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Vasoactivity of diadenosine polyphosphates in human small renal resistance arteries

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Abstract

Background. We examined for the first time the vascular effects of purinergic agents that contribute to the regulation of peripheral vascular resistance in human small renal resistance arteries (hRRAs).

Methods and Results. Diadenosine polyphosphates (ApnAs, $n = 3$–6) and ATP, mounted in a microvessel myograph, caused vasoconstriction in hRRAs (rank order of potency: Ap5A > Ap6A = Ap4A > Ap3A = ATP). ADP, AMP and adenosine had less contractile potency than ApnA, suggesting that the observed effects were not induced by ApnA degradation products. The ApnA agent, Ap5A, but not Ap4A, induced vasoconstrictions that were inhibited by pyridoxal phosphate-6-azophenyl-2′,4′-disulfonic acid (PPADS; a P2X purinoceptor antagonist), but not by ADP3′5′ (a P2Y purinoceptor antagonist). In precontracted hRRAs, all of the ApnA agents caused vasorelaxation, and the potencies did not differ from each other. The ApnA degradation products had less vasorelaxing potencies than ApnA, suggesting that the vasorelaxation was caused by the ApnA agents themselves. Ap4A-induced vasorelaxation was inhibited by ADP3′5′ and PPADS. In contrast, Ap5A-induced vasorelaxation was not antagonized by ADP3′5′, but was antagonized more strongly by PPADS than was Ap4A.

Conclusions. We found that the tone of resistance arteries in human kidneys can be considerably influenced by these purinergic agonists, and most potently by ApnAs. Ap5A-induced vasoconstriction appeared to be mediated by P2X purinoceptors, whereas constriction due to Ap4A was caused by a different purinoceptor. Vasorelaxation due to Ap4A, but not Ap5A, appeared to be mediated by P2Y purinoceptors.

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Keywords: ApnA; diadenosine polyphosphate; human kidney; human renal resistance artery; purinoceptor

Introduction

Previous studies have demonstrated differential effects of diadenosine polyphosphates (ApnAs) on rat kidney vasculature [1,2]. These agents act as both purinergic humoral signal transducers and neurotransmitters. They are co-released from the adrenal medulla along with ATP and catecholamines, and are also stored in and released from human platelets [3]. ApnAs can modify mesangial cell proliferation, such as during glomerular disease [4]. The systemic concentrations of ApnAs measured in whole blood do not surpass 1 μM, but much higher concentrations can accumulate in the vascular lumen after degranulation of thrombocytes [5]. Although purinergic agents and purinoceptors may play a minor role in (patho)physiological processes, they appear to have potent modulating effects on other hormonal systems, including the adrenergic system. Phylogenetically, they constitute an early developed endo- or paracrine system [5].

A number of studies have shown the effects of ApnAs on animal arteries. We recently evaluated the effects of ApnAs on human mesenteric resistance arteries (hMRAs) [6], and found that findings from human studies may differ from experiments using animal models. This indicates that experiments with ApnAs should be performed with human tissues whenever possible. Accordingly, we investigated for the first time the vasodynamic effects of ApnAs on isolated human renal resistance arteries (hRRAs). This vascular bed is of greater importance for regulation of systemic blood pressure than the mesenteric arteries because the small renal (interlobular) arteries precede the glomerular vessels containing the juxtaglomerular apparatus, and therefore can modulate the activity of the renin–angiotensin–aldosterone system.

Although renal vascular resistance is largely regulated by afferent arterioles, these small arteries contribute to the regulation of pre-capillary resistance since up to 50% of the pre-capillary drop in blood pressure may occur in arteries of this size [7].

Vascular effects of ApnAs depend on initial vessel tone since they induce vasoconstriction at basal vessel tone and vasodilation at raised vessel tone. The potency of ApnA-induced vasoactivity on rat isolated vessel preparations and on hMRAs is dependent upon the number of phosphate groups of the ApnA molecules [8]. In anaesthetized rats, ApnAs decreased renal blood flow and increased renal vascular resistance [9]. One of these polyphosphates, Ap4A, has already been used for treatment of high blood pressure in humans during anaesthesia [10], emphasizing the growing importance of these physiological purinergic substances for clinical application.

ApnAs, like other purinergic substances, act via purinoceptors [2,11]. In the present study, we performed pharmacological analysis with purinoceptor antagonists to identify purinoceptors in hRRAs that are activated by ApnAs. In these studies, we antagonized Ap5A- and Ap4A-induced effects on hRRA using pyridoxal phosphate-6-azophenyl-2′,4′-disulfonic acid (PPADS) a compound that reportedly acts as a P2X purinoceptor antagonist [12]. There are, however, additional studies suggesting that PPADS also binds to $P2Y_1$ purinoceptors [13]. We also used ADP3′5′ which has been described as a $P2Y_1$ purinoceptor antagonist [14]. Ap5A was chosen because of its high potency and because previous detailed studies with animal resistance arteries would allow direct comparisons between results from human and animal tissues. Ap4A was chosen because of its demonstrated clinical potential in a previous study with humans [10]. Finally we evaluated the effects of ATP, ADP, AMP and adenosine on hRRAs to clarify the potential impact of ApnA degradation products on renovascular tone [15,16].

Subjects and methods

Human tissue preparations

Small specimens (~1–2 cm^3) of renal parenchymal tissue were dissected from resected kidneys during interventions in our local urology clinic. These nephrectomies were necessary because of malignant tumours. The ethical board of the medical faculty of the University of Münster, Germany, 'Ethikkommission der Medizinischen Fakultät und der Ärztekammer Westfalen-Lippe' approved this study (Reg.No. 1IVStei) and we obtained written consent from all participating patients. The patients suffering from hypernephroma underwent nephrectomy at different stages of the disease. We excluded renal tissue from patients suffering from hypertension due to tumour renin excretion and from patients with chronic renal failure. We also excluded nephrectomies from patients with advanced disease or kidneys that had large and obvious tumour infiltrations. Surgeons from the Department of Urology were asked to dissect renal tissue samples that were not in the direct vicinity of the tumour. In these samples, a distance of a few centimetres separated the dissected arteries from the tumorous tissue. Macroscopic examination revealed that we received only normal renal tissue specimens. These samples were immediately stored in cooled physiological Krebs–Ringer bicarbonate solution.

Vessel isolation

Isolation of small arteries was performed immediately after receipt of the tissue samples from the operating theatre. The samples were placed in a Petri dish coated with sylgard (Dow Corning, Seneffe, Belgium), which was filled with Krebs–Ringer bicarbonate solution. Connective tissue overlaying and surrounding the arterial structures was carefully removed under a dissection microscope, and 2 mm long segments of the renal resistance size arteries were isolated. The arterial vascular segments that we dissected stemmed from the interlobular renal arteries arising from the arcuate arteries. These were the smallest arterial segments that we were able to dissect and mount into the myograph. From each renal tissue sample, up to six interlobular arteries were dissected and mounted into the myograph. To determine the role of the endothelium in ApnA vasoactivity, the endothelium was mechanically removed by pulling a human hair through the vascular lumen of selected arterial preparations. The effectiveness of this procedure was demonstrated from the non-responsiveness of denuded arteries to acetylcholine.

Tension measurements

Arteries were mounted on two stainless steel wires (diameter 40 μm) as ring segments on an isometric myograph (Myograph-Model 410A, J.P. Trading, Aarhus, Denmark); one wire was connected to a force transducer (Kistler Morse DSC6, Seattle, WA) for recording isometric force development [7] and the other was connected to a displacement device. The arteries were stretched to their optimal luminal diameter using an active length tension protocol that included 125 mmol/l K^+ as the activating stimulus [17]. The optimal luminal diameter was established when depolarization of the vascular smooth muscle cells with high K^+ (125 mmol/l) was maximal. During experimentation, the vessels were kept in a Krebs–Ringer bicarbonate solution that was maintained at 37°C and aerated with 95% O_2 and 5% CO_2. After establishing the optimal lumen diameter which standardized the different arterial preparations, we demonstrated intact function of the endothelium by inducing vasorelaxion with acetylcholine (10 μmol/l) in each arterial preparation.

Experimental protocols

In previous experiments with animal tissues, we found a considerable desensitizing effect of purinergic substances. In the current study, we therefore performed preliminary experiments to determine the minimum time necessary to obtain identical vascular responses from two applications of the same concentration of the purinergic agent to the same vessel. We found that 30 min intervals between 100 μmol/l of ApnAs or 300 μmol/l of adenosine, AMP, ADP or ATP caused the same vascular effects during four repeated exposures of

hRRAs. For each purinergic agent, this effect was demonstrated in three vascular preparations, each derived from different patients. Because this time interval of 30 min was comparable with the intervals obtained in our previous animal tissue experiments, we performed the experiments with human arteries according to the same pattern, creating discontinuous concentration response curves that allowed 30 min intervals between applications of purinergic agents. The effects of vasorelaxing agonists were evaluated during pre-contraction induced by 10 μmol/l phenylephrine, which provided a stable vasoconstriction of ~115% of the force evoked by the high K^+ solution. Inhibitory effects of the purinoceptor antagonists were evaluated after the vessels had been incubated with the respective antagonist for 10 min. Vascular effects of Ap5A and Ap4A were antagonized with at least two different antagonist concentrations. Up to two concentration–response curves were created per vessel preparation. In total, 21 renal tissue samples were used for creating the concentration–response curves for ApnA and for the degradation products, and 10 renal tissue samples were used for the experiments with ApnA and purinoreceptor antagonists.

Drugs and solutions

The composition of Krebs–Ringer bicarbonate solution was (in mmol/l): NaCl, 119.1; KCl, 4.7; $MgSO_4$, 1.2; KH_2PO_4, 1.2; $NaHCO_3$, 25.0; $CaCl_2$, 2.5; and glucose, 5.5. In the high K^+ solution (125 mmol/l K^+), all of the NaCl was replaced by an equimolar concentration of KCl. All agonists and pharmacological tools were obtained from Sigma Chemical Co. (Deisenhofen, Germany) except for PPADS which was obtained from Research Biochemicals Int. (Natick, MA). All stock solutions were prepared on the day of use in double-distilled water.

Data analysis

Contractile reactivity was measured as active wall tension (active force divided by twice the vessel segment length) and was expressed as a percentage of the tissue response to 125 mM K^+ at the beginning of the experimental protocols. Whenever possible, concentration–response curves were analysed in terms of potency ($pD_2 = -\log EC_{50}$), determined by least square sigmoidal curve fitting of individual curves (Graphpad Prism 1.00, San Diego, CA), and pD_2 values were calculated when concentration–response curves reached the maximal effect. However, maximal effects were not always reached with the maximal agonist concentration (1 mmol/l). Differences between agonist responses and between control and inhibition responses were evaluated by Mann–Whitney tests, with $P < 0.05$ denoting statistical significance. Data are shown as means ± SEM, if not indicated otherwise.

Results

Phenylephrine (10 μmol/l) induced a stable and lasting vasoconstriction in isolated hRRAs that was similar in magnitude to vasoconstrictions evoked by the 125 mmol/l K^+ Krebs–Ringer bicarbonate solution. Discontinuous concentration–response curves were generated to avoid interference with the desensitizing effect of ApnAs. The general tissue characteristics of the hRRAs are depicted in Table 1.

Table 1. General tissue characteristics of hRRAs

Optimal lumen diameter (μm)	277 ± 5 (STD) ($n = 162$)
Wall tension induced by 125 mM K^+ (N/m)	4.58 ± 0.23 (STD) ($n = 162$)
Wall tension induced by phenylephrine (10 μM) (% of response to 125 mM K^+)	105 ± 3 ($n = 91$)
Age of patients with nephrectomy (years)	59 ± 3 ($n = 31$)
Male:female ratio	1.39:1

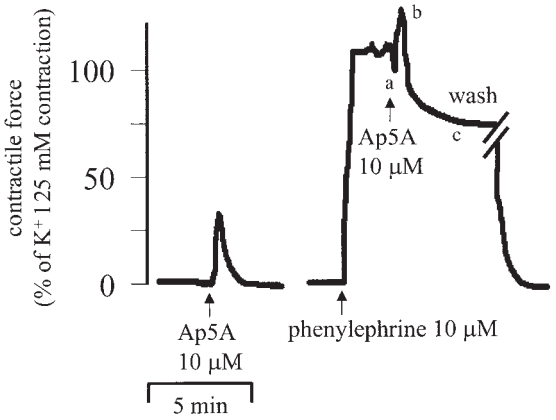

Fig. 1. Representative original records of myograph experiments with human renal resistance arteries (hRRAs) at basal tone (left) showing transient contractile force development by Ap5A (10 μmol/l). At raised tone (right), Ap5A (10 μmol/l) caused a brief relaxation (**a**) followed by a further increase in tone (**b**) which was rapidly followed by a lasting and marked relaxation (**c**).

Effects of Ap3A, Ap4A, Ap5A and Ap6A on the basal tone of hRRAs

In hRRAs, all of the ApnA analogues caused concentration-dependent, transient contractions. A representative rapid and brief vasoconstriction evoked by Ap5A (10 μmol/l) is shown in Figure 1 (left). The maximal contractile force occurred within 10–30 s. Contractions were followed by immediate and complete fading of active vascular tone. The rank order of contractile potency was: Ap5A > Ap6A = Ap4A > Ap3A (Figure 2a). Table 2 (left column) shows the corresponding pD_2 values. The maximum ApnA-induced vasoconstrictions were ~40–60% of the maximal contractile force induced by 125 mmol/l high K^+ solution. A concentration of 1 mmol/l of Ap4A induced a maximal contraction.

Effects of adenosine, AMP, ADP and ATP on the basal tone of hRRAs

We performed experiments with adenosine, AMP, ADP and ATP to determine whether the effects of ApnAs

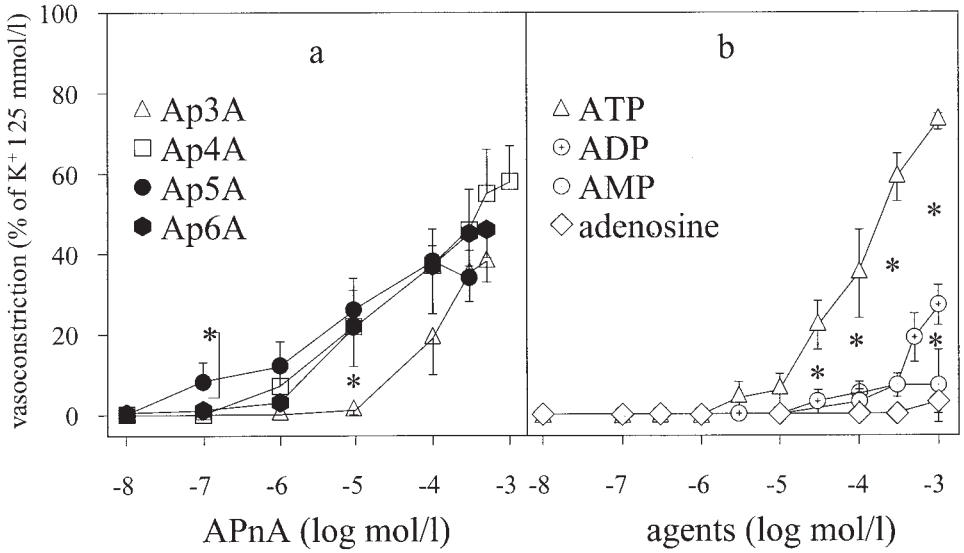

Fig. 2. Concentration–response curves for the contractile effects of Ap3A, Ap4A, Ap5A and Ap6A (left panel) and of ATP, ADP, AMP and adenosine (right panel) in hRRAs at basal tone. Effects were calculated as a percentage of the response to 125 mmol/l K^+ and are shown as means ± SEM ($n = 7$–8). *$P < 0.05$.

Table 2. pD_2 (–log M of EC_{50}) of contractile and relaxing effects of ApnAs in human renal hRRAs ($n = 7$–8) and comparison with previously studied effects in hMRAs ($n = 6$–9) [6]

Agent	hRRA	hMRA		
	Contractile effects	Relaxing effects	Contractile effects	Relaxing effects
Ap6A	4.56 ± 0.27	[a]	5.49 ± 0.17	5.45 ± 0.41
Ap5A	5.46 ± 0.08	5.07 ± 0.25	6.28 ± 0.12	5.48 ± 0.14
Ap4A	4.92 ± 0.19	5.01 ± 0.33	4.9 ± 0.03	5.47 ± 0.13
Ap3A	3.75 ± 0.23	5.54 ± 0.29	[a]	5.54 ± 0.26

[a]The maximum of the concentration response curve was not reached

on hRRAs could be evoked by their degradation products (Figure 2b). The rank order of potency was: Ap5A > Ap6A=Ap4A > Ap3A=ATP > ADP > AMP > adenosine, with the latter having only marginal influence on vascular tone. Within the concentration range tested (10–1000 µmol/l), the concentration–response curves of ATP and ADP did not reach their maxima of contractile force. As with ApnA, these contractions were transient in nature. In general, the potencies of the degradation products of ApnAs were lower than those of ApnAs, and only Ap3A-induced contractions were comparable with those of ATP.

Vasorelaxation in hRRAs induced by Ap3A, Ap4A, Ap5A and Ap6A

Pre-contraction of the arteries was initiated with phenylephrine (10 µmol/l). Application of ApnAs produced a triphasic vascular response (Figure 1, right), consisting of an initial reduction in vascular tone for seconds (Figure 1, right, a), followed by a short increase that exceeded the level of pre-contraction (Figure 1, right, b), and ending in a terminal long-lasting vasorelaxation (Figure 1, right, c). This terminal vasorelaxation (c) was measured for evaluation of the vasorelaxing potency of the agents. We found that the potencies of the ApnA agents were not significantly different from each other (Figure 3a). Table 2 (second column) shows the corresponding pD_2 values. These were calculated whenever the maximum of the induced effects was reached within the tested range of concentrations. The vasorelaxation of vessels induced by 100 µM Ap5A or Ap4A was not significantly altered after mechanically removing the endothelium by pulling a human hair through the vascular lumen.

Vasorelaxation in hRRAs induced by adenosine, AMP, ADP and ATP

After pre-contraction with phenylephrine (10 µmol/l), the ApnA degradation products caused a similar triphasic vascular response to that described for ApnAs. ATP showed a greater vasorelaxing potency than adenosine, ADP and AMP, which were not different from each other (Figure 3b). These data showed that all ApnAs were more potent vasorelaxants than their degradation products.

Antagonism of the contractile effects of Ap4A and Ap5A with PPADS and ADP3′5′

In hRRAs, neither PPADS (1–100 µmol/l) nor ADP3′5′ (10–100 µmol/l) had an influence on basal vessel tone.

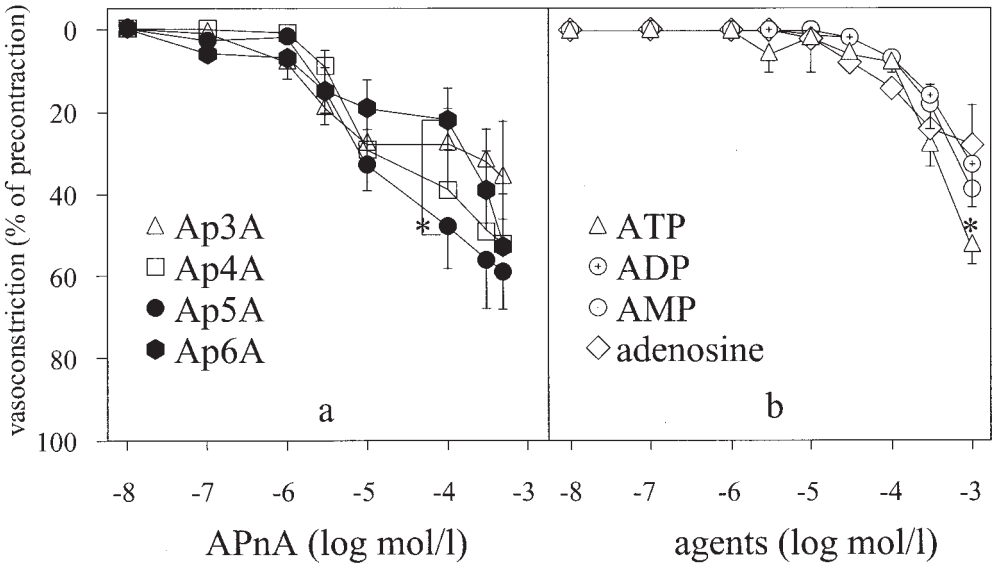

Fig. 3. Relaxing effects of Ap3A, Ap4A, Ap5A and Ap6A (left panel) and of ATP, ADP, AMP and adenosine (right panel) in hRRAs at a phenylephrine (10 µmol/l) pre-contraction. Each point is the mean of 5–7 determinations ± SEM. *$P < 0.05$.

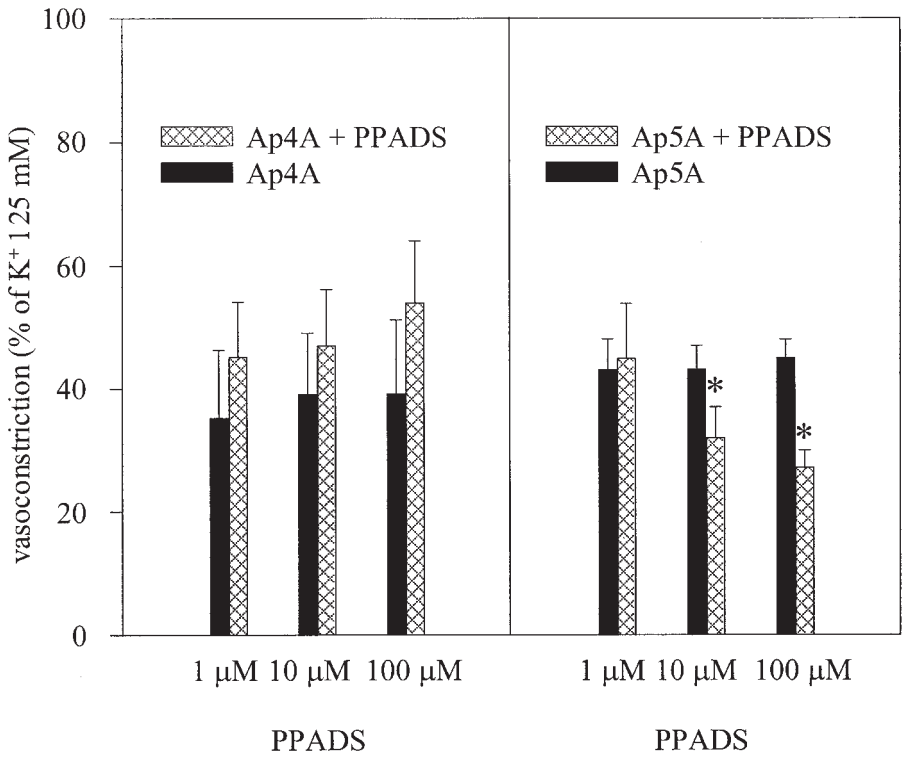

Fig. 4. Contractile effects of Ap4A (100 µmol/l) (left panel) and of Ap5A (100 µmol/l) (right panel) in hRRAs at basal tone presented as a percentage of the response to 125 mmol/l K^+ (control) as well as contractile effects after pre-incubation with PPADS (1, 10 and 100 µmol/l). Each column represents the mean of 6–10 determinations ± SEM. *$P < 0.05$.

Vasoconstriction caused by Ap5A (100 µmol/l) but not Ap4A (100 µmol/l) was inhibited in a concentration-dependent manner by the P2X antagonist PPADS (10 or 100 µmol/l) (Figure 4). PPADS appeared to enhance the response to Ap4A. In the presence of the selective $P2Y_1$ purinoceptor antagonist ADP3′5′ (10 or 100 µmol/l), there were no alterations in vascular contractions induced by Ap4A or Ap5A (data not shown).

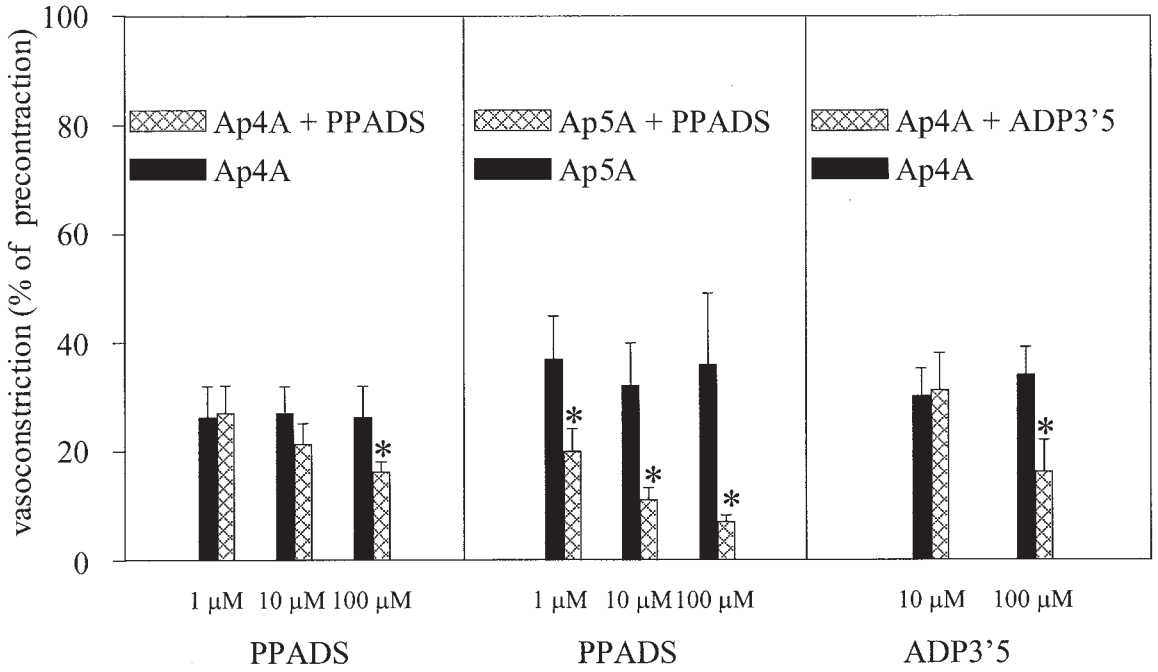

Fig. 5. Relaxing effect of 10 μM Ap4A (left panel) and Ap5A (middle panel) in hRRAs at a phenylephrine (10 μM) pre-contraction (control), and relaxing effect after pre-incubation with PPADS (1, 10 and 100 μM). Right panel: relaxing effect of 10 μM Ap4A in hRRAs at a phenylephrine (10 μM) pre-contraction (control), and relaxing effect after pre-incubation with ADP3′5′ (10 and 100 μM). Each column represents the mean of 7–11 determinations ± SEM. $*P < 0.05$.

Antagonism of the vasodilating effects of Ap4A and Ap5A in hRRAs by PPADS and ADP3′5′

In hRRAs, neither PPADS nor ADP3′5′ had an influence on the degree of pre-contraction induced by 10 μM phenylephrine. Pre-incubation with the P2X purinoceptor antagonist PPADS (1, 10 or 100 μmol/l) significantly inhibited vasorelaxation induced by Ap5A (10 μmol/l) (Figure 5). Vasorelaxation induced by Ap4A (10 μmol/l) was antagonized only by the highest concentration of PPADS (100 μmol/l). In contrast, vasorelaxation caused by Ap4A (10 μmol/l) was inhibited in a dose-dependent manner by the $P2Y_1$ purinoceptor antagonist ADP3′5′ (10 or 100 μmol/l) (Figure 5), but this antagonist did not influence Ap5A-induced vasorelaxation in hRRAs (data not shown).

Discussion

The present study, performed in renal resistance arteries, represents a continuation of experiments providing detailed knowledge about the effects of ApnAs on vascular tone in animal resistance arteries [11] and in hMRAs [6]. The principal aim of this study was to examine the influence of ApnAs on vascular tone in hRRAs. These vessels precede the renin-producing juxtaglomerular apparatus, which play an important part in the regulation of systemic blood pressure [7] and therefore are of great medical interest. The present experiments also examined purinoceptor antagonists to determine which purinoceptors are crucial for mediating the vascular effects of ApnAs in human kidneys. Finally, we also tested vascular effects of adenosine, AMP, ADP and ATP, which arise by asymmetric cleavage of ApnAs and also display purinergic activity in the kidney vasculature [16].

Vasoconstriction

The rank order contractile potency of ApnA agents was Ap5A > Ap6A = Ap4A > Ap3A. Vasoconstriction due to Ap5A but not Ap4A was inhibited by the P2X purinoceptor antagonist PPADS [12]. In contrast, neither constrictions to Ap4A nor to Ap5A were inhibited by the $P2Y_1$ purinoceptor antagonist ADP3′5′ [14]. Thus, activation of P2X receptors seems to be responsible for Ap5A-induced vasoconstriction in hRRA. The finding that Ap5A induced comparable contractile effects in hRRAs, in hMRAs [6] and in rat MRAs [11] suggests a similar mechanism of vasoconstriction in these diverse vascular beds. Because Ap4A-induced vasoconstriction was not antagonized by PPADS, this effect was independent from Ap5A-mediated contraction. It is possible that the $P2X_6$ purinoceptor or a heteromeric $P2X_{4+6}$ receptor mediated Ap4A responses since these receptor formations are blocked only by relatively high concentrations of PPADS [18].

The finding that the degradation products of ApnAs had generally lower potencies than the original nucleotides indicates that the contractile properties of ApnAs are not due primarily to their degradation

products. The greater contractile potency of ApnAs compared with their degradation products may be due to their considerably higher stability in the blood. For instance, ATP, which is the prototype purinergic agonist, is cleaved into adenosine and phosphate within seconds [15]. In general, experiments with isolated organs require higher concentrations of agonists and antagonists to induce purinergic effects than experiments with single cells or cell cultures because the potency of certain agonists and antagonists is greatly decreased by the breakdown by ectonucleotidases and other enzymes present in vascular tissue [19]. In myograph preparations, the access of agonists to receptors is restricted in the vessels because the vascular lumen is not perfused and remnants of surrounding connective tissue are present. Therefore, *in vivo* actions of these substances may occur at even lower agonist concentrations than observed in the current studies. This effect has been shown indirectly with perfused vascular preparations where the concentrations of ApnAs to achieve maximal effects were lower than in the organ bath of a myograph [2,8].

The rank order contractile potency of ApnA agents in hRRAs was comparable with that seen in hMRA [6], rat MRA [11], the perfused rat kidney [2] and mesenteric [8] artery. These observations are compatible with the findings of Gabriëls *et al.* [1], who found stronger vasoconstrictions with Ap5A than with Ap3A in interlobar arteries of hydronephrotic rat kidneys. A number of studies with animal vascular preparations showed that purinergically mediated vasoconstrictions are P2X purinoceptor dependent [2,8,11]. Von Kügelgen *et al.* [20] found marked differences in responsiveness between human saphenous vein, renal artery and renal vein strips to the selective P2X purinoceptor agonist β,γ-methylene-l-ATP, suggesting tissue differences in the density of operational P2X purinoceptors. Antagonist selectivity is still a critical problem in purinoceptor research, e.g. PPADS, apart from antagonizing P2X, also shows P2Y inhibitory effects [13]. The current findings suggest that ApnA-induced vasoconstriction in hRRAs is P2X purinoceptor dependent. This is because vasoconstriction in hRRA was not inhibited by the $P2Y_1$ antagonist ADP3′5′, and because the extremely rapid course of vasoconstriction in hRRAs indicated an ionotropic process at the ion channel-gated P2X receptors, which contrasts with the slow metabotropic process of vasorelaxation mediated by G-protein-coupled P2Y receptors.

Vasorelaxation

Pharmacological pre-contraction of arteries is necessary to quantify vasorelaxation of isolated hRRAs mounted in a small vessel myograph. In pre-contracted hRRAs, ApnAs caused a triphasic vascular response that included a terminal long-lasting vasorelaxation. The different ApnA agents caused vasorelaxations that were not different in potency. Vasodilations induced by Ap4A but not those by Ap5A were inhibited concentration dependently by the $P2Y_1$ purinoceptor antagonist ADP3′5′. Furthermore, only the highest antagonist PPADS concentration (100 μmol/l) inhibited Ap4A-induced vasorelaxation in hRRAs, which contrasted with very effective inhibition of Ap5A-induced vasorelaxation at only 1 μmol/l PPADS.

Thus, Ap4A-induced vasorelaxations in hRRA are obviously mediated by $P2Y_1$ purinoceptors, and Ap5A evokes vasorelaxation through mechanisms that are independent of Ap4A. The $P2Y_6$ purinoceptor may mediate Ap5A-induced vasorelaxation since this receptor is readily blocked by PPADS [21]. The lack of difference in vasorelaxing potencies of the ApnA agents in hRRAs is in agreement with our previous data in hMRA [6]. A similar lack of difference was reported for vasorelaxing potencies of Ap3A and Ap5A in interlobar arteries of rat kidneys *in vivo* [1].

The weaker potencies of the ApnA degradation products (ATP, ADP, AMP and adenosine) suggest that the primary effects are due to ApnAs. It was demonstrated previously that renal vascular and mesangial cells respond to extracellular ATP via mechanisms that are distinct from those elicited by adenosine. For instance, ATP vasoconstricted afferent but not efferent arterioles, whereas adenosine constricted both vascular segments. Rat renal microvascular (arteriolar) responses to adenosine include vasoconstriction at low concentrations and vasodilation at higher concentrations [16]. In our study, however, we showed in hRRAs that adenosine induced vasorelaxation at lower concentrations that normally cause vasoconstriction, indicating once more that purinergic agents stimulate a diversity of responses that depend on the renovascular domain or on the animal species.

Purinergic renovascular vasodilation has been shown to be mediated via P2Y purinoceptors [1,2]. In hRRAs in the present study, the $P2Y_1$ purinoceptor antagonist ADP3′5′ blocked vasodilation caused by Ap4A but not Ap5A. Identical results were revealed in human MRA [6]. We also found that the purinoceptor antagonist PPADS only slightly inhibited Ap4A-induced vasorelaxation in hRRAs, but strongly inhibited relaxation induced by Ap5A. In striking contrast, in human MRA, PPADS caused a stronger inhibition of Ap4A relaxation but no inhibition of Ap5A-induced vasorelaxation [6]. Thus, Ap5A apparently activates different sets of purinoceptors in hRRAs and hMRAs to cause vasorelaxation. The inhibition of the Ap4A- and Ap5A-induced vasorelaxation by PPADS may reflect the limited P2X selectivity of PPADS [13]. The selectivity of ADP3′5′ for $P2Y_1$ purinoceptors has been shown repeatedly [14].

In conclusion, the capacity of different ApnA agents to activate purinoceptors in the kidney vasculature is heterogeneous because Ap4A and Ap5A evoke similar contractile or vasorelaxing effects by activating different renovascular purinoceptors. Furthermore, Ap4A induces vasoconstriction and Ap5A induces vasorelaxation through a set of purinoceptors in

hRRAs that are different from those in the previously examined hMRAs. This diverging pattern of Ap4A and Ap5A antagonism effects in hMRA [6] and hRRA illustrates that individual vascular beds within a species display characteristic patterns of purinoceptor expression [22].

The findings of Gabriëls *et al*. [1], demonstrating differential effects of ApnAs on subsequent segments of rat intrarenal vasculature, suggest that a diversity of responses may also be present in human kidneys. Therefore, additional studies are warranted to gain further knowledge of and insight into the pharmacology of ApnA in the human renal and cardiovascular systems. Ap4A has already been used for treatment of high blood pressure in humans during anaesthesia [10], and offers the advantage of applying a physiologically existing and easily metabolized pharmacological agent. The heterogeneous distribution of purinoceptor subtypes in different vascular beds and the ability of ApnAs to stimulate diverging purinoceptor subtypes in intrarenal resistance vessels suggest the possibility of using ApnAs in hypertensive or other patients to provide selective and differentiated pharmacological regulation of both blood pressure and kidney perfusion. More specific purinoceptor agonists or antagonists or antisense gene transfer techniques will be necessary to firmly establish the role that these purinoceptor subtypes play in stimulating contractile, relaxing or both effects.

Although there are already a variety of antihypertensive agents that affect intrarenal microvasculature, the introduction of purinergic agents, especially ApnAs, into therapeutic practice [10] may offer the unique advantage of differential activation of purinergic receptors in the renal microvascular tree producing a fine tuning of intrarenal vascular tone. In the two human vascular beds that we have examined, the vasorelaxing qualities of ApnAs were dominant. In contrast, animal vascular preparations [11], such as in the epigastric artery supplying the muscular abdominal wall, have shown that purinergic stimulation may result in stable vasoconstriction. If, as these findings suggest, ApnA agents facilitate renal perfusion while reducing peripheral muscular perfusion, they may constitute a desirable vasoactive agent to favour renal blood flow. For example, they could be applied for prophylaxis of radiocontrast-induced nephropathy. Experiments in animal kidneys have shown that administration of contrast medium causes increased oxygen consumption and decreases in ATP that result in adenosine accumulation and subsequent renal vasoconstriction [23]. ApnAs may protect kidneys by inducing renal vasorelaxation and providing the lacking ATP. However, these perspectives are speculative and will clearly require further studies of differential effects of purinergic agents in the human microvasculature to determine potential therapeutic applications.

Conflict of interest statement. None declared.

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