In Vivo Cardiovascular Pharmacology of 2',3'-cAMP, 2'-AMP, and 3'-AMP in the Rat

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ABSTRACT

The naturally occurring purine 2',3'-cAMP is metabolized in vitro to 2'-AMP and 3'-AMP, which are subsequently metabolized to adenosine. Whether in vivo 2',3'-cAMP, 2'-AMP, or 3'-AMP are rapidly converted to adenosine and exert rapid effects via adenosine receptors is unknown. To address this question, we compared the cardiovascular and renal effects of 2',3'-cAMP, 2'-AMP, 3'-AMP, 3',5'-cAMP, 5'-AMP, and adenosine in vivo in the rat. Purines were infused intravenously while monitoring mean arterial blood pressure (MABP), heart rate (HR), cardiac output, and renal and mesenteric blood flows. Total peripheral (TPR), renal vascular (RVR), and mesenteric vascular (MVR) resistances were calculated. Urine was collected for determination of urine excretion rate [urine volume (UV)]. When sufficient urine was available, the sodium excretion rate (Na^+ ER) and glomerular filtration rate (GFR) were determined. 2',3'-cAMP, 2'-AMP, and 3'-AMP dose-dependently and profoundly reduced MABP, HR,

TPR, and MVR with efficacy and potency similar to adenosine and 5'-AMP. These effects of 2',3'-cAMP, 2'-AMP, and 3'-AMP were attenuated by blockade of adenosine receptors with 1,3-dipropyl-8-(p-sulphophenyl)xanthine. 2',3'-cAMP, 2'-AMP, 3'-AMP, adenosine, and 5'-AMP variably affected RVR, but profoundly (nearly 100%) decreased UV at higher doses. GFR and Na^+ ER could be measured at the lower doses and were suppressed by 2',3'-cAMP, 2'-AMP, and 3'-AMP, but not by adenosine or 5'-AMP. 2',3'-cAMP increased urinary excretion rates of 2'-AMP, 3'-AMP, and adenosine. 3',5'-cAMP exerted no adverse hemodynamic effects yet increased urinary adenosine as efficiently as 2',3'-cAMP. Conclusions: In vivo 2',3'-cAMP is rapidly converted to adenosine. Because both cAMPs increase adenosine in the urinary compartment, these agents may provide unique therapeutic opportunities.

Introduction

By use of high-performance liquid chromatography–tandem mass spectrometry to analyze venous perfusate from isolated, perfused kidneys, we discovered that intact kidneys produce and release into the extracellular compartment 2',3'-cAMP (Jackson et al., 2009; Ren et al., 2009), a positional isomer of the second messenger 3',5'-cAMP. In addition, we found that kidneys, both in vitro and in vivo, can metabolize 2',3'-cAMP to 2'-AMP plus 3'-AMP and that these AMPs can be further metabolized to adenosine (Jackson et al., 2009). More recently, we have shown the existence of 2',3'-cAMP, 2'-AMP, and 3'-AMP in vivo in mice and humans (Verrier et al., 2012).

Although our previously published studies show that exogenous 2',3'-cAMP, 2'-AMP, and 3'-AMP alter vascular smooth muscle cell, endothelial cell, and epithelial cell proliferation via conversion to adenosine, those experiments were performed by incubating cells in vitro for several days with these compounds (Jackson et al., 2010, 2011a,b; Jackson and Gillespie, 2012). Thus it is unknown whether conversion of 2',3'-cAMP, 2'-AMP, or 3'-AMP to adenosine occurs rapidly

enough in vivo to yield significant biologic effects. To our knowledge the only in vivo study that addressed this question was published in 1963 (Denatale et al., 1963), was limited in scope, and indicated that the in vivo metabolism of 2',3'-cAMP, 2'-AMP, and 3'-AMP to adenosine might be sluggish, because it was reported that in cats these compounds only affect blood pressure after a delay following intravenous injection. The goal of the present study was to determine whether 2',3'-cAMP, 2'-AMP, and 3'-AMP are so rapidly converted to adenosine in vivo that they give cardiovascular and renal responses that are essentially identical to adenosine and blocked by adenosine receptor antagonism. Also, because 3',5'-cAMP (similar to 2',3'-cAMP) can be metabolized to its corresponding AMP, in this case 5'-AMP, which is also an adenosine precursor, we chose to compare the effects of 2',3'-cAMP, 2'-AMP, 3'-AMP, 3',5'-cAMP, 5'-AMP, and adenosine in a head-to-head study.

Materials and Methods

2',3'-cAMP, 2'-AMP, 3'-AMP, 3',5'-cAMP, 5'-AMP, 3'-AMP, and 1,3-dipropyl-8-(p-sulphophenyl)xanthine (DPSPX) were purchased from Sigma-Aldrich (St. Louis, MO).

Animals. This study employed 118 male Sprague-Dawley rats (Charles River Laboratories, Wilmington, MA) weighing 464 ± 8

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ABBREVIATIONS: AKI, acute kidney injury; CO, cardiac output; DPSPX, 1,3-dipropyl-8-(p-sulphophenyl)xanthine; GFR, glomerular filtration rate; HR, heart rate; MABP, mean arterial blood pressure; MBF, mesenteric blood flow; MVR, mesenteric vascular resistance; PE, polyethylene; RBF, renal blood flow; RVR, renal vascular resistance; TPR, total peripheral resistance; UV, urine volume.

(mean $\pm$ S.E.M.) grams. The Institutional Animal Care and Use Committee approved all procedures. The investigation conforms to the *Guide for the Care and Use of Laboratory Animals* (NIH Publication No. 85-23, revised 1996).

Protocol 1 (Low-Dose Study). Rats were anesthetized with Inactin (thiobutabarbital; 90 mg/kg i.p.), placed on an isothermal pad, monitored for body temperature with a rectal probe thermometer, and kept at 37°C with a heat lamp. The trachea was cannulated with polyethylene (PE)-240 to facilitate respiration, and a PE-50 cannula was inserted into the left femoral artery and connected to a digital blood pressure analyzer (Micro-Med, Inc., Louisville, KY) for continuous measurement of mean arterial blood pressure (MABP) and heart rate (HR). Two PE-50 cannulas were inserted into the jugular vein, and an infusion of 0.9% saline at 25 μ l/min was initiated via one of the cannulas. The other jugular vein cannula was used for injection of saline for cardiac output (CO) measurements. A PE-10 tubing was placed in the left ureter for urine collection. A thermomicroprobe was placed in the aortic arch via the left carotid artery and was connected to a Cardiotherm-55-AC-R cardiac output computer (Columbus Instruments International Corp., Columbus, OH). Noncannulating, transit-time flow probes (Transonic Systems, Inc., Ithaca, NY) were placed on the left renal (1 mm) and mesenteric (2 mm) arteries and were connected to a two-channel small animal transit-time flowmeter (model T-206; Transonic Systems, Inc.) for measurement of renal blood flow (RBF) and mesenteric blood flow (MBF). After a 1-hour stabilization period, urine was collected for 30 minutes while MABP and HR were time-averaged during this clearance period. RBF and MBF were recorded at 10, 20, and 30 minutes, and cardiac output was measured at 10, 20, and 30 minutes by injecting 0.15 ml of room-temperature saline. A 0.2-ml blood sample was taken during the middle of the 30-minute urine collection, centrifuged and obtained for measurement of plasma creatinine. In some rats, the intravenous

saline infusion (saline group) was continued, and in other rats the intravenous saline infusion was changed to either 2'-AMP, 3'-AMP, 5'-AMP, 2',3'-cAMP, 3',5'-cAMP, or adenosine at 0.03 μ mol/kg per minute. Each rat received only a single substance (either saline, 2'-AMP, 3'-AMP, 5'-AMP, 2',3'-cAMP, 3',5'-cAMP, or adenosine; i.e., seven groups). The infusions were continued for 40 minutes, and during the last 30 minutes of the experimental period, all measurements described above were repeated. This procedure was then repeated with two additional 40-minute experimental periods using 0.1 and then 0.3 μ mol/kg per minute of 2'-AMP, 3'-AMP, 5'-AMP, 2',3'-cAMP, 3',5'-cAMP, or adenosine.

Protocol 2 (High-Dose Study). This protocol, which was performed in seven additional groups of rats, was the same as described for Protocol 1 (Low-Dose Study) except that the doses of 2'-AMP, 3'-AMP, 5'-AMP, 2',3'-cAMP, 3',5'-cAMP, and adenosine were 1, 3, and 10 μ mol/kg per minute.

Protocol 3 (Antagonist Study). Rats were instrumented as described above, then half the animals were administered DPSPX (30 mg/kg i.v. bolus followed by continuous intravenous infusion of 0.5 mg/kg per minute for the remainder of the protocol). We previously showed that this dose of DPSPX antagonizes the cardiovascular effects of adenosine in rats (Holycross et al., 1989; Kuan et al., 1990). After a 30-minute stabilization period, urine was collected for 30 minutes, and MABP and HR were time-averaged. RBF, MBF, and CO were each measured at 10, 20, and 30 minutes. Next, rats received an intravenous infusion of either 2'-AMP, 3'-AMP, 2',3'-cAMP, or adenosine (3 μ mol/kg per minute). Each rat received only a single treatment (i.e., eight groups: saline + 2'-AMP, saline + 3'-AMP, saline + 2',3'-cAMP, saline + adenosine, DPSPX + 2'-AMP, DPSPX + 3'-AMP, DPSPX + 2',3'-cAMP, and DPSPX + adenosine). The infusions were continued for 40 minutes, and measurements were repeated for the last 30 minutes of the experimental period.

Fig. 1. Bar graphs show the percentage change in MABP induced by 2',3'-cAMP, 2'-AMP, 3'-AMP, 5'-AMP, adenosine, and 3',5'-cAMP at the indicated doses. The left and right panels summarize the results of the low-dose and high-dose studies, respectively. In both studies, a vehicle/time control group (saline) was included. In the low-dose study, basal values for MABP were 113 ± 5 , 116 ± 4 , 108 ± 5 , 114 ± 4 , 111 ± 6 , 96 ± 5 , and 108 ± 5 mm Hg for the saline, 2',3'-cAMP, 2'-AMP, 3'-AMP, 5'-AMP, adenosine, and 3',5'-cAMP groups, respectively. The basal values in the high-dose study were 106 ± 3 , 106 ± 4 , 113 ± 4 , 105 ± 6 , 103 ± 9 , 105 ± 4 , and 111 ± 4 mm Hg, for the saline, 2',3'-cAMP, 2'-AMP, 3'-AMP, 5'-AMP, adenosine, and 3',5'-cAMP groups, respectively. Values are presented as mean $\pm$ S.E.M. for the indicated number of animals (n). The letters "a" and "b" indicate $P < 0.05$ (Fisher's least significant difference test) compared with the saline and adenosine groups, respectively. ANOVA, analysis of variance.

Analytical Measurements. Creatinine was measured using a creatinine autoanalyzer (Creatinine Analyzer 2; Beckman Instruments, Inc., Fullerton, CA), and sodium was measured by flame photometry (Model IL-943; Instrumentations Laboratory, Inc., Lexington, MA). In some urine samples, 2'-AMP, 3'-AMP, 5'-AMP, 2',3'-cAMP, 3',5'-cAMP, and adenosine were measured using high-performance liquid chromatography–tandem mass spectrometry as previously described (Jackson et al., 2009).

Statistics. Data are presented as mean $\pm$ S.E.M. Statistical analysis was performed using analysis of variance, unpaired Student's *t* test, or Fisher's least significant difference test, as appropriate. A value of *P* < 0.05 was considered statistically significant.

Results

Effects of Purines on MABP. As shown in Fig. 1, 2',3'-cAMP, 2'-AMP, 3'-AMP, 5'-AMP, and adenosine dose-dependently decreased MABP. In this regard, the threshold for reducing MABP was remarkable low (0.1 $\mu\text{mol/kg}$ per minute), and the effects at 10 $\mu\text{mol/kg}$ per minute were profound (60–70% reduction in MABP). In general, the hypotensive effects of 2'-AMP and 3'-AMP exceeded those of adenosine. Notably, 3',5'-cAMP only reduced MABP at the higher doses and then only moderately (27% at the highest dose). These findings indicate that 2',3'-cAMP, 2'-AMP, and 3'-AMP are at least as effective as or more effective than adenosine as hypotensive agents and that 3',5'-cAMP, in contrast, has little or only moderate effects on arterial blood pressure.

Effects of Purines on HR. 2',3'-cAMP, 2'-AMP, 3'-AMP, 5'-AMP, and adenosine also dose-dependently decreased HR

(Fig. 2), with 2',3'-cAMP, 2'-AMP, and adenosine exhibiting threshold effects at 0.03 $\mu\text{mol/kg}$ per minute. At several concentrations, the effects of 2',3'-cAMP, 2'-AMP, and 3'-AMP exceeded those of adenosine. In contrast, 3',5'-cAMP had little, if any, effect on HR. Thus, as with MABP, 2',3'-cAMP, 2'-AMP, and 3'-AMP are at least as effective or more effective than adenosine as bradycardic agents, whereas 3',5'-cAMP, in contrast, has little effect on HR.

Effects of Purines on CO. Although variable, 2',3'-cAMP, 2'-AMP, 3'-AMP, 5'-AMP, and adenosine tended to decrease CO; however, these effects reached significance only for some doses of 2',3'-cAMP, 2'-AMP, and 3'-AMP; and for adenosine only at the highest dose (Fig. 3). As with MABP and HR, 3',5'-cAMP had little, if any, effect on CO. These data suggest that the hypotensive effects of these purines are only partially explained by decreases in CO.

Effects of Purines on Total Peripheral Resistance (TPR) and Mesenteric Vascular Resistance (MVR). The higher doses of 2',3'-cAMP, 2'-AMP, 3'-AMP, 5'-AMP, and adenosine reduced TPR, whereas 3',5'-cAMP did not (Fig. 4). The mesenteric vascular bed was particularly sensitive to the vasodilatory effects of these purines as evidenced by the decreases in MVR beginning at 0.1–0.3 $\mu\text{mol/kg}$ per minute (Fig. 5). Once again, 3',5'-cAMP had little, if any, effect. These data suggest that systemic vasodilation plays an important role in the hypotensive effects of these purines.

Effects of Purines on Renal Vascular Resistance. Neither adenosine, 5'-AMP, nor 3',5'-cAMP significantly

Fig. 2. Bar graphs show the percentage change in heart rate induced by 2',3'-cAMP, 2'-AMP, 3'-AMP, 5'-AMP, adenosine, and 3',5'-cAMP at the indicated doses. The left and right panels summarize the results of the low-dose and high-dose studies, respectively. In both studies, a vehicle/time control group (saline) was included. In the low-dose study, basal values for HR were 370 ± 6 , 400 ± 11 , 388 ± 15 , 379 ± 12 , 398 ± 18 , 374 ± 15 , and 391 ± 5 beats/min for the saline, 2',3'-cAMP, 2'-AMP, 3'-AMP, 5'-AMP, adenosine, and 3',5'-cAMP groups, respectively. The basal values in the high-dose study were 369 ± 9 , 395 ± 8 , 399 ± 20 , 432 ± 14 , 402 ± 20 , 413 ± 18 , and 399 ± 24 beats/min, for the saline, 2',3'-cAMP, 2'-AMP, 3'-AMP, 5'-AMP, adenosine, and 3',5'-cAMP groups, respectively. Values are presented as mean $\pm$ S.E.M. for the indicated number of animals (*n*). The letters "a" and "b" indicate *P* < 0.05 (Fisher's least significant difference test) compared with the saline and adenosine groups, respectively. ANOVA, analysis of variance.

Fig. 3. Bar graphs show the percentage change in cardiac output induced by 2',3'-cAMP, 2'-AMP, 3'-AMP, 5'-AMP, adenosine, and 3',5'-cAMP at the indicated doses. The left and right panels summarize the results of the low-dose and high-dose studies, respectively. In both studies, a vehicle/time control group (saline) was included. In the low-dose study, basal values for CO were 122 ± 11 , 130 ± 11 , 150 ± 20 , 136 ± 15 , 149 ± 9 , 129 ± 14 , and 117 ± 14 ml/min for the saline, 2',3'-cAMP, 2'-AMP, 3'-AMP, 5'-AMP, adenosine, and 3',5'-cAMP groups, respectively. The basal values in the high-dose study were 128 ± 12 , 141 ± 15 , 116 ± 8 , 130 ± 11 , 135 ± 2 , 128 ± 10 , and 136 ± 9 ml/min for the saline, 2',3'-cAMP, 2'-AMP, 3'-AMP, 5'-AMP, adenosine, and 3',5'-cAMP groups, respectively. Values are presented as mean $\pm$ S.E.M. for the indicated number of animals (*n*). The letters "a" and "b" indicate $P < 0.05$ (Fisher's least significant difference test) compared with the saline and adenosine groups, respectively. ANOVA, analysis of variance.

altered renal vascular resistance (RVR) (Fig. 6). The effects of 2',3'-cAMP, 2'-AMP, and 3'-AMP were biphasic with low doses tending to decrease RVR and high doses increasing RVR, particularly 2'-AMP.

Effects of Purines on Urine Volume. 2',3'-cAMP, 2'-AMP, and 3'-AMP had little effect on urine volume (UV) at low doses, but profoundly (in some cases 100%) decreased UV at higher doses (Fig. 7). The effects of 5'-AMP and adenosine

Fig. 4. Bar graphs show the percentage change in total peripheral resistance (calculated as mean arterial blood pressure $\div$ cardiac output) induced by 2',3'-cAMP, 2'-AMP, 3'-AMP, 5'-AMP, adenosine, and 3',5'-cAMP at the indicated doses. The left and right panels summarize the results of the low-dose and high-dose studies, respectively. In both studies, a vehicle/time control group (saline) was included. In the low-dose study, basal values for TPR were 0.96 ± 0.09 , 0.92 ± 0.06 , 0.78 ± 0.09 , 0.89 ± 0.09 , 0.76 ± 0.08 , 0.77 ± 0.07 , and 0.98 ± 0.09 mm Hg/(ml/min) for the saline, 2',3'-cAMP, 2'-AMP, 3'-AMP, 5'-AMP, adenosine, and 3',5'-cAMP groups, respectively. The basal values in the high-dose study were 0.92 ± 0.13 , 0.80 ± 0.12 , 0.99 ± 0.06 , 0.82 ± 0.05 , 0.76 ± 0.07 , 0.85 ± 0.09 , and 0.82 ± 0.06 mm Hg/(ml/min), for the saline, 2',3'-cAMP, 2'-AMP, 3'-AMP, 5'-AMP, adenosine, and 3',5'-cAMP groups, respectively. Values are presented as mean $\pm$ S.E.M. for the indicated number of animals (*n*). The letters "a" and "b" indicate $P < 0.05$ (Fisher's least significant difference test) compared with the saline and adenosine groups, respectively. ANOVA, analysis of variance.

Fig. 5. Bar graphs show the percentage change in mesenteric vascular resistance, calculated as mean arterial blood pressure ÷ mesenteric blood flow) induced by 2',3'-cAMP, 2'-AMP, 3'-AMP, 5'-AMP, adenosine, and 3',5'-cAMP at the indicated doses. The left and right panels summarize the results of the low-dose and high-dose studies, respectively. In both studies, a vehicle/time control group (saline) was included. In the low-dose study, basal values for MVR were 11 ± 1 , 10 ± 1 , 10 ± 1 , 11 ± 1 , 10 ± 2 , 9 ± 1 , and 10 ± 2 mm Hg/(ml/min) for the saline, 2',3'-cAMP, 2'-AMP, 3'-AMP, 5'-AMP, adenosine, and 3',5'-cAMP groups, respectively. The basal values in the high-dose study were 10 ± 2 , 15 ± 2 , 14 ± 2 , 12 ± 1 , 15 ± 2 , 9 ± 1 , and 12 ± 1 mm Hg/(ml/min), for the saline, 2',3'-cAMP, 2'-AMP, 3'-AMP, 5'-AMP, adenosine, and 3',5'-cAMP groups, respectively. Values are presented as mean $\pm$ S.E.M. for the indicated number of animals (*n*). The letters "a" and "b" indicate $P < 0.05$ (Fisher's least significant difference test) compared with the saline and adenosine groups, respectively. ANOVA, analysis of variance.

were biphasic, with low doses increasing and high doses decreasing UV (Fig. 7). 3',5'-cAMP only slightly decreased UV at the highest dose.

Effects of Purines on Glomerular Filtration Rate and Na^+ ER. Because of the profound suppression of UV by the higher doses of purines (Fig. 7), it was only possible to measure glomerular filtration rate (GFR) and Na^+ ER with the low doses of purines (Fig. 8). At these lower doses, adenosine and 5'-AMP had little effect on GFR or Na^+ ER. 2',3'-cAMP did not affect GFR, but it decreased Na^+ ER;

whereas 2'-AMP and 3'-AMP suppressed both GFR and Na^+ ER. In contrast, 3',5'-cAMP did not affect GFR, and it increased Na^+ ER.

Effects of Purines on Urinary Excretion Rate of Purines. Sufficient urine was collected during the low-dose study to perform mass spectrometry analysis of the effects of purine infusions on the urinary excretion rate of purines. Intravenous infusion of purines did not affect the urinary excretion rate of 2',3'-cAMP, 3',5'-cAMP, or 5'-AMP (unpublished data). 2',3'-cAMP increased the urinary excretion

Fig. 6. Bar graphs show the percentage change in renal vascular resistance (calculated as mean arterial blood pressure ÷ renal blood flow) induced by 2',3'-cAMP, 2'-AMP, 3'-AMP, 5'-AMP, adenosine, and 3',5'-cAMP at the indicated doses. The left and right panels summarize the results of the low-dose and high-dose studies, respectively. In both studies, a vehicle/time control group (saline) was included. In the low-dose study, basal values for RVR were 15 ± 2 , 16 ± 2 , 14 ± 2 , 15 ± 2 , 13 ± 2 , 14 ± 1 , and 12 ± 1 mm Hg/(ml/min) for the saline, 2',3'-cAMP, 2'-AMP, 3'-AMP, 5'-AMP, adenosine, and 3',5'-cAMP groups, respectively. The basal values in the high-dose study were 16 ± 2 , 16 ± 1 , 14 ± 2 , 15 ± 2 , 15 ± 2 , 12 ± 2 , and 15 ± 1 mm Hg/(ml/min), for the saline, 2',3'-cAMP, 2'-AMP, 3'-AMP, 5'-AMP, adenosine, and 3',5'-cAMP groups, respectively. Values are presented as mean $\pm$ S.E.M. for the indicated number of animals (*n*). The letters "a" and "b" indicate $P < 0.05$ (Fisher's least significant difference test) compared with the saline and adenosine groups, respectively. ANOVA, analysis of variance.

Fig. 7. Bar graphs show the percentage change in UV, an indicator of urine excretion rate, induced by 2',3'-cAMP, 2'-AMP, 3'-AMP, 5'-AMP, adenosine, and 3',5'-cAMP at the indicated doses. The left and right panels summarize the results of the low-dose and high-dose studies, respectively. In both studies, a vehicle/time control group (saline) was included. In the low-dose study, basal values for UV were 116 ± 19 , 127 ± 20 , 100 ± 13 , 87 ± 8 , 93 ± 17 , 89 ± 18 , and 98 ± 17 $\mu\text{l}/30$ minute for the saline, 2',3'-cAMP, 2'-AMP, 3'-AMP, 5'-AMP, adenosine, and 3',5'-cAMP groups, respectively. The basal values in the high-dose study were 100 ± 17 , 99 ± 19 , 100 ± 20 , 98 ± 33 , 119 ± 35 , 84 ± 21 , and 115 ± 18 $\mu\text{l}/30$ minutes for the saline, 2',3'-cAMP, 2'-AMP, 3'-AMP, 5'-AMP, adenosine, and 3',5'-cAMP groups, respectively. Values are presented as mean $\pm$ S.E.M. for the indicated number of animals (n). The letters "a" and "b" indicate $P < 0.05$ (Fisher's least significant difference test) compared with the saline and adenosine groups, respectively. ANOVA, analysis of variance.

of 2'-AMP, 3'-AMP, and adenosine. Importantly, even though 3',5'-cAMP did not adversely affect systemic or renal hemodynamics, 3',5'-cAMP increased the urinary excretion of adenosine as much as did 2',3'-cAMP and more so than adenosine per se (Fig. 9). Therefore, 3',5'-cAMP may be an effective therapeutic approach to increase adenosine selectively in the urinary compartment while avoiding systemic and renal hemodynamic changes.

Effects of DPSPX on Responses to Purines. To determine whether the effects of 2',3'-cAMP, 2'-AMP, and 3'-AMP were mediated by adenosine, rats were pretreated with either saline or DPSPX (a potent adenosine receptor antagonist) dissolved in saline, and responses to 3 $\mu\text{mol}/\text{kg}$ per minute of these purines were examined. To make sure that the dose of DPSPX was sufficient, groups treated with adenosine were also included. As shown in Fig. 10, DPSPX similarly suppressed the hypotensive and bradycardic effects

of 2',3'-cAMP, 2'-AMP, 3'-AMP, and adenosine; and as demonstrated in Fig. 11, DPSPX similarly blocked the vasodilatory effects of 2',3'-cAMP, 2'-AMP, 3'-AMP, and adenosine on the mesenteric vascular bed and system circulation. In the absence of DPSPX, 2',3'-cAMP, 2'-AMP, and 3'-AMP variably increased RVR by 18 ± 16 , 36 ± 11 , and $21 \pm 22\%$, respectively; and in the presence of DPSPX, 2',3'-cAMP, 2'-AMP, and 3'-AMP did not affect RVR (-10 ± 10 , -21 ± 9 , and $-4 \pm 13\%$, respectively). The dose of DPSPX, however, was insufficient to overcome the large reduction in urine volume triggered by adenosine, 2',3'-cAMP, 2'-AMP, and 3'-AMP (unpublished data).

Discussion

Our recent discoveries that 2',3'-cAMP is formed in intact organs, both in vitro and in vivo, and is metabolized to 2'-AMP,

Fig. 8. Bar graphs show the percentage change in glomerular filtration rate (left panel) and sodium excretion rate (Na^+ER ; right panel) induced by 2',3'-cAMP, 2'-AMP, 3'-AMP, 5'-AMP, adenosine, and 3',5'-cAMP at the indicated doses. A vehicle/time control group (saline) was included. Basal values for GFR were 2.4 ± 0.2 , 2 ± 0.4 , 2.4 ± 0.6 , 2.6 ± 0.3 , 2.0 ± 0.2 , 2.4 ± 0.6 , and 2.2 ± 0.1 ml/min for the saline, 2',3'-cAMP, 2'-AMP, 3'-AMP, 5'-AMP, adenosine, and 3',5'-cAMP groups, respectively. The basal values for Na^+ER were 3.1 ± 1.2 , 1.8 ± 0.7 , 1.5 ± 0.4 , 1.1 ± 0.4 , 3.1 ± 2.1 , 1.0 ± 0.3 , and 1.1 ± 0.3 mEq/30 minutes for the saline, 2',3'-cAMP, 2'-AMP, 3'-AMP, 5'-AMP, adenosine, and 3',5'-cAMP groups, respectively. Values are presented as mean $\pm$ S.E.M. for the indicated number of animals (n). The letters "a" and "b" indicate $P < 0.05$ (Fisher's least significant difference test) compared with the saline and adenosine groups, respectively. ANOVA, analysis of variance.

3'-AMP, and adenosine indicate the need to understand the pharmacology of these interesting endogenous compounds. However, currently, very little is known about the pharmacology of 2',3'-cAMP, 2'-AMP, or 3'-AMP (Jackson, 2011). In particular, it is unknown whether 2',3'-cAMP, 2'-AMP, and 3'-AMP are so rapidly converted to adenosine in vivo that they are essentially adenosine prodrugs. Although Denatale et al. (Denatale et al., 1963) report that intravenous boluses of 2'-AMP, 3'-AMP, and 2',3'-cAMP to cats cause a reduction in arterial blood pressure, these authors report a delayed effect (achieving maximum in about 5 minutes) with reflex tachycardia followed by a gradual recovery over 20–30 minutes. Their findings suggest rather sluggish metabolism of 2'-AMP, 3'-AMP, and 2',3'-cAMP to adenosine. Our previous experiments demonstrate that 2',3'-cAMP, 2'-AMP, and 3'-AMP potently and efficaciously inhibit the proliferation of microvascular smooth muscle cells (Jackson et al., 2010, 2011a), macrovascular smooth muscle cells (Jackson et al.,

2011b), and glomerular mesangial cells (Jackson et al., 2010, 2011a), yet stimulate the proliferation of vascular endothelial cells (Jackson and Gillespie, 2012) and renal tubular epithelial cells (Jackson and Gillespie, 2012). All these effects are mediated mostly by conversion of 2',3'-cAMP, 2'-AMP, and 3'-AMP to adenosine, followed by stimulation of adenosine receptors. Thus, at least in vitro, 2',3'-cAMP, 2'-AMP, and 3'-AMP appear to be adenosine precursors that are converted to adenosine. However, in vitro inhibition of cell proliferation by 2',3'-cAMP, 2'-AMP, and 3'-AMP requires incubating the cells for several days with the purines; thus these experiments do not address the question of whether 2',3'-cAMP, 2'-AMP, and 3'-AMP are rapidly converted to adenosine in vivo.

The present study unequivocally demonstrates the concepts that 2',3'-cAMP, 2'-AMP, and 3'-AMP are biologically active in vivo and that their biologic activity is mediated by rapid conversion to adenosine. These conclusions are based on our observations that 2',3'-cAMP, 2'-AMP, and 3'-AMP dose-dependently

Fig. 9. Bar graphs show the excretion rate of 2'-AMP (top panel), 3'-AMP (middle panel), and adenosine (bottom panel) in rats infused with either 2',3'-cAMP, 2'-AMP, 3'-AMP, 5'-AMP, adenosine, or 3',5'-cAMP. A vehicle/time control group (saline) was included. During period 1, all groups received only saline. During periods 2, 3, and 4, the groups infused with a purine received 0.03, 0.1, and 0.3 $\mu\text{mol/kg}$ per minute of the indicated purine; whereas the saline group only received saline for periods 1–4. Values are presented as mean $\pm$ S.E.M. for the indicated number of animals (n). The letters “a” and “b” indicate $P < 0.05$ (Fisher’s least significant difference test) compared with the period 1 value of the corresponding group and compared with the saline group of the corresponding period, respectively. ANOVA, analysis of variance.

and profoundly reduce MABP, HR, TPR, and MVR with efficacies and potencies equal to or greater than those for adenosine and 5'-AMP; and blockade of adenosine receptors with DPSPX attenuates the effects of 2',3'-cAMP, 2'-AMP, and 3'-AMP. These findings are important for at least two reasons. First, this settles the issue once and for all that 2',3'-cAMP, 2'-AMP, and 3'-AMP in vivo are extremely rapidly converted to adenosine in the cardiovascular system. Second, this indicates that the enzymes that metabolize these compounds to adenosine are richly expressed in vivo, which supports the concept that these are indeed important naturally occurring purines.

It is important to note that both 2'-AMP and 3'-AMP seem to be more potent than even adenosine with respect to affecting cardiovascular parameters. Likely, 2'-AMP and 3'-AMP are a better adenosine “delivery system” because they represent an adenosine prodrug that, unlike adenosine, is not subjected to the extremely rapid adenosine clearance mechanisms. Thus, more 2'-AMP and 3'-AMP may reach the site of action, whereas adenosine is rapidly transported into red blood cells and metabolized.

The present results are also important because they suggest a novel therapeutic opportunity using 2',3'-cAMP. Past studies suggest that adenosine would be useful for

Fig. 10. Bar graphs show the percentage change in mean arterial blood pressure (left panel) and percentage change in heart rate (right panel) induced by 2',3'-cAMP, 2'-AMP, 3'-AMP, or adenosine (3 μ mol/kg per minute) in control rats (CONTROL) and rats treated with DPSPX (30 mg/kg i.v. bolus followed by continuous intravenous infusion of 0.5 mg/kg per minute). Basal values for MABP in the CONTROL/2',3'-cAMP group, DPSPX/2',3'-cAMP group, CONTROL/2'-AMP group, DPSPX/2'-AMP group, CONTROL/3'-AMP group, DPSPX/3'-AMP group, CONTROL/adenosine group, and the DPSPX/adenosine group were 106 ± 5 , 108 ± 7 , 112 ± 8 , 113 ± 2 , 112 ± 5 , 113 ± 7 , 107 ± 4 , and 115 ± 2 mm Hg, respectively. Basal values for HR in the CONTROL/2',3'-cAMP group, DPSPX/2',3'-cAMP group, CONTROL/2'-AMP group, DPSPX/2'-AMP group, CONTROL/3'-AMP group, DPSPX/3'-AMP group, CONTROL/adenosine group, and DPSPX/adenosine group were 375 ± 16 , 402 ± 5 , 362 ± 17 , 397 ± 14 , 391 ± 14 , 427 ± 17 , 368 ± 6 , and 413 ± 12 beats/min, respectively. Values are presented as mean $\pm$ S.E.M. for the indicated number of animals (*n*).

producing controlled hypotension in surgical patients in whom a temporary reduction in blood pressure is required (Sollevi et al., 1984). Our studies show that 2',3'-cAMP can, similar to adenosine, produce rapid-onset hypotension. However, unlike adenosine, 2',3'-cAMP increases urinary levels of adenosine. Since adenosine in the urinary compartment is renoprotective (Okusa et al., 1999, 2000, 2001; Lee and Emala, 2000, 2002; Okusa, 2002; Day et al., 2005; Grenz et al., 2007a,b, 2008, 2012; Lee et al., 2007; Kim et al., 2009; Bauerle et al., 2011), 2',3'-cAMP could be a drug that allows for controlled hypotension while delivering renoprotective adenosine to the renal tubules. Moreover, a study in perfused

canine coronary arteries shows that 2',3'-cAMP does not affect coronary vascular tone (Nakane and Chiba, 1993), suggesting that the conversion of 2',3'-cAMP to adenosine in the coronary circulation is minimal. Thus, 2',3'-cAMP might be useful for controlled hypotension without causing coronary steal in patients with coronary vascular disease. Finally, if 2',3'-cAMP is not converted to adenosine in the carotid body chemoreceptors, adverse effects of adenosine related to stimulation of chemoreceptors could be avoided. Taken together, our present findings indicate that 2',3'-cAMP should be investigated for utility as an agent to produce controlled hypotension.

Fig. 11. Bar graphs show the percentage change in mesenteric vascular resistance (left panel) and percentage change in total peripheral resistance (right panel) induced by 2',3'-cAMP, 2'-AMP 3'-AMP, or adenosine (3 μmol/kg per minute) in control rats (CONTROL) and rats treated with DPSPX (30 mg/kg i.v. bolus followed by continuous intravenous infusion of 0.5 mg/kg per minute). Basal values for MVR in the CONTROL/2',3'-cAMP group, DPSPX/2',3'-cAMP group, CONTROL/2'-AMP group, DPSPX/2'-AMP group, CONTROL/3'-AMP group, DPSPX/3'-AMP group, CONTROL/adenosine group, and the DPSPX/adenosine group were 9 ± 1 , 12 ± 1 , 12 ± 2 , 12 ± 4 , 9 ± 1 , 12 ± 2 , 11 ± 1 , and 14 ± 3 mm Hg/(ml/min), respectively. Basal values for TPR in the CONTROL/2',3'-cAMP group, DPSPX/2',3'-cAMP group, CONTROL/2'-AMP group, DPSPX/2'-AMP group, CONTROL/3'-AMP group, DPSPX/3'-AMP group, CONTROL/adenosine group, and DPSPX/adenosine group were 0.72 ± 0.10 , 0.85 ± 0.10 , 0.98 ± 0.28 , 0.76 ± 0.07 , 0.83 ± 0.10 , 0.83 ± 0.06 , 0.79 ± 0.10 , and 0.81 ± 0.08 mm Hg/(ml/min), respectively. Values are presented as mean $\pm$ S.E.M. for the indicated number of animals (*n*).

By engaging A_1 , A_{2A} , and A_{2B} receptors, adenosine protects the kidneys from ischemia/reperfusion injury as well as other forms of acute kidney injury (AKI) (Okusa et al., 1999, 2000, 2001; Lee and Emala, 2000, 2002; Okusa, 2002; Day et al., 2005; Grenz et al., 2007a,b, 2008, 2012; Lee et al., 2007; Kim et al., 2009; Bauerle et al., 2011). However, adenosine and adenosine receptor agonists exert systemic effects (e.g., hypotension and bradycardia) that likely would limit their clinical utility for treatment/prevention of AKI. Our previous work shows that the kidney converts extracellular 3',5'-cAMP to extracellular 5'-AMP, which in turn is metabolized to adenosine (Jackson, 2011). Moreover, our previously published findings show that intravenously infused 3',5'-cAMP increases both renal interstitial adenosine and urinary

adenosine excretion (Jackson et al., 2007). A potentially important aspect of the present findings is that intravenous 3',5'-cAMP increases urinary adenosine excretion more so than does an equivalent dose of adenosine, 5'-AMP, 3'-AMP, or 2'-AMP. But unlike these other compounds, 3',5'-cAMP has slight, if any, hemodynamic or cardiac effects. It appears, therefore, that the ecto-phosphodiesterase that converts 3',5'-cAMP to adenosine is more highly expressed in the kidneys than in other tissues or organ systems, thus allowing for 3',5'-cAMP to increase renal adenosine levels without raising system levels that would stimulate extrarenal receptors. Although 2',3'-cAMP and 3',5'-cAMP similarly increase urinary adenosine, 2',3'-cAMP causes hypotension, suggesting that the ecto-phosphodiesterase that metabolizes 2',3'-cAMP

to 2'-AMP and 3'-AMP are more ubiquitously expressed. It is conceivable, therefore, that 3',5'-cAMP could be used to selectively increase adenosine levels in the kidney to prevent or treat AKI without lowering renal perfusion pressure.

Authorship Contributions

Participated in research design: Jackson, Mi.

Conducted experiments: Mi.

Performed data analysis: Jackson.

Wrote or contributed to the writing of the manuscript: Jackson, Mi.

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