CHARACTERIZATION OF RENAL ECTO-PHOSPHODIESTERASE

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**List of Abbreviations:**
- phosphodiesterase, PDE; ecto-phosphodiesterase, ecto-PDE; ecto-5'-nucleotidase, ecto-5'-NT; α,β-methylene-adenosine-5'-diphosphate, AMPCP; 8-methoxymethyl-3-isobutyl-1-methylxanthine, mmIBMX; 1, 3-isobutyl-1-methylxanthine, IBMX; 1,3-dipropyl-8-p-sulfophenylxanthine, DPSPX; erythro-9-(2-hydroxy-3-nonyl)adenine, EHNA; 5-nitro-2,N,N-trimethylbenzenesulfonamide, BRL-50481; 4-(3-butoxy-4-methoxybenzyl)imidazolidin-2-one, RO 20-1724.
ABSTRACT

In kidneys, stimulation of adenylyl cyclase causes egress of cAMP, conversion of cAMP to AMP by ecto-phosphodiesterase and metabolism of AMP to adenosine by ecto-5'-nucleotidase. Although much is known about ecto-5'-nucleotidase, the renal ecto-phosphodiesterase remains uncharacterized. We administered cAMP (10 μmol/L in the perfusate) to 12 different groups of perfused kidneys. AMP was measured in perfusate using ion trap mass spectrometry. In control kidneys (n=17), basal renal secretion rate of AMP was 0.49 ± 0.08 and increased to 3.0 ± 0.2 nmoles AMP per gram kidney weight per min during administration of cAMP. A broad spectrum PDE inhibitor (1,3-isobutyl-1-methylxanthine, 300 μmol/L, n=6) and an ecto-phosphodiesterase inhibitor (1,3-dipropyl-8-sulfophenylxanthine, 1 mmol/L, n=6) significantly attenuated cAMP-induced AMP secretion by 60% and 74%, respectively. Blockade of PDE1 (8-methoxymethyl-3-isobutyl-1-methylxanthine, 100 μmol/L), PDE2 (erythro-9-(2-hydroxy-3-nonyl)adenine, 30 μmol/L), PDE3 (milrinone, 10 μmol/L; cGMP, 10 μmol/L), PDE4 (RO 20-1724, 100 μmol/L), PDE5 and PDE6 (zaprinast, 30 μmol/L) and PDE7 (BRL-50481, 10 μmol/L) did not alter renal ecto-phosphodiesterase activity. Administration of a concentration (100 μmol/L) of dipyridamole that blocks PDE8 inhibited ecto-phosphodiesterase activity (by 44%). However, a lower concentration of dipyridamole (3 μmol/L) that blocks PDE9, PDE10 and PDE11, but not PDE8, did not inhibit ecto-phosphodiesterase activity. These data support the conclusion that renal ecto-phosphodiesterase activity is not mediated by PDE1, PDE2, PDE3, PDE4, PDE5, PDE6, PDE7, PDE9, PDE10 or PDE11, and is inhibited by high concentrations of dipyridamole. Ecto-phosphodiesterase has some pharmacological characteristics similar to PDE8.
INTRODUCTION

Multiple biochemical pathways provide for the biosynthesis of adenosine. The well-characterized pathways include the intracellular ATP pathway (intracellular dephosphorylation of ATP to adenosine) (Schrader, 1991), the extracellular ATP pathway (metabolism of released adenine nucleotides to adenosine by ecto-enzymes) (Gordon, 1986) and the transmethylation pathway (the hydrolysis of S-adenosyl-L-homocysteine (SAH) to L-homocysteine and adenosine by the enzyme SAH-hydrolase) (Lloyd et al., 1988). The intracellular ATP pathway is activated when energy demand exceeds energy supply (Schrader, 1991), the extracellular ATP pathway is engaged when adenine nucleotides are released during sympathoadrenal activation, platelet aggregation or activation of cardiovascular cells by clotting factors, neutrophil interactions, and catecholamines (Pearson and Gordon, 1979; Pearson et al., 1980; LeRoy et al, 1984), and the transmethylation pathway is triggered by methylation reactions involving S-adenosyl-L-methionine as the methyl donor (Lloyd et al., 1988; Deussen et al., 1989). These three well-described routes of adenosine biosynthesis are not well suited for physiological modulation of extracellular levels of adenosine because the intracellular and extracellular ATP pathways of adenosine production require crisis events and the transmethylation pathway is mostly constitutive.

We postulate a fourth pathway, the cAMP-adenosine pathway, for adenosine production that would be more amenable to physiological modulation of adenosine levels by hormones (Jackson, 1991, 1997, 2001; Jackson and Dubey, 2001, 2004). In this regard, our hypothesis is that stimulation of adenylyl cyclase activates the cAMP-adenosine pathway, which has both intracellular and extracellular sites of adenosine production. The intracellular arm involves
metabolism of cAMP to AMP and AMP to adenosine via cytosolic phosphodiesterase (PDE) and cytosolic 5'-nucleotidase (5'-NT), respectively. The adenosine thus formed would reach the extracellular space by way of facilitated transport. However, the quantitative importance of the intracellular cAMP-adenosine pathway would be diminished by the competition of cytosolic 5'-NT and adenylate kinase for AMP and by the competition of transport mechanisms with adenosine kinase for adenosine. Therefore, the extracellular limb of the cAMP-adenosine pathway may be quantitatively more important.

We hypothesize that the extracellular cAMP-adenosine pathway is mediated by three spatially-linked processes: egress of cAMP, metabolism of cAMP to AMP by ecto-phosphodiesterase (ecto-PDE) and metabolism of AMP to adenosine by ecto-5'-nucleotidase (ecto-5'-NT). Activation of adenylyl cyclase is always associated with egress of cAMP into the extracellular space by an active transport mechanism (King and Mayer, 1974; Barber and Butcher, 1981), and ecto-5'-NT is a ubiquitous enzyme that is tethered to the extracellular face of the plasma membrane via a lipid-sugar linkage (Misumi et al., 1990; Zimmermann, 1992). Because cAMP transport is robust and ecto-5'-NT efficiently metabolizes AMP to adenosine, activation of adenylyl cyclase would trigger the extracellular metabolism of cAMP to AMP and hence to adenosine, provided that sufficient levels of ecto-PDE exist. Because these reactions would take place in a highly localized environment, this newly formed adenosine could then act in an autocrine and/or paracrine fashion to amplify, inhibit, and/or expand the local response to hormonal stimulation of adenylyl cyclase. In this regard, modest increases in cAMP production could significantly increase adenosine concentrations at the cell surface. Indeed, our studies
demonstrate that hormone stimulation increases adenosine via the extracellular cAMP-adenosine pathway (Mi and Jackson, 1998; Dubey et al., 2000, 2001; Jackson et al., 2003, 2006).

Our studies in the perfused rat renal vascular bed demonstrate that infusion of cAMP causes a concentration-dependent increase in the renal secretion rates of AMP, adenosine, and inosine, and the increases in AMP and adenosine secretion are inhibited by 1,3-isobutyl-1-methylxanthine (IBMX; broad spectrum PDE inhibitor) and 1,3-dipropyl-8-p-sulfophenylxanthine (DPSPX; ecto-PDE inhibitor at high concentrations), whereas the increases in adenosine, but not AMP, secretion are blocked by α,β-methylene-adenosine-5'-diphosphate (AMPCP; ecto-5'-NT inhibitor) (Mi and Jackson, 1995). Thus, our previous studies indicate that the kidney contains ecto-PDE. However, the renal ecto-PDE that mediates the conversion of extracellular cAMP to AMP remains uncharacterized.

It is conceivable that ecto-PDE is one of the currently know 11 different families of PDE. These PDE families can be distinguished by sensitivity to PDE inhibitors (Bender and Beavo, 2006). Therefore, the purpose of this study was to characterize pharmacologically the renal ecto-PDE by comparing the effects of a panel of PDE inhibitors on ecto-PDE activity in the isolated, perfused rat kidney.
METHODS

Seventy-six adult male Sprague-Dawley rats obtained from Charles River (Wilmington, MA) were housed at the University of Pittsburgh Animal Facility and fed Prolab RMH 3000 (PMI Feeds, Inc., St. Louis, MO) containing 0.26% sodium and 0.82% potassium. All studies received prior approval by the University of Pittsburgh Animal Care and Use Committee.

Rats were anesthetized (sodium pentobarbital 45 mg/kg, intraperitoneal injection), a midline incision was made, and the left kidney, left renal artery, abdominal aorta, and left ureter were dissected free from surrounding tissue. The left ureter was cannulated with polyethylene (PE)-10 tubing, the abdominal aorta below the left kidney was cannulated (PE-50 tubing), the suprarenal aorta was ligated, and the left kidney was flushed with 2.5 ml/min of oxygenated Tyrode's solution containing 100 units/ml of heparin. While maintaining perfusion, the left kidney was isolated and mounted in a water-jacketed organ chamber. The organ chamber was maintained at 37°C with a thermostatically-controlled water circulation (Thermocirculator, Harvard Apparatus, South Natick, MA). Kidneys were perfused (5 ml/min) using a Harvard model 1210 peristaltic pump with Tyrode's solution (composition in mM: NaCl, 137; KCl, 2.7; CaCl₂, 1.8; MgCl₂, 1.1; NaHCO₃, 12; NaH₂PO₄, 0.42; D(+)-glucose, 5.6) that was heated to 37°C with a warming coil, gassed with 95% O₂ and 5% CO₂ and a passed through a bubble trap. A Statham pressure transducer (model P23ID, Statham Division, Gould Inc., Oxnard, CA) was connected to an access port located in the perfusion line immediately before the kidney so that perfusion pressure could be continuously monitored (Grass model 79D polygraph, Grass Instruments, Quincy, MA). The perfused kidneys were allowed to equilibrate for 105 minutes to obtain a stable baseline renal venous AMP secretion rate. Next, 100 μmol/L of AMPCP, a
specific inhibitor of ecto-5'-nucleotidase (Zimmermann, 1992), was added to the perfusate to prevent the metabolism of AMP to adenosine. Our previously published results demonstrate that this concentration of AMPCP blocks AMP metabolism to adenosine in the isolated, perfused rat kidney (Mi and Jackson, 1995).

The kidneys were assigned to one of twelve groups: 1) control group (n=17); 2) group treated with IBMX [300 µmol/L, n=6, a broad spectrum PDE inhibitor (Beavo et al., 1970)]; 3) group treated with DPSPX [1 mmol/L, an inhibitor of ecto-PDE, but not intracellular PDEs (Mi and Jackson, 1995)]; 4) group treated with 8-methoxymethyl-3-isobutyl-1-methylxanthine [mmIBMX, 100 µmol/L, n=6, a selective PDE1 inhibitor (Wells and Miller, 1988)]; 5) group treated with erythro-9-(2-hydroxy-3-nonyl)adenine [EHNA, 30 µmol/L, n=6, selective inhibitor of PDE2 (Benter and Beavo, 2006)]; 6) group treated with cGMP [10 µmol/L, n=6, selective inhibitor of PDE3 (Benter and Beavo, 2006)]; 7) group treated with milrinone [10 µmol/L, n=6, a selective PDE3 inhibitor (Harrison et al., 1986)]; 8) group treated with RO 20-1724 [100 µmol/L, n=6, a selective PDE4 inhibitor (Beavo and Reifsnyder, 1990)]; 9) group treated with zaprinast [30 µmol/L, n=6, a selective PDE5 (Benter and Beavo, 2006) and PDE6 inhibitor (Ballard et al., 1998)]; 10) group treated with 4-(3-butoxy-4-methoxybenzyl)imidazolidin-2-one [BRL-50481, 10 µmol/L, n=6, selective inhibitor of PDE7 (Benter and Beavo, 2006)]; 11) group treated with high concentration of dipyridamole [100 µmol/L, n=7, inhibits PDE8, PDE9, PDE10 and 11 (Fawcett et al., 2000, Gamanuma et al., 2003)]; and 12) group treated with low concentration of dipyridamole [3 µmol/L, n=6, inhibits PDE9, PDE10 and PDE11, but not PDE8 (Fawcett et al., 2000, Gamanuma et al., 2003)]. The concentrations of mmIBMX, EHNA,
milrinone, RO 20-1724, zaprinast and BRL 40418 were selected on the basis of their IC50s for their respective PDE subtype targets. In this regard, a concentration approximately 30 times the IC50 was employed. The concentrations of AMPCP, IBMX and DPSPX were selected on the basis of our previous studies (Mi and Jackson, 1995). The low concentration of dipyridamole was selected to be 1 to 30 times above the IC50 for PDE9, PDE10 and PDE11, while being 0.1 to 0.3 of the IC50 for PDE8. The high concentration of dipyridamole was selected to be approximately three times the IC50 for PDE8. Higher concentrations of dipyridamole were precluded by lack of solubility.

In each kidney, the treatment was added directly to the perfusate, and four minutes later the perfusate exiting the renal vein was collected for one minute. Then cAMP (10 μmol/L) was added to the perfusate, and four minutes later, the venous perfusate was again collected for one minute. Perfusate was immediately placed on ice and then frozen at -40°C for later analysis of AMP.

The concentration of AMP in the perfusate were measured using a ThermoFinnigan high pressure liquid chromatographic system coupled to a ThermoFinnigan LCQ Duo ion trap mass spectrometer equipped with an electrospray ionization source (Thermo Electron Corporation, Waltham, MA) as recently described (Jackson et al., 2006). The renal venous secretion rate of AMP was calculated by multiplying the concentration of each substance in the venous perfusate by the perfusion rate.

Within each group, the secretion rates of AMP were compared in the absence and presence of AMP with a paired Student’s t-test. The secretion rates of AMP during administration of cAMP were compared among the 12 groups by one-factor ANOVA. Multiple
comparisons were performed by a Fisher LSD test if the overall ANOVA indicated that some of the means were different. All statistical analyses were performed using the Number Cruncher Statistical System (Kaysville, Utah), and all values in the text and figures refer to means ± SEM.
RESULTS

Basal perfusion pressure was similar in all 12 groups (approximately 50 mm Hg), and cAMP infusions did not alter perfusion pressure (data not shown). Figure 1 illustrates the effects of cAMP on renal AMP secretion in control kidneys (top panel), in kidneys pretreated with IBMX (a broad-spectrum PDE inhibitor; middle panel) and in kidneys treated with DPSPX (inhibitor of ecto-PDE; lower panel). Both IBMX and DPSPX significantly attenuated the cAMP-induced increase in renal secretion of AMP by 60% and 74%, respectively.

As shown in Figure 2, inhibition of PDE1 with mmIBMX (top panel) or PDE2 with EHNA (middle panel) did not alter cAMP-induced renal AMP secretion. Also, inhibition of PDE3 with either cGMP (Figure 2, bottom panel) or milrinone (Figure 3, top panel), PDE4 with RO 20-1724 (Figure 3, middle panel), PDE5 and PDE6 with zaprinast (Figure 3, bottom panel) or PDE7 with BRL-50481 (Figure 4, top panel) did not alter cAMP-induced renal AMP secretion.

Importantly, administration of a concentration of dipyridamole (100 μmol/L) that inhibits PDE8, PDE9, PDE10 and PDE11 significantly reduced cAMP-induced renal AMP secretion by 44% (Figure 4, middle panel). However, a concentration of dipyridamole (3 μmol/L) that inhibits PDE9, PDE10 and PDE11, but not PDE8, did not significantly attenuate cAMP-induced renal AMP secretion.
DISCUSSION

The extracellular cAMP-adenosine pathway may participate importantly in a number of physiological processes. For example, Hong et al. (1999) report that the extracellular cAMP-adenosine pathway exists in rat pial arteries and contributes to the regulation of cerebral vascular dilation in response to hypotension. Finnegan and Carey (1998) demonstrate the existence of the extracellular cAMP-adenosine pathway in adipocytes and suggest a possible role for this pathway in the regulation of fat metabolism. Our own work implicates the extracellular cAMP-adenosine pathway in the regulation of vascular smooth muscle cell (Dubey et al., 1996) and cardiac fibroblast (Dubey et al., 2001) growth, in the regulation of renin release (Jackson, 1991) and as a mediator of some of the cellular effects of 17β-estradiol (Dubey et al., 2000). These findings indicate the importance of elucidating the nature of the ecto-PDE that mediates the cAMP-adenosine pathway.

The purpose of this investigation was to determine whether ecto-PDE is pharmacologically similar to one or more of the known PDEs. The PDEs represent a group of enzymes with considerable molecular diversity. In mammals there are more than 20 different PDE genes and, due to splice variants and alternative start sites, more than 50 different molecularly distinct PDEs (Lugnier, 2006). However, PDEs can be classified into 11 different families according to their structure, substrate preference, modalities of regulation and sensitivity to pharmacological inhibitors (Bender and Beavo, 2006). The PDE1 family is stimulated by calcium-calmodulin and inhibited by mmIBMX (Wells and Miller, 1988). Some isoforms of PDE1 hydrolyze cGMP more efficiently than cAMP, whereas at least one isoform hydrolyzes cAMP and cGMP with equal efficiency (Rybalkin and Bornfeldt, 1999). PDE2 is a cGMP-
stimulated PDE that hydrolyzes both cAMP and cGMP. In contrast, PDE3 is a cGMP-inhibited PDE that hydrolyzes cAMP and cGMP and is potently inhibited by drugs such as milrinone (Harrison et al., 1986). PDE4 is a cAMP-specific PDE that is not regulated by calcium-calmodulin nor by cGMP and is specifically inhibited by drugs such as RO 20-1724 (Beavo et al., 1990). PDE5 is a cGMP-preferring enzyme that is regulated by phosphorylation, and PDE6 is also a cGMP-preferring enzyme but is regulated by transducin (Lugnier, 2006). PDE7 and PDE8 are cAMP-preferring and insensitive to rolipram, whereas PDE9 hydrolyzes preferably cGMP. PDE10 and 11 hydrolyze both cAMP and cGMP (Lugnier, 2006).

In the present investigation, we tested, using an array of pharmacological inhibitors, whether ecto-PDE activity in the kidney is pharmacologically similar to one or more of the known families of PDEs. There are several important conclusions that can be deduced from this experimental series. First, renal ecto-PDE activity is inhibited by high concentrations of IBMX, a finding that supports the concept that renal ecto-PDE activity is related to the classical PDE family. Second, inhibition of ecto-PDE by DPSPX is in accordance with our previous observations (Mi and Jackson, 1995), and confirms that ecto-PDE activity is indeed an extracellular PDE since DPSPX is negatively charged at physiological pH and does not penetrate cell membranes to any significant extent (Tofovic et al., 1991). Third, ecto-PDE activity is not inhibited by high concentrations (approximately 30 times the IC₅₀s of the inhibitors for their respective PDE targets) of mmIBMX, EHNA, milrinone, cGMP, RO 20-1724, zaprinast or BRL-50481. These findings virtual exclude the possibility that PDE1, PDE2, PDE3, PDE4, PDE5, PDE6 or PDE7 contribute to ecto-PDE activity in the kidney. Fourth, a concentration of dipyridamole that blocks PDE9, PDE10 and PDE11 does not inhibit renal ecto-PDE activity, a
finding that also eliminates these PDE isoforms as candidates for ecto-PDE activity.

Our findings in the perfused rat kidney are entirely consistent with the findings of Zacher and Carey (1999). These investigators reported that swine adipocyte plasma membranes metabolized cAMP to AMP and adenosine via PDE and 5'-NT, respectively. Although maximal activity of microsomal membrane PDE was seven times greater than that of plasma membrane PDE, microsomal membrane PDE was completely inhibited by the PDE3B inhibitor cilostamide, while plasma membrane PDE was unaffected by this PDE3B inhibitor. Moreover, PDE1, PDE2, PDE4 and PDE5 inhibitors did not attenuate plasma membrane PDE activity. As in our study, Zacher and Carey found that 1 mM DPSPX inhibited plasma membrane PDE activity by 72%.

Our previous studies demonstrate in isolated perfused rat kidneys that stimulation of adenylyl cyclase with the beta-adrenoceptor agonist isoproterenol increases renal cAMP secretion and that this response is enhanced by RO 20-1724, but not by milrinone nor by mmIBMX (Jackson et al., 1997). Thus, the intracellular metabolism of endogenous cAMP in the renal vasculature appears to be mediated mostly by PDE4, whereas the metabolism of extracellular cAMP is mediated by a non-PDE4 enzyme.

Another conclusion from this study is that ecto-PDE in the kidney is pharmacologically similar to PDE8. Although our results do not imply that ecto-PDE is identical to PDE8, it is conceivable that ecto-PDE activity is mediated in part by PDE8 because PDE8 is expressed in the kidney (Bender and Beavo, 2006). Even though PDE8 does not contain putative transmembrane spanning domains, it contains a PAS domain which mediates binding to other proteins which could tether PDE8 to the cell membrane (Lugnier, 2006). Indeed, even though PDE is a cytosolic enzyme, PDE8 is partially localized to the membrane compartment (Bender
and Beavo, 2006). Adenosine deaminase serves as an important example of how an enzyme without membrane anchors can function as an ecto-enzyme. Although adenosine deaminase is considered an intracellular enzyme, it is secreted by cells and binds avidly to CD26 (dipeptidyl peptidase IV) on cell surfaces (Resta et al., 1998). Nonetheless, it is also possible that ecto-PDE is not authentic PDE8 but another PDE that is pharmacologically similar to PDE8. For example, the slime mold \textit{Dictyostelium discoideum} secretes cAMP as a signaling molecule. Importantly, a recent study identified and characterized an ecto-PDE in \textit{Dictyostelium discoideum} that has a catalytic domain with a high degree of homology with that of mammalian PDE8 (Bader et al., 2006). A mammalian enzyme similar to the \textit{Dictyostelium discoideum} ecto-PDE remains to be identified.

In summary, the present study indicates that the renal ecto-PDE enzyme is not PDE1, PDE2, PDE3, PDE4, PDE5, PDE6, PDE7, PDE9, PDE10 or PDE11. Moreover, taken together with our previous published work in the perfused rat kidney, the present results demonstrate that the ecto-PDE responsible for hydrolyzing extracellular cAMP to AMP is not the same as the intracellular enzyme found in the renal circulation. We conclude that ecto-PDE has pharmacological characteristics similar to PDE8, although the exact identity of ecto-PDE remains undefined.
REFERENCES


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LEGENDS FOR FIGURES

Figure 1: Effect of cAMP (10 μmol/L) on AMP secretion by isolated, perfused rat kidneys in the absence and presence of 1,3-isobutyl-1-methylxanthine (IBMX; broad spectrum PDE inhibitor) and 1,3-dipropyl-8-sulfophenylxanthine (DPSPX; ecto-phosphodiesterase inhibitor). Kidneys were pretreated with α,β-methylene-adenosine-5'-diphosphate (AMPCP; ecto-5'-nucleotidase inhibitor; 100 μmol/L). AMPCP and the PDE inhibitors were added to the perfusate beginning 5 minutes before adding cAMP to the perfusate. Basal AMP secretion was determined during the one minute before adding cAMP, and AMP secretion in the presence of cAMP was determined from four to five minutes after adding cAMP. The horizontal line indicates the level of AMP achieved in the control group. a$p<0.05$ compared with basal; b$p<0.05$ compared with cAMP in control group.

Figure 2: Effect of cAMP (10 μmol/L) on AMP secretion by isolated, perfused rat kidneys in the presence of 3,8-methoxymethyl-3-isobutyl-1-methylxanthine (mmIBMX; selective PDE1 inhibitor), erythro-9-(2-hydroxy-3-nonyl)adenine (EHNA; selective PDE2 inhibitor) and cGMP (PDE3 inhibitor). See Legend to Figure 1 for details. a$p<0.05$ compared with basal.

Figure 3: Effect of cAMP (10 μmol/L) on AMP secretion by isolated, perfused rat kidneys in the presence of milrinone (selective PDE3 inhibitor), 4-(3-butoxy-4-methoxybenzyl)imidazolidin-2-one (RO 20-1724; selective PDE4 inhibitor) and zaprinast (selective PDE5 and PDE6 inhibitor). See Legend to Figure 1 for details. a$p<0.05$ compared
Figure 4: Effect of cAMP (10 μmol/L) on AMP secretion by isolated, perfused rat kidneys in the presence of 5-nitro-2,N,N-trimethylbenzenesulfonamide (BRL-50481; selective PDE7 inhibitor), a high concentration of dipyridamole (inhibits PDE8, PDE9, PDE10 and PDE11) and a low concentration of dipyridamole (inhibits PDE9, PDE10 and PDE11). See Legend to Figure 1 for details. \(^{a}p<0.05\) compared with basal; \(^{b}p<0.05\) compared with cAMP in control group.
Figure 1

AMP SECRETION (nmoles per gram kidney per min)

Control

(n = 19)

IBMX (300 micromol/L)

(n = 6)

DPSPX (1 mmol/L)

(n = 6)
Figure 2

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mmIBMX (100 micromol/L)

EHNA (30 micromol/L)

cGMP (10 micromol/L)
**Figure 3**

**Basal cAMP**

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(n = 6)

**Milrinone**

(10 micromol/L)

**RO 20-1724**

(100 micromol/L)

**Zaprinast**

(30 micromol/L)

AMP SECRETION (nmoles per gram kidney per min)
Figure 4

BRL-50481 (10 micromol/L)

Dipyridamole (100 micromol/L)

Dipyridamole (3 micromol/L)

AMP SECRETION (nmoles per gram kidney per min)

(n = 6)

(n = 7)

(n = 6)