

Research Overview

P2Y₂ Receptors Regulate Multiple Signal Transduction Pathways in Monocytic Cells

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Strategy, Management and Health Policy				
Venture Capital Enabling Technology	Preclinical Research	Preclinical Development Toxicology, Formulation Drug Delivery, Pharmacokinetics	Clinical Development Phases I–III Regulatory, Quality, Manufacturing	Postmarketing Phase IV

ABSTRACT Activation of P2Y₂ receptors by extracellular nucleotides induces cellular responses required for differentiation of human promonocytic U937 cells. Multiple signal transduction pathways are independently coupled to P2Y₂ receptors in U937 cells, including stimulation of phospholipase C (PLC)-dependent calcium mobilization and phosphorylation of mitogen-activated protein (MAP) kinases. In U937 cells, P2Y₂ receptors couple to the MAP kinases MEK1/2 and ERK1/2 via phosphatidylinositol 3-kinase (PI3-K) and c-Src. ERK1/2 phosphorylation induced by UTP was inhibited by the PI3-K inhibitors wortmannin and LY294002, the c-Src inhibitors radicicol and PP2, and the inhibitor of actin polymerization, cytochalasin D, but not by inhibitors of calcium-dependent protein kinase C isoforms. The phosphorylation of ERK1/2 induced by UTP was independent of calcium mobilization, since chelation of intracellular calcium with BAPTA had no effect. The importance of multiprotein complexes in mediating G-protein-coupled receptor signaling has recently been recognized. Studies with an epitope-tagged P2Y₂ receptor expressed in human 1321N1 astrocytoma cells indicated that activation of the P2Y₂ receptor with UTP causes it to colocalize with the EGF receptor. The P2Y₂ receptor also contains the consensus integrin-binding motif arginine-glycine-aspartic acid (RGD) in its first extracellular loop that was hypothesized to promote interactions with integrins. Results of immunofluorescence and peptide binding experiments suggest that wild-type, RGD-containing receptors, but not RGE mutant receptors, can form complexes with $\alpha_v\beta_3/\beta_5$ integrins and the integrin-associated thrombospondin receptor (CD47). Compared to wild-type P2Y₂ receptors, RGE mutant receptors required 1,000-fold higher concentrations of ATP or UTP to activate PLC-dependent calcium mobilization and the phosphorylation of focal adhesion kinase (FAK) and ERK1/2. Competition studies indicated that an RGDS peptide inhibited the P2Y₂ receptor-mediated phosphorylation of FAK and ERK1/2. These findings suggest that the RGD domain of the P2Y₂ receptor is required to promote efficient coupling to intracellular signaling pathways. Thus, P2Y₂ receptors regulate divergent signal transduction pathways that are dependent on the formation of multiprotein complexes including EGF receptors and integrins. Our studies encourage further attempts to develop strategies to independently regulate these pathways at steps proximal to P2Y₂ receptors. *Drug Dev. Res.* 53:186–192, 2001. © 2001 Wiley-Liss, Inc.

Key words: purinergic receptors; monocytes; MAP kinases; P2Y₂ receptors; immune system; nucleotides

INTRODUCTION

Human promonocytic U937 cells have been used to study monocyte to macrophage differentiation caused by physiological and pharmacological agents, including, phorbol esters, vitamin D, and cytokines [Harris et al., 1985]. The ability of extracellular nucleotides including ATP and UTP to activate G-protein-coupled P2Y nucle-

otide receptors in U937 cells has been recognized [Cowen et al., 1991]. In this cell line, P2Y receptor activation by extracellular adenosine 5'-triphosphate (ATP) and uridine

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5'-triphosphate (UTP) can inhibit cell proliferation, enhance the action of cytokines, activate phospholipase D, and increase the expression of type 1 and 3 complement receptors [Cowen et al., 1989, 1991; Kusner et al., 1993]. Since ATP and UTP can activate phospholipase C (PLC) in U937 cells, leading to an inositol 1,4,5-trisphosphate (IP₃)-dependent increase in the intracellular calcium concentration [Cowen et al., 1989, 1991], many of the cellular responses to nucleotides could be regulated through calcium mobilization. However, recent studies indicate that activation by ATP or UTP of a G-protein-coupled P2Y₂ nucleotide receptor subtype expressed in U937 cells also causes the stimulation of intracellular mitogen-activated protein (MAP) kinase cascades, as well as PLC [Weisman et al., 1998a; Santiago-Pérez et al., 2001]. This review describes our studies on P2Y₂ receptor-mediated signal transduction in U937 cells and related studies with a recombinant P2Y₂ nucleotide receptor expressed in a heterologous cell system, human 1321N1 astrocytoma cells, that normally lacks G-protein-coupled P2Y nucleotide receptors.

P2Y₂ RECEPTOR SIGNAL TRANSDUCTION PATHWAYS IN U937 CELLS

Addition of ATP or UTP to U937 monocytic cells causes an increase in the phosphorylation of the MAP kinases MEK1/2 that reaches a maximum within 1 min of nucleotide exposure [Weisman et al., 1998a; Santiago-Pérez et al., 2001]. Subsequently, the MAP kinases ERK1/2 are phosphorylated (maximal response within 2 min of nucleotide exposure), an effect that is inhibited by the MEK1/2 inhibitor PD 098059 [Weisman et al., 1998a; Santiago-Pérez et al., 2001]. These results suggest that a P2Y₂ receptor in U937 cells causes the sequential activation of MEK and ERK MAP kinases that has been shown to regulate monocytic cell differentiation by phorbol esters and ceramide [Ragg et al., 1998], most likely involving the phosphorylation of a TATA-binding protein [Biggs et al., 1998]. The ability of ATP and UTP to activate both intracellular calcium mobilization [Cowen et al., 1991; Santiago-Pérez et al., 2001] and MAP kinases [Santiago-Pérez et al., 2001] with similar EC₅₀s suggests that a P2Y₂ nucleotide receptor subtype mediates these responses in U937 cells. Among the cloned P2Y nucleotide receptors, only the P2Y₂ receptor subtype is activated by ATP and UTP with equipotency and equiefficacy [Lustig et al., 1993; Parr et al., 1994; Weisman et al., 1998b]. U937 cells express mRNA for both P2Y₂ and P2Y₆ receptors [Jin et al., 1998; Santiago-Pérez et al., 2001]. However, calcium mobilization and MAP kinase activation were not detected upon incubation of U937 cells with the P2Y₆ receptor agonist uridine 5'-diphosphate (UDP) [Santiago-Pérez et al., 2001].

The activation of ERK1/2 by UTP in U937 cells

was not inhibited by preloading the cells with BAPTA, a calcium chelator. Thus, it appears that the increase in the intracellular calcium concentration caused by P2Y₂ receptor activation in U937 cells is not the cause of the phosphorylation and activation of MAP kinases. In other cell types, ERK1/2 phosphorylation by the P2Y₂ receptor has been found to occur by the calcium-independent activation of protein kinase C δ and phospholipase D [Neary et al., 1999] and/or by the calcium-dependent transactivation of the EGF receptor [Soltoff, 1998]. Our results in U937 cells indicated that an inhibitor of calcium-dependent protein kinase C (PKC), GF 109203X, did not decrease UTP-induced ERK1/2 activation [Weisman et al., 1998a; Santiago-Pérez et al., 2001]. However, Ro31-8220, which affects both calcium-dependent and calcium-independent PKC isoforms, significantly inhibited ERK1/2 phosphorylation in U937 cells (unpublished results), suggesting that a calcium-independent isoform of PKC, such as PKC δ , PKC ζ , or PKC λ , participates in transduction of the signal from the P2Y₂ receptor to the MAP kinase cascade. Since the formation of IP₃ from phosphatidylinositol 4,5-bisphosphate (PIP₂) would also generate the PKC activator, diacylglycerol (DAG), a role for a PKC isoform in P2Y₂ receptor function is plausible. Inhibitors of c-Src, including radicicol and PP2, also decreased ERK1/2 phosphorylation by UTP in U937 cells [Santiago-Pérez et al., 2001].

Furthermore, inhibitors of phosphatidylinositol 3-kinase (PI3-K), LY 294002 and wortmannin, also prevented UTP-induced ERK1/2 phosphorylation, suggesting that both c-Src and PI3-K lie upstream of MEK1/2 and ERK1/2 in the P2Y₂ receptor signal transduction pathway. Interestingly, pertussis toxin, an inhibitor of the G_i/G_o family of G-proteins, decreased ERK1/2 phosphorylation, but not calcium mobilization, coupled to P2Y₂ receptor activation in U937 cells. Earlier studies have suggested that IP₃-dependent calcium mobilization caused by P2Y₂ receptor activation is mediated by a pertussis-toxin-insensitive Gq protein [Boarder et al., 1995; Weisman et al., 1998b]. Therefore, these results with U937 cells suggest that G α and Gq α proteins are involved in P2Y₂ receptor-mediated MAP kinase activation and IP₃-dependent calcium mobilization, respectively. The results of these studies on P2Y₂ receptor signal transduction pathways present in U937 cells are summarized in Figure 1.

SIGNAL TRANSDUCTION PATHWAYS COUPLED TO A RECOMBINANT P2Y₂ RECEPTOR IN 1321N1 CELLS

Maudsley et al. [2000] have described a model for the activation of MAP kinases by the G-protein-coupled β 2-adrenergic receptor (β 2-AR) that depends upon the formation of a multiprotein complex involving EGF receptors (EGFR) and β -arrestins. In this model, activated

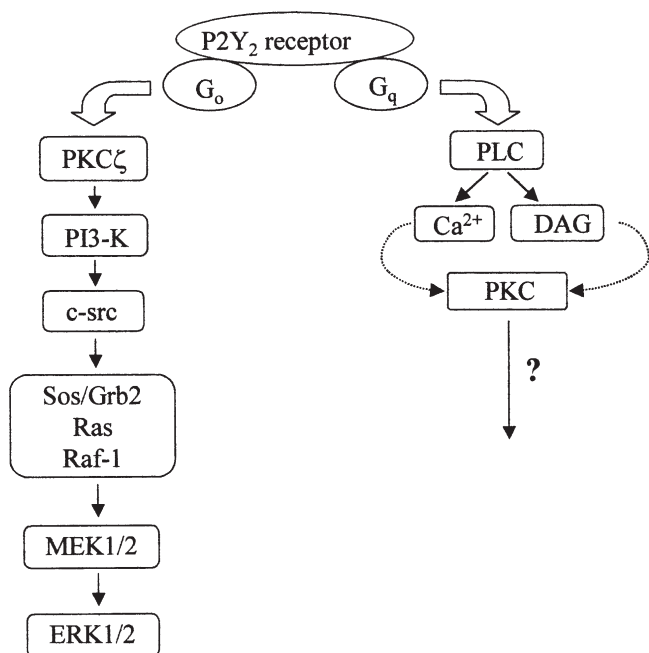


Fig. 1. Signal transduction pathways of the G-protein-coupled P2Y₂ receptor in human U937 monocytic cells.

β₂-AR, after its phosphorylation by G-protein-coupled receptor kinase 2, associates with β-arrestins, which then bind c-Src. Activated β₂-AR also induces phosphorylation of EGFR, which then binds β₂-AR. Upon sequestration of the β₂-AR/EGFR/β-arrestin/c-Src complex in clathrin-coated pits, the receptor can couple to the MAP kinase cascade in a Shc-dependent manner.

To determine whether the P2Y₂ receptor activates MAP kinases through a similar EGFR-dependent pathway, we utilized the recombinant P2Y₂ receptor expressed in human 1321N1 astrocytoma cells, a cell line that lacks functional P2Y receptors [Parr et al., 1994; Erb et al., 1995]. Results indicate that activation of the P2Y₂ receptor expressed in 1321N1 cells induces phosphorylation of both EGFR and ERK1/2, responses that are inhibited by the c-Src inhibitor PP2 (Griffin et al., in prep.). In contrast, phosphorylation of ERK1/2, but not EGFR, was inhibited by the PI3-K inhibitor LY 294002. Similarly, a variety of inhibitors of calcium-dependent and calcium-independent PKC isoforms decreased P2Y₂ receptor-mediated phosphorylation of ERK1/2, but not EGFR. These results suggest that P2Y₂ receptors in 1321N1 cells can utilize at least two pathways for the phosphorylation of the MAP kinases, ERK1/2 (Fig. 2). First, the P2Y₂ receptor can activate ERK1/2 via the transactivation of EGFR in a c-Src-dependent manner, similar to the pathway described for transactivation of EGFR and phosphorylation of MAP kinases by a P2Y₂ receptor expressed in pheochromocytoma (PC-12) cells [Soltoff et al., 1998; Soltoff, 1998]. Second, the P2Y₂ receptor can activate

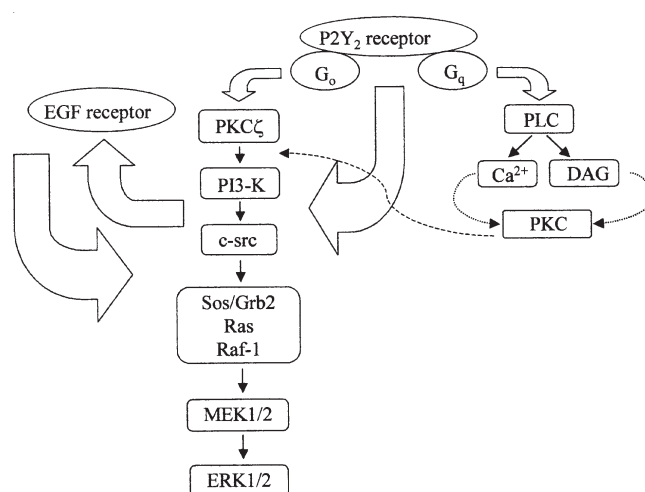


Fig. 2. Signal transduction pathways of the G-protein-coupled P2Y₂ receptor in human 1321N1 astrocytoma cells.

ERK1/2 via c-Src through a pathway that is dependent upon PKC and PI3-K (Fig. 2). Since calcium-dependent PKC isoforms participate in this pathway, a role for IP₃-dependent calcium mobilization in this mechanism is implied. Accordingly, preloading of 1321N1 cells with the calcium chelator BAPTA partially inhibited UTP-dependent ERK1/2 phosphorylation, consistent with the participation of both calcium-dependent and calcium-independent pathways in the activation of MAP kinases by P2Y₂ receptors (Erb et al., 2001).

Although β-arrestin binding and receptor sequestration regulates MAP kinase activity coupled to the β₂-AR [Maudsley et al., 2000], a similar pathway does not appear to exist for P2Y₂ receptors. Previous studies have shown that deletion of the C-terminal domain of the P2Y₂ receptor containing potential phosphorylation sites for G-protein-coupled receptor kinase (GRK) (Fig. 3) produces a receptor that is resistant to agonist-induced desensitization and receptor internalization when expressed in 1321N1 cells [Garrad et al., 1998]. As compared to the wild-type receptor, this C-terminal truncation mutant of the P2Y₂ receptor exhibits similar concentration- and time-dependent changes in UTP-induced ERK1/2 activation in 1321N1 cell transfectants. These data suggest that ERK1/2 activation occurs independent of P2Y₂ receptor internalization and the binding of β-arrestin to C-terminal GRK phosphorylation sites. Sequence analysis of the C-terminal domain of the P2Y₂ receptor indicates the presence of two Src-homology (SH3) binding domains just upstream of the GRK phosphorylation sites (Fig. 3). The SH3 binding domains contain 5 prolines in a 10 amino acid stretch and include two consensus phosphorylation sites for MAP kinases, suggesting that the P2Y₂ receptor may be subject to feedback inhibition by MAP kinases. SH3 binding domains are found in the C-termini or the third intra-

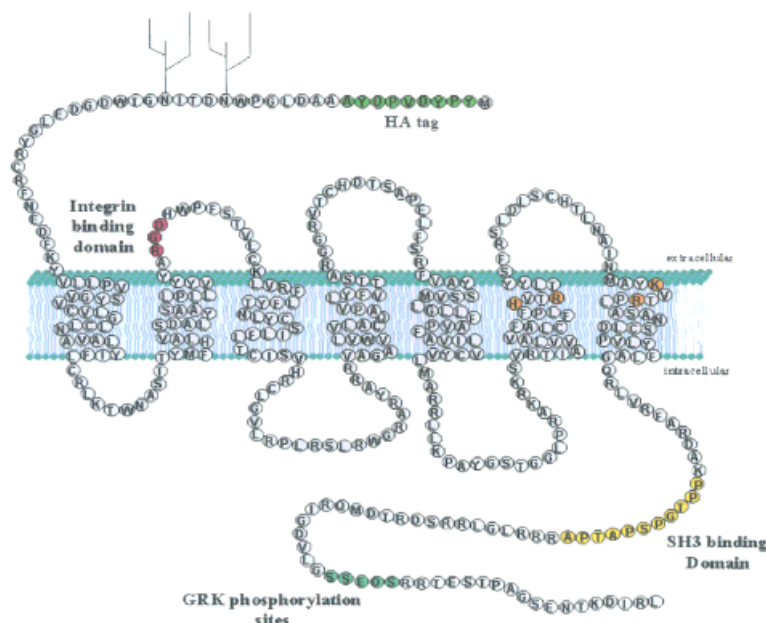


Fig. 3. The putative secondary structure of the human P2Y₂ receptor. Domains of functional significance are highlighted (e.g., residues in the

sixth and seventh transmembrane domains that are postulated to participate in ligand binding [Erb et al., 1995].

cellular loop of ~70% of the human mitogenic G-protein-coupled receptors (GPCRs) that have been cloned to date (GenBank). Although the physiological significance of the SH3 binding domains in GPCRs has not been delineated, it is hypothesized that the SH3 binding domain within the P2Y₂ receptor may enable direct interactions with c-Src to facilitate tyrosine phosphorylation of proteins known to be involved in activation of the downstream MAP kinase signaling cascade (Fig. 4). Other results indicate that P2Y₂ receptor activation by UTP promotes colocalization of EGFR and P2Y₂ receptors (Griffin et al., in prep.), as demonstrated in immunofluorescence experiments using antibodies to EGFR and a hemagglutinin (HA) epitope tag incorporated at the extracellular N-terminus of the P2Y₂ receptor (Fig. 4), as previously described [Erb et al., 1995; Garrad et al., 1998]. It remains to be determined whether the SH3 binding domain is required for colocalization of EGFR and P2Y₂ receptors and whether this domain also regulates the UTP-induced activation of MAP kinases via PKC and PI3-K (Fig. 2).

COMPLEX FORMATION BETWEEN P2Y₂ RECEPTORS AND INTEGRINS

Other studies indicate that the P2Y₂ receptor may be relatively unique among G-protein-coupled receptors in that its activity is regulated through cell surface interactions with integrins, adhesion molecules that regulate a variety of intracellular signaling pathways [Clark and Brugge, 1995]. The first extracellular loop of the P2Y₂

receptor contains an arginine-glycine-aspartic acid (RGD) sequence (Fig. 3), a known integrin-binding motif [Pierschbacher and Ruoslahti, 1984]. In the G-protein-coupled receptor superfamily, this RGD sequence is present only in the first extracellular loop of several species homologs of the P2Y₂ and P2Y₆ nucleotide receptors and the third extracellular loop of the H₂ histamine receptor (GPCR database: www.gpcr.org). The relevance of the RGD domain to P2Y₂ receptor signal transduction was evaluated in a series of experiments with 1321N1 cell transfectants expressing wild-type or mutant P2Y₂ receptors in which the RGD domain was replaced with

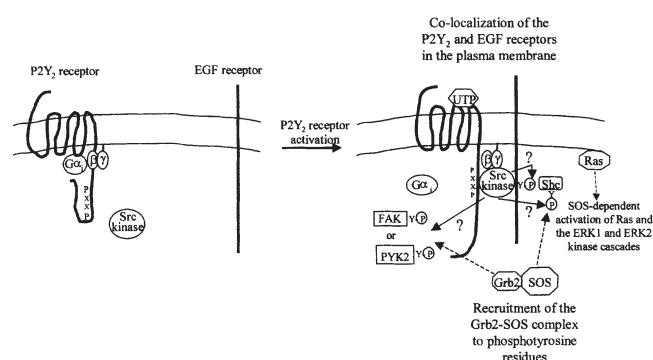


Fig. 4. A possible role of the P2Y₂ receptor SH3 binding site in mitogenesis. We hypothesize that a proline-rich, Src-homology (SH3)-binding motif in the intracellular C-terminal domain of the P2Y₂ receptor may bind directly to c-Src. c-Src could then phosphorylate tyrosine residues in proteins known to be involved in P2Y₂ receptor-mediated activation of ERK1/2, including FAK, Pyk2, Shc, and the EGF receptor.

RGE, a motif that has a low affinity for integrins. Results indicate that the EC_{50} for calcium mobilization is $\sim 1,000$ -fold greater for RGE-P2Y₂ than wild-type P2Y₂ receptors. This result was not due to differences in cell surface receptor expression levels between wild-type and RGE-mutant P2Y₂ receptors, as verified by fluorescence-activated cell sorting (FACS) using antibodies against the HA epitope incorporated at the N-terminus of the receptor [Erb et al., 2001]. Furthermore, the receptor domain containing the RGD sequence is not believed to be directly involved in agonist binding, as determined in ligand-binding site modeling studies with P2Y₁ and P2Y₂ receptors [Erb et al., 1995; Van Rhee et al., 1998]. Thus, it is hypothesized that integrin interactions with the P2Y₂ receptor allow the receptor to maintain a high affinity agonist binding state and/or promotes localization to a protein signaling complex for optimum signal transduction.

Attempts were made to identify the type of integrin(s) that binds the P2Y₂ receptor. These studies employed a human erythroleukemia cell line, K562 cells, in which we detected expression of α_5 , α_v , β_1 , β_3 , and β_5 integrin subunits [Erb et al., 2001]. We assessed the potential for interactions between the P2Y₂ receptor and specific integrins in studies with an 18 amino acid peptide encompassing the RGD domain of the P2Y₂ receptor. Parallel studies were performed with a similar peptide in which the RGD domain was replaced with RGE. The peptides were coupled to aldehyde-modified fluorescent beads and incubated with K562 cells. The results indicated that 8-fold higher levels of RGD peptide-coated beads bound to K562 cells as compared to RGE peptide-coated beads. Beads coupled with the established integrin receptor ligands, fibronectin and vitronectin, exhibited 6-fold higher levels of binding as compared to control human serum albumin (HSA)-coated beads [Erb et al., 2001]. Preincubation of cells with soluble RGD peptide, but not RGE peptide or the fibronectin receptor ligand, GRGDSP, inhibited K562 cell binding of RGE peptide- and vitronectin-coated beads [Erb et al., 2001], suggesting that the RGD-containing peptide was interacting with an integrin that binds vitronectin. Using integrin subunit-specific antibodies, RGD-coated bead binding to K562 cells was inhibited by monoclonal antibodies to the $\alpha_v\beta_3$ integrin (vitronectin receptor), but not by antibodies to the $\alpha_5\beta_1$ integrin (fibronectin receptor) [Erb et al., 2001]. In addition, antibodies against the thrombospondin receptor (CD47), a protein that associates with the $\alpha_v\beta_3$ integrin [Lindberg et al., 1996], inhibited RGD peptide-coated bead binding to K562 cells.

It was determined with 1321N1 cell transfectants that wild-type P2Y₂ receptors colocalized with integrins containing the α_v subunit in immunofluorescence experiments using antibodies against α_v and the N-terminal HA epitope incorporated in the P2Y₂ receptor [Erb et al.,

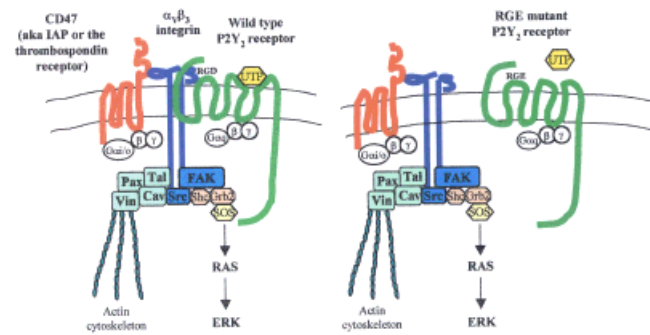


Fig. 5. RGD-dependent P2Y₂ receptor and $\alpha_v\beta_3$ interactions regulate FAK and ERK1/2 phosphorylation induced by nucleotides.

2001]. Expression of RGE mutant P2Y₂ receptors in 1321N1 cells reduced colocalization with $\alpha_v\beta_3$ to only $\sim 10\%$ the level seen with wild-type, RGD-containing P2Y₂ receptors. In contrast to colocalization of P2Y₂ receptors with EGFR (Griffin et al., in prep.), colocalization with α_v integrins does not require P2Y₂ receptor activation [Erb et al., 2001]. These results suggest that integrin/P2Y₂ receptor interactions are required for inducing the active state of the receptor, whereas interactions between EGFR and P2Y₂ receptors function to transduce a signal from the activated P2Y₂ receptor to intracellular protein cascades.

Previous studies have shown that P2Y₂ receptor activation induces tyrosine phosphorylation of focal adhesion kinase (FAK) [Soltoff et al., 1998], a protein known to be associated with integrins, focal adhesions, and cytoskeletal proteins [Clark and Brugge, 1995] (Fig. 5). Our studies indicate that replacement of the RGD motif with RGE inhibits P2Y₂ receptor-mediated activation of focal adhesion kinase (FAK) [Erb et al., 2001]. Similar to the UTP concentration response curves for calcium mobilization, the EC_{50} s for FAK phosphorylation and ERK1/2 activation are $\sim 1,000$ -fold greater for the RGE mutant than the wild-type receptor. FAK phosphorylation is known to be calcium dependent and, accordingly, preloading of 1321N1 cell transfectants with BAPTA inhibited UTP-induced phosphorylation of FAK by both RGE mutant and wild-type receptors [Erb et al., 2001]. These data also indicate the calcium-dependence of ERK1/2 activation in 1321N1 cells, in contrast to the calcium-independence of ERK1/2 activation by UTP in U937 monocytes. Preloading of 1321N1 cell transfectants with BAPTA partially inhibited ERK1/2 phosphorylation by wild-type P2Y₂ receptors and indicates that a calcium-independent pathway for MAP kinase activation likely coexists in these cells. This effect of BAPTA on ERK1/2 phosphorylation induced by UTP was less pronounced with RGE mutant than wild-type P2Y₂ receptors, suggesting that integrin interactions affect primarily the calcium-dependent pathway for MAP kinase activation via

P2Y₂ receptors in 1321N1 cells. Consistent with the existence of multiple pathways for P2Y₂ receptor-mediated MAP kinase activation in 1321N1 cells is the finding that both calcium-dependent and calcium-independent isoforms of PKC apparently couple the P2Y₂ receptor to c-Src and the MAP kinase cascade (Fig. 2; Griffin et al., in prep.).

Other results indicate that a soluble RGDS peptide inhibits the P2Y₂ receptor-mediated activation of FAK and ERK1/2 [Erb et al., 2001], suggesting that reagents that interfere with P2Y₂ receptor/ $\alpha_v\beta_3$ interactions would be effective modulators of P2Y₂ receptor functions in vivo. The RGDS peptide has been shown to inhibit the signaling activity of other G-protein-coupled receptors [Della Rocca et al., 1999], suggesting that these receptors rely on integrin-based focal adhesion complexes for proper function. We also examined the ability of pertussis toxin to modulate UTP-induced calcium mobilization by wild-type and RGE mutant P2Y₂ receptors expressed in 1321N1 cells. In contrast to results with U937 cells in which calcium mobilization induced by UTP was pertussis toxin-insensitive, wild-type P2Y₂ receptors in 1321N1 cells exhibited a partial sensitivity to pertussis toxin, indicating a contribution of G_o proteins to the classical G_q-mediated pathway for stimulation of phospholipase C by P2Y₂ receptors [Boarder et al., 1995]. Interestingly, the RGE mutant receptors were insensitive to pertussis toxin, suggesting that failure to bind integrins inhibits the ability of the P2Y₂ receptor to activate G_o proteins. The thrombospondin receptor (CD47) has been shown to form a complex with integrins and proteins in the G_{i/o} family [Frazier et al., 1999]. Thus, the finding that the G_{i/o} inhibitor pertussis toxin reduced Ca²⁺ signaling by the wild-type P2Y₂ receptor, but not the RGE mutant receptor, suggests that the P2Y₂ receptor requires integrin/CD47 interactions to access G_o (Fig. 5). There are potentially many other components of integrin complexes that could contribute to P2Y₂ receptor functions. A potential contribution to P2Y₂ receptor signaling of actin cytoskeleton, a component of the integrin complex (Fig. 5), is suggested by the ability of the cytoskeletal disrupter cytochalasin D to inhibit UTP-induced ERK1/2 activation in U937 cells (Fig. 6).

CONCLUSIONS

This report describes a series of studies indicating that P2Y₂ receptors can couple to multiple signal transduction pathways in human U937 monocytes and 1321N1 astrocytoma cells. Studies suggest that by virtue of several different amino acid motifs within the receptor protein, the P2Y₂ receptor can interact with integrins, EGF receptors, several G-proteins, G-protein-coupled receptor kinases, c-Src, and perhaps even MAP kinases. The complexity of interactions for P2Y₂ receptors suggests that

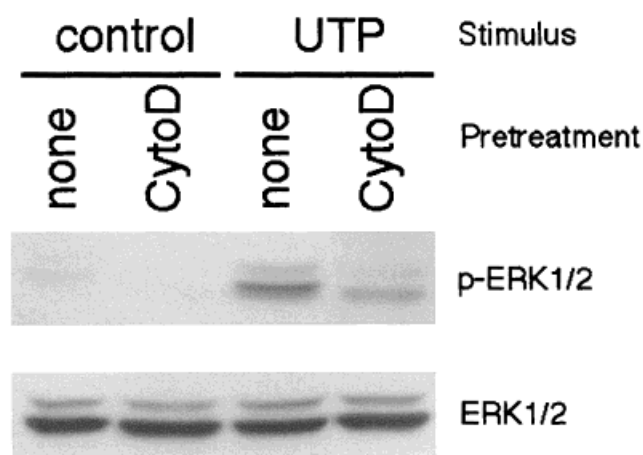


Fig. 6. Effect of the actin depolymerizing agent, cytochalasin D, on P2Y₂ receptor-mediated ERK1/2 phosphorylation. The phosphorylation of ERK1/2 was detected by Western analysis of cell extracts from U937 cells treated in the presence or absence of 10 μ M cytochalasin D for 1 h followed by incubation in the presence or absence of 100 μ M UTP for 2 min. The phosphorylated forms of ERK1/2 were detected using antiphospho p42/p44 MAPK IgG. Total ERK1/2 protein in cell extracts (lower panel) was detected by reprobing the blot with anti-p42/p44(ERK1/2) MAPK IgG.

it may be plausible to devise methods for modulating receptor functions selectively by masking specific receptor domains. Considering that the P2Y₂ receptor has been shown to be upregulated in response to tissue injury [Turner et al., 1997, 1998] and as an immediate early gene response in activated thymocytes [Koshiba et al., 1997], and downregulated during differentiation of human myeloid leukocytes [Martin et al., 1997], P2Y₂ receptor-mediated signal transduction may have particular relevance to tissue development and wound healing. Clearly, more work is needed to determine the full nature of the P2Y₂ receptor complexes and signaling events involving this unique type of G-protein-coupled receptor.

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