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SPECIAL REPORT

Cloned and transfected $P2Y_4$ receptors: characterization of a suramin and PPADS-insensitive response to UTP

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The P2Y family of receptors are G protein-coupled receptors for ATP, ADP, UTP and UDP. Recently several members of this family have been cloned, including the $P2Y_4$, which is activated by UTP but not by ATP. In the present report, using receptors stably transfected into 1321N1 cells, we show that suramin acts as an antagonist at cloned $P2Y_1$ and (less potently) $P2Y_2$ receptors, but not at the cloned $P2Y_4$ receptor. Furthermore, PPADS (pyridoxal-phosphate-6-azophenyl-2',4'-disulphonic acid), a potent antagonist at the $P2Y_1$ receptor, is a relatively inneffective antagonist at the cloned $P2Y_4$ receptor. This work moves us closer to the goal of classifying the native P2Y receptors on the basis of agonist and antagonist profiles.

Keywords: P2Y receptors; P_{2Y}-purinoceptors; purinoceptors; pyrimidinoceptors

Introduction Recently a number of G protein-coupled P2 receptors (receptors for adenine and uridine nucleotides) have been cloned, including $P2Y_1$ (also called P_{2Y}) (e.g. Filtz *et al.*, 1994); $P2Y_2$ (P_{2U}) (e.g. Parr *et al.*, 1994); $P2Y_3$ (preferentially activated by UDP) (Webb *et al.*, 1996a); $P2Y_4$ (activated by UTP but not ATP) (e.g. Nguyen *et al.*, 1995); $P2Y_5$ (Webb *et al.*, 1996b); and $P2Y_6$ (UDP but not ATP) (Communi *et al.*, 1996). One of the current challenges in the P2 receptor field is to relate the cloned receptors to the diverse native responses using the relative potencies of various agonists and antagonists. Here we show that when compared with transfected $P2Y_1$ and $P2Y_2$ receptors, the transfected $P2Y_4$ receptor has a unique profile with respect to antagonist activity. This provides the potential to relate further native and cloned receptors, and shows that previously described suramin-insensitive responses to UTP may be due to action at $P2Y_4$ receptors.

Methods Procedures were essentially as described in Charlton *et al.* (1996). Lines of 1321N1 cells stably transfected with turkey $P2Y_1$ receptors (t-$P2Y_1$-1321N1 cells), human $P2Y_2$ receptors (h-$P2Y_2$-1321N1 cells) or human $P2Y_4$ receptors (h-$P2Y_4$-1321N1 cells) have been described previously (Parr *et al.*, 1994; Filtz *et al.*, 1994; Nguyen *et al.*, 1995; Charlton *et al.*, 1996). Cells were labelled for 24 h with [2-^{3}H]-inositol (1 μCi ml^{-1}, 0.5 ml per well) in medium M199 and stimulated by addition of agonists to this medium. When included, antagonists were present as described previously, except that in some cases the preincubation period was for 1 h instead of 10 min. No difference in results was seen between these two procedures. Total [^{3}H]-inositol (poly)phosphates ([^{3}H]$InsP_x$) were extracted into 0.5 M trichloroacetic acid, and after ether washes to remove the acid, were purified on small Dowex-1 (Cl^-) columns. The details of the experimental procedures and data analysis were as in Charlton *et al.* (1996); curve fitting and parameter derivation were by Graph-Pad Prism.

Results Figure 1 shows the concentration-response curves for stimulation of [^{3}H]-$InsP_x$ by UTP, UDP, ATP, ADP and 2MeSATP acting at the h-$P2Y_4$-1321N1 cells. The potency of UTP (log $EC_{50} = -5.94 \pm 0.07$; $EC_{50} = 1.17$ μM) was considerably greater than that for the other agonists. UDP was significantly less potent than UTP, and ATP did not reach a maximal response with the highest concentration used.

Figure 2 (a-c) compares suramin as an antagonist at $P2Y_1$, $P2Y_2$ and $P2Y_4$ receptors. Concentration-response curves for stimulation of the t-$P2Y_1$-1321N1 cells by 2MeSATP (Figure 2a) and h-$P2Y_2$-1321N1 cells by UTP (Figure 2b) generated Schild plots with slopes of 0.86 ± 0.04 and 0.66 ± 0.05, and pA_2 values of 5.75 ± 0.46 and 4.26 ± 1.09 respectively. UTP concentration-response curves for stimulation of h-$P2Y_4$-1321N1 cells in the presence of increasing concentrations of suramin are shown in Figure 2c. There was no effect of suramin on maximum responses, and pooled across 3 separate experiments the log EC_{50} values for the UTP response were -6.21 ± 0.13, -6.70 ± 0.38, -6.63 ± 0.16, and -6.58 ± 0.06 (0, 30, 100 and 300 μM suramin respectively). There were no significant effects of suramin, as determined by analysis of variance and Dunnett's multiple range tests. The tendency of suramin to shift curves slightly to the left may be due to inhibition of UTP breakdown in the absence of any antagonism at the receptor. We have shown with these cells a very small effect of agonist breakdown by ectonucleotidases on the concentration effect curves (Charlton *et al.*, 1996). The main conclusion from this series of experiments is that suramin is not an antagonist at the transfected h-$P2Y_4$ receptor.

Figure 2 (d-e) examines the effect of PPADS on the h-$P2Y_4$ receptor. Pooled across 3 separate experiments the log EC_{50} values for UTP with h-$P2Y_4$-1321N1 cells were 6.15 ± 0.005 in the absence of PPADS and 6.82 ± 0.17 in the presence of 30 μM PPADS ($P < 0.05$; Student's paired t test), showing that PPADS produced a slight shift of the curve to the left (Figure 2d). There was also a small effect on the maximal response (to 100 μM UTP), which was reduced to 82.5% by 30 μM PPADS ($P < 0.025$; Student's t test). This is consistent with the effect of increasing concentrations of PPADS when the h-$P2Y_4$-1321N1 cells were stimulated with 10 μM UTP (Figure 2e).

Discussion The nature of the receptor transfected into the h-$P2Y_4$-1321N1 cells was confirmed by data in Figure 1 showing a rank order of agonist potency of UTP > UDP > ATP, consistent with the results of Nguyen *et al.* (1995). This unique rank order of agonist potencies confirms this receptor as a pyrimidinoceptor. Thus, responses to UTP in native systems may be at receptors for both ATP and UTP, such as the $P2Y_2$ receptors, or alternatively at receptors for UTP but not ATP, such as $P2Y_4$. We have shown in this report that these two P2Y receptor subtypes may be distinguished from

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each other, and from $P2Y_1$, by the antagonist profile of suramin and PPADS.

The PPADS data presented here extends earlier reports that the $P2Y_1$ receptor was sensitive to antagonism by PPADS with a pA_2 of 6, while the $P2Y_2$ receptor was unaffected by the presence of 30 μM PPADS (Brown *et al.*, 1995; Charlton *et al.*, 1996; Ralevic & Burnstock, 1996). Here we show that PPADS has a modest influence on the cloned and transfected $P2Y_4$ receptor, producing both a small shift of the concentration-response curve to the left and a small reduction in the maximum response. The reduction in EC_{50} indicates that the compound lacks any competitive antagonist action, and may be due to a residual effect on attenuating agonist breakdown, as explored in Charlton *et al.* (1996). These results show that PPADS can distinguish responses at the $P2Y_4$ receptor from those at $P2Y_1$ but not those at $P2Y_2$ receptors.

We show here that suramin acts as an antagonist at $P2Y_1$ and $P2Y_2$ receptors with pA_2 values of 5.7 and 4.3, respectively, confirming the differential potency of suramin at these two receptors reported by Charlton *et al.* (1996). In this report we also show that suramin is not an antagonist at the $P2Y_4$ receptor. This distinguishes the $P2Y_4$ receptor from the recently cloned receptor for UTP ($P2Y_3$), where suramin had an estimated pA_2 value of 5 (Webb *et al.*, 1996a), and from the possibly related $P2Y_6$ receptor, which has also been reported to show some suramin sensitivity (Chang *et al.*, 1995). Thus, the characterization of the cloned receptors so far indicates that where ATP and UTP are both effective agonists and suramin is a low potency antagonist, this is likely to be the $P2Y_2$ receptor. When UTP/UDP but not ATP are effective agonists a suramin-insensitive response is likely to be $P2Y_4$, but a suramin-sensitive response may be $P2Y_3$, or $P2Y_6$. The $P2Y_4$ and $P2Y_3$/$P2Y_6$ receptors may also be distinguished by the relative potency of UTP and UDP.

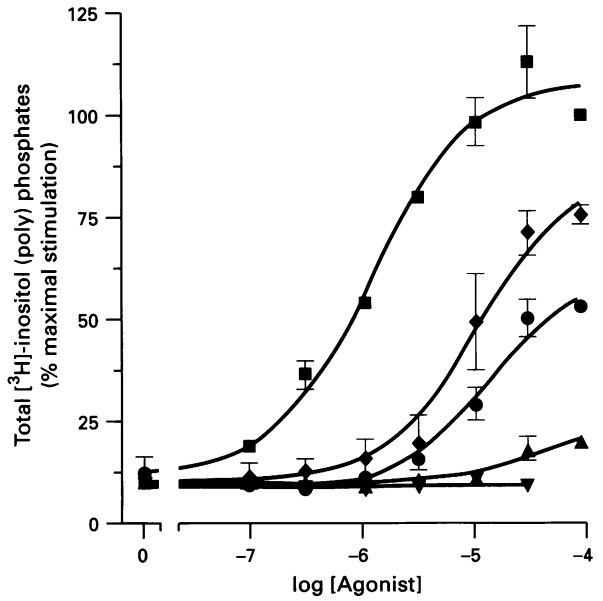

Figure 1 Concentration-response curves for various agonists at the transfected $P2Y_4$ receptor: UTP (■), UDP (◆), ATP (●), ADP (▲), and 2MeSATP (▼). Data are mean ± s.e.mean from 3 separate experiments each in triplicate.

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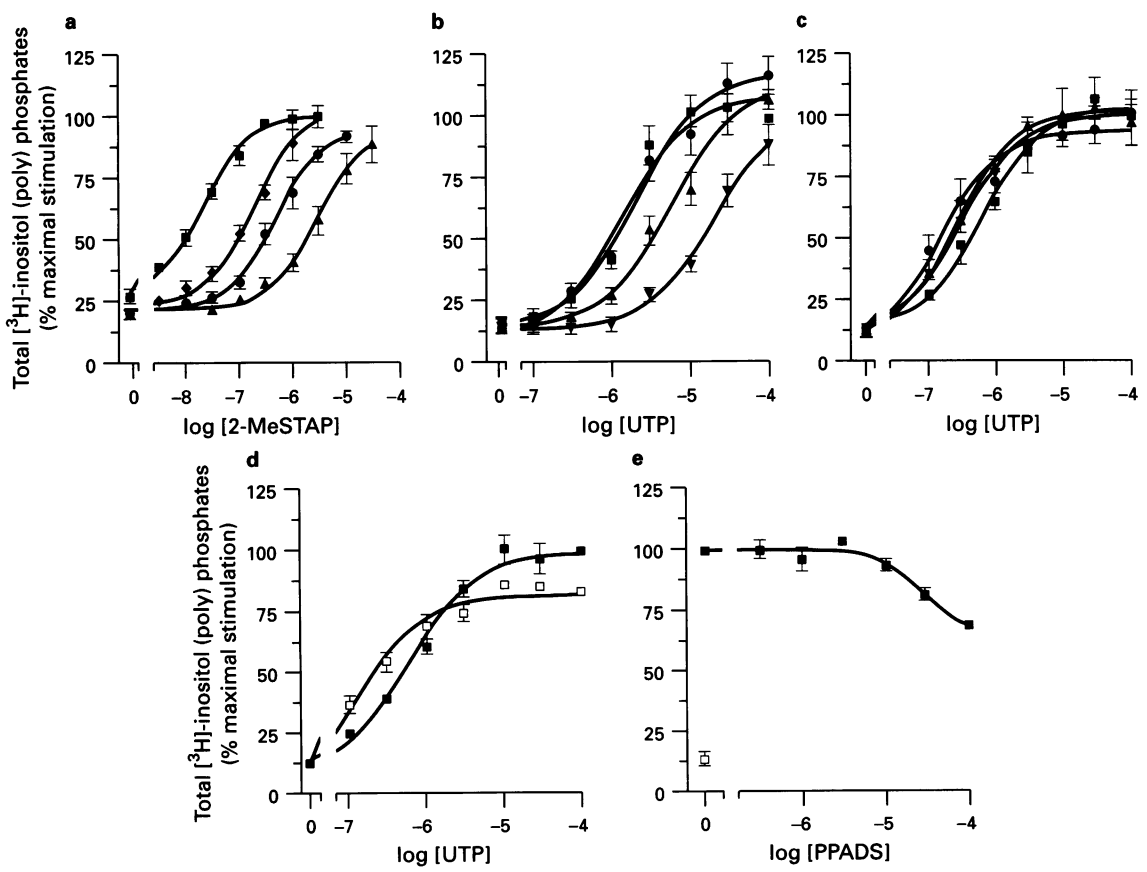

Figure 2 Antagonism by suramin and PPADS at transfected P2Y purinoceptors. (a–c) Show concentration-response curves for 2-MeSATP at $P2Y_1$ (a), and UTP at $P2Y_2$ (b) and $P2Y_4$ (c) receptors with no antagonist (■) or in the presence of suramin at 10 (◆), 30 (●), 100 (▲) or 300 (▼) μM. (d) Concentration-response curves to UTP at the $P2Y_4$ receptor in the presence (□) or absence (■) of 30 μM PPADS; (e) response to 10 μM UTP in the presence of increasing concentrations of PPADS (■); data from no UTP and no PPADS control are also shown (□). In each case data are pooled from 3 or 4 separate experiments (mean ± s.e.mean) each in triplicate.

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