranesic for stimulating comments on the manuscript.

References

[1] Burnstock, G. (1996) Drug Dev. Res. 39, 204–242.
[2] Barnard, E.A., Simon, J. and Webb, T.E. (1997) Mol. Neurobiol. 15, 103–129.
[3] Fredholm, B.B., Abbracchio, M.P., Burnstock, G., Dubyak, G.R., Harden, T.K., Jacobson, K.A., Schwabe, U. and Williams, M. (1997) Trends Pharmacol. Sci. 18, 79–82.
[4] Harden, T.K., Boyer, J.L. and Nicholas, R.A. (1995) Annu. Rev. Pharmacol. Toxicol. 35, 541–579.
[5] Gandia, L., Garcia, A.G. and Morad, M. (1993) J. Physiol. (Lond.) 470, 55–72.
[6] Ikeuchi, Y., Nishizaki, T., Mori, M. and Okada, Y. (1996) Eur. J. Pharmacol. 304, 191–199.
[7] Lim, W., Kim, S.J., Yan, H.D. and Kim, J. (1997) Pflüg. Arch. 435, 34–42.
[8] Motte, S., Pirotton, S. and Boeynaems, J.M. (1993) Circ. Res. 72, 504–510.
[9] Schulze-Lohoff, E., Bitzer, M., Ogilvie, A. and Sterzel, R.B. (1995) Renal Physiol. Biochem. 18, 219–230.
[10] Webb, T.E., Feolde, E., Vigne, P., Neary, J.T., Runberg, A., Frelin, C. and Barnard, E.A. (1996) Br. J. Pharmacol. 119, 1385–1392.
[11] Boyer, J.L., Lazarowski, E.R., Chen, X.H. and Harden, T.K. (1993) J. Pharmacol. Exp. Ther. 267, 1140–1146.
[12] Matsuura, H., Sakaguchi, M., Tsuruhara, Y. and Ehara, T. (1996) J. Physiol. (Lond.) 490, 659–671.
[13] Friel, D.D. and Bean, B.P. (1990) Pflüg. Arch. 415, 651–657.
[14] Clapham, D.E. and Neer, E.J. (1997) Annu. Rev. Pharmacol. Toxicol. 37, 167–203.
[15] Communi, D., Motte, S., Boeynaems, J.M. and Pirotton, S. (1996) Eur. J. Pharmacol. 317, 383–389.
[16] Communi, D., Govaerts, C., Parmentier, M. and Boeynaems, J.M. (1997) J. Biol. Chem. 272, 31969–31973.
[17] Parr, C.E., Sullivan, D.M., Paradiso, A.M., Lazarowski, E.R., Burch, L.H., Olsen, J.C., Erb, L., Weisman, G.A., Boucher, R.C. and Turner, J.T. (1994) Proc. Natl. Acad. Sci. USA 91, 3275–3279.
[18] Filippov, A.K., Webb, T.E., Barnard, E.A. and Brown, D.A. (1997) Br. J. Pharmacol. 121, 849–851.
[19] King, B.F., Wang, S. and Burnstock, G. (1996) J. Physiol. (Lond.) 494, 17–28.
[20] Lustig, K.D., Shiau, A.K., Brake, A.J. and Julius, D. (1993) Proc. Natl. Acad. Sci. USA 90, 5113–5117.
[21] Maier, R., Glatz, A., Mosbacher, J. and Bilbe, G. (1997) Biochem. Biophys. Res. Commun. 240, 298–302.
[22] Gudermann, T., Schoneberg, T. and Schultz, G. (1997) Annu. Rev. Neurosci. 20, 399–427.
[23] Sharon, D., Vorobiov, D. and Dascal, N. (1997) J. Gen. Physiol. 109, 477–490.
[24] Communi, D., Raspe, E., Pirotton, S. and Boeynaems, J.M. (1995) Circ. Res. 76, 191–198.
[25] Saugstad, J.A., Segerson, T.P. and Westbrook, G.L. (1996) J. Neurosci. 16, 5979–5985.
[26] Milligan, G. (1993) Trends Pharmacol. Sci. 14, 239–244.
[27] Vallar, L., Muca, C., Magni, M., Albert, P., Bunzow, J., Meldolesi, J. and Civelli, O. (1990) J. Biol. Chem. 265, 10320–10326.
[28] Raymond, J.R., Albers, F.J., Middleton, J.P., Lefkowitz, R.J., Caron, M.G., Obeid, L.M. and Dennis, V.W. (1991) J. Biol. Chem. 266, 372–379.
[29] Hung, D.T., Wong, Y.H., Vu, T.K. and Coughlin, S.R. (1992) J. Biol. Chem. 267, 20831–20834.