The UDP-sugar-sensing P2Y14 receptor promotes Rho-mediated signaling and chemotaxis in human neutrophils

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Submitted 19 April 2012; accepted in final form 1 June 2012

Sesma JI, Kreda SM, Steinckwich-Besancon N, Dang H, García-Mata R, Harden TK, Lazarowski ER. The UDP-sugar-sensing P2Y14 receptor promotes Rho-mediated signaling and chemotaxis in human neutrophils. Am J Physiol Cell Physiol 303: C490–C498, 2012. First published June 6, 2012; doi:10.1152/ajpcell.00138.2012.—The Gi-coupled nucleotide-sugar. HL60 human promyelocytic leukemia cells do not glucosylate glucose for up to 1 h resulted in negligible metabolism of the rapidly hydrolyzed extracellular ATP, but incubation with UDP-glucose resulted in cytoskeleton rearrangement, change of cell shape, and enhanced cell migration. We also demonstrate that UDP-glucose promotes rapid, robust, and concentration-dependent activation of RhoA in these cells. Ecto-nucleotidases expressed on neutrophils hydrolyzed UDP-glucose in ERK phosphorylation in these cells, but this response was had no effect (43). UDP-glucose also evoked a modest increase of forskolin-stimulated cyclic AMP formation in neutrophils, activation in PMNs remain undefined. Scrivens and Dickinson (43) reported that UDP-glucose promoted a modest inhibition of forskolin-stimulated cyclic AMP formation in neutrophils, but other UDP-sugars known to promote P2Y14-R activation had no effect (43). UDP-glucose also evoked a modest increase in ERK phosphorylation in these cells, but this response was observed only at high micromolar/submillimolar concentrations and was not mimicked by other UDP-sugars (43). UDP-glucose failed to induce elastase secretion from human PMNs (43). Thus, unambiguous association of the expression of P2Y14-R transcripts with P2Y14-R-mediated responses in PMNs remains to be established.

In the present study, we used isolated human neutrophils and neutrophil-like HL60 human promyelocytic leukemia cells to illustrate that UDP-glucose evokes activation of Rho GTPases in a P2Y14-R expression-dependent manner. Rho activation was accompanied by cytoskeleton remodeling and enhanced cell motility.1

MATERIALS AND METHODS

Human neutrophils and cell cultures. Peripheral neutrophils were isolated from fresh venous blood samples from healthy volunteers with written informed consent and the approval of the University of North Carolina Institutional Review Board (IRB). Neutrophils were isolated (>98% purity) using Ficoll-paque Plus (GE Healthcare) and 3% dextran as described previously (29, 52). HL60 cells were grown in RPMI-1640 medium supplemented with 10% fetal bovine serum at a cell density of 3 × 105 cells/ml, as previously described (16). Neutrophil differentiation of HL60 (dHL60) cells was achieved by

1 This article is the topic of an Editorial Focus by Scott M. O’Grady (40a).
inclusion of 1.3% DMSO in the culture medium for 5 days (16). Neutrophils and HL60 cells were rinsed and, except when indicated otherwise, were incubated (1–10 × 10⁶ cells/ml) in HEPES-buffered (pH 7.4) Hanks’ balanced salt solution supplemented with 1.6 mM CaCl₂ and 0.8 mM MgCl₂ (HBSS). Apyrase (1 U/ml) was added to PMN suspensions during the isolation steps. Inclusion of apyrase ensures the removal of ATP and UTP potentially released from damaged or mechanically activated neutrophils, e.g., during cell washes and centrifugations (28), and thus, avoids unwanted activation added to PMN suspensions during the isolation steps. Inclusion of 1/1000 to 1/100,000 diluted antiserum to P2Y2 or P2X1 receptors expressed in these cells (32, 51, 52). HPLC washes and centrifugations (28), and thus, avoids unwanted activation added to PMN suspensions during the isolation steps. Inclusion of 100 μM ATP and 100 μM UTP were completely hydrolyzed by 1 U/ml apyrase in <5 min, while 100 μM UDP-glucose remained essentially unchanged after 90 min (not shown).

**Extracellular metabolism of UDP-glucose and ATP.** Neutrophils (2 × 10⁶/ml) were incubated in HBSS (supplemented as above) in the presence of 100 μM UDP-glucose or 100 μM ATP, or in the presence of 0.1 μCi UDP-[³H]glucose (26 Ci/mmol) or 0.1 μCi [³H]ATP (37 Ci/mmol). At the times indicated, cells were centrifuged and nucleotides present in the supernatant were separated by HPLC (Waters) via a Nova-Pack C18 column. Absorbance at λ = 260 nm and radioactivity were quantified on-line as previously described (27, 31).

**Stable expression of P2Y₁₄-R in HL60 cells.** Retroviruses encoding the human P2Y₁₄-R were produced using the pLXSN expression vector, as previously described (16). HL60 cells were infected with P2Y₁₄-R-bearing retroviruses and selected in medium containing 0.5 mg/ml G418.

**RhoA pull-down assays.** GTP-bound RhoA was measured using the Rho Activation Assay Biochem Kit, following the manufacturer’s instructions. Briefly, neutrophils or 12-h serum-starved HL60 cells were rinsed and suspended in HBSS (10⁷ cells/ml), preincubated for 45 min at 37°C, and incubated for 1 min (or the indicated times) with vehicle or agonists. Incubations were terminated by addition of lysis buffer (supplied in the kit) followed by rapid centrifugation, and aliquots of supernatants were incubated with Rhotekin-RBD beads for 1 h at 4°C. RhoGTP from pull-down assays and total RhoA were quantified on-line as previously described (27, 31).

**RESULTS**

**Stability of UDP-glucose on human neutrophils.** The capacity of human neutrophils to rapidly hydrolyze extracellular ATP and other nucleotides is well established (11, 25, 42), but the extent to which these cells metabolize UDP-glucose is not known. Thus, we quantified the stability of this nucleotide-sugar in neutrophil suspensions using HPLC analysis. Incubation of neutrophils with 100 μM UDP-glucose for 60 min resulted in negligible metabolism of the nucleotide-sugar (Fig. 1A), i.e., <1% of the initial UDP-glucose was recovered as UMP. In contrast, neutrophils rapidly hydrolyzed 100 μM ATP, displaying an apparent hydrolysis rate of Vₜₜₜ = 7.3 nmol/min × 10⁶ cells (Fig. 1A). ADP and AMP were the major products of ATP metabolism (not shown). Stability of nucleotides in the presence of PMNs also was monitored using UDP-[³H]glucose or [³H]ATP with high specific activity radiotracers. As shown in Fig. 1B, [³H]ATP decayed with an apparent half-life of 2.5 min, while UDP-[³H]glucose remained essentially unchanged after 60 min. These results are in agreement with previous reports indicating that NTPDase1, which hydrolyzes ATP and other NTPs and NDPs, but not UDP-sugars, is expressed on the surface of human neutrophils (11, 25, 42). The data also indicate that little or no ecto-nucleotide pyrophosphatase activity (which hydrolyzes UDP-sugars) is present on these cells.

**UDP-glucose promotes actin cytoskeleton reorganization, cell migration, and Rho activation in human neutrophils.** We tested the hypothesis that UDP-glucose acts as an extracellular signaling molecule for neutrophils by first assessing its effect on cell shape and actin cytoskeleton reorganization, visualized via differential interference contrast (DIC) and fluorescence microscopy. In the absence of external stimulus, most neutrophils displayed round shape and phalloidin uniformly stained the cortical actin fibers (Fig. 2, A and B, left). Incubation of neutrophils for 15 min with 100 μM UDP-glucose resulted in changes in cell shape (i.e., from round to elongated shape) that were accompanied by a sharp increase in the intensity of the cortical actin staining and the formation of a broad ruffle-like structure in one cellular pole (Fig. 2, A and B, right). These morphological changes were observed in 56.0 ± 4% of the cells incubated with UDP-glucose, as opposed to 15.2 ± 3% in...
untreated cells (means ± SE). The data suggest that UDP-glucose promotes changes in the organization of actin fibers in neutrophils.

Observation of UDP-glucose-dependent cell shape change and cytoskeleton rearrangement suggests that this nucleotide-sugar acts as an extracellular signaling molecule to regulate cellular functions associated with neutrophil motility. Studies using a modified Boyden chamber illustrated that UDP-glucose promotes PMN migration (Fig. 3). That is, enhanced cell migration was observed when UDP-glucose was added to the lower compartment of the chemotaxis chamber, but not when the concentration gradient of agonist was abolished by addition of UDP-glucose to both compartments (Fig. 3A). Thus, UDP-glucose enhances neutrophil chemotaxis but has no effect on random migration as illustrated by its lack of effect in the absence of a concentration gradient. In agreement with our HPLC analysis indicating that UDP-glucose stock solutions contained no detectable ATP or UTP (not shown), chemotaxis was not affected by inclusion of apyrase in the lower compartment of the chamber (Fig. 3B). However, UDP-glucose-evoked

Fig. 1. Stability of UDP-glucose (UDP-Glc) in human neutrophils. Neutrophils were incubated for the indicated times with 100 μM UDP-glucose or ATP (A) or trace amounts (0.1 μCi) of UDP-[3H]glucose or [3H]ATP (B). Nucleotides were separated and quantified by HPLC. The data are representative of two independent experiments performed in duplicate.
chemotaxis was partially reduced when apyrase was added to both lower and upper compartments, suggesting that ATP/UTP released from neutrophils contributed, at least in part, to this response.

Chemotaxis was observed in response to the addition of 1/10262 M UDP-glucose and was maximal with 1/10262 M UDP-glucose (Fig. 3C). These nominal concentration values overestimate the actual concentration of agonist reaching the cells, since only ~20% of the UDP-glucose added to the lower compartment was recovered in the upper compartment after 60 min (Fig. 3C, inset). Based on the concentration of agonist estimated to reach the upper compartment at the time of measurement (see inset). Inset: time course for the diffusion of 100 μM UDP-[3H]glucose through the filter membrane (means ± SD, n = 3; representative of two experiments). D: RhoA activation was measured in neutrophils that were incubated for 1 min in the presence of the indicated concentrations of UDP-glucose. The data are presented as means ± SE of results from three separate experiments.

We used the Rhotekin-RBD pull-down assay to examine the extent to which UDP-glucose promotes activation of RhoA in HL60 cells. Addition of UDP-glucose to isolated neutrophils resulted in rapid (1 min) formation of RhoA-GTP, which was evident with 100 nM UDP-glucose (EC50 = 0.9 μM; Fig. 3D), consistent with UDP-glucose acting through the P2Y14-R (21).

Collectively, the data in Figs. 1–3 indicate that UDP-glucose is a potent and highly stable agonist of the P2Y14-R endogenously expressed by human neutrophils. Activation of this G protein-coupled receptor promotes RhoA activation, cell shape changes, and chemotaxis in these cells.
shown), but robust formation of RhoA-GTP occurred in the presence of ATP (Fig. 4A), likely reflecting activation of endogenous P2X1 and/or P2Y2 receptors (4, 5, 10, 52).

Differentiation of HL60 cells with DMSO (dHL60 cells) results in P2Y14-R mRNA expression (5, 16), and as is illustrated in Fig. 4B, RhoA activation in dHL60 cells was observed with low micromolar concentrations of UDP-glucose (EC50 = 0.7 μM), consistent with the concentration dependence of a P2Y14-R. To support the conclusion that activation of RhoA occurs through an extracellular signaling action of UDP-glucose, the effects of Crotalus adamanteus nucleotide pyrophosphatase, a phosphodiesterase (PDE) that hydrolyzes nucleotide-sugars and nucleotides (31), and apyrase, which hydrolyzes nucleotides but not nucleotide-sugars (31), were examined. Activation of RhoA in the presence of UDP-glucose was virtually eliminated by preincubation of UDP-glucose with 1 U/ml PDE (Fig. 4C), but not by 1 U/ml apyrase (Fig. 4D). UDP-galactose and UDP-N-acetyl-glucosamine also promoted RhoA activation in dHL60 cells (Fig. 4E).

To further assess the involvement of the P2Y14-R in UDP-glucose-evoked RhoA activation in HL60 cells, the human P2Y14-R was stably expressed in these cells (P2Y14-HL60 cells) via retroviral infection (16). In contrast to the lack of effect observed in undifferentiated HL60 cells (see Fig. 4A), UDP-glucose promoted RhoA activation in undifferentiated P2Y14-HL60 cells (Fig. 5). UDP-glucose-evoked RhoA activation was robust between 1 and 15 min following agonist addition, and it faded after 30 min (Fig. 5A). UDP-glucose-promoted RhoA activation exhibited an EC50 value of 0.4 μM (Fig. 5B). UDP-galactose and UDP-N-acetyl-glucosamine also promoted RhoA-GTP formation in undifferentiated P2Y14-HL60 cells (Fig. 5C).

Pertussis toxin and phosphoinositide 3-kinase inhibition abolish P2Y14-R-promoted Rho activation in HL60 cells. Several studies from our and other groups support the notion that the human P2Y14-R signals through Gi (6, 15–17, 31). In agreement with this concept, preincubation of dHL60 (Fig. 6A) with pertussis toxin abolished UDP-glucose-promoted RhoA activation in dHL60 cells. RhoA activation was measured in undifferentiated HL60 cells incubated with vehicle, 100 μM UDP-glucose, or 100 μM ATP. B: concentration-effect relationship for UDP-glucose-evoked RhoA activation in dHL60 cells. Error bars indicate differences to the mean from two independent experiments. C: the effect of 100 μM UDP-glucose on dHL60 cells was abolished by pretreatment (15 min) of the nucleotide-sugar with 1 U/ml nucleotide-pyrophosphatase [a phosphodiesterase (PDE)]. D: apyrase (1 U/ml, 15 min) had no effect on UDP-glucose-evoked RhoA activation in dHL60 cells. E: RhoA activation was measured in dHL60 cells incubated with vehicle or 100 μM UDP-glucose, UDP-galactose (UDP-Gal), or UDP-N-acetyl-glucosamine (UDP-GlcNAc). All incubations (A–E) were for 1 min.

Fig. 4. UDP-sugars promote RhoA activation in differentiated HL60 (dHL60) cells. A: RhoA activation was measured in undifferentiated HL60 cells incubated for 1 min with vehicle, 100 μM UDP-glucose, or 100 μM ATP. B: concentration-effect relationship for UDP-glucose-evoked RhoA activation in dHL60 cells. Error bars indicate differences to the mean from two independent experiments. C: the effect of 100 μM UDP-glucose on dHL60 cells was abolished by pretreatment (15 min) of the nucleotide-sugar with 1 U/ml nucleotide-pyrophosphatase [a phosphodiesterase (PDE)]. D: apyrase (1 U/ml, 15 min) had no effect on UDP-glucose-evoked RhoA activation in dHL60 cells. E: RhoA activation was measured in dHL60 cells incubated with vehicle or 100 μM UDP-glucose, UDP-galactose (UDP-Gal), or UDP-N-acetyl-glucosamine (UDP-GlcNAc). All incubations (A–E) were for 1 min.

Fig. 5. P2Y14 receptor (P2Y14-R) confers UDP-sugar-promoted RhoA activation to undifferentiated HL60 cells. A: undifferentiated P2Y14-HL60 cells were incubated for the time indicated with 100 μM UDP-glucose. The data are presented as means ± SE of results from three separate experiments. *Significantly different from vehicle, P < 0.05 by 2-way ANOVA. B: cells were incubated for 1 min with the indicated concentrations of UDP-glucose. The data are presented as means ± SE of results from three separate experiments. C: cells were incubated for 1 min with vehicle or 100 μM of UDP-glucose, UDP-galactose, or UDP-N-acetyl-glucosamine; the results are representative of three experiments.
activation. A possible mechanism whereby Gi-coupled receptors promote RhoA activation in HL60 cells involves activation of phosphoinositide 3-kinase (PI-3-kinase) (44). Consistent with this hypothesis, incubation of dHL60 cells with wortmannin, a selective inhibitor of PI-3-kinase, abolished UDP-glucose-promoted RhoA activation (Fig. 6B).

**UDP-glucose promotes dHL60 cell migration.** Incubation of dHL60 cells with 100 μM UDP-glucose (added to the lower compartment of the Boyden chamber) resulted in enhanced cell migration. As shown above with neutrophils, cell migration was not observed when the concentration gradient of agonist was abolished by addition of UDP-glucose to both compartments of the chamber, and it was inhibited by the Rho kinase inhibitors H1152 and Y27632 (Fig. 7A). As predicted, UDP-glucose-evoked dHL60 cell migration was abolished by preincubation of UDP-glucose with nucleotide pyrophosphatase, but not by apyrase (Fig. 7A). Addition of apyrase to the lower compartment of the chamber had no effect on dHL60 cell chemotaxis, but UDP-glucose-evoked chemotaxis was partially reduced when apyrase was also present in the upper chamber (Fig. 7B). The concentration-effect relationship for UDP-glucose-promoted chemotaxis in dHL60 is illustrated in Fig. 7C. Based on the diffusion rate of agonist through the chemotaxis membrane (see Fig. 3C), the estimated EC50 value for UDP-glucose-promoted dHL60 cell chemotaxis was 0.5 μM (Fig. 7C).

![Graphs showing RhoA activation](image)

**Fig. 6.** Pertussis toxin (PTX) and a phosphoinositide 3-kinase inhibitor abolish P2Y14-R-promoted Rho activation in HL60 cells. dHL60 cells were preincubated overnight with vehicle or 100 ng/ml of pertussis toxin (A) or 15 min with vehicle or 100 nM wortmannin (Wort) (B) and stimulated for 1 min with 100 μM UDP-glucose. The data are presented as means ± SE of results from four separate experiments. *,#Significantly different from vehicle and UDP-glucose, respectively, P < 0.05 by 2-way ANOVA.

**Fig. 7.** UDP-glucose promotes chemotaxis in dHL60 cells. A: chemotaxis of dHL60 cells was measured in response to vehicle or 100 μM UDP-glucose added either to the lower or to the upper compartment of the Boyden chamber, as indicated. UDP-glucose was preincubated with 1 U/ml of apyrase or nucleotide pyrophosphatase (PDE) for 15 min before addition of the agonist to the lower compartment, and dHL60 cells were preincubated with vehicle, 1 μM H1152, or 5 μM Y27632 for 15 min. Results are means ± SE from three separate experiments, each one performed in quadruplicate. *,#Significantly different from vehicle and UDP-glucose (lower), respectively, P < 0.05 by 2-way ANOVA. B: dHL60 cells migration in response to vehicle or 100 μM UDP-glucose added to the lower compartment. Apyrase (5 U/ml) was included in the lower and/or upper compartment, as indicated. Results are means ± SE from three separate experiments, each one performed in sextuplicate. *,#Significantly different from vehicle and UDP-glucose without apyrase, respectively, P < 0.05 by 2-way ANOVA. C: concentration-effect relationship for UDP-glucose-promoted chemotaxis in dHL60 cells; the values on the x-axis indicate the concentration of agonist added to the lower compartment (added), and the concentration of agonist calculated to reach the upper compartment (calc), as in Fig. 3C. The results represent means ± SE from two separate experiments, each performed in quadruplicate.
DISCUSSION

P2Y14-R mRNA is highly abundant in circulating neutrophils (37), and UDP-glucose promotes ERK1/2 phosphorylation in these cells (43). Involvement of P2Y14-R activation in neutrophil function has, nonetheless, remained poorly defined. In the present study, we demonstrate that addition of UDP-glucose to human neutrophils results in rapid and robust activation of RhoA, and this response occurs with concentration dependence consistent with a P2Y14-R-mediated response. UDP-glucose-evoked Rho activation was accompanied by changes in cell shape (with the formation of ruffle-like structures in one cellular pole), cytoskeleton rearrangements, and enhanced neutrophil chemotaxis. Furthermore, using the HL60 human promyelocytic leukemia cells, we demonstrate that UDP-glucose-evoked responses occurred in a P2Y14-R-dependent manner. That is, UDP-glucose had no effect on HL60 cells lacking P2Y14-R mRNA expression, but strong UDP-glucose-evoked RhoA activity was observed after either inducing endogenous expression of the receptor via DMSO differentiation (dHL60 cells) or stably expressing the P2Y14-R via retroviral infection (P2Y14-HL60 cells).

A caution was introduced by Brautigam et al. (3) for studies of inflammatory and other effects potentially regulated by the P2Y14-R. That is, although commercial sources of UDP-glucose induced inflammatory effects in N9 microglia, this action was only observed with high micromolar (>75 μM) concentrations of bacterially derived but not synthetic UDP-glucose; other nucleotide sugars such as UDP-galactose also had no effect (3). The conclusion that actions of UDP-sugars on neutrophils reflected activation of P2Y14-R is firmly supported by the following observations: 1) HPLC analysis of UDP-glucose indicated no detectable contamination with ATP or any other nucleotide signaling molecule; moreover, the nucleotide di- and tri-phosphohydrolase apyrase (1 U/ml) was included in the PMN suspension medium during the Rho assay to minimize neutrophil activation by ATP/UTP potentially released during PMN isolation (28); 2) both RhoA activation and cell migration in the presence of UDP-glucose were virtually eliminated by preincubation of UDP-glucose with nucleotide pyrophosphatase, which hydrolyzes UDP-sugars (31), but not by apyrase; 3) neutrophil activation occurred with concentrations of UDP-glucose consistent with P2Y14-R activation; and 4) UDP-glucose, UDP-galactose, and UDP-N-acetylgalcosamine promoted RhoA activation in dHL60 cells, which natively express P2Y14-R (16), and in undifferentiated HL60 cells expressing recombinant P2Y14-R, but not in wild-type undifferentiated HL60 cells, which do not express P2Y14-R (16).

Extensive literature associates P2Y14-R-promoted responses with activation of heterotrimeric G proteins of the G12 subfamily (reviewed in Ref. 21). Our results illustrating that pertussis toxin abolishes UDP-glucose-promoted RhoA activation in dHL60 and P2Y14-HL60 cells are consistent with this notion. We also showed that RhoA activation was inhibited by a selective inhibitor of PI-3-kinase, a downstream effector of G12 (20, 49). Based on these results, we propose that the P2Y14-R promotes activation of G12, resulting in Gβγ-evoked PI-3-kinase-mediated formation of phosphatidylinositol(3,4,5)trisphosphate, recruitment of a Rho G protein exchange factor to the plasma membrane, and facilitation of RhoGTP formation. Such a signaling pathway was previously illustrated with formyl peptide-stimulated monocytes (7).

The present study provides evidence for a functional role of the neutrophil P2Y14-R, i.e., modulation of neutrophil shape change and motility. Furthermore, our observation that Rho kinase inhibitors markedly reduced neutrophil motility are in line with studies indicating that activation of RhoA/Rho kinase results in myosin activation, rear contraction, and development of cell polarity in migrating neutrophils (32, 40, 54, 55). RhoA activation in neutrophils and dHL60 cells was robust after 1 min of UDP-glucose addition, and time course measurements in dHL60 cells indicated that this response persisted for up to 15 min and faded after 30 min. These observations are consistent with studies indicating that rapid, transient increase of RhoA activity in the first minutes after agonist treatment is necessary for migration of leukocytes (1, 55) and other cells (19, 50, 56). They are also in agreement with the notion that RhoA activity is subjected to negative feedback by Rho-GTPase activating proteins (GAPs) and Rho GDP dissociation inhibitor (RhoGDI) (13, 48). Whether additional signaling pathways associated with chemoattractant-evoked responses, e.g., Rac1, Rac2, and Cdc42 (34, 39, 40), contribute to P2Y14-R-regulated neutrophil shape change and locomotion remain to be investigated.

ATP release from stimulated phagocytes provides autocrine regulation of directional migration (12). For example, formyl peptides, interleukin 8, C5a complement, and leukotriene LTB4 promote neutrophil migration, which was accompanied by ATP release and inhibited by apyrase or P2Y2-R antagonists (8, 9). In line with these reports, UDP-glucose evoked chemotaxis of neutrophils and dHL60 cells was partially reduced when apyrase was included in the upper level of the Boyden chamber (Figs. 3B and 7B). Thus, endogenous ATP likely contributed, at least in part, to UDP-glucose-evoked neutrophil chemotaxis.

Recent data from our group demonstrated that, in addition to UDP-sugars, the P2Y14-R is activated by UDP (53). Since UDP is also a potent agonist at the P2Y6-R and P2Y6-R transcripts are endogenously expressed in neutrophils and HL60 cells (22), the relative contribution of P2Y14-R to UDP-promoted signaling in these cells could not be readily assessed. The effect of UDP on P2Y14-R-mediated responses was not investigated in the present study.

Our findings differ from those previously published by Arase et al. (2). These authors reported that 1 μM UDP-glucose had no direct effect on PMN chemotaxis, assessed via the classic transwell filter staining method (2). Addition of 1 μM UDP-glucose to the lower compartment of the chemotaxis chamber in our studies resulted in a relatively minor response, as compared with the response observed with 10 μM UDP-glucose (Fig. 3C). However, measurement of UDP-[1H]glucose diffusion through the filter membrane indicated that the concentration of UDP-glucose in the upper compartment of the chamber at the time of quantification of chemotaxis (1 h) was only 20% of the nominal concentration of agonist added to the lower chamber (Fig. 3C, inset). We suggest that the sensitive quantification provided by the automated, fluorescence-based chemotaxis assay used here as well as the difficulty of accurate estimation of the effective agonist concentration in situ account for the apparent discrepancy between the two studies.

AJP-Cell Physiol • doi:10.1152/ajpcell.00138.2012 • www.ajpcell.org
UDP-glucose promotes activation of human neutrophils

The concept that the P2Y<sub>14</sub>-R plays (patho)physiologically relevant roles is supported by studies demonstrating that UDP-glucose, UDP-galactose, and UDP-N-acetylglucosamine are released from cells in a regulated manner (14, 23, 24, 26, 31, 41, 46). Moreover, unlike other proinflammatory molecules that require activation of biosynthetic pathways before their release from cells, UDP-glucose is highly abundant both in the cytosol and secretory pathway of most cells, and it is poised for rapid release together with other nucleotides and nucleotide-sugars from activated and/or damaged cells (30). Importantly, in contrast to short-lived ATP/UTP find-me signals released during sterile inflammatory processes (30, 35, 36), extracellular UDP-glucose is highly stable (31) and is not degraded by neutrophil ecto-nucleotidases (Fig. 1). Thus, UDP-glucose likely is a novel, important local contributor to sterile neutrophil inflammation.

Our present findings are particularly relevant to human airway pathophysiology. That is, we recently illustrated that Ca<sup>2+</sup>-regulated exocytosis of specialized mucin granules from goblet cell-reach airway epithelia is accompanied by robust release of UDP-glucose (23, 41). We also have shown that lung goblet cell-reach airway epithelia is accompanied by robust release from cells, UDP-glucose is highly abundant both in the cytosol and secretory pathway of most cells, and it is poised for rapid release together with other nucleotides and nucleotide-sugars from activated and/or damaged cells (30). Importantly, in contrast to short-lived ATP/UTP find-me signals released during sterile inflammatory processes (30, 35, 36), extracellular UDP-glucose is highly stable (31) and is not degraded by neutrophil ecto-nucleotidases (Fig. 1). Thus, UDP-glucose likely is a novel, important local contributor to sterile neutrophil inflammation.

In summary, we demonstrate that UDP-glucose is a highly stable proinflammatory mediator that promotes P2Y<sub>14</sub>-R-regulated RhoA activation, cytoskeleton rearrangements, and chemotaxis in human neutrophils.

**ACKNOWLEDGMENTS**

We are grateful to Dr. Rob Tarran for the use of the Leica SP5 confocal microscopy.

**GRANTS**

Research from the authors’ laboratories was supported by National Institutes of Health Grants GM-38123 and P01-HL-0343223.

**DISCLOSURES**

No conflicts of interest, financial or otherwise, are declared by the author(s).

**AUTHOR CONTRIBUTIONS**


**REFERENCES**


