Available online at www.sciencedirect.com
SCIENCE DIRECT®
European Journal of Pharmacology 468 (2003) 9–14
www.elsevier.com/locate/ejphar

Short communication

P2Y receptor specific for diadenosine tetraphosphate in lung: selective inhibition by suramin, PPADS, Ip_5I, and not by MRS-2197

Werner Laubinger[a], Hong Wang[a], Tobias Welte[b], Georg Reiser[a,*]

[a] *Institut für Neurobiochemie, Fakultät für Medizin, Otto-von-Guericke Universität, Leipziger Str. 44, 39120, Magdeburg, Germany*
[b] *Klinik für Kardiologie, Angiologie und Pneumologie, Fakultät für Medizin, Otto-von-Guericke Universität, Leipziger Str. 44, 39120, Magdeburg, Germany*

Received 10 February 2003; received in revised form 10 March 2003; accepted 14 March 2003

Abstract

Extracellular dinucleotides, which act as signaling molecules in a variety of systems, may regulate fluid homeostasis in the human lung by activation of a specific P2Y receptor subtype. Previously, we presented evidence for a G protein-coupled P2Y receptor with high affinity for dinucleotides in both rat and human lung tissue. In a human bronchial epithelial cell line (HBE-1), diadenosine polyphosphates (Ap_nA, n=2–6) increase intracellular Ca^{2+}. The aim of the present work was to find additional evidence that, in these cells, the receptors selectively activated by diadenosine polyphosphates are distinct from already known P2Y receptors, which are activated by the mononucleotides ATP or UTP. We tested antagonists suitable to classify P2Y receptor subtypes. The $P2Y_1$ receptor-selective antagonist 2′-deoxy-N^6-methyl adenosine 3′,5′-diphosphate (MRS-2197) did not affect Ca^{2+} mobilization induced by diadenosine tetraphosphate (Ap_4A). However, suramin, pyridoxal phosphate-6-azo(benzene-2,4-disulfonic acid) (PPADS) and diinosine pentaphosphate (Ip_5I) inhibited the Ca^{2+} response by 96%, 92% and 32%, respectively. Moreover, these results were confirmed by assessing the specific binding of [^{3}H]Ap_4A to membranes from human and rat lung. Suramin (100 μM), PPADS (400 μM) and Ip_5I (200 μM), reduced [^{3}H]Ap_4A binding in lung membrane preparations by 66%, 77% and 80%, respectively. The Ap_4A-induced Ca^{2+} response in HBE-1 cells was inhibited to a much greater extent by these antagonists than the ATP- or UTP-evoked Ca^{2+} rise. Thus, Ap_4A in lung epithelial cells also activates a still unidentified P2Y receptor that is specific for dinucleotides over mononucleotides.

Keywords: Ca^{2+} mobilization; Diadenosine polyphosphate; Diinosine pentaphosphate; Evans blue; G protein-coupled receptor; MRS-2197; P2 receptor; PPADS; Reactive blue 2; Suramin

1. Introduction

P2 receptors, which are activated by the extracellular nucleotides ATP, UTP, ADP or UDP, play a crucial role in the regulation of a variety of cellular signaling processes. They are classified into two families: ligand-gated ion channels (P2X) and G protein-coupled receptors (P2Y), which frequently stimulate phospholipase C (Dubyak and el Moatassim, 1993). In lung physiology, chloride transport, secretion of mucin and stimulation of ciliary activity are regulated through $P2Y_2$ receptors located in the apical membrane of epithelial cells. $P2Y_2$ receptors are equipotently activated by ATP and UTP (Morse et al., 2001; Yamaya et al., 1996). UTP also activates the $P2Y_4$ receptor subtype and ATP the $P2Y_{11}$ receptor subtype, whereas the $P2Y_1$, $P2Y_{12}$ and $P2Y_{13}$ subtypes are potently activated by the nucleoside diphosphate ADP, and $P2Y_6$ by UDP (Ralevic and Burnstock, 1998).

In the last few years, P2-like receptors specific for diadenosine polyphosphates have been found in several tissues, such as brain, lung, heart and blood vessels (Blouse et al., 1998; Hohage et al., 1996; Laubinger and Reiser, 1999; Pintor and Miras-Portugal, 1995). Dinucleotides, like diadenosine tetraphosphate (Ap_4A) or diadenosine pentaphosphate (Ap_5A), are a novel class of relatively stable extracellular signaling nucleotides (Kisselev et al., 1998; Ogilvie et al., 1996). They are supposed to be also responsible for regulatory processes in the lung, such as mucociliary clearance, ion transport and control of fluid homeostasis. Extracellular nucleotides are of potential clinical use for the treatment of genetic defects, such as those in cystic fibrosis. In cystic fibrosis, the cystic fibrosis transmembrane con-

* Corresponding author. Tel.: +49-391-67-13088; fax: +49-391-6713097.
E-mail address: Georg.Reiser@medizin.uni-magdeburg.de (G. Reiser).

0014-2999/03/$ - see front matter © 2003 Elsevier Science B.V. All rights reserved.
doi:10.1016/S0014-2999(03)01624-8

ductance regulator (CFTR) has a molecular defect in the inbuilt Cl^- channels (Hwang et al., 1996; Olivier et al., 1996). Furthermore, diadenosine polyphosphates can cause vasodilation or vasoconstriction in blood vessels, according to the receptor type activated (Schlüter et al., 1994; Steinmetz et al., 2000). For this reason, receptors specific for diadenosine polyphosphates in lung are of special interest as therapeutic target in pathophysiological conditions with disturbed ion fluxes or hypertonicity. However, despite extensive efforts during the last decade, so far, convincing evidence for the existence of a distinct P2Y receptor subtype for diadenosine polyphosphates is still lacking (Lazarowski et al., 1995).

Recently, we identified receptor binding sites with high affinity for diadenosine polyphosphates both in lung tissue and in cell lines derived from the respiratory system. [^{3}H]Ap_4A binding sites in rat and human lung were found to be coupled to G proteins and showed pharmacological characteristics different from those of P2X or $P2Y_2$ receptors (Laubinger and Reiser, 1998, 1999; Laubinger et al., 1999). In human bronchial epithelial cells (HBE-1), we were able to show that besides the P2Y receptors already described ($P2Y_2$, $P2Y_4$ and $P2Y_6$) (Communi et al., 1999), there are also P2Y receptors which are activated by diadenosine polyphosphates (Laubinger et al., 2001). Although the characteristics of the Ca^{2+} rise evoked by Ap_4A were different from those evoked by ATP or UTP, in those investigations, it was not possible to conclude unequivocally that a distinct receptor for diadenosine polyphosphates exists in these cells. Therefore, the goal of our present experiments was to extend previous studies of a P2Y receptor activated by diadenosine polyphosphates in lung, by using P2 receptor antagonists (Von Kügelgen and Wetter, 2000). Our results provide further evidence for the existence of a distinct P2Y receptor for diadenosine polyphosphates in lung.

2. Materials and methods

2.1. Materials

ATP, diadenosine tetraphosphate (Ap_4A), diinosine pentaphosphate (Ip_5I), UTP, suramin, Evans blue and pyridoxal phosphate-6-azo(benzene-2,4-disulfonic acid) (PPADS) were from Sigma. Fura-2 acetoxymethylester (Fura-2/AM) was from Alexis. Reactive blue 2 and 2′-deoxy-N^6-methyl adenosine 3′,5′-diphosphate (MRS-2197) were from RBI.

2.2. Cell culture and Ca^{2+} measurement

The HBE-1 cell line derived from human bronchial epithelial cells has been described in detail elsewhere (Yankaskas et al., 1993). Cells were cultured with 5% CO_2 at a temperature of 37 °C in medium containing Dulbecco's Modified Eagle's Medium/NUT MIX F12 (1:1) as described earlier (Laubinger et al., 2001). Cells were grown to 70–90% confluency and then subcultured.

HBE-1 cells were seeded onto glass coverslips and cultured for 5–6 days. Cells with 40–60% confluency were used for single-cell measurement of $[Ca^{2+}]_i$. Before measurement, cells were preincubated with 2 μM Fura-2/acetoxymethylester in Na–HBS (HEPES-buffered saline; 145 mM NaCl, 5.4 mM KCl, 1.8 mM $CaCl_2$, 1 mM $MgCl_2$, 25 mM glucose, 20 mM HEPES/Tris pH 7.4) for 30 min at 37 °C. Fluorescence was recorded alternately at 340 and 380 nm excitation and 520 nm emission. Changes were monitored at 37 °C in a perfusion chamber on a fluorescence imaging system from TILL Photonics with a X40 immersion objective and a flow rate of 2 ml/min, as described earlier (Ubl et al., 1998).

2.3. Binding of [^{3}H]Ap_4A to lung membranes

Membranes from rat or human lung were prepared as described earlier in detail (Laubinger and Reiser, 1998). The study using the human material was approved by the Ethics Committee of the University of Magdeburg and was done in accordance with the principles laid down in the Declaration of Helsinki. Binding of [^{3}H]Ap_4A was measured by incubation of lung membranes (50 μg of protein) in incubation medium containing 25 mM HEPES, pH 7.4, 50 mM NaCl and 5 mM KCl for 35 min at 4 °C, as described before (Laubinger and Reiser, 1999). Unless stated otherwise, experiments were performed with triplicate determinations.

3. Results

In a previous investigation of the P2Y receptor-mediated increase of intracellular Ca^{2+} in human bronchial epithelial cells (HBE-1), we found evidence for the existence of a P2Y receptor subtype activated by Ap_4A which is different from the $P2Y_2$ receptor. It is known that the latter can also be activated by Ap_4A (Lazarowski et al., 1995). Fig. 1 compares the magnitude of the Ca^{2+} responses induced by the agonists ATP (10 μM), UTP (10 μM) or Ap_4A (100 μM) in the absence (control) or presence of antagonists. Both suramin and PPADS were very effective antagonists of the putative diadenosine polyphosphate receptor, with 96% inhibition for suramin and 92% inhibition for PPADS, respectively. The $[Ca^{2+}]_i$ rise evoked by Ap_4A was inhibited more than the Ca^{2+} rise evoked by ATP or UTP. In the presence of 100 μM suramin, the intracellular Ca^{2+} rise evoked by ATP or UTP was inhibited only by 38% and 45%, respectively, and in the presence of 400 μM PPADS, it was inhibited by 41% and 47%, respectively. Addition of MRS-2179, an antagonist which is reported to be selective for the $P2Y_1$ receptor subtype (Baurand et al., 2001), did not affect significantly the rise of intracellular Ca^{2+} evoked by Ap_4A, whereas the $[Ca^{2+}]_i$ rise caused by ATP or UTP was moderately decreased, by 28% and 16%, respectively. Ip_5I,

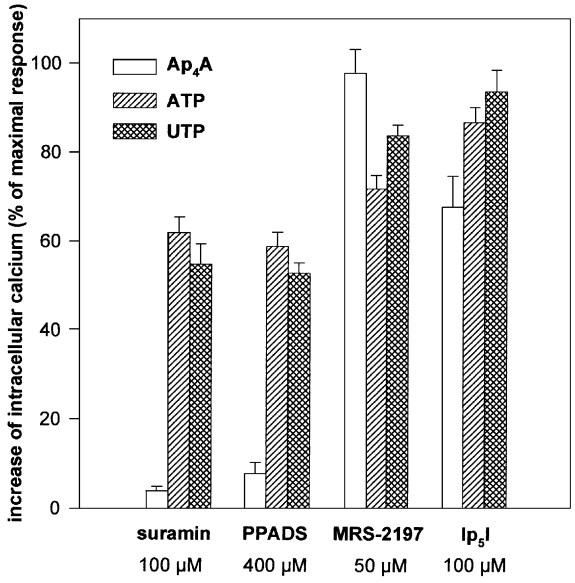

Fig. 1. P2 receptor antagonists block intracellular Ca^{2+} rise in HBE-1 cells evoked by Ap_4A, ATP or UTP. HBE-1 cells were treated in absence or presence of suramin, PPADS, MRS-2179 or Ip_5I for 30 min at 37 °C and then stimulated for 1 min with 100 µM Ap_4A (white bar), 10 µM ATP (hatched bar) or 10 µM UTP (checked bar). Cells were superfused with buffer (control) or antagonists during Ca^{2+} measurement. Influence of the agonists is given as percentage of the maximal response induced by the respective nucleotides in the absence of antagonist (F_{340}/F_{380} of the maximal response was 0.837±0.130, 1.413±0.234 and 1.560±0.154 for stimulation of the cells with Ap_4A, ATP and UTP, respectively). Data are from single cell Ca^{2+} recordings and represent the mean±S.E. for at least 50 cells for each value.

a dinucleotide described to be a selective antagonist for some receptors activated by diadenosine polyphosphates (Pereira et al., 2000), diminished the Ca^{2+} increase evoked by Ap_4A by 32%, whereas the Ca^{2+} increase evoked by the mononucleotides ATP or UTP was only slightly inhibited, by 13% and 6%, respectively. The dinucleotide Ip_5I itself was not able to evoke a Ca^{2+} response when applied to HBE-1 cells in the absence of Ap_4A (data not shown). Thus, the difference in sensitivity to various P2 receptor antagonists of responses activated by mono- and dinucleotides provides evidence for the existence of a distinct receptor subtype specific for diadenosine polyphosphates in HBE-1 cells.

To determine the mechanism by which the Ca^{2+} rise was reduced in the presence of suramin, PPADS or Ip_5I, we examined whether these antagonists directly act on the Ap_4A-activated receptor. In human and rat lung membranes, we recently identified high-affinity binding sites for [^{3}H]Ap_4A (K_d approximately 90 nM). These binding sites were coupled to G proteins and showed pharmacological characteristics different from those of the $P2Y_2$ receptor which is also present in lung tissue (Laubinger and Reiser, 1998, 1999; Laubinger et al., 1999). In the present study, we investigated the effect of suramin on this Ap_4A binding site (Fig. 2A). Rat lung membranes (50 µg of protein) were incubated with [^{3}H]Ap_4A in the absence and in the presence of the antagonist suramin. The maximal binding of the radioactive ligand at a concentration of 10 nM was reduced by about 50% in the presence of 100 µM suramin. Displacement curves with unlabelled Ap_4A showed that half of the [^{3}H]Ap_4A bound was displaced from the binding site at a concentration between 100 and 200 nM of unlabelled Ap_4A.

Binding of [^{3}H]Ap_4A to rat or human lung membranes was reduced concentration-dependently by suramin (Fig. 2B). In rat lung membranes, 10 µM suramin caused a weak inhibition only (about 30%), whereas maximal inhibition (50–60% of total specific binding) was reached at an antagonist concentration between 50 and 100 µM. In human lung membranes, inhibition occurred already at lower con-

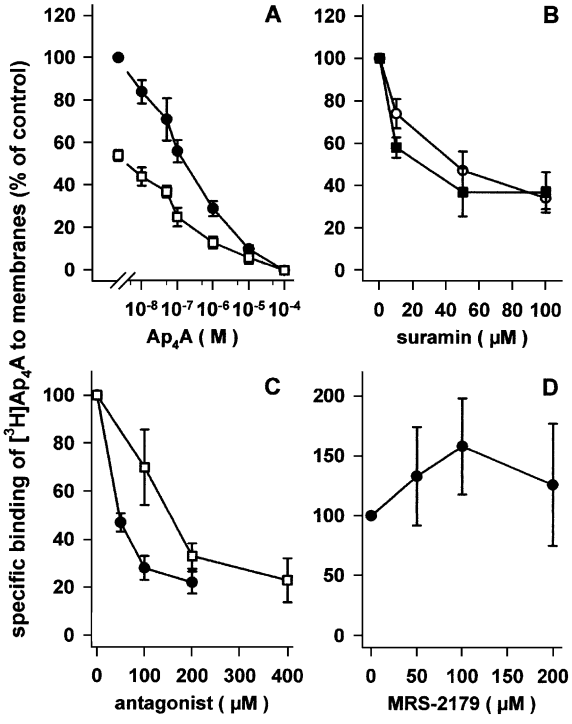

Fig. 2. Influence of P2 receptor antagonists on [^{3}H]Ap_4A binding in membranes from rat and human lung. (A) Influence of suramin on [^{3}H]Ap_4A binding in rat lung membranes. Lung membranes (50 µg of protein) were incubated with 10 nM [^{3}H]Ap_4A and increasing concentrations of Ap_4A in the absence (●) or presence of 100 µM suramin (□), as described in Materials and methods. Data shown are mean values±S.D. from five independent experiments. (B) Effect of suramin on Ap_4A binding in membranes from human and rat lung. Membranes from rat (○) or human (■) lung were incubated in the presence of 10 nM [^{3}H]Ap_4A with increasing amounts of suramin, as described in Materials and methods. Data points represent mean values from five (human lung) and ten (rat lung) independent experiments. Error bars indicate S.D. (C) Binding of [^{3}H]Ap_4A to lung membranes in the presence of the P2 receptor antagonists PPADS and Ip_5I. Lung membranes (50 µg of protein) were incubated in the presence of 10 nM [^{3}H]Ap_4A and increasing amounts of PPADS (□) or Ip_5I (●), as described in Materials and methods. Data are mean values±S.D. from four independent experiments with human (□) or three independent experiments with rat (●) lung membrane preparations. (D) Influence of MRS-2179 on [^{3}H]Ap_4A binding in human lung membranes. Membranes prepared from human lung (50 µg of protein) were incubated with 10 nM [^{3}H]Ap_4A in the presence of increasing amounts of MRS-2179. The data shown are mean values±S.D. derived from three independent experiments.

centrations of suramin, but the maximal inhibition at 100 μM suramin was identical with that obtained in rat lung membranes. [^{3}H]Ap_4A binding was strongly inhibited in the presence of PPADS, another P2 receptor antagonist with limited selectivity (Fig. 2C). Addition of 400 μM PPADS reduced [^{3}H]Ap_4A binding to human lung membranes to 23% of the control value.

Next, Ip_5I was used in the binding experiments. [^{3}H]Ap_4A binding to rat lung membranes was inhibited by about 80% at 200 μM Ip_5I (Fig. 2C). A similar inhibition of Ap_4A binding in the presence of Ip_5I was seen when human lung membranes were used (data not shown). Consistent with the results obtained from Ca^{2+} response measurements with Ap_4A, MRS-2179 at concentrations between 50 and 200 μM did not inhibit [^{3}H]Ap_4A binding to lung membranes, but on the contrary even displayed a slight stimulatory effect (Fig. 2D). This apparent increase was, however, not statistically significant. Further, nonselective P2 receptor antagonists were also tested. Evans blue had no inhibitory effect on [^{3}H]Ap_4A binding, but rather stimulated binding compared with the control values, and Reactive blue 2 proved to be a partial antagonist, reducing [^{3}H]Ap_4A binding by about 50% at concentrations between 100 and 200 μM (data not shown).

4. Discussion

Dinucleotides like Ap_4A are known to act as intracellular and extracellular messenger molecules in a broad spectrum of signal transduction processes (Kisselev et al., 1998; Ogilvie et al., 1996). Dinucleotides can act as neurotransmitters (Castillo et al., 1992). They are also released from stores in human platelets, thus, regulating vascular tone (Schlüter et al., 1994). Furthermore, diadenosine polyphosphates are supposed to be important activators of P2 receptors in pathophysiologal regulatory processes in the lung (Lazarowski et al., 1995). Nevertheless, the nature of the receptor on which they act is still far from clear. Several purinoceptors can be activated by various adenine and/or uridine nucleotides and their analogues, according to a subtype-specific agonist profile. Moreover, in many cell types, several P2 receptor subtypes are co-expressed, which makes it difficult to attribute agonist or antagonist effects to only one distinct receptor subtype. Diadenosine polyphosphates are, most probably, also able to activate various purinoceptor subtypes.

In a preceding study, we provided evidence that dinucleotides activate a G protein-coupled P2Y receptor in lung that is different from the $P2Y_2$ receptor (Laubinger and Reiser, 1999; Laubinger et al., 2001). Evidence for a distinct receptor for diadenosine polyphosphates was also found in the heart (Walker et al., 1993). Other authors showed that the cloned $P2Y_2$ receptor subtype expressed in a heterologous system can be activated by Ap_4A with a potency equal to that of ATP or UTP (Lazarowski et al., 1995). Additionally, diadenosine polyphosphates are supposed to be potent agonists of some subtypes of the P2X receptor family (Pintor et al., 1997).

Unfortunately, so far, investigation of purinoceptors is hampered by the fact that there is a lack of antagonists that are specific for only one receptor subtype (Von Kügelgen and Wetter, 2000). This fact makes it difficult to distinguish distinct P2 receptor subtypes. Nevertheless, application of known antagonists is in some cases helpful to classify or distinguish P2 receptor subtypes. Thus, suramin is an unspecific inhibitor of nucleotide receptors and antagonizes Ca^{2+} responses evoked by ATP, UTP or ADP (Sabala et al., 2001). PPADS has been described as a selective antagonist of the $P2Y_1$ receptor (Schachter et al., 1997). However, PPADS is also a potent antagonist of the $P2X_7$ receptor in humans and rats, and a weak antagonist in mice (Hibell et al., 2001). In brain, suramin abolished P2 receptor-mediated responses to ATP in the midbrain and in the cerebellum, but not responses to dinucleotides. In cortical preparations, however, suramin at a concentration of 100 μM blocked responses both to mono- and dinucleotides (Pintor et al., 1997). In mouse neuroblastoma x rat glioma hybrid cell line, NG108-15, suramin, Reactive blue 2 and PPADS antagonized the phospholipase C response to both UTP and UDP (Sak et al., 2001). These species and tissue differences in antagonist potency in some cases make the interpretation of data difficult.

In the central nervous system, diadenosine polyphosphates act as neuromodulators, releasing acetylcholine mainly through activation of diadenosine polyphosphate receptors. However, in this case, neither suramin (100 μM) nor PPADS (10 μM) cause a remarkable inhibition (only 18–24% inhibition) (Pereira et al., 2000). Sometimes there are also differences between the effects of suramin and PPADS, as shown for the cloned $P2Y_{11}$ receptor, which is the only P2Y receptor known so far which is positively coupled to the adenylyl cyclase pathway. In stably transfected haematopoietic cell lines, the ATP-induced elevation of cAMP was inhibited by 500 μM suramin but not by 100 μM PPADS (Van der Weyden et al., 2000).

Recently, we have shown that in human bronchial epithelial cells (HBE-1), there are receptors which can be activated by mononucleotides (ATP, UTP) and dinucleotides (Ap_4A) in a different manner: (1) In single cell measurements, cells were less sensitive to diadenosine polyphosphates than to ATP or UTP, (2) the EC_{50} value for Ap_4A (17 μM) was one order of magnitude higher than that for ATP or UTP (1.5 and 1.8 μM, respectively), (3) a small part of the Ap_4A-induced Ca^{2+} rise resulted from Ca^{2+} flow through the plasma membrane, whereas Ca^{2+} mobilized by ATP or UTP exclusively came from internal Ca^{2+} stores. Therefore, we concluded that there is a receptor for diadenosine polyphosphates that is distinct from the $P2Y_2$ receptor (Laubinger et al., 2001). Additionally, in these cells, [^{3}H]Ap_4A was displaced from membrane binding sites by unlabelled Ap_4A, but was only partially displaced by ATP

and not at all by UTP. Testing here the effects of P2 receptor antagonists on [^{3}H]Ap_4A binding in lung membranes and on Ca^{2+} release in HBE-1 cells, we found that PPADS and suramin were potent antagonists of the putative Ap_4A receptor. However, Reactive blue 2 was only a partial antagonist and Evans blue had no inhibitory effect at all on this receptor. Our results are compatible with reports on [^{3}H]Ap_4A binding sites in cultured vascular smooth muscle cells and endothelial cells, where [^{3}H]Ap_4A was displaced from its binding site by high concentrations of suramin or PPADS, respectively (Verspohl et al., 1999). From these data, it was assumed that, besides the $P2Y_2$ receptor subtype, either further subtypes of P2Y receptors or P2X receptors were involved in the binding of diadenosine polyphosphates in lung cells.

In HBE-1 cells, MRS-2179, a potent $P2Y_1$ receptor antagonist (Baurand et al., 2001; Nandanan et al., 2000), clearly had no effect on the Ca^{2+} rise evoked by Ap_4A and only a minimal inhibitory effect on that evoked by ATP or UTP. As HBE-1 cells have been reported to possess no $P2Y_1$ receptors (Communi et al., 1999), MSR-2179 might also be a partial antagonist of the $P2Y_2$ receptor but not of the putative Ap_4A-specific P2Y receptor. The effect of the antagonists suramin and PPADS on [^{3}H]Ap_4A binding in lung membranes was confirmed by our functional experiments using HBE-1 cells. In the present work, we could show that receptors on HBE-1 cells activated by diadenosine polyphosphates were much more sensitive to suramin, PPADS or Ip_5I than those receptors activated by ATP or UTP. This clear difference excludes $P2Y_2$ receptors from being responsible for Ap_4A activity in HBE-1 cells.

In the past, Ip_5I has been described as an antagonist especially of the diadenosine polyphosphate-activated receptor subtype of the P2X receptor family ($P2X_3$ receptor) (Dunn et al., 2000; Pereira et al., 2000). In this study, we show that the binding of [^{3}H]Ap_4A to the G protein-coupled putative $P2Y_{Ap4A}$ receptor in lung was also strongly inhibited by Ip_5I. Furthermore, the Ca^{2+} increase evoked by Ap_4A was partially diminished in the presence of this antagonist. However, the decrease in Ca^{2+} response in the presence of Ip_5I was considerably smaller than would be expected from the strong inhibition of Ap_4A binding. A possible explanation for this discrepancy is a difference in the expression pattern of P2Y and P2X receptor subtypes between HBE-1 cells and lung membranes. If P2X receptor subtypes which also bind Ap_4A exist in the lung, the strong inhibition of [^{3}H]Ap_4A binding by Ip_5I could be attributed to there being more than one P2 receptor subtype.

By showing different effects of antagonists on Ca^{2+} mobilization caused by either the dinucleotide Ap_4A or the mononucleotides ATP or UTP, we here provide further evidence for the hypothesis that distinct receptors for diadenosine polyphosphates exist in the lung. The antagonists used directly act on the binding of Ap_4A to this membrane receptor. In contrast to other P2 receptors specific for diadenosine polyphosphates which were reported to belong to the P2X receptor family, this putative diadenosine polyphosphate receptor in the lung is G protein-coupled and therefore belongs to the P2Y receptor family. However, it is not identical with the $P2Y_2$ receptor that is also activated by Ap_4A. Thus, the present work is a further step in the identification of several P2 receptors activated by diadenosine polyphosphates in the lung.

Acknowledgements

HBE-1 cells were a generous gift from Dr. T. Meyer/Prof. Dr. L. Pott, Institut für Physiologie, Ruhr-Universität Bochum, Germany. We thank Dr. A. Schneider for culturing of HBE-1 cells. The work was supported BMBF (FZ 01ZZ0107), Deutsche Forschungsgemeinschaft (grant Re 563/11-1) and Fonds der Chemischen Industrie.

References

Baurand, A., Raboisson, P., Freund, M., Leon, C., Cazenave, J.P., Bourguignon, J.J., Gachet, C., 2001. Inhibition of platelet function by administration of MRS2179, a $P2Y_1$ receptor antagonist. Eur. J. Pharmacol. 412, 213–221.

Blouse, G.E., Liu, G., Hilderman, R.H., 1998. Characterization of ATP and P_2 agonists binding to the cardiac plasma membrane P^1, P^4-diadenosine 5′-tetraphosphate receptor. Biochim. Biophys. Acta 1375, 61–72.

Castillo, C.J., Moro, M.A., Del Valle, M., Sillero, A., Garcia, A.G., Sillero, M.A., 1992. Diadenosine tetraphosphate is co-released with ATP and catecholamines from bovine adrenal medulla. J. Neurochem. 59, 723–732.

Communi, D., Paindavoine, P., Place, G.A., Parmentier, M., Boeynaems, J.M., 1999. Expression of P2Y receptors in cell lines derived from the human lung. Br. J. Pharmacol. 127, 562–568.

Dubyak, G.R., el Moatassim, C., 1993. Signal transduction via P2-purinergic receptors for extracellular ATP and other nucleotides. Am. J. Physiol., Cell Physiol. 265, C577–C606.

Dunn, P.M., Liu, M., Zhong, Y., King, B.F., Burnstock, G., 2000. Diinosine pentaphosphate: an antagonist which discriminates between recombinant $P2X_3$ and $P2X_{2/3}$ receptors and between two P2X receptors in rat sensory neurones. Br. J. Pharmacol. 130, 1378–1384.

Hibell, A.D., Thompson, K.M., Xing, M., Humphrey, P.P., Michel, A.D., 2001. Complexities of measuring antagonist potency at P2X7 receptor orthologs. J. Pharmacol. Exp. Ther. 296, 947–957.

Hohage, H., Reinhardt, C., Borucki, U., Enck, G., Schluter, H., Schlatter, E., Zidek, W., 1996. Effects of diadenosine polyphosphates on renal function and blood pressure in anesthetized Wistar rats. J. Am. Soc. Nephrol. 7, 1216–1222.

Hwang, T.H., Schwiebert, E.M., Guggino, W.B., 1996. Apical and basolateral ATP stimulates tracheal epithelial chloride secretion via multiple purinergic receptors. Am. J. Physiol., Cell Physiol. 270, C1611–C1623.

Kisselev, L.L., Justesen, J., Wolfson, A.D., Frolova, L.Y., 1998. Diadenosine oligophosphates (Ap_nA), a novel class of signalling molecules? FEBS Lett. 427, 157–163.

Laubinger, W., Reiser, G., 1998. Differential characterization of binding sites for adenine and uridine nucleotides in membranes from rat lung as possible tools for studying P2 receptors in lung. Biochem. Pharmacol. 55, 687–695.

Laubinger, W., Reiser, G., 1999. Evidence for a G protein-coupled diadenosine-5′,5‴-P^1,P^4-tetraphosphate (Ap_4A) receptor binding site in lung membranes from rat. Eur. J. Pharmacol. 366, 93–100.

Laubinger, W., Welte, T., Streubel, G., Schäfer, H.J., Reiser, G., 1999. In human and rat lung membranes [^{35}S]GTPγS binding is a tool for pharmacological characterization of G protein-coupled dinucleotide receptors. Life Sci. 65, PL183–PL190.

Laubinger, W., Streubel, G., Reiser, G., 2001. Physiological evidence for a P2Y receptor responsive to diadenosine polyphosphates in human lung via Ca^{2+} release studies in bronchial epithelial cells. Biochem. Pharmacol. 61, 623–629.

Lazarowski, E.R., Watt, W.C., Stutts, M.J., Boucher, R.C., Harden, T.K., 1995. Pharmacological selectivity of the cloned human P_{2U}-purinoceptor: potent activation by diadenosine tetraphosphate. Br. J. Pharmacol. 116, 1619–1627.

Morse, D.M., Smullen, J.L., Davis, C.W., 2001. Differential effects of UTP, ATP, and adenosine on ciliary activity of human nasal epithelial cells. Am. J. Physiol., Cell Physiol. 280, C1485–C1497.

Nandanan, E., Jang, S.Y., Moro, S., Kim, H.O., Siddiqui, M.A., Russ, P., Marquez, V.E., Busson, R., Herdewijn, P., Harden, T.K., Boyer, J.L., Jacobson, K.A., 2000. Synthesis, biological activity, and molecular modeling of ribose-modified deoxyadenosine bisphosphate analogues as $P2Y_1$ receptor ligands. J. Med. Chem. 43, 829–842.

Ogilvie, A., Blasius, R., Schulze-Lohoff, E., Sterzel, R.B., 1996. Adenine dinucleotides: a novel class of signalling molecules. J. Auton. Pharmacol. 16, 325–328.

Olivier, K.N., Bennett, W.D., Hohneker, K.W., Zeman, K.L., Edwards, L.J., Boucher, R.C., Knowles, M.R., 1996. Acute safety and effects on mucociliary clearance of aerosolized uridine 5′-triphosphate+/−amiloride in normal human adults. Am. J. Respir. Crit. Care Med. 154, 217–223.

Pereira, M.F., Hernandez, M.D., Pintor, J., Miras-Portugal, M.T., Cunha, R.A., Ribeiro, J.A., 2000. Diadenosine polyphosphates facilitate the evoked release of acetylcholine from rat hippocampal nerve terminals. Brain Res. 879, 50–54.

Pintor, J., Miras-Portugal, M.T., 1995. P2 purinergic receptors for diadenosine polyphosphates in the nervous system. Gen. Pharmacol. 26, 229–235.

Pintor, J., Puche, J.A., Gualix, J., Hoyle, C.H., Miras-Portugal, M.T., 1997. Diadenosine polyphosphates evoke Ca^{2+} transients in guinea-pig brain via receptors distinct from those for ATP. J. Physiol. 504, 327–335.

Ralevic, V., Burnstock, G., 1998. Receptors for purines and pyrimidines. Pharmacol. Rev. 50, 413–492.

Sabala, P., Czajkowski, R., Przybyek, K., Kalita, K., Kaczmarek, L., Baranska, J., 2001. Two subtypes of G protein-coupled nucleotide receptors, $P2Y_1$ and $P2Y_2$ are involved in calcium signalling in glioma C6 cells. Br. J. Pharmacol. 132, 393–402.

Sak, K., Samuel, K., Kelve, M., Webb, T.E., 2001. Pharmacological characterisation of pyrimidinoceptor responses in NG108-15 cells. Eur. J. Pharmacol. 415, 127–133.

Schachter, J.B., Sromek, S.M., Nicholas, R.A., Harden, T.K., 1997. HEK293 human embryonic kidney cells endogenously express the $P2Y_1$ and $P2Y_2$ receptors. Neuropharmacology 36, 1181–1187.

Schlüter, H., Offers, E., Bruggemann, G., van der Giet, M., Tepel, M., Nordhoff, E., Karas, M., Spieker, C., Witzel, H., Zidek, W., 1994. Diadenosine phosphates and the physiological control of blood pressure. Nature 367, 186–188.

Steinmetz, M., Bierer, S., Hollah, P., Rahn, K.H., Schlatter, E., 2000. Heterogenous vascular effects of AP5A in different rat resistance arteries are due to heterogenous distribution of P2X and $P2Y_1$ purinoceptors. J. Pharmacol. Exp. Ther. 294, 1182–1187.

Ubl, J.J., Vöhringer, C., Reiser, G., 1998. Co-existence of two types of $[Ca^{2+}]_i$-inducing protease-activated receptors (PAR-1 and PAR-2) in rat astrocytes and C6 glioma cells. Neuroscience 86, 597–609.

Van der Weyden, L., Adams, D.J., Luttrell, B.M., Conigrave, A.D., Morris, M.B., 2000. Pharmacological characterisation of the $P2Y_{11}$ receptor in stably transfected haematological cell lines. Mol. Cell. Biochem. 213, 75–81.

Verspohl, E.J., Johannwille, B., Kaiserling-Buddemeier, I., Schlüter, H., Hagemann, J., 1999. Diadenosine polyphosphates in cultured vascular smooth-muscle cells and endothelium cells—their interaction with specific receptors and their degradation. J. Pharm. Pharmacol. 51, 1175–1181.

Von Kügelgen, I., Wetter, A., 2000. Molecular pharmacology of P2Y-receptors. Naunyn-Schmiedeberg's Arch. Pharmacol. 362, 310–323.

Walker, J., Bossman, P., Lackey, B.R., Zimmerman, J.K., Dimmick, M.A., Hilderman, R.H., 1993. The adenosine 5′,5‴,P1,P4-tetraphosphate receptor is at the cell surface of heart cells. Biochemistry 32, 14009–14014.

Yamaya, M., Sekizawa, K., Kakuta, Y., Ohrui, T., Sawai, T., Sasaki, H., 1996. P_{2u}-purinoceptor regulation of chloride secretion in cultured human tracheal submucosal glands. Am. J. Physiol., Lung Cell Mol. Physiol. 270, L979–L984.

Yankaskas, J.R., Haizlip, J.E., Conrad, M., Koval, D., Lazarowski, E., Paradiso, A.M., Rinehart, C.A., Sarkadi, B., Schlegel, R., Boucher, R.C., 1993. Papilloma virus immortalized tracheal epithelial cells retain a well-differentiated phenotype. Am. J. Physiol., Cell Physiol. 264, C1219–C1230.