J. Pharm. Pharmacol. 1999, 51: 1175–1181
Received March 24, 1999
Accepted May 5, 1999

Diadenosine Polyphosphates in Cultured Vascular Smooth-muscle Cells and Endothelium Cells—Their Interaction with Specific Receptors and their Degradation

E. J. VERSPOHL, B. JOHANNWILLE, I. KAISERLING-BUDDEMEIER, H. SCHLÜTER AND J. HAGEMANN

Department of Pharmacology, Institute of Pharmaceutical Chemistry, University of Münster, Münster, Germany

Abstract

The role of diadenosine polyphosphates (Ap_nA, where ''A'' denotes ''adenosine'' and ''n'' denotes the number of phosphate groups ''p'') as vasoconstrictors of smooth-muscle cells and as blood-pressure regulating and insulin-releasing compounds has been described. It was the aim of this study to investigate whether specific receptors for these compounds, mediating the above mentioned effects, occur in cultured vascular smooth-muscle cells (VSMC) and in endothelium cells, and whether these compounds are degraded during incubation.

Saturable binding sites for diadenosine polyphosphate [^{3}H]Ap_4A with an extremely quick saturation equilibrium, even at low temperature (4°C), are present in vascular smooth-muscle cells. Diadenosine polyphosphates at micromolar concentrations displaced [^{3}H]Ap_4A from binding sites; the ranking order was $Ap_4A > Ap_3A > Ap_5A \approx Ap_6A$. Compounds interacting with purinergic P_{2X} receptors such as suramin, α,β-methylene ATP and pyridoxalphosphate-6-azophenyl-2′,4′-disulphonic acid (PPADS), albeit at high concentrations, displaced [^{3}H]Ap_4A from its binding sites. Surprisingly, at low concentrations the compounds tested increased the binding of [^{3}H]Ap_4A, which might imply the occurrence of positive receptor cooperativity or inhibition of [^{3}H]Ap_4A degradation. By use of thin-layer chromatography it was observed that [^{3}H]Ap_4A was quickly degraded (half-life approx. 12 min) in the extracellular medium to (mainly) adenosine and inosine. [^{3}H]Ap_4A and its degradation products were quickly taken up by the cells. Degradation can be inhibited by Ap_6A, α,β-methylene ATP or PPADS. Rather similar degradation and uptake results were also obtained when endothelium cells were used.

These data indicate that specific binding sites for [^{3}H]Ap_4A are present in vascular smooth-muscle cells and that diadenosine polyphosphates at physiological concentrations displace binding. The receptors involved might be distinct diadenosine polyphosphate receptors, although the involvement of others, such as P_{2X} receptors, is also possible. Ap_4A is quickly degraded in the extracellular space and compounds that inhibit degradation result in an increase in [^{3}H]Ap_4A binding. It should be remembered that when diadenosine polyphos-phates are being investigated in physiological and pathophysiological studies of their impact on smooth-muscle cell proliferation and on vasoconstriction (blood-pressure regulation), results obtained from long-term incubations might be critical.

Diadenosine polyphosphates (Ap_3A, Ap_4A, Ap_5A and Ap_6A—where ''A'' denotes ''adenosine'' and ''3'', ''4'', ''5'' or ''6'' denotes the number of phosphate groups ''p'') belong to a group of ubiquitous compounds formed by two adenosine molecules bridged by three to six phosphate groups. They are present, and stored in, for example, the dense granules of platelets, chromaffin cells, and neuronal cells, and they are released into the extracellular space. It has been suggested that some are involved in blood-pressure regulation (Schlüter et al 1994; Davies et al 1995) or are even important in the development of essential hyper-

Correspondence: E. J. Verspohl, Department of Pharmacology, Institute of Pharmaceutical Chemistry, Hittorfstraße 58–62, 48149 Münster, Germany.

tension (Schlüter et al 1996). Binding sites for diadenosine polyphosphates have already been shown in different cells, for example heart (Walker et al 1993; Vahlensieck et al 1996), brain (Hilderman et al 1991; Rodriguez-Pascual et al 1997), liver (Edgecombe et al 1996) and insulin secreting cells (Verspohl & Johannwille 1998). Some effects are mediated mainly via their action on intracellular calcium (Pintor & Miras-Portugal 1995; Tepel et al 1995, 1997) or K_{ATP} channels (Ripoll et al 1996).

Vascular smooth muscle and endothelium cells regulate vascular tone, and their proliferation is under the control of various compounds including diadenosine polyphosphates. Though the influence of some purinergic compounds, including their pathophysiological impact, is well known (Yu et al 1996; Erlinge 1998), it is not known whether effects on vascular smooth-muscle cells are mediated via specific diadenosine polyphosphate receptors or other purinergic receptors, or whether physiological effects and receptor binding are disturbed by specific degradation kinetics.

Materials and Methods

Diadenosine polyphosphates, α,β-methylene ATP, adenosine and inosine were from Sigma (Deisenhofen, Germany) and pyridoxalphosphate-6-azophenyl-2′,4′-disulphonic acid (PPADS) and suramin were supplied by Dr Lambrecht (Frankfurt, Germany). [^{3}H]Ap_4A labelled in the adenosine part of the molecule (sp. act. 237 Gbq mmol^{-1}) was purchased from Amersham (Braunschweig, Germany). Tritiated samples were counted in a scintillation counter (Tri Carb 300 CD, Packard, Frankfurt, Germany) by use of Quickscint 212 (Zinsser Analytic, Frankfurt, Germany). Other compounds (analytical grade) were from Baker (Griesheim, Germany) or Merck (Darmstadt, Germany).

Cell culture (vascular smooth-muscle cells)

A primary culture of vascular smooth-muscle cells was prepared essentially as described by Meyer-Lehnert & Schrier (1989). In brief, aortas and inferior vena cavas were removed from two to four female Sprague–Dawley rats under sterile conditions and placed in Dulbecco's modified Eagle's medium containing foetal calf serum (10%), penicillin (100 units mL^{-1}), and streptomycin (0·1 mg mL^{-1}). With the use of a binocular microscope, the adventitia and outer media were stripped off. The vessels were opened by a longitudinal cut, and the intima was removed by scraping. The vessels were placed in fresh Dulbecco's modified Eagle's medium, including supplements, and minced. After incubation for two weeks the tissues were disrupted, centrifuged gently, and the dissociated cells were then resuspended in Dulbecco's modified Eagle's medium, including supplements. The resulting suspension was then plated in 75-mL flasks and cultured at 37°C in humidified 5% CO_2–95% air until cells reached confluence (7–10 days). vascular smooth-muscle cells were passaged at least five times before use. Cell viability was tested by exclusion of trypan blue (> 95%).

Cell culture (endothelium cells)

Primary cell cultures from endothelium cells of the hog aorta were incubated until confluence in Dulbecco's modified Eagle's medium with foetal calf serum (10%), sodium hydrogen carbonate (0·37%), penicillin (100 units mL^{-1}), and streptomycin (0·1 mg mL^{-1}). Only the second and third passage were used for the experiments.

Binding experiments

Half confluent cells (grown in 75-mL culture flasks for 6 days) were washed with Krebs–Ringer buffer containing HEPES (2-(4-(2-hydroxyethyl)-1-piperazinyl)ethanesulphonic acid; 10 mM) and bovine serum albumin (0·5%), pH 7·35 (KRH buffer) and harvested by scraping. After spinning, cells were resuspended in KRH buffer and homogenized with a Dounce homogenizer. The particulate fraction (2600 *g*) was washed with KRH buffer and resuspended in KRH buffer (400 μL). This suspension (30 μL) was incubated at 22 or 4°C, as indicated in the legends of figures, with [^{3}H]Ap_4A (104 nM) in the absence or presence of increasing concentrations of either different unlabelled diadenosine polyphosphates or purinoceptor agonists and antagonists. The final incubation volume was 90 μL. To determine non-specific binding, the incubation was performed in the presence of unlabelled Ap_4A (0·48 mM). The incubation was terminated at the indicated time points by cooling (4°C), high-speed centrifugation, and washing twice with ice-cold KRH buffer. The pellet was finally lysed with sodium dodecyl sulphate (SDS; 0·5%, 50 μL) and counted as described under uptake experiments.

Uptake experiments

Half-confluent vascular smooth-muscle or endothelium cells grown in microwells for five or six days were washed twice with ice-cold KRH buffer and then incubated for up to 300 min at 4°C in

KRH buffer (300 μL) containing [^{3}H]Ap_4A (31 nM). Incubation was terminated at the times indicated by washing the cells twice with ice-cold KRH buffer. Samples from the supernatant were counted to determine the total amount of radioactivity added. Cells were lysed with SDS (0·5%; 400 μL) for 30 min at 37°C, and the wells were again washed with SDS (300 μL). The combined samples were counted in a scintillation counter after adding scintillation cocktail (5 mL).

Determination of [^{3}H]Ap_4A degradation products

The original radioactively labelled compound and its degradation products were determined in the extracellular space of vascular smooth muscle and in endothelium cells by use of thin-layer chromatography (TLC). Half-confluent cells were incubated for up to 90 min in KRH buffer containing [^{3}H]Ap_4A (310 nM). At the times indicated in the figure legends samples were taken from the incubation medium, mixed with the appropriate unlabelled standards (ATP, ADP, Ap_4A, AMP, inosine or adenosine), transferred to aluminium-backed silica gel TLC plates (Merck, Darmstadt, Germany), and developed for 2 h in dioxane–ammonia–water, 6 : 1 : 4. Chromatographic spots of unlabelled standards were visualized under ultraviolet light and cut from the plates. Their radioactivity was counted in a liquid scintillation counter as described above.

Results

Binding experiments

Binding experiments with [^{3}H]Ap_4A were performed on a particulate fraction of vascular smooth-muscle cells (Figure 1). Binding was rapid with maximum binding occurring within seconds; steady-state binding could not be established at 22°C (Figure 1A) and was 4·91% of total radioactivity within 7·5–15 min at 4°C (Figure 1B). At both temperatures non-specific binding (determined in the presence of unlabelled Ap_4A (0·48 mM)) was less than 1% of added radioactivity. Further binding experiments were performed under steady-state conditions with a 15 min incubation period at 4°C.

All diadenosine polyphosphates tested inhibited specific [^{3}H]Ap_4A binding to vascular smooth-muscle cells (Figures 2A, B), although inhibition of binding by unlabelled Ap_4A (Figure 2A) and Ap_3A, Ap_5A, and Ap_6A (Figure 2B) was obvious only at higher concentrations with the order of potency being $Ap_4A > Ap_3A > Ap_5A \approx Ap_6A$. In contrast,

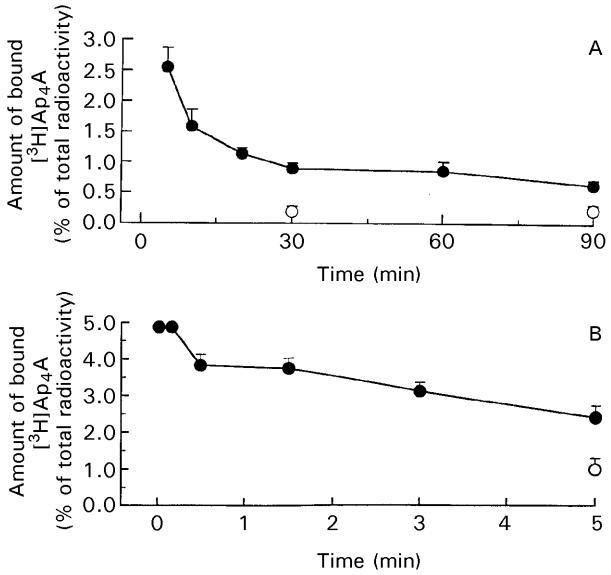

Figure 1. The time-course of [^{3}H]Ap_4A binding to a vascular smooth-muscle cell homogenate. The homogenate was incubated either for up to 90 min at 22°C (A) or for up to 5 h at 4°C in 90 μL KRH buffer containing [^{3}H]Ap_4A (104 nM) (B). Results are expressed as amount bound as a percentage of total radioactivity. Each value is the mean ± s.e.m. of results from three separate experiments run in quadruplicate. ●, total binding; ○, non-specific binding (presence of 0·48 nM unlabelled Ap_4A).

at low diadenosine polyphosphate concentrations (up to 1 or 10 μM) the displacement experiments showed that binding increased. As shown in Figure 2C the tested purinoceptor agonist α,β-methylene ATP and both antagonists suramin and PPADS had the same effect on specific [^{3}H]Ap_4A binding to vascular smooth-muscle cells, i.e. increase at low concentrations and displacement at high concentrations.

Degradation and uptake experiments

Degradation of [^{3}H]Ap_4A was determined in the incubation medium of vascular smooth-muscle cells. Figure 3A shows the effect of degradation during a 90 min incubation. [^{3}H]Ap_4A was degraded with a half-life of 12 min (approx.). The major degradation products were adenosine and inosine. Figure 3B shows uptake of [^{3}H]Ap_4A or its degradation products by vascular smooth-muscle cells over 300 min. The tritiated compounds are taken up to a substantial extent.

Possible inhibition of [^{3}H]Ap_4A degradation mediated by different compounds was then tested. Table 1 shows the effect of 10 μM Ap_6A, α,β-methylene ATP, or PPADS on concentrations of [^{3}H]Ap_4A and its degradation products, for example tritiated ATP, ADP, AMP, adenosine, or inosine. Within 10 min at 4°C large amounts of

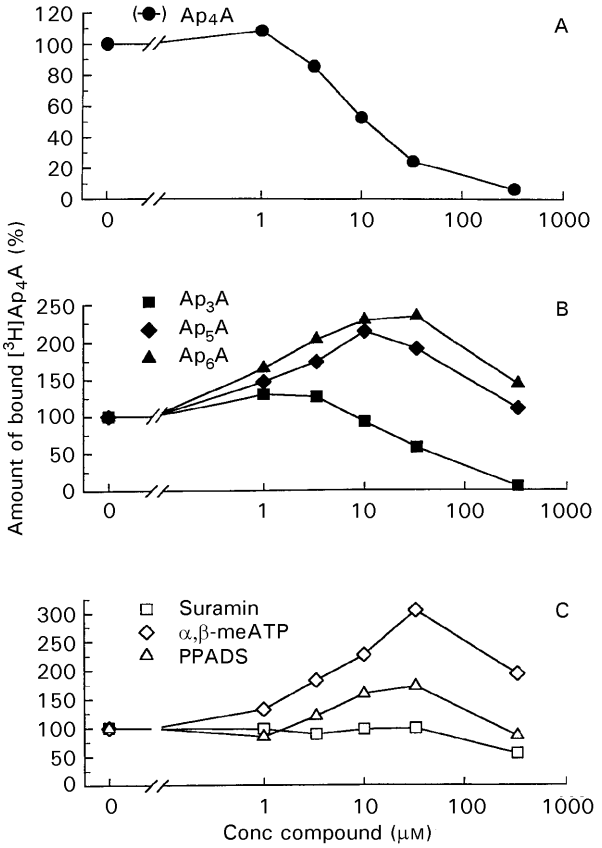

Figure 2. Inhibition of [^{3}H]Ap_4A binding to a vascular smooth-muscle cell homogenate by various diadenosine polyphosphates. The homogenate was incubated with [^{3}H]Ap_4A (104 nM) for 15 min at 4°C in Krebs–Ringer–HEPES buffer (90 μL), and increasing concentrations of various diadenosine polyphosphates (A. Ap_4A; B. ■ Ap_3A, ◆ Ap_5A and ▲ Ap_6A; C. □ suramin, ◇ α,β-methylene ATP and △ pyridoxalphosphate-6-azophenyl-2′,4′-disulphonic acid). Results are expressed as the amounts (%) of maximum bound radioactivity in the absence of unlabelled compounds. Non-specific binding (presence of 0·48 mM unlabelled Ap_4A) is already subtracted. Each value is the mean ± s.e.m. of results from three separate experiments run in quadruplicate.

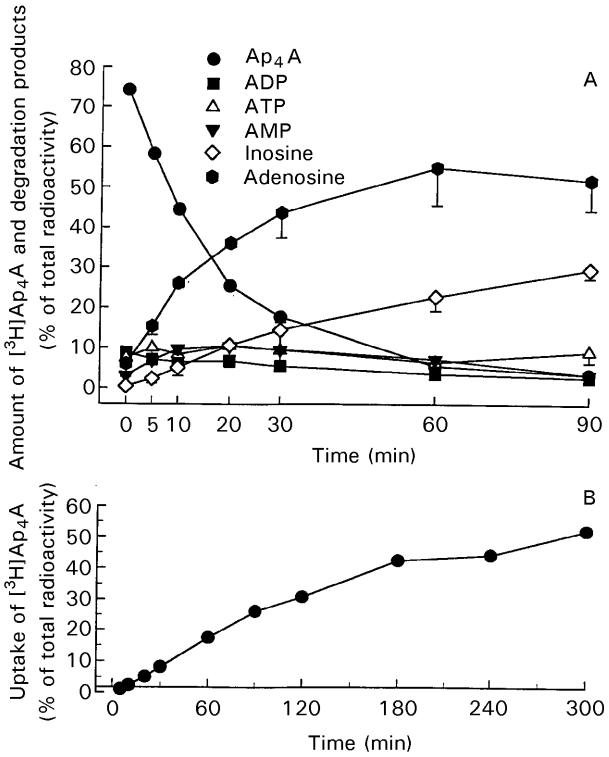

Figure 3. Degradation and uptake of [^{3}H]Ap_4A by vascular smooth-muscle cells. Degradation in the incubation medium (A) and uptake by vascular smooth-muscle cells (B) were investigated at 4°C for 90 and 300 min, respectively. A. Cells were incubated with [^{3}H]Ap_4A (310 nM) in Krebs–Ringer–HEPES buffer. At the times indicated samples from the incubation medium were analysed for degradation products by thin-layer chromatography: ● Ap_4A, ■ ADP, △ ATP, ▼ AMP, ◇ inosine, ⬢ adenosine. Results are means ± s.e.m. from three separate experiments. B. Cells were incubated with [^{3}H]Ap_4A (310 nM) in Krebs–Ringer–HEPES buffer. At the times indicated the radioactivity of lysed cells was counted; it is given as a percentage of the total radioactivity added. Each value is the mean of results from two separate experiments.

[^{3}H]Ap_4A were degraded compared with control (0 min incubation). 10 μM Ap_6A strongly inhibited degrad-ation; 10 μM PPADS or α,β-methylene ATP were less potent. In both experiments adenosine was the most prominent degradation product.

Additional experiments with endothelium cells were performed to check for general uptake of Ap_4A by other cells closely located to vascular smooth-muscle cells, and because Brandts et al (1998) observed rapid disappearance of Ap_4A after an in-vitro infusion to a myocardial vessel preparation when pre- and postinfusion media were compared. Degradation of [^{3}H]Ap_4A was also determined in the incubation medium of endothelium cells. Figure 4A shows the effect of degradation after 90 min incubation. [^{3}H]Ap_4A was degraded with a half-life of 12 min (approx.). The major degradation product was adenosine. Figure 4B shows uptake of [^{3}H]Ap_4A, including its degradation products, by endothelium cells over a period of 90 min. The tritiated compounds were largely taken up. The data resemble those observed for vascular smooth-muscle cells.

Discussion

Diadenosine polyphosphates are known to be released from cells, and to be present in blood and ubiquitous in the extracellular space. Their interaction with binding sites and their degradation were investigated in the current study of binding to vascular smooth-muscle cells. Binding sites for labelled Ap_4A are present because [^{3}H]Ap_4A binding is time-dependent and saturable in vascular smooth-muscle cells.

Table 1. Inhibition of [^{3}H]Ap_4A degradation in vascular smooth-muscle cells by diadenosine polyphosphates, purinergic compounds, adenosine or inosine.

Addition	Ap_4A	ATP	ADP	AMP	Adenosine	Inosine
Control	95·30 ± 1·21	0·82 ± 0·091	1·97 ± 0·089	0·47 ± 0·003	0·61 ± 0·008	0·82 ± 0·012
None	2·290 ± 0·13	4·52 ± 0·067	3·01 ± 0·045	2·27 ± 0·045	60·53 ± 0·792	27·42 ± 1·06
Ap_6A	69·98 ± 0·14	4·01 ± 0·11	7·78 ± 0·134	0·79 ± 0·034	15·12 ± 0·912	2·34 ± 0·034
α,β-methylene ATP	13·34 ± 0·98	19·27 ± 1·14	14·92 ± 0·987	24·59 ± 0·989	22·84 ± 0·456	5·03 ± 0·089
Pyridoxalphosphate-6-azophenyl-2′,4′-disulphonic acid	31·69 ± 1·32	14·71 ± 1·07	11·07 ± 0·970	1·14 ± 0·062	35·32 ± 0·128	6·06 ± 0·023

Homogenate of vascular smooth-muscle cells was incubated for 10 min at 4°C in Krebs–Ringer–HEPES buffer (90 μL) containing [^{3}H]Ap_4A (104 nM), and Ap_6A, α,β-methylene ATP, or pyridoxalphosphate-6-azophenyl-2′,4′-disulphonic acid (10 μM). Results (means ± s.e.m. from three separate experiments) are expressed as a percentage of compound radioactivity in the presence of added substance.

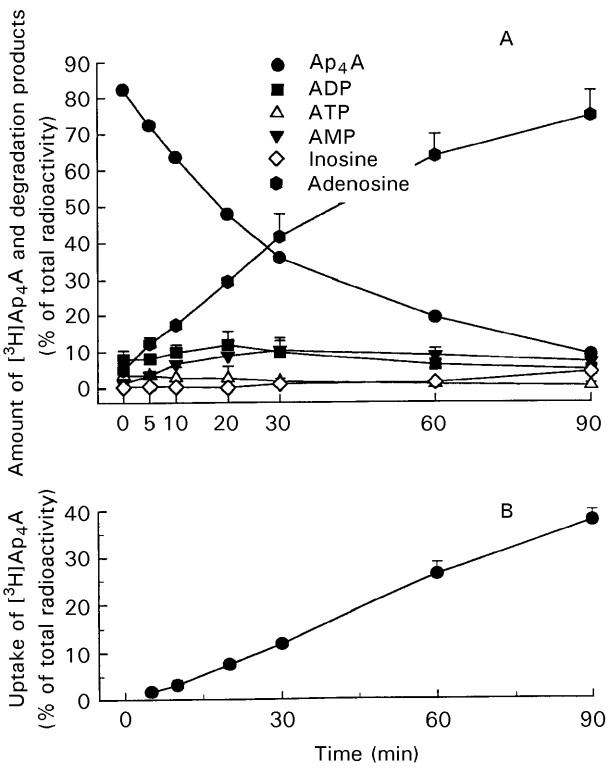

Figure 4. Degradation and uptake of [^{3}H]Ap_4A by endothelium cells. Degradation in the incubation medium (A) and uptake by vascular smooth-muscle cells (B) were investigated at 4°C for 90 min. A. Cells were incubated with [^{3}H]Ap_4A (310 nM) in Krebs–Ringer–HEPES buffer. At the times indicated samples from the incubation medium were analysed for degradation products by thin-layer chromatography: ● Ap_4A, ■ ADP, △ ATP, ▼ AMP, ◇ inosine, ⬢ adenosine. Results are means ± s.e.m. of results from three separate experiments. B. Cells were incubated with [^{3}H]Ap_4A (310 nM) in Krebs–Ringer–HEPES buffer. At the times indicated the radioactivity of lysed cells was counted and is given as percentage of the total radioactivity added. Each value is the mean ± s.e.m. of results from three separate experiments.

The observed inhibition of [^{3}H]Ap_4A binding by various diadenosine polyphosphates at concentrations of 10 μM or higher probably reflects physiological concentrations. It has been calculated that, assuming complete release from platelet dense granules, a local concentration of 100 μM (approx.) is possible and, after distribution in blood, a concentration of 1 μM could result (Ogilvie 1992). Extracellular concentrations of 27 μM can be estimated for diadenosine polyphosphates after secretion from chromaffin cells (Pintor et al 1991; Miras-Portugal et al 1994). The concentration in blood (resulting from storage in platelets) can be estimated as 0·14–0·3 μM (recalculated from the data of Lüthje et al (1987) and Floodgard & Klenlow (1982)). Thus these concentrations observed in the literature are well in the range eliciting half-maximum inhibition of binding in our experiments. These high and regulated concentrations of diadenosine polyphosphates in blood imply an important function because of signalling via specific receptors on vascular smooth-muscle cells, for example, mediating proliferation.

The increase in [^{3}H]Ap_4A binding when using either unlabelled diadenosine polyphosphate or purinergic receptor agonists and antagonists at low concentration implies positive receptor cooperativity or inhibition of [^{3}H]Ap_4A degradation. This implication is substantiated by the observation of degradation products, mainly adenosine and inosine, at high concentrations. Because the increase in binding is more prominent after addition of Ap_6A than, for example, PPADS, it can be speculated that it might be because of the production of ATP, which was already shown by others to inhibit Ap_4A degradation (Ogilvie et al 1989).

Determining the type of binding site with which Ap_4A interacts on vascular smooth-muscle cells is not easy. The binding sites do not clearly discriminate between various diadenosine polyphosphates, although a ranking order is apparent. Therefore, a specific diadenosine polyphosphate receptor cannot be excluded. Binding sites for diadenosine polyphosphates have already been shown in a variety of cells: heart (Walker et al

1993), brain (Hilderman et al 1991; Rodriguez-Pascual et al 1997), liver (Edgecombe et al 1996), INS-1 cells (Verspohl & Johannwille 1998) and rat pancreatic islets (Verspohl & Johannwille 1998). Occasionally binding sites resemble adenosine receptors (heart (Vahlensieck et al 1996)), or either adenosine or P_2 receptors (guinea pig vas deferens (Hoyle et al 1995), follicular oocytes (Pintor et al 1996), heart (Hoyle et al 1996), kidney (Van der Giet et al 1997) and INS-1 cells (Verspohl & Johannwille 1998)).

The cell proliferation induced by ATP and its effect on intracellular Ca^{2+} correlate well in vascular smooth-muscle cells (Erlinge et al 1993), thus a purinergic receptor is probably present in these cells. Because several compounds such as UTP, ITP, GTP, ADP and UDP have proliferative effects similar to those of Ap_4A (Erlinge 1998), it is possible that all compounds act via the same (purinergic?) receptor(s) in vascular smooth-muscle cells. In fact proliferation (mitogenic effects) and cell growth of vascular smooth-muscle cells have been shown to be mediated by the purinergic receptor subtype P_{2Y} (Yu et al 1996; Erlinge 1998). However, the diadenosine polyphosphates Ap_4A, Ap_5A and Ap_6A at 10 μM increase intracellular Ca^{2+} in vascular smooth-muscle cells probably via P_{2X} receptors (Tepel et al 1997) when used at an early passage; at a later stage vascular smooth-muscle cells can lose their P_{2X} receptors. In addition interactions of diadenosine polyphosphates with the P_{2X} receptor subtype have been shown for cells and tissues such as fibroblasts, urinary bladder, mesangial cells and vas deferens (Westfall et al 1997). In our experiments the involvement of a P_{2X} receptor cannot be excluded for vascular smooth-muscle cells because PPADS, which is selective for several P_{2X} receptors (P_{2X1-3}, P_{2X7} and possibly also P_{2Y1}), competed with [^{3}H]Ap_4A binding, as did the non-selective P_{2X}/P_{2Y} receptor antagonist suramin. Interpretation on the basis of ranking orders is difficult because specific ranking orders for diadenosine polyphos-phates and purinergics are not known and have not been tested in artificial cells overexpressing only one cloned receptor subtype. Adenosine receptors which might be addressed by the Ap_4A degradation product adenosine are known to be present in vascular smooth-muscle cells and regulate cAMP concentrations (Zhao et al 1997). Because adenosine transport mechanisms are known, it is conceivable that mainly adenosine is taken up by vascular smooth-muscle cells when being incubated with [^{3}H]Ap_4A in our experiments.

The observation of Brandts et al (1998) of rapid disappearance of diadenosine polyphosphates after in-vitro infusion to a myocardial vessel preparation when pre- and postinfusion media were compared is substantiated by the short half-life of [^{3}H]Ap_4A in our endothelium cell preparation. Thus results from in-vitro experiments with high local concentrations of Ap_4A might differ from those obtained from in-vivo experiments with large amounts of the degradation products that are quantified in our experiments.

In conclusion, our data indicate the presence of specific binding sites for diadenosine polyphosphates in vascular smooth-muscle cells. The type of receptor is unclear but might be a specific diadenosine polyphosphate receptor or a P_{2X} receptor. The interaction is short-lived because degradation of [^{3}H]Ap_4A to adenosine and inosine occurs quickly. Interpretation of binding data is complicated because compounds that inhibit degradation increase binding of (intact) [^{3}H]Ap_4A. The exact role of diadenosine polyphosphates in physiological and pathophysiological terms warrants detailed evaluation. Results after long-term incubations, however, should be interpreted with caution.

Acknowledgements

We thank Dr C. B. Wollheim, Geneva, for supplying INS-1 cells, and Dr G. Lambrecht (Frankfurt, Germany) for supplying PPADS and suramin. This research was supported by the Deutsche Diabetes Gesellschaft, Germany (E. J. Verspohl) and the Deutsche Forschungsgemeinschaft, Germany (H. Schlüter). We thank Dr P. Vischer (Institute of Atherosclerosis, Münster, Germany) for supplying endothelium cells.

References

Brandts, B., Brandts, A., Wellner-Kienitz, M. C., Zidek, W., Schlüter, H., Pott, L. (1998) Non-receptor-mediated activation of $I_{K(ATP)}$ and inhibition of $I_{K(Ach)}$ by diadenosine polyphosphates in guinea-pig atrial myocytes. J. Physiol. 512: 407–420

Davies, G., MacAllister, R. J., Bogle, R. G., Vallance, P. (1995) Effects of diadenosine polyphosphates on human umbilical vessels: novel platelet-derived vasoconstrictors. Br. J. Clin. Pharmacol. 40: 170–172

Edgecombe, M., McLennan, A. G., Fisher, M. J. (1996) Characterization of the binding of diadenosine 5′, 5″-P^1, P^4-tetraphosphate (Ap_4A) to rat liver cell membranes. Biochem. J. 314: 687–693

Erlinge, D. (1998) Extracellular ATP: a growth factor for vascular smooth-muscle cells. Gen. Pharmacol. 31: 1–8

Erlinge, D., Yoo, H., Edvisson, L., Reis, D. J., Wahlstedt, C. (1993) Mitogenic effects of ATP on vascular smooth-muscle cells vs. other growth factors and sympathetic cotransmitters. Am. J. Physiol. 265: H1089–H1097

Floodgard, H., Klenlow, H. (1982) Abundant amounts of diadenosine 5′, 5″-P^1, P^4-tetraphosphate are present and releasable, but metabolically inactive, in human platelets. Biochem. J. 208: 737–742

Hilderman, R. H., Martin, M., Zimmerman, J. K., Pivorun, E. B. (1991) Identification of a unique membrane receptor for adenosine 5′, 5″-P_1, P_4-tetraphosphate. J. Biol. Chem. 266: 6915–6918

Hoyle, C. H. V., Postorino, A., Burnstock, G. (1995) Pre- and postjunctional effects of diadenosine polyphosphates in the guinea-pig vas deferens. J. Pharm. Pharmacol. 47: 926–931

Hoyle, C. H. V., Ziganshin, A. U., Pintor, J., Burnstock, G. (1996) The activation of P_1- and P_2-purinoceptors in the guinea-pig left atrium by diadenosine polyphosphates. Br. J. Pharmacol. 118: 1294–1300

Lüthje, J., Miller, D., Ogilvie, A. (1987) Unproportionally high amounts of diadenosine triphosphate (Ap_3A) and diadenosine tetraphosphate (Ap_4A) in heavy platelets. Blut 54: 193–200

Meyer-Lehnert, H., Schrier, R. W. (1989) Potential mechanism of cyclosporine A-induced vascular muscle contraction. Hypertension Dallas 13: 352–360

Miras-Portugal, M. T., Pintor, J., Castro, E., Rodriguez-Pascual, F., Torres, M. (1994) Diadenosine polyphosphates from neuro-secretory granules: the search for receptors, signals and function. In: Municio, A. M., Miras-Portugal, M. T. (eds) Cell Signal Transduction, Second Messengers, and Protein Phosphorylation in Health and Disease, Plenum Press, New York

Ogilvie, A. (1992) Extracellular functions for Ap_nA. In: McLennan, A. G. (ed.) Ap_4A and other Dinucleoside Polyphosphates, CRC press, Boca Raton, pp 230–273

Ogilvie, A. J., Lüthje, J., Pohl, U., Busse, R. (1989) Identification and partial characterization of an adenosine(5′)adenosine hydrolase on intact bovine aortic endothelial cells. Biochem. J. 259: 97–103

Pintor, J., Miras-Portugal, M. T. (1995) A novel receptor for diadenosine polyphosphates coupled to calcium increase in rat midbrain synaptosomes. Br. J. Pharmacol. 115: 895–902

Pintor, J., Torres, M., Miras-Portugal, M. T. (1991) Carbachol induced release of diadenosine polyphosphates—Ap_4A and Ap_5A—from perfused bovine adrenal medulla and isolated chromaffin cells. Life Sci. 48: 2317–2324

Pintor, J., King, B. F., Ziganshin, A. U., Miras-Portugal, M. T., Burnstock, J. (1996) Diadenosine polyphosphate-activated inward and outward currents in follicular oocytes of *Xenopus laevis*. Life Sci. 59: 179–184

Ripoll, C., Martin, F., Rovira, J. M., Pintor, J., Miras-Portugal, M. T., Soria, B. (1996) Diadenosine polyphosphates: a novel class of glucose-induced intracellular messengers in the pancreatic β-cell. Diabetes 45: 1431–1434

Rodriguez-Pascual, F., Cortes, R., Torres, M., Palacios, J. M., Miras-Portugal, M. T. (1997) Distribution of [^{3}H]diadenosine tetraphosphate binding sites in rat brain. Neuroscience 77: 247–255

Schlüter, H., Offers, E., Brüggemann, 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: 187–188

Schlüter, H., Tepel, M., Zidek, W. (1996) Vascular actions of diadenosine polyphosphates. J. Autonom. Pharmacol. 16: 357–362

Tepel, M., Bachmann, J., Schlüter, H., Zidek, W. (1995) Diadenosine polyphosphate-induced increase in cytosolic free calcium in vascular smooth muscle cells. J. Hypertension 13: 1686–1688

Tepel, M., Jankowski, J., Schlüter, H., Bachmann, J., van der Giet, M., Rüss, C., Terliesner, J., Zidek, W. (1997) Diadenosine polyphosphates' action on calcium and vessel contraction. Am. J. Hypertens. 10: 1404–1410

Vahlensieck, U., Boknik, P., Knapp, J., Linck, B., Müller, F. U., Neumann, J., Herzig, S., Schlüter, H., Zidek, W., Deng, M. C., Scheld, H. H., Schmitz, W. (1996) Negative chronotropic and inotropic effects by diadenosine hexaphosphate (Ap_6A) via A_1-adenosine receptors. Br. J. Pharmacol. 119: 835–844

Van der Giet, M., Khattab, M., Börgel, J., Schlüter, H., Zidek, W. (1997) Differential effects of diadenosine phosphates on purinoceptors in the rat isolated perfused kidney. Br. J. Pharmacol. 120: 1453–1460

Verspohl, E. J., Johannwille, B. (1998) Diadenosine polyphosphates in insulin-secreting cells: interaction with specific receptors and degradation. Diabetes 47: 1727–1734

Walker, J., Bossman, P., Lackey, B. R., Zimmermann, J. K., Dimmick, M. A., Hilderman, R. H. (1993) The adenosine 5′, 5″, P_1, P_4-tetraphosphate receptor is at the cell surface of heart cells. Biochemistry 32: 14009–14014

Westfall, T. D., McIntyre, C. A., Obeid, S., Bowes, J., Kennedy, C., Sneddon, P. (1997) The interaction of diadenosine polyphates with P_{2x}-receptors in the guinea-pig isolated vas deferens. Br. J. Pharmacol. 121: 57–62

Yu, S. M., Chen, S. F., Lau, Y. T., Yang, C. M., Chen, J. C. (1996) Mechanism of extracellular ATP-induced proliferation of vascular smooth-muscle cells. Mol. Pharmacol. 50: 1000–1009

Zhao, Z., Francis, C. E., Ravid, K. (1997) An A3-subtype adenosine receptor is highly expressed in rat vascular smooth-muscle cells: its role in attenuating adenosine-induced increase in cAMP. Microvasc. Res. 54: 243–252