RENSAL HAEMODYNAMICS AND NATRIURETIC RESPONSES TO INTRAVENOUS ADMINISTRATION OF DIADENOSINE TETRAPHOSPHATE (AP4A) AND NICOTINAMIDE ADENINE DINUCLEOTIDE (NAD) IN RAT

Effects of Ap4A and NAD - precursor of adenosine, on renal plasma flow (RPF), glomerular filtration rate (GFR) and urine excretion were determined in the anaesthetised rats. Infusion of Ap4A or NAD (i.v., bolus - 1 µmol/kg followed by 10 nmol/min/kg) decreased RPF and GFR (by 30 and 40%, respectively). In spite of GFR reduction during Ap4A infusion, the significant increase in sodium excretion and urine flow was noticed: fractional sodium (FE_{Na}) and urine excretion (FE_{urine}) rose 15-fold and 2.5-fold in comparison with the control value, respectively. In contrast to Ap4A, NAD-induced decrease in GFR was associated with parallel decrease in sodium and urine excretion, thus the FE_{Na} and FE_{urine} did not significantly change. Pre-treatment with adenosine deaminase (adenosine degrading enzyme, 2 U/min/kg) or theophylline (P1-receptors antagonist, 0.2 mmol/min/kg) ceased responses to NAD, whereas Ap4A-induced changes were not affected. Pre-treatment with suramin (P2-receptors antagonist, (i.v., bolus - 12 mg/kg followed by 1.2 mg/min/kg) completely abolished the renal effects of Ap4A. We conclude that Ap4A may exert specific action on renal function. It acts different from NAD that modified renal function through its hydrolysis product - adenosine. Ap4A might reduce glomerular filtration rate and evoke natriuresis and diuresis, and its effects are probably mediated through stimulation of P2-receptors.

Key words: GFR, NAD, diadenosine tetraphosphate.
INTRODUCTION

Diadenosine polyphosphates (ApnA; n=3-7 of phosphate groups) have been detected in various mammalian cells (1,2). However, considerable amounts of these dinucleotides, mainly diadenosine tetraphosphate (Ap4A), are stored in and released from platelets dense granules (3,4,5), adrenal gland (6,7) and brain synaptosomes (6,8,9). There is growing evidence indicating that these agents may play an important role as extracellular messengers. Diadenosine polyphosphates affect directly the target cells through activation of P1- and P2-receptors (2,10). The length of the polyphosphate chain plays a crucial role in their activity and interactions with receptors (10,11). Moreover, the extracellular dinucleodides are metabolised by soluble enzymes in the blood plasma as well as membrane-bound ectoenzymes of different cell types (12-15). The enzymatic degradation of ApnA leads to generation the others purinergic active molecules such as ATP, ADP and adenosine (16-19). The products of ApnA hydrolysis may modulate biological effects of ApnA (15).

It has been shown that ApnA have biphasic vasomotor activity and influence vascular bed in different way. It has been described that Ap4A induces endothelium-independent vasoconstriction and endothelium-dependent vasodilatation in rabbit mesenteric arteries (11). Moreover, Ap4A has been used in humans causing a sustained reduction in blood pressure (20). The renal system is apparently an important target for diadenosine polyphosphates. The influence of ApnA on renal hemodynamics and urine excretion is currently discussed (10,21-24). Despite the number of reports concerning ApnA action on the renal vascular system only limited information about Ap4A action on sodium and urine excretion is available. Intravenous single injection of Ap3A reduced whereas Ap6A increased urinary flow and sodium excretion without detectable changes in glomerular filtration rate. However, any changes in sodium and water excretion after Ap4A injection has not been recorded (25). Although diadenosine polyphosphates are metabolised in extracellular space, most studies so far have examined renal effects of ApnA using single injection of these agents.

In the present study we have evaluated the effects of continuous infusion of diadenosine tetraphosphate (Ap4A) and NAD as a reference adenosine precursor, on renal hemodynamic and urinary excretion. The potential role of adenosine in Ap4A- and NAD-induced changes were investigated by comparison the effects of these dinucleotides on GFR, renal plasma flow, sodium and potassium excretion and urine flow before and after the administration of adenosine deaminase (approach to adenosine degradation) and nonselective antagonist of adenosine receptors - theophylline. Furthermore, we have tested the influence of Ap4A on renal function after pre-treatment with suramin - P2-receptors antagonist.
MATERIALS AND METHODS

The clearance experiments were performed with male Wistar rats weighting 240-280g, maintained on a standard diet (Altromin, Lage, Germany). The animals had free access to food and water until the day of study. Before the experiments the animals were anesthetised with Inactin (i.p., 100 mg/kg body wt). A polyethylene tube was placed in the trachea to ensure free airways. The femoral vein was cannulated for saline and drug infusion. Aortic blood pressure was recorded by a catheter introduced into the femoral artery and connected with pressure transducer. The same catheter was also used for sampling blood. The urinary bladder was exposed by an abdominal incision and cannulated to drain urine. To collect urine completely, the urethra was ligated. During the surgical preparation and experimental procedures the animals were placed on a thermostatically controlled heated table and the rectal temperature was maintained at about 37°C. In order to compensate for fluid losses during surgical procedures 3% bovine albumin in isotonic saline was infused at 0.5 ml/min.

Experimental procedures

Directly after surgical procedure, the infusion of isotonic saline containing 0.2% sodium p-aminohippurate, 5 µCi/ml [3H]-inulin and 5 u.i./ml heparine was started. The infusion rate was kept constant at 45 µl/min. After 60 min equilibration period, timed urine collections were started. Arterial blood samples (about 100 µl) was collected in the mid-point of clearance periods. The first three collecting periods were used as baseline value (control period). After control period, a priming dose of 1 µmole of Ap4A or NAD in 0.2 ml isotonic saline was given i.v. and three 20 min urine collections (experimental period) were made immediately while the nucleotides were infused at 10 nmol/kg/min. After stopping infusion of nucleotides one additional 20 min urine collection was made (recovery period).

The protocols of next experiments differed from previous one in that the nucleotides were given during the infusion of 2 U/min/kg adenosine deaminase or 0.2 mmol/min/kg theophylline. In another group of animals, the Ap4A infusion was superimposed on i.v. administration of suramin (bolus of 12 mg/kg) and continuos its infusion of 1.2 mg/min/kg.

Analytical methods and calculations

The glomerular filtration rate (GFR) was calculated as clearance of [3H]-inulin given by the urine to plasma activity ratio multiplied by urine flow. Plasma and urine samples were counted in duplicate in liquid scintillation counter. Renal plasma flow (RPF) was calculate as clearance of p-aminohippurate (PAH). PAH concentration in plasma and urine was measured by Marshall's method, sodium and potassium by potenciometry. Fractional excretion (FE) was calculate as percent of filtered load.

Data analysis

Statistical analyses were performed using a one-way analysis of variance followed by the Dunnett's multiple comparison test. Values of p<0.05 were considered as significant. Data are shown as the mean ± SEM.

Materials

NAD, theophylline, suramin, adenosine deaminase were purchased from Sigma (St Louis, MO, USA) and Inactin from RBI (Natick, USA). [3H]-Inulin was obtained from DuPont NEN Products (Boston, MA, USA). All other agents were purchased from POCh (Gliwice, Poland).
RESULTS

The effects of dinucleotides (Ap4A and NAD) on hemodynamics and renal excretion are summarised in Table 1. The i.v. bolus injection of Ap4A and its followed infusion induced progressive reduction in mean arterial pressure (MAP; maximal Δ 10 mmHg) and rapid decrease in renal plasma flow (RPF, measured as $C_{\text{PAH}}$) and glomerular filtration rate (GFR, measured as $C_{\text{in}}$) by 40 and 30% (p<0.05 vs. baseline), respectively. In contrast to the changes in renal hemodynamics parameters, there was slight but significant (1.4-fold) increase in urine flow (V) and marked (4.4-fold) increase in sodium excretion ($U_{\text{Na}}V$). These effects were observed without significant changes in potassium excretion ($U_{\text{K}}V$).

As expected, the i.v. bolus injection of NAD and its followed infusion resulted in marked decrease in RPF and GFR by 48 and 40% in comparison to control period, respectively (Table 1). MAP was significantly decreased (Δ 20 mmHg) during NAD infusion but remained within the renal blood flow auto-regulatory range. The natriuretic and diuretic responses to NAD quite differed from effects of Ap4A: $U_{\text{Na}}V$ decreased 2-3-fold during NAD infusion. However, less pronounced changes in

| Table 1. Hemodynamics and renal excretion responses to i.v. infusion of Ap4A and NAD (both 1μmol/kg bolus then 10 nmol/min/kg). |
|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| Clearance period (min) | Baseline | Ap4A infusion | Baseline | NAD infusion |
| MAP (mmHg) | -20-0 | 0-20 | 20-60 | -20-0 | 0-20 | 20-60 |
| RPF (ml/min) | 116±4 | 108±5 | 106±3* | 120±5 | 108±3* | 100±3* |
| GFR (ml/min) | 10.25±1.70 | 6.40±1.50* | 7.21±1.30 | 8.05±0.31 | 4.60±0.15* | 4.88±0.92* |
| FF (%) | 19±3.0 | 20±2.6 | 16±2.5 | 24±1.0 | 22±1.5 | 20±2.2 |
| V (μl/min) | 10.1±1.1 | 12.7±1.4 | 16.0±2.5* | 12.0±0.5 | 6.1±0.7* | 5.5±0.9* |
| FE$_{\text{urine}}$ (%) | 0.6±0.05 | 1.0±0.1 | 1.4±0.05* | 0.6±0.04 | 0.6±0.05 | 0.5±0.10 |
| $U_{\text{Na}}V$ (μmol/min) | 0.5±0.1 | 1.5±0.5* | 2.2±0.7* | 0.9±0.2 | 0.3±0.1* | 0.2±0.1* |
| FE$_{\text{Na}}$ (%) | 0.1±0.05 | 0.8±0.1* | 1.5±0.1* | 0.3±0.1 | 0.2±0.05 | 0.1±0.05 |
| $U_{\text{K}}V$ (μmol/min) | 3.0±0.5 | 3.0±0.4 | 2.4±0.6 | 2.7±0.4 | 2.4±0.3 | 2.0±0.2 |

Abbreviations: MAP - mean arterial blood pressure; RPF - renal plasma flow (measured as p-aminohippurate clearance, $C_{\text{PAH}}$); GFR - glomerular filtration rate (measured as inulin clearance, $C_{\text{in}}$); FF - filtration fraction; V - urinary flow; FE$_{\text{urine}}$ - fractional urine excretion; $U_{\text{Na}}V$ - sodium excretion; FE$_{\text{Na}}$ - fractional sodium excretion; $U_{\text{K}}V$ - potassium excretion. Values are mean ± S.E.M., n = 7. *P< 0.05 vs baseline (control period).
urine excretion were in parallel with those in sodium excretion. As shown in Figure 1, the hemodynamic alternations in response to tested dinucleotides were transitory; in the first period of urine collection after termination of Ap4A or NAD infusions GFR (Fig.1A) and RPF (Fig.1B) returned to the baseline value (p>0.05 vs. control).

To answer the question whether Ap4A- as well as NAD-induced changes in the renal hemodynamics are resulted of adenosine-generated action, the effects of dinucleotides were evaluated during intravenous infusion of adenosine deaminase (ADA; enzyme which metabolises adenosine to inosine) or theophylline (nonselective antagonist of P1-receptors). As shown in Figure 2, the administration of ADA abolished NAD-induced decrease in GFR (Fig.2A) and RPF (Fig.2B), however, did not change the effects of Ap4A on either GFR

Fig.1. Changes of GFR (A) and RPF (B) during the infusion of Ap4A (o) and NAD (●) and after stopping the infusion of the nucleotides Both nucleotides were given i.v. as a bolus 1µmol/kg, followed by continuous infusion of 10 nmol/min/kg (n = 9, in each group). Data are presented as a mean ± S.E.M. *P< 0.05 vs baseline.
(Fig. 2A) or RPF (Fig. 2B). Similar to effects of ADA, administration of the theophylline abolished only NAD-induced but not Ap4A-induced decrease in RPF and GFR (Fig. 3A and B).

*Figure 4* shows the effects of Ap4A on diuresis, natriuresis and GFR in the presence of suramin, non-selective antagonist of P2-receptors. Pre-treatment with suramin before an intravenous infusion of Ap4A abolished the Ap4A-induced increase in urine and sodium excretion. Furthermore, no alternation in GFR was detected during co-infusion of Ap4A and suramin.

To demonstrate the stability of renal function, GFR, RPF and solute excretion were measured during control period with saline infusion. There was no significant change in any of these parameters during observation period of 30 min.

![Graph showing effects of Ap4A and NAD on GFR and RPF](image)

*Fig. 2. Effects of Ap4A (○) and NAD (●) on GFR (A) and RPF (B) during adenosine deaminase infusion.*

Adenosine deaminase was continuously i.v. infused at 2 U/min/kg. After 50 min of its infusion, nucleotides were given i.v. as a bolus 1µmol/kg, followed by continuous infusion of 10 nmol/min/kg (n = 6, in each group). Data are presented as a mean ± S.E.M. *P< 0.05 vs baseline.*
DISCUSSION

The present study compares the effects of intravenous infusion of Ap4A with equivalent dose of NAD (precursor of adenosine which is regarded as a reference substance) on renal hemodynamics and urine excretion in anaesthetised rats. Our results document that both nucleotides profoundly affect renal function; Ap4A similarly to NAD, has decreased GFR and RPF but in contrast to NAD, unexpectedly significantly has increased urine and sodium excretion. In this study we have indicated for the first time that Ap4A has induced natriuresis with simultaneous inhibition of glomerular filtration. As it was previously shown, both Ap4A and NAD induced hypotensive response in anesthetized rats. However, there is difference in time of response to Ap4A

Fig. 3. Effects of Ap4A (○) and NAD (●) on GFR (A) and RPF (B) during theophylline infusion
Theophylline was continuously i.v. infused at 0.2 mmol/min/kg. After 50 min of its infusion, nucleotides were given i.v. as a bolus 1μmol/kg, followed by continuous infusion of 10 nmol/min/kg (n = 7, in each group). Data are presented as a mean ± S.E.M. *P< 0.05 vs baseline
Our results indicate that NAD-induced decrease in renal hemodynamic is similar to this induced by adenosine (26-28). It was previously shown that systemic infusion of NAD led to decrease in renal hemodynamics. Those effects were abolished by theophylline and intensified by dipyridamole - inhibitor of cellular uptake of adenosine (26). Our observations support the hypothesis that extracellular NAD apparently reduces the renal function by action of adenosine which is released in the results of enzymatic NAD hydrolysis. These results stay in line with already published observations, where adenosine has caused decrease in renal blood flow and GFR by an interaction with adenosine A1 receptors (29,30).

Renal vascular effects induced by Ap4A in several models have been reported to be mediated by P1-receptors as well as P2-receptors (2,24,31). In the present study, the renal effects evoked by Ap4A administration, in contrast to NAD, were not altered by theophylline - non-selective antagonist of P1-receptors. This finding suggests that Ap4A does not change renal hemodynamic via activation of P1-receptors. Moreover, to answer the question whether adenosine i.e. product of Ap4A hydrolysis has induced changes in GFR and RPF a new approach with adenosine deaminase (ADA), which leads to a degradation of extracellular adenosine, has been used. Intravenous infusion of ADA has not influenced renal effects of Ap4A. These results suggest that Ap4A-induced hypotension manifests much later than NAD-induced one.

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mediated via product of its degradation i.e. adenosine. Next the involvement of P2-receptors in Ap4A action has been checked. The effects of Ap4A have been abolished by suramine which is known as a non-selective P2-receptor antagonist. Taken together, these observations support the hypothesis that Ap4A reduces GFR and RPF via P2-receptors.

The effects of Ap4A and NAD on sodium, potassium and water excretion have also been compared. In contrast to Ap4A, we have observed that NAD has induced decrease in sodium, potassium and water excretion. These alternations of tubular function induced by NAD accord with results of adenosine action (29-32). However, Ap4A has induced slight but significant (1.4-fold) increase in urine flow (V) and marked (4.4-fold) increase in sodium excretion (U_{Na}V) without significant changes in potassium excretion (U_{K}V). These effects have been abolished in the presence of suramin. Since the increase of renal excretion of urine and sodium occurred with simultaneous decrease in GFR, the fractional excretion of urine (FE_{urine}) and sodium (FE_{Na}) has increased 2.3-fold and 15-fold in comparison to control period (baseline), respectively. The distinction between sodium and water excretion suggest that Ap4A inhibits reabsorption of sodium and stimulates reabsorption of water in kidney. The cellular mechanism of Ap4A action is under investigation now.

Thus, it seems to be possible that P2-receptors are involved in the renal effects of Ap4A. The mechanism of Ap4A action should be elucidated. It is known that Ap4A is an agonist of P2-receptors (17,23,31). Moreover, it is well documented that Ap4A may be hydrolised to ATP and ADP in extracellular space (12-15). Thus, it is possible that observed renal effects are mediated by ATP and ADP. To our knowledge it is not feasible to differentiate between effects evoked by Ap4A per se and ATP or ADP in vivo.

The results of present study suggest possibility that local release of Ap4A in the kidney may profoundly alter glomerular and tubular function. It has been shown that activation of platelets lead to the release of a variety of vasoactive nucleotides (3-5). Moreover, the concentration of dinucleotides following platelet aggregation, namely Ap4A, at local microenvironment of the cell surface may be at least in order of magnitude higher than 0.5 - 3 µM (15).

In conclusion, our results provide evidence that Ap4A may play an important role in the regulation of renal glomerular and tubular function, mainly as a natriuretic substance. We anticipate that modulation of renal function may become a field of pharmacotherapeutic interventions in the future, especially in renal sodium retaining states e.g. cirrhotic ascites, congestive heart failure or nephrotic syndrome.

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