Pharmacological effects mediated by UDP-glucose that are independent of P2Y$_{14}$ receptor expression

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Abstract

In transfected cells, the P2Y$_{14}$ receptor reportedly couples to pertussis toxin-sensitive G$_{i/o}$-proteins. However, the functional coupling of endogenously expressed P2Y$_{14}$ receptors to the inhibition of adenylyl cyclase activity has not been reported. Therefore, the primary aim of this study was to investigate the effects of uridine 5′-diphosphoglucose (UDP-glucose) on forskolin-stimulated cyclic AMP (cAMP) accumulation in two cell lines that reportedly express P2Y$_{14}$ receptor mRNA, namely human neuroblastoma SH-SY5Y cells and human astrocytoma U373 MG cells. In U373 MG cells, UDP-glucose inhibited forskolin-stimulated cAMP accumulation in a concentration-dependent manner (pEC$_{50}$ = 4.5 ± 0.3). Furthermore, treatment with pertussis toxin abolished the inhibitory effects of UDP-glucose on forskolin-stimulated cAMP accumulation in U373 MG cells. In SH-SY5Y cells, UDP-glucose had no significant effect on forskolin-stimulated cAMP accumulation. To confirm the expression of P2Y$_{14}$ receptor mRNA in U373 MG and SH-SY5Y cells, we performed reverse transcriptase polymerase chain reaction (RT-PCR) analysis. However, RT-PCR did not detect the expression of P2Y$_{14}$ receptor mRNA in SH-SY5Y cells or surprisingly in U373 MG cells. In conclusion, we have shown that although UDP-glucose inhibits forskolin-stimulated cAMP accumulation in human U373 MG astrocytoma cells, we did not detect P2Y$_{14}$ receptor mRNA in these cells. These results would suggest that the effects of UDP-glucose in U373 MG cells are independent of P2Y$_{14}$ receptor expression. Thus, results obtained with UDP-glucose should be interpreted with caution, since they clearly may not necessarily reflect the involvement of the P2Y$_{14}$ receptor.
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1. Introduction

Uridine 5′-diphosphoglucose (UDP-glucose) is widely known to function as a glycosyl donor in the biosynthesis of carbohydrates. However, it has recently been shown that UDP-glucose and related sugar nucleotides, UDP-galactose, UDP-glucuronic acid and UDP-*N*-acetylglucosamine are all potent agonists of the orphan human G protein-coupled receptor (GPCR) KIAA0001 [1]. This orphan GPCR was originally cloned from the human immature myeloid cell line KG-1 [2]. More recently, the rat and mouse orthologs of the human KIAA0001 receptor have subsequently been cloned and show 80 and 83% amino acid identity, respectively [3], and in line with GPCR nomenclature, KIAA0001, and the

rodent orthologs were renamed GPR105. A fragment of a novel GPCR, which had previously been identified from rat ventral tegmentum (termed VTR 15–20) shares 80% amino acid identity with the first 293 amino acids of the human GPR105 and thus represented a truncated version of the rat GPR105 [4]. Since the UDP-glucose receptor shares approximately 45% amino acid identity with the human P2Y$_{12}$ and P2Y$_{13}$ receptors, it has recently been included as a member of the P2Y receptor family and renamed the P2Y$_{14}$ receptor [5].

The P2Y$_{14}$ receptor appears to couple G$_{i/o}$-proteins, since pertussis toxin treatment (which blocks G$_{i/o}$-protein coupling) completely inhibited UDP-glucose-stimulated GTPγS binding to membranes prepared from transfected HEK 293 cells [1]. GPCRs that couple to G$_{i/o}$-proteins are classically associated with the inhibition of adenylyl cyclase activity; however, the functional coupling of endogenously expressed

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P2Y$_{14}$ receptors to inhibition of adenylyl cyclase activity has not been reported. However, UDP-glucose has been shown to stimulate increases in intracellular Ca^{2+} concentration in rat cortical astrocytes and human immature monocyte-derived dendritic cells (MDDC) [6,7]. Interestingly, UDP-glucose-mediated Ca^{2+} responses observed in immature MDDCs were partially sensitive to pertussis toxin suggesting coupling of the P2Y$_{14}$ receptor to G$_{i/o}$-proteins in an endogenous system. However, it is important to note that although both rat cortical astrocytes and human immature MDDCs express P2Y$_{14}$ receptors, it is conceivable that the Ca^{2+} responses to UDP-glucose in these cells are not mediated via the P2Y$_{14}$ receptor [6,7].

The physiological function of the P2Y$_{14}$ receptor is unknown at present, although it is widely expressed in human tissue with highest expression levels in placenta, adipose tissue, stomach and intestine and moderate levels in the brain, spleen, lung and heart [1]. In the rat, Northern blot analysis revealed that P2Y$_{14}$ receptor mRNA is present at high levels in the kidney and spleen, with lower levels in several CNS regions including the cerebral cortex, hippocampus, hypothalamus and cerebellum [4]. These authors also reported the expression of P2Y$_{14}$ receptor mRNA in a variety of cell lines including human neuroblastoma (SH-SY5Y), astrocytoma (U373 MG) and promyelocytic (HL-60) cells. More recently, the P2Y$_{14}$ receptor has been detected in rat cortical astrocytes, bone marrow hematopoietic stem cells and immature MDDCs [6–8].

As detailed above, the functional coupling of endogenously expressed P2Y$_{14}$ receptors to inhibition of adenylyl cyclase activity has not been reported. Therefore, the primary aim of this study was to investigate the effects of UDP-glucose on forskolin-stimulated cyclic AMP accumulation in human neuroblastoma (SH-SY5Y) and astrocytoma (U373 MG) cells.

2. Materials and methods

2.1. Materials

[2,8-^{3}H] Adenine was from Amersham International (Aylesbury, Bucks). Dulbecco's modified Eagles medium, Dulbeco's modified Eagles Medium/Nutrient Mix F-12, foetal calf serum, forskolin, N^6-cyclopentyladenosine (CPA), pertussis toxin, UDP and UDP-glucose were obtained from Sigma Chemical Co. (Poole, Dorset, UK). Rolipram was purchased from Calbiochem (Nottingham, UK). DAMGO and HU 210 were purchased from Tocris Cookson Ltd. All molecular biology reagents including RQ1 RNase-free DNase, M-MLV reverse transcriptase, SV total RNA isolation kit and random primers were obtained from Promega. Primers for RT-PCR analysis were synthesised by Sigma–Genosys (Pampisford, Cambridgeshire, UK). Lipofectamine was from Life Technologies. All other chemicals were of analytical grade.

2.2. Cell culture

Parent CHO-K1 cells, human neuroblastoma (SY-SY5Y) and astrocytoma (U373 MG) cells were obtained from the European Collection of Animal Cell Cultures (Porton Down, Salisbury, UK). SH-SY5Y cells were cultured in Dulbecco's modified Eagles medium (DMEM) supplemented with 2 mM L-glutamine and 10% (v/v) foetal calf serum. U373 MG and CHO-K1 cells were cultured in DMEM/Nutrient F12 (1:1) supplemented with 2 mM L-glutamine and 10% (v/v) foetal calf serum. Cells were maintained at 37 °C in a humidified 5% CO$_2$ atmosphere until confluency and subcultured (1:5 split ratio) using trypsin (0.05%, w/v)/EDTA (0.02%, w/v).

2.3. Expression of recombinant human P2Y$_{14}$ receptors in Chinese hamster ovary cells

The pCDN expression vector (in-house vector that contains a CMV promoter and a neomycin resistance marker) containing the human P2Y$_{14}$ receptor cDNA was a generous gift from GlaxoSmithKline. Chinese hamster ovary (CHO-K1) cells were transfected with the human P2Y$_{14}$ receptor using lipofectamine. Stably transfected CHO-K1 cells were selected using the neomycin analogue geneticin (G418; 400 μg/ml) for 2 weeks. CHO-K1 cells resistant to G418 were subsequently cloned using the dilution cloning method. RT-PCR analysis revealed significant mRNA expression for the human P2Y$_{14}$ in clone 9 (CHO-hP2Y$_{14}$ cells).

2.4. Measurement of [^{3}H] cAMP accumulation

SH-SY5Y and U373 MG cells were grown in 24-well plate cluster dishes and, when 80–90% confluent, were incubated for 2 h at 37 °C with 500 μl of Hanks/HEPES buffer (pH 7.4) containing [^{3}H] adenine (37 kBq/well). [^{3}H] adenine-labelled cells were washed once and then incubated in 1 ml/well Hanks/HEPES buffer containing the cyclic AMP phosphodiesterase inhibitor, rolipram (10 μM) for 15 min at 37 °C. Agonists were added (in 10 μl of medium) 5 min prior to incubation with 3 μM forskolin (10 min). Incubations were terminated by the addition of 50 μl concentrated HCl. [^{3}H] cAMP was isolated by sequential Dowex–alumina chromatography as previously described [9]. After elution, the levels of [^{3}H] cAMP were determined by liquid scintillation counting.

2.5. RT-PCR analysis of P2Y$_{14}$ receptor mRNA expression

Total RNA was isolated from U373 MG, SH-SY5Y and CHO-hP2Y$_{14}$ cells using the Promega SV total RNA isolation kit according to the manufacturer's instructions. During the isolation procedure, mRNA was routinely treated with RQ1 DNase (1 U/μl) in order to remove genomic DNA. First-strand complementary DNA (cDNA) was synthesised utilising random primers and M-MLV reverse

transcriptase. PCR was performed using the following P2Y$_{14}$ receptor gene-specific primer sequences: forward one (F1), 5′-CGACAAACGCTCACTGGGCAAA-3′; forward two (F2), 5′-CGCAACAATTCAGCATCGTGT-3′ and reverse one (R1), 5′-CAAAGTATCTGTGCTTTCAAG-3′. It should be noted that F1 and R1 amplify across an intron (in order to discriminate between cDNA and genomic DNA) but F2 and R1 do not. GAPDH primers (501 bp product) were forward 5′-ACTCCACTCACGGCAAATTC-3′ and reverse 5′-CCTTCCACAATGCCAAAGTT-3′. PCR conditions for the P2Y$_{14}$ receptor were 40 or 60 cycles of 94 °C for 1 min, 50 °C for 1.5 min and 72 °C for 2 min. PCR conditions for GAPDH were 30 cycles of 94 °C for 1 min, 58 °C for 1 min and 72 °C for 1 min. PCR products were subjected to 2% (w/v) agarose gel electrophoresis and visualised by ethidium bromide staining. Running PCR reactions prior to cDNA synthesis using GAPDH primers controlled for potential contamination by genomic DNA.

2.6. Data analysis

Agonist pEC$_{50}$ values ($-\log$ EC$_{50}$; concentration of drug producing 50% of the maximal response) were obtained by computer assisted curve fitting by use of the computer programme Prism (GraphPAD, CA, USA). Statistical significance was determined by Student's unpaired t-test ($P < 0.05$ was considered statistically significant). All data are presented as mean ± S.E.M., where n refers to the number of separate experiments.

3. Results

3.1. Effects of UDP-glucose on forskolin-stimulated cyclic AMP accumulation

As detailed in Section 1, the functional coupling of endogenously expressed P2Y$_{14}$ receptors to inhibition of adenylyl cyclase activity has not been reported. Therefore, in this study, we investigated the effects of UDP-glucose on forskolin-stimulated cyclic AMP accumulation in human SH-SY5Y neuroblastoma cells and human U373 MG astrocytoma cells; two cell lines that have previously been reported to express P2Y$_{14}$ receptor mRNA [4]. In U373 MG cells, UDP-glucose inhibited forskolin-stimulated cAMP accumulation in a concentration-dependent manner (pEC$_{50}$ = 4.5 ± 0.3; n = 5; Fig. 1). UDP-glucose (100 μM) inhibited 43 ± 5% (n = 5) of the cAMP response induced by 3 μM forskolin. For comparison, activation of the G$_{i/o}$-protein-coupled CB$_1$ cannabinoid receptor (which is endogenously expressed in U373 MG cells; [10]) with HU 210 inhibited forskolin-stimulated cAMP accumulation in a concentration-dependent manner (pEC$_{50}$ = 9.4 ± 0.3; n = 5; Fig. 1). The maximal effective concentration of HU 210 (1 μM) inhibited the forskolin (3 μM) response by 65 ± 12% (n = 5). In contrast, UDP (1, 10 and 100 μM; Fig. 1) had no significant effects on forskolin-stimulated cAMP accumulation suggesting that the inhibition
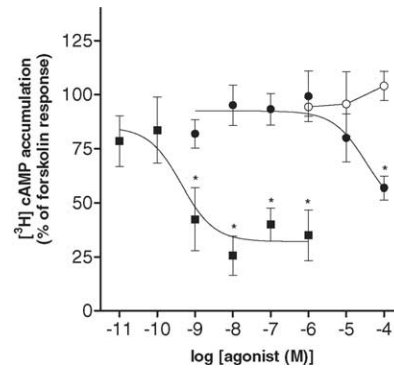

Fig. 1. Effect of UDP-glucose, UDP and HU 210 on forskolin-stimulated cyclic AMP accumulation in U373 MG cells. Cells were pre-stimulated for 5 min with the indicated concentrations of UDP-glucose (●), UDP (○) and HU 210 (■) prior to stimulation with 3 μM forskolin for 10 min in the continued presence of UDP-glucose, UDP and HU 210. Data are expressed as the percentage of the forskolin response (in the absence of agonist = 100%) and represent the mean ± S.E.M. from five independent experiments each performed in triplicate; $^*P < 0.05$ vs. forskolin alone.

of cAMP accumulation observed with UDP-glucose is not due to UDP-glucose breakdown and subsequent activation of the G$_q$-coupled P2Y$_6$ receptor. There is evidence for G$_q$-coupled receptor-mediated inhibition of cAMP accumulation in U373 MG cells [11]. Finally, pre-treatment with pertussis toxin (100 ng/ml for 16 h) abolished the inhibitory effects of UDP-glucose (100 μM) and HU 210 (1 μM) on forskolin-stimulated cAMP accumulation in U373 MG cells (Fig. 2). It is also notable that UDP-glucose and HU 210 both augmented forskolin-induced cAMP responses in the presence of pertussis toxin.
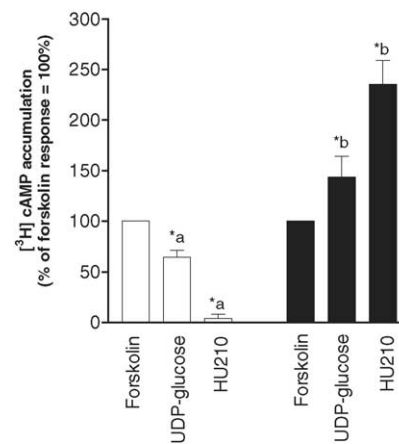

Fig. 2. Effect of pertussis toxin on UDP-glucose and HU 210-mediated inhibition of forskolin-stimulated cyclic AMP accumulation in U373 MG cells. Cells were pre-treated with pertussis toxin (PTX; 100 ng/ml) for 16 h; control (□) and PTX-treated cells (■) were then pre-stimulated for 5 min with UDP-glucose (100 μM) and HU 210 (1 μM) prior to stimulation with 3 μM forskolin for 10 min in the continued presence of UDP-glucose and HU 210. Data are expressed as the percentage of the forskolin response (in the absence of agonist = 100%) and represent the mean ± S.E.M. from three independent experiments each performed in triplicate; ($^*a$) $P < 0.05$ vs. forskolin alone in the absence of PTX and ($^*b$) $P < 0.05$ vs. forskolin alone in the presence of PTX.
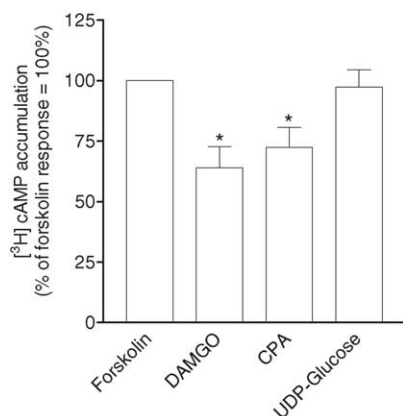
Fig. 3. Effect of UDP-glucose on forskolin-stimulated cyclic AMP accumulation in SH-SY5Y cells. Cells were pre-stimulated for 5 min with UDP-glucose (100 μM), CPA (1 μM) and DAMGO (1 μM) prior to stimulation with 3 μM forskolin for 10 min in the continued presence of the agonists. Data are expressed as the percentage of the forskolin response (in the absence of agonist = 100%) and represent the mean $\pm$ S.E.M. from five independent experiments each performed in triplicate; $^*P < 0.05$ vs. forskolin alone.

As shown in Fig. 3, UDP-glucose (100 μM) did not inhibit forskolin-stimulated cAMP accumulation in SH-SY5Y cells. In contrast, N^6-cyclopentyladenosine (CPA; 1 μM) and DAMGO (1 μM), selective adenosine A_1 receptor and μ-opioid receptor agonists, respectively, significantly inhibited forskolin-stimulated cyclic AMP responses in SH-SY5Y cells (Fig. 3). Previous studies have shown that adenosine A_1 receptor and μ-opioid receptors are functionally expressed in SH-SY5Y cells [12,13].

3.2. RT-PCR analysis of P2Y$_{14}$ receptor mRNA expression

Previous studies have shown that SH-SY5Y neuroblastoma cells and human U373 MG astrocytoma cells express P2Y$_{14}$ receptor mRNA [4]. However, using RT-PCR analysis, we did not detect P2Y$_{14}$ receptor mRNA in these two cell lines after 40 PCR cycles (Fig. 4). Similar results were obtained after 60 cycles (data not shown). CHO-K1 cells stably transfected with the human P2Y$_{14}$ receptor (CHO-hP2Y$_{14}$) were employed, as a positive control (see lane 7, Fig. 4), to confirm that the primers used are able to detect P2Y$_{14}$ mRNA expression. The absence of P2Y$_{14}$ receptor mRNA in U373 MG cells was particularly interesting given the effects of UDP-glucose on forskolin-stimulated cAMP accumulation (Fig. 1). These results clearly suggest that the effects of UDP-glucose in U373 MG cells are independent of P2Y$_{14}$ receptor expression.

4. Discussion

At present, the signalling pathways activated by the UDP-glucose receptor (recently renamed the P2Y$_{14}$ receptor) are poorly understood. Studies using transfected
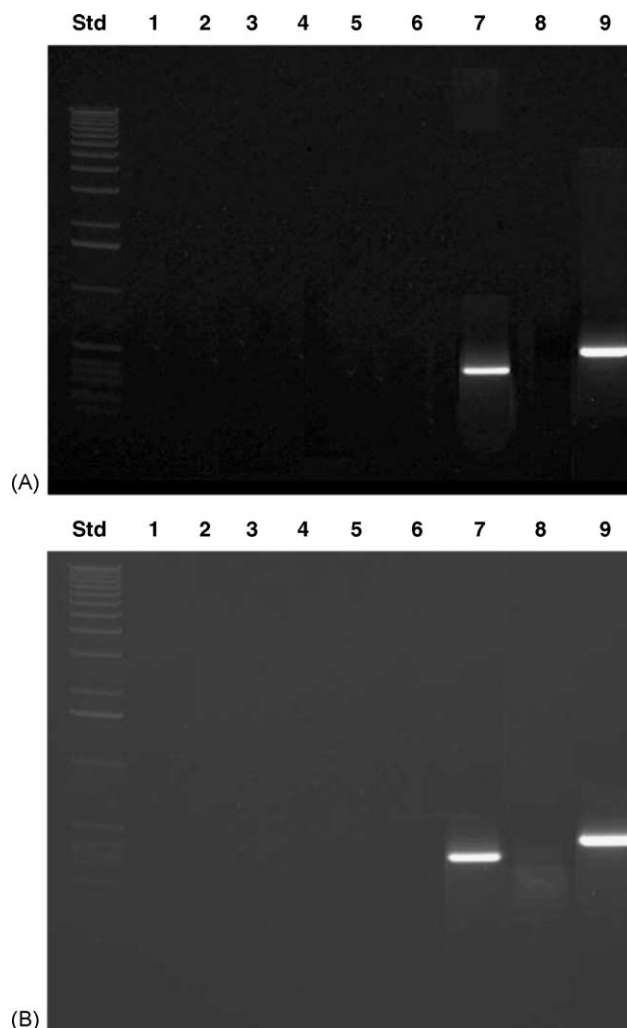

Fig. 4. Determination of P2Y$_{14}$ receptor mRNA expression in U373 MG, SH-SY5Y and transfected CHO-K1 cells. mRNA was isolated from (A) U373 MG cells and (B) SH-SY5Y cells was subjected to RT-PCR using P2Y$_{14}$ receptor gene specific primers. *Std:* 1-kb DNA standard; *lane 1:* RNA control using GAPDH primers; *lane 2:* no DNA control; *lane 3:* primers F2 and R1; *lane 4:* no DNA control; *lane 5:* primers F1 and R1; *lane 6:* no DNA control; *lane 7:* primers F2 and R1 using mRNA isolated from CHO-hP2Y$_{14}$ cells (318 bp product); *lane 8:* no DNA control; *lane 9:* GAPDH primers (501 bp product). The results presented are representative of three separate experiments performed on different cell cultures.

cells suggest that the P2Y$_{14}$ receptor couples to members of the G$_{i/o}$-protein family, since pertussis toxin treatment (which blocks G$_{i/o}$-protein coupling) completely inhibited UDP-glucose-stimulated GTPγS binding to membranes prepared from HEK 293 cells [1]. Similarly, studies using HEK 293 cells co-transfected with P2Y$_{14}$ and either Gα_{qo5}, Gα_{qi5} or Gα_{qs5} have revealed that the P2Y$_{14}$ receptor couples to G$_{i/o}$-proteins and not G$_s$ or G$_q$ [13]. The chimeric Gα subunits used by Moore et al. [14] were constructed by replacing the five C-terminal amino acids of human Gα_q with the corresponding residues from Gα_s, Gα_o or Gα_i. Overall, these studies suggest that in transfected cell lines, at least, the P2Y$_{14}$ receptor couples to members of

the $G_{i/o}$-protein family. However, the functional coupling of endogenously expressed $P2Y_{14}$ receptors to inhibition of adenylyl cyclase activity (which represents classical $G_{i/o}$-protein-mediated signaling) has not been reported. Therefore, the aim of this study was to investigate the effect of UDP-glucose on forskolin-stimulated cAMP accumulation in human SH-SY5Y neuroblastoma and human U373 MG astrocytoma cells. Since Northern blot analysis revealed that the presence of $P2Y_{14}$ receptor mRNA in these two cell lines, they may represent useful model systems for studying endogenous $P2Y_{14}$ receptor-mediated signaling [4]. The data presented clearly indicate that UDP-glucose inhibits forskolin-stimulated cAMP accumulation in U373 MG astrocytoma cells. Furthermore, the effects of UDP-glucose on forskolin-stimulated cyclic AMP accumulation were inhibited by pertussis toxin clearly suggesting the involvement of $G_{i/o}$-proteins. This observation is important, since previous studies have shown that the G_q-protein-coupled histamine H_1 receptor inhibits forskolin and isoprenaline-stimulated cAMP accumulation in U373 MG cells via a Ca^{2+}-dependent pathway [11]. In addition, UDP alone did not inhibit forskolin-induced cAMP responses thus eliminating the possibility that the inhibition observed with UDP-glucose is not simply a consequence of UDP-glucose degradation. However, in contrast to previous studies reporting the expression of $P2Y_{14}$ receptor mRNA in U373 MG cells, we did not detect $P2Y_{14}$ mRNA using RT-PCR analysis [4]. The reasons for this discrepancy is not entirely clear, but it could represent clonal variation (this is often a problem when using established cell lines obtained from different sources) or the use of U373 MG cells at different passage numbers, which may influence $P2Y_{14}$ receptor expression. However, despite the apparent absence of $P2Y_{14}$ receptor mRNA expression in our U373 MG cells, we still observed an inhibition of forskolin-stimulated cAMP accumulation by UDP-glucose. These data suggest that UDP-glucose may also mediate cellular effects independent of the $P2Y_{14}$ receptor. Thus, caution is clearly required when interpreting functional responses of cells to UDP-glucose, since such responses may not be linked to $P2Y_{14}$ receptor activation. The apparent inhibition of forskolin-stimulated cAMP accumulation by UDP-glucose in U373 MG cells may reflect the activation of other putative nucleotide GPCR family members, namely GPR87 and/or GPR91. At present, GPR87 and GPR91 are orphan receptors, which interestingly, along with $P2Y_1$, $P2Y_{13}$ and $P2Y_{14}$ are located on human chromosome 3 [15].

It is notable that the EC_{50} value for UDP-glucose-mediated inhibition of forskolin-stimulated cAMP accumulation in U373 MG cells (*circa* 30 μM) is significantly higher than the EC_{50} values reported for UDP-glucose induced Ca^{2+} responses (80 nM) in HEK 293 cells stably co-transfected with the human $P2Y_{14}$ receptor and $G_{\alpha16}$ and also UDP-glucose-stimulated $GTP\gamma S$ binding (234 nM) to membranes prepared from transfected HEK 293 cells without recombinant G proteins [1]. Furthermore, in HEK 293 cells transiently co-transfected with the human $P2Y_{14}$ receptor and either $G\alpha_{qo5}$, $G\alpha_{qi5}$ or $G\alpha_{16}$, UDP-glucose-stimulated intracellular Ca^{2+} release with EC_{50} values of 29, 32 and 191 nM, respectively [14]. However, the EC_{50} value obtained in this study is comparable to the EC_{50} for UDP-glucose-mediated Ca^{2+} responses (11.5 μM) in immature MDDCs [7]. In view of the data presented in this study, it is conceivable that the Ca^{2+} responses to UDP-glucose in immature MDDCs are not mediated via the $P2Y_{14}$ receptor.

We also investigated the effects of UDP-glucose on forskolin-stimulated cAMP accumulation in human SH-SY5Y neuroblastoma cells. However, in contrast to U373 MG cells, we did not observe any significant inhibition of forskolin-stimulated cAMP accumulation by UDP-glucose. However, CPA and DAMGO, selective adenosine A_1 receptor and μ-opioid receptor agonists, respectively, inhibited forskolin-stimulated cyclic AMP accumulation in SH-SY5Y cells. In addition, and in contrast to Charlton et al. [4], RT-PCR analysis did not reveal $P2Y_{14}$ receptor expression in SH-SY5Y cells. During the preparation of this manuscript, Moore et al. [14] also reported the absence of $P2Y_{14}$ receptor expression (using Taq-Man quantitative RT-PCR) in both differentiated and undifferentiated SH-SY5Y cells. Overall, these observations suggest that human neuroblastoma SH-SY5Y cells do not express functional $P2Y_{14}$ receptors.

In conclusion, the RT-PCR data presented in this study would indicate that human astrocytoma U373 MG cells and human neuroblastoma SH-SY5Y cells do not express the $P2Y_{14}$ receptor. However, in U373 MG cells, UDP-glucose did inhibit forskolin-stimulated cyclic AMP accumulation in a pertussis toxin-sensitive manner. No effects of UDP-glucose on forskolin-induced cyclic AMP responses were observed in SH-SY5Y cells. Overall, these observations suggest that UDP-glucose may also mediate cellular effects that are independent of the $P2Y_{14}$ receptor.

References

[1] Chambers JK, Macdonald LE, Sarau HM, Ames RS, Freeman K, Foley JJ, et al. A G protein-coupled receptor for UDP-glucose. J Biol Chem 2000;275:10767–71.

[2] Nomura N, Miyajima N, Sazuka T, Tanaka A, Kawarabayasi Y, Sato S, et al. Prediction of the coding sequences of unidentified human genes. Part I: the coding sequences of 40 new genes (KIAA0001-KIAA0040) deduced by analysis of randomly sampled cDNA clones from human immature myeloid cell line KG-1. DNA Res 1994;1:27–35.

[3] Freeman K, Tsui P, Moore D, Emson PC, Vawter L, Naheed S, et al. Cloning, pharmacology, and tissue distribution of G-protein-coupled receptor GPR105 (KIAA0001) rodent orthologs. Genomics 2001;78:124–8.

[4] Charlton ME, Williams AS, Fogliano M, Sweetman PM, Duman RS. The isolation and characterization of a novel G protein-

coupled receptor regulated by immunologic challenge. Brain Res 1997;764:141–8.

[5] Abbracchio MP, Boeynaems J-M, Barnard EA, Boyer JL, Kennedy C, Miras-Portugal MT, et al. Characterization of the UDP-glucose receptor (re-named here the P2Y$_{14}$ receptor) adds diversity to the P2Y receptor family. Trends Pharmacol Sci 2003;24:52–5.

[6] Fumagalli M, Brambilla R, D'Ambrosi N, Volonte C, Matteoli M, Verderio C, et al. Nucleotide-mediated calcium signaling in rat cortical astrocytes: role of P2X and P2Y receptors. Glia 2003;43:218–30.

[7] Skelton L, Cooper M, Murphy M, Platt A. Human immature monocyte-derived dendritic cells express the G protein-coupled receptor GP105 (KIAA0001, P2Y$_{14}$) and increase intracellular calcium in response to its agonist, uridine diphosphoglucose. J Immunol 2003;171:1941–9.

[8] Lee BC, Cheng T, Adams GB, Attar EC, Miura N, Lee SB, et al. P2Y-like receptor, GPR105 (P2Y$_{14}$), identifies and mediates chemotaxis of bone-marrow hematopoietic stem cells. Genes Dev 2003;17:1592–604.

[9] Donaldson J, Brown AM, Hill SJ. Influence of rolipram on the cyclic $3',5'$-adenine monophosphate response to histamine and adenosine in slices of guinea-pig cerebral cortex. Biochem Pharmacol 1988;37:715–23.

[10] Bouaboula M, Bourrie B, Rinaldi-Carmona M, Shire D, Le Fur G, Casellas P. Stimulation of cannabinoid receptor CB1 induces krox-24 expression in human astrocytoma cells. J Biol Chem 1995;270:13973–80.

[11] Wong MPM, Cooper DMF, Young KW, Young JM. Characteristics of the Ca^{2+}-dependent inhibition of cyclic AMP accumulation by histamine and thapsigargin in human U373 MG astrocytoma cells. Br J Pharmacol 2000;130:1021–30.

[12] Salim H, Ferre S, Dalal A, Peterfreund RA, Fuxe K, Vincent JD, et al. Activation of adenosine A1 and A2A receptors modulates dopamine D2 receptor-induced responses in stably transfected human neuroblastoma cells. J Neurochem 2000;74:432–9.

[13] Kazmi SMI, Mishra RK. Comparative pharmacological properties and functional coupling of μ- and δ-opioid receptor sites in human neuroblastoma SH-SY5Y cells. Mol Pharmacol 1987;32:109–18.

[14] Moore DJ, Murdock PR, Watson JM, Faull RLM, Waldvogel HJ, Szekeres PG, et al. GPR105 a novel $G_{i/o}$-coupled UDP-glucose receptor expressed on brain glia and peripheral immune cells, is regulated by immunologic challenge: possible role in neuroimmune function. Mol Brain Res 2003;118:10–23.

[15] Wittenberger T, Schaller HC, Hellebrand S. An expressed sequence tag (EST) data mining strategy succeeding in the discovery of new G-protein-coupled receptors. J Mol Biol 2001;307:799–813.