Published in final edited form as:
Prostaglandins Other Lipid Mediat. 2012 August ; 98(0): 39–47. doi:10.1016/j.prostaglandins.2011.12.002.

Role of the adenosine $_{2A}$ receptor-epoxyeicosatrienoic acid pathway in the development of salt-sensitive hypertension

Mairéad A. Carroll
Department of Pharmacology, New York Medical College, Valhalla, New York

Abstract

Activation of rat adenosine $_{2A}$ receptors (A_{2A} R) dilates preglomerular microvessels, an effect mediated by epoxyeicosatrienoic acids (EETs). High salt (HS) intake increases epoxygenase activity and adenosine levels and greater vasodilator response to a stable adenosine analog, 2-chloroadenosine (2-CA), was seen in kidneys obtained from HS-fed rats which was mediated by increased EET release. Because this pathway is antipressor, we examined the role of the A_{2A} R-EET pathway in a genetic model of salt-sensitive hypertension, the Dahl salt-sensitive (SS) rats. Dahl S resistant (R) rats fed a HS diet demonstrated a greater renal vasodilator response to 2-CA. In contrast, Dahl SS rats did not exhibit a difference in the vasodilator response to 2-CA whether fed normal salt (NS) or HS diet. In Dahl SR but not Dahl SS rats, HS intake significantly increased purine flux, augmented the protein expression of A_{2A} R and cytochrome P450 2C23 and 2C11 epoxygenases, and elevated the renal efflux of EETs. Thus the Dahl SR rat is able to respond to HS intake by recruiting EET formation, whereas the Dahl SS rat appears to have exhausted its ability to increase EET synthesis above the levels observed on NS intake. In vivo inhibition of the A_{2A} R-EET pathway in Dahl SR rats fed a HS diet results in reduced renal EETs levels, diminished natriuretic capacity and hypertension, thus supporting a role for the A_{2A} R-EET pathway in the adaptive natriuretic response to modulate blood pressure during salt loading. An inability of Dahl SS rats to upregulate the A_{2A} R-EET pathway in response to salt loading may contribute to the development of salt-sensitive hypertension.

Keywords

Cytochrome P450; Epoxyeicosatrienoic acids; Adenosine; Kidney; Salt-sensitive hypertension

1. Introduction

Salt-sensitivity, as defined by blood pressure elevation in response to a dietary salt load, is not only a causative factor for a subgroup of humans with essential hypertension, but has also been reported to be an independent cardiovascular risk factor in patients with hypertension (1). Therefore, understanding the mechanisms that contribute to the development of salt-sensitivity and identifying potential therapeutic targets for the management of salt-sensitive hypertension should provide novel approaches to treat elevated blood pressure. It has been recognized for many years that sodium chloride intake is one of

Corresponding Author: Tel.: 914 594-4664, mairead_carroll@nymc.edu.

the main environmental factors responsible for the development of hypertension (2). Increased sodium chloride intake results in increased renal sodium chloride excretion. This adaptive process prevents progressive salt retention and volume expansion, with elevation of blood pressure; thus, an impaired ability of the kidney to excrete sodium requires an increase in blood pressure to increase natriuresis and correct the sodium balance, resulting in hypertension (3).

2. Renal Adenosine Receptors

The physiological effects of adenosine, a metabolite of ATP, are observed in nearly every tissue and modulates cellular and organ function by binding to specific cell-surface P1 purinergic receptors (R), of which there are four known subtypes (A_1, A_{2A}, A_{2B} and A_3) (4). These receptors are members of the large family of seven transmembrane spanning heterotrimeric G protein-coupled receptors (5).

The renal localization of the adenosine receptor subtypes is well documented, but the reported distribution throughout the kidney vasculature and tubular segments varies depending on the technique employed. A_1 R have a high affinity for adenosine and are expressed in preglomerular microvessels (PGMV), including the afferent arterioles, in glomeruli including mesangial cells, juxtaglomerular cells and vasa recta (6;7). The A_1 R mRNA is expressed throughout every nephron segment (8–11). The A_{2A} R and the A_{2B} R, which possess a high and a low adenosine affinity, respectively, have been demonstrated in whole kidney preparations (6) and in glomeruli of rat and mouse kidney, in the outer medullary descending vasa recta and in the papilla (9;12;13). Although expression of A_{2A} R was not detected in rat PGMV (7), using primer-specific polymerase chain reaction A_{2A} R were found to be expressed in microdissected mouse efferent arterioles (14). Expression of A_{2B} R was reported in rat PGMV (7) and have been implicated in the efferent arteriole tubular glomerular feedback (TGF) response (14–16). In addition, A_{2B} R mRNA has been detected in the cortical thick ascending loop of Henle (TALH) and in the distal convoluted tubule as well as in the outer medullary descending vasa recta (9;12;13). There is scant information on the distinct intrarenal localization of A_3 R, although both the mRNA and protein are present in whole kidney preparations of various species (6). The A_3 R protein and mRNA was undetectable in PGMV (7), but was found in rat cortical and medullary tissue (17).

The kidney plays an integral role in the maintenance of extracellular fluid volume and electrolyte balance and thus, contributes to the long-term control of arterial pressure (18). In the kidney, adenosine plays a critical role in the regulation of renal vascular tone and reactivity, and additionally affects tubular transport (19;20). Adenosine also inhibits renin release, sympathetic neurotransmission, platelet aggregation, and lipolysis (20). Stimulation of A_1 R constricts the renal vasculature and inhibits renin release and enhances proximal tubular NaCl reabsorption (21;22). The vasoconstrictor effects of A_1 R activation in the afferent arteriole is a major focus as adenosine is a primary mediator of TGF (22–25). Stimulation of A_2 R results in endothelium-dependent relaxation via stimulation of adenylyl cyclase (26–28), thus increasing renal blood flow (29) and decreasing blood pressure (30). Stimulation of A_{2A} R promotes natriuresis by reducing NaCl reabsorption in the TALH and collecting duct (31–33) and A_2 R activation attenuated TGF responses by stimulation of endothelial NOS (34). In afferent arterioles, A_2R activation has been shown to counteract the A_1 R-induced constriction leading to dilation and decreased autoregulation (35;36). Activation of A_{2B} R dilates renal arteries in a NO-dependent manner (37) and in juxtamedullary afferent arterioles, functional expression of both A_{2A} R and A_{2B} R was observed, but the opposing vasodilator effect during adenosine-mediated afferent arteriolar vasoconstriction was predominantly via activation of A_{2B} R (38). The renal function of the

A_3 R is poorly characterized. It may play a role in sodium and fluid balance by regulating the Na+/H+ exchanger (39) and may exacerbate renal ischemia-reperfusion injury (40). Interestingly A_3 R expression increase with age and expression upregulated by high salt (HS) intake (6;17).

As production of adenosine is increased under stressful conditions such as hypoxia, ischemia, and inflammation, adenosine has traditionally been implicated in the renal functional responses to pathological events (41;42). However, there is now evidence supporting the contribution of adenosine to renal mechanisms that respond to nonpathological challenges to renal function. Adenosine levels have been shown to correlate with salt intake; switching rats from a normal salt (NS) to a HS or low-salt diet results in parallel changes in renal interstitial and urinary adenosine levels (43). The increase in adenosine concentration during HS intake may contribute to a reduction of macula densa-mediated renin secretion and enhance sodium excretion (44).

3. Cytochrome P450-derived arachidonic acid metabolites

The cytochrome P450 (CYP)-derived arachidonic acid (AA) metabolites, 20-hydroxyeicosatetraenoic acids (20-HETE) and four regioisomeric cis-epoxyeicosatrienoic acids (EETs), 5,6-, 8,9-, 11,12- and 14,15-EETs, generated by hydroxylases and epoxygenases, respectively, occupy a key position in the regulation of renal vascular tone (45;46). The constrictor effect of 20-HETE on PGMV established its importance as a modulator of TGF and renal autoregulation (47). In contrast, EETs are important modulators of cardiovascular function and have been recognized for their vasodilator, antiinflammatory, antiproliferative, and profibrinolytic properties (48–53). These lipid derived metabolites mediate/modulate the vascular responses of many vasoactive peptides, for example, angiotensin II (54;55), endothelin-1 (56) and bradykinin (57;58).

Furthermore, the contribution of EETs to blood pressure regulation has been established in several different animal models (59–61). It is well documented that the activity of renal epoxygenase is increased with dietary salt loading (62;63). EETs are thought to be natriuretic by virtue of their ability to dilate the renal vasculature (54;64;65), as well as regulating Na^+ transport in proximal and distal tubules (66–68). Thus, an increase in the production of natriuretic EETs is one of the significant components of the kidney's adaptive response to prevent elevation of blood pressure in response to HS intake (60;69).

Although there is much evidence showing an increase in epoxygenase activity and subsequent increased production of antihypertensive EETs in response to salt loading, the stimulus for this increased epoxygenase activity has not been identified. We have linked A_{2A} R activation to EET production in rat arcuate arteries (70) and have reported that activation of A_{2A} R is coupled to EET release upstream of adenylyl cyclase activation and that EETs stimulate mono-ADP ribosyltransferase resulting in Gs_α protein activation (28).

4. The renal A_{2A} R – EET pathway

As adenosine levels are increased by dietary salt intake, we proposed that adenosine is the stimulus for increased renal epoxygenase activity in response to salt loading. More specifically, we hypothesized that HS intake increases the renal response to adenosine, resulting in increased epoxygenase activity and EET levels (71).

We examined the renal response to adenosine in isolated, perfused kidneys obtained from Spraque-Dawley (SD) rats fed a 4% HS diet or NS diet for 7 days (71). To define the vascular responses to adenosine, we used a stable adenosine analog, 2-chloroadenosine (2-CA) that is not subject to inactivation by either adenosine deaminase/kinase or rapid

removal by nucleoside carriers. Bolus injections of 2-CA elicited biphasic responses: a transient vasoconstriction followed by a prolonged dilation (Fig. 1). The dilator responses to 2-CA were dose-dependent and were exaggerated in kidneys from rats fed a HS diet compared with those receiving a NS diet for 7 days. Under both conditions of NS and HS, 2-CA resulted in dilation, but the magnitude of the response was enhanced in kidneys obtained from rats fed HS for 7 days. As seen in Fig. 1, the inhibitory effect of a selective epoxygenase inhibitor, *N*-methylsulfonyl-6-(2-propargyloxyphenyl)hexanamide (MS-PPOH), was greater under HS conditions. However, MS-PPOH did not completely abolish the response; thus EETs may not be the sole mediators of adenosine-induced dilation in isolated, perfused rat kidneys. Our study was performed in the presence of nitric oxide synthetase (NOS) and cyclooxygenase (COX) inhibition, which eliminated the contribution of NO and prostaglandins (i.e., PGI_2 and PGE_2) to the 2-CA response. In a previous study, we showed that the renal responses to 2-CA in the perfused kidney were not dependent on NO or PG synthesis (70). A possible candidate mediator of the epoxygenase-independent dilation in response to adenosine is carbon monoxide (CO), a product of heme degradation by heme oxygenase. An interaction between CO and adenosine in the nucleus of the solitary tract of rats has been reported: adenosine receptor antagonism attenuated the vasodepressor effect of hemin, while heme oxygenase inhibition attenuated the vasodepressor effect of adenosine (72).

As salt loading increases renal epoxygenase activity (62;63), we assessed the role of EETs in the enhanced dilator response to 2-CA seen in kidneys from HS-fed rats (Fig. 2). Although EETs can be metabolized via a number of different pathways (73;74), the rapid hydrolysis to their corresponding dihydroxyeicosatrienoic acids (DHTs) by soluble epoxide hydrolase (sEH) seems to be the dominant pathway in the kidney (75;76). Thus total EET and DHT release was measured, to account for total epoxygenase activity. Compared with NS intake, salt loading significantly augmented epoxygenase activity, as reflected by an increase in EET and DHT release, without affecting 20-HETE levels, in response to 10 μg of 2-CA. Epoxygenase inhibition abolished this increase, suggesting that de novo synthesis of EETs, rather than release of preformed EETs from storage in phospholipids (54), is responsible for this enhanced renal response.

Because all four adenosine receptor subtypes are expressed within the kidney (6) and 2-CA is a nonselective agonist of the adenosine receptors, it was necessary to elucidate which receptor isoform mediated the 2-CA-induced vasodilation and EET stimulation in the isolated, perfused rat kidney. Previously, we showed that the A_{2A} R-selective agonist, CGS-21680, induced dilation of rat renal PGMV was mediated by stimulation of EET synthesis (70). Adenosine-dependent vasodilation is mediated by increased cAMP levels via stimulation of A_2 R subtypes (A_{2A} R and/or A_{2B} R) (28). Our data revealed that as with the pressurized arcuate artery preparation (70), 2-CA-induced vasodilation of the isolated, perfused rat kidney was also mediated through activation of A_{2A} R, a finding confirmed by experiments with ZM-241385, a selective A_{2A} R antagonist. As seen in Fig. 3A in the presence of ZM-241385, the dilator response to 2-CA was abolished; in fact, only a transient vasoconstriction was observed. This suggests that 2-CA-induced dilation in the isolated, perfused rat kidney is solely mediated by A_{2A} R activation. The abrogated dilator response was accompanied by a significant reduction in total EET and DHT release after ZM-241385 (Fig. 3B). As activation of A_{2B} R dilates renal arteries in a NO-dependent manner (37), we eliminated this pathway as a mediator of 2-CA-induced dilation by inhibition of NOS. The association between A_{2A} R activation and EET release in arcuate arteries and isolated, perfused kidney experiments was based on the pharmacological studies using an A_{2A} R-selective agonist, CGS-21680 and A_{2A} R antagonism with ZM-241385. It is feasible that ZM-241385 may antagonize both A_{2A} R and A_{2B} R, under our experimental conditions. The expression of A_2 receptors in arcuate arteries has not has been addressed and further studies

exploring the contribution of the A_{2A} R and A_{2B} R are required to reconcile the differences in the functional responses to adenosine in the arcuate artery versus the afferent arteriole (38;70). The vasodilator link between A_{2A} R and EETs was confirmed with A_{2A} R knockout mice. Relaxation responses to A_{2A} R agonists of aorta obtained from wild type mice were abrogated in aorta of A_{2A} R knockout mice and epoxygenase expression and activity was also reduced in A_{2A} R knockout mice (77). Further, greater dilation to A_{2A} R agonists was observed with HS intake and A_1 R was downregulated, whereas A_{2A} R was upregulated with HS compared with NS intake (78).

5. Upregulation of CYP2C23 and A_{2A} R under conditions of salt loading

Most renal EET biosynthesis has been attributed to the CYP2C and 2J epoxygenase families (79–81). In the rat kidney, CYP2C23 has been identified as the major 2C arachidonate epoxygenase and the isoform of the 2C family that is subject to regulation by dietary salt (79). In agreement with other studies (79;82), renal CYP2C23 expression was upregulated with increased dietary salt intake. We showed that HS increased medullary CYP2C23 protein expression, without affecting levels of cortical CYP2C23. In contrast to Zhao et al. (82), who reported that an 8% NaCl diet for 14 days increased CYP2C11 cortical protein expression, we did not detect changes in either cortical or medullary homogenates under our experimental conditions of 4% NaCl intake for 7 days. Changes in adenosine receptor protein expression in response to dietary salt intake have also been observed (17). Under conditions of low salt, the renal expression of A_1 R was increased, whereas HS diet downregulated A_1 R expression (17;73). Similarly, we showed that the expression of cortical A_1 R protein was decreased by ~20%, whereas in medullary homogenates, A_{2A} R expression was increased from HS-fed rats compared with NS-fed rats (71). Thus the enhanced dilator response to 2-CA seen in kidneys obtained from HS-fed rats is mediated by increased EET and DHT release and is associated with a downregulation of cortical A_1 R protein expression and increased medullary A_{2A} R and CYP2C23 protein expression.

In summary in normotensive SD rats, HS intake augmented renovascular responses to a non-selective adenosine receptor agonist, 2-CA, associated with increased renal protein expression of the A_{2A} R and CYP2C23, a salt-inducible epoxygenase, that promotes increased renal efflux of EETs and the hydrolysis products of EETs and DHTs.

6. Inability of Dahl salt-sensitive (SS) rats to upregulate the A_{2A} R-EET pathway may contribute to the development of salt-sensitive hypertension

We have reported that adenosine-activated renovascular dilatation is mediated by stimulating the A_{2A} R that is linked to increasing EET synthesis, the latter mediating adenosine actions on the renal vasculature and that A_{2A} R-EET pathway is upregulated by HS intake in normotensive rats (71). As this pathway is antipressor, we examined the role of the adenosine-epoxygenase pathway in Dahl salt-sensitive (SS) hypertensive rats. The Dahl SS rat is a genetic model of salt-dependent hypertension (83), that exhibits a rightward shift in the pressure-natriuresis curve (84), the hallmark of salt-sensitive hypertension. A common underlying adaptation in hypertension is an increase in vascular resistance, although the mechanisms of the changes of vascular reactivity induced by a HS intake are not fully known. Many mechanisms have been proposed to contribute to the development of hypertension; enhanced reactivity to constrictors such as norepinephrine, endothelin and angiotensin II as well as increased tissue oxygen delivery have been reported, whereas, endothelium-dependent relaxations to hypoxia, acetylcholine, and sodium nitroprusside are depressed (85). In addition, in Dahl SS rats, a deficiency of 20-HETE production by TALH (59), an inability to increase renal epoxygenase activity in response to dietary salt intake (60;86) has been documented, as well as an inability to produce or release eicosanoid

precursors from phospholipid stores in response to dietary salt (87). Interactions between NO and CYP metabolites have been described; both NO and CYP metabolites contribute to the regulation of kidney function and blood pressure control, however, the role of 20-HETE and EETs increases with HS intake or after NOS inhibition (88). 20-HETE has been shown to result in endothelium dysfunction by reducing NO release and increasing superoxide production (89), whereas, CYP epoxygenases are directly inhibited by hydrogen peroxide, and this interaction may modulate vascular EET bioavailability (90). Increased NO production may suppress 20-HETE synthesis yet permit EETs-dependent vasodilation (91).

We studied Dahl salt-resistant (SR) and SS rats with the expectation of demonstrating abnormalities in the adenosine A_{2A} R-epoxygenase pathway in the Dahl SS rat (92). Dahl SS rats challenged with HS intake, in contrast to Dahl SR rats, failed to respond to 2-CA elicited renal vasodilation above the levels produced by 2-CA in Dahl SS rats on NS intake. Thus, the renal vasodilator dose-response curves to 2-CA and the efflux of EETs in response to 2-CA did not differ in Dahl SS rats irrespective of salt intake, whether fed NS or HS. Moreover, differences in the renal vasodilator effect of 2-CA demonstrated by Dahl SS and SR rats on either NS or HS intake were entirely accounted for by their ability to release EETs because inhibition of epoxygenase activity with MS-PPOH eliminated differences in renal vasodilator responses to 2-CA observed in Dahl SR rats on NS vs. HS intake, as well as differences in the renal vasodilator responses to 2-CA occurring between Dahl SR rats and Dahl SS rats. Thus, inhibiting EET synthesis obliterated all differences in renal vasodilator responses between NS and HS-fed Dahl SS rats produced by 2-CA as well as those between Dahl SR and SS rats, demonstrating the crucial role of EETs as mediators of renovascular responses on activating the renal adenosine system. The dilator response to 2-CA was abolished in both Dahl SR and SS rats in the presence of A_{2A} R antagonism with ZM 241385.

The role of adenosine in cardiovascular regulation of blood pressure has been evaluated in the Dahl SS model of hypertension (93). Plasma adenosine concentrations were significantly higher in Dahl SS than in Dahl SR rats, irrespective of the NaCl content of the diet and adenosine levels positively correlated with blood pressure, data indicating that an abnormality in adenosine signaling may play a role in the pathophysiology of hypertension of Dahl SS rats. In our study, total purines, which reflect increased flux through the adenosine pathway, were also higher in Dahl SS rats than in Dahl SR rats on NS intake (Fig. 4) and Dahl SS rats exhibited an inability to upregulate the A_{2A} R, suggesting that an abnormality in adenosine signaling contributes to the development of hypertension in Dahl SS rats. In Dahl SR rats, HS intake did increase urinary purine excretion in contrast to the unresponsiveness to additional increases of urinary purine excretion that occurred in Dahl SS rats on HS intake (92). As renal purine excretion increased in Dahl SR rats on HS intake, the upregulation of A_{2A} R was unexpected. Adenosine receptors are G-protein coupled receptors, which typically exhibit desensitization. However, an increased A_{2A} R expression in the face of increased purine levels in Dahl SR rats does not conform to typical G-protein coupled receptor pharmacology, and instead displays a feed-forward mechanism. Expression A_{2B} R was not significantly affected by salt intake in Dahl SR rats or in Dahl SS rats. In agreement with other studies (17), the renal expression of A_1 R decreased with HS intake in both cortex and medulla of Dahl SS rats but not in Dahl SR rats. Spontaneously hypertensive rats (SHR) develop tolerance to chronic administration of a selective A_1 R agonist, but not a selective A_{2A} R agonist, over the course of 21 days (94). Although the functional relevance remains unknown, HS intake has also been shown to increase the expression of A_3 R (17).

Renal efflux of EETs and DHTs in response to 2-CA was greater in Dahl SR rats fed a HS diet than a NS diet (Fig. 5). Epoxygenase inhibition abolished the HS-induced increase in

EET/DHT release in Dahl SR rats, indicating that *de novo* synthesis of EETs rather than release of preformed EETs from storage in phospholipids (54), was responsible for the enhanced renovascular response. In contrast to Dahl SR rats, salt-loading of Dahl SS rats failed to increase the renal protein expression of either CYP2C23 or CYP2C11. Rather, levels of medullary CYP2C23 and CYP2C11 tended to decrease with HS intake in Dahl SS rats. The functional significance of this reduction in CYP2C protein is unknown, as the renovascular responsiveness of Dahl SS kidneys to 2-CA mediated by EETs was not decreased with HS feeding compared to NS intake.

As we did not observe any significant change in the EET to DHT ratios with salt-loading, it is unlikely that the increased EET levels seen with HS intake in Dahl SR rats can be accounted for by decreased sEH activity. In view of the antagonistic effects of 20-HETE on EET-induced renal vasodilation (64), we also examined the effects of HS diet on 2-CA elicited renal release of 20-HETE. Salt-loading did not affect 20-HETE renal release in response to 2-CA thereby eliminating the potential contribution of changes in 20-HETE production to the altered renal vasodilator effects of 2-CA in Dahl SS and SR rats. Deficient medullary TALH 20-HETE production associated with deficient cortical EETs have been proposed to contribute to hypertension in Dahl SS rats (86). A 20-HETE deficiency in the outer medulla of Dahl SS rats may promote increased TALH sodium and chloride reabsorption with elevation of blood pressure (59). In contrast to our findings, the production of 20-HETE and EETs by renal cortical microsomes actually fell in Dahl SS rats fed a HS diet (86). Further, we did not find differences in renal 20-HETE production by Dahl SR and SS rats, which reflect critical differences in the assays between our study and that of Ma *et al.*, (59). Namely, we measured renal efflux of CYP-AA metabolites from the isolated, perfused kidney in response to an adenosine analog, whereas they measured the synthesis of EETs and 20-HETE by renal microsomes in the presence of AA, which is an index of CYP450 enzyme synthetic capability rather than an index of the response of endogenous CYP products to experimental conditions, such as changes in salt intake.

Our findings suggest a ceiling imposed on both Dahl SR and SS rats that limited the ability of Dahl SS rats to recruit epoxygenase activity as indicated by renal EET release/efflux. Several factors that relate to the adenosine-epoxygenase system have been identified in our study that may explain the inability of HS intake to mobilize increased EET production in the Dahl SS rat, in response to HS intake. Namely: 1) purine levels reflecting activity of the adenosine pathway were fixed at levels found in NS-fed Dahl SS rats; 2) protein expression of the preeminent CYP2C isozyme, CYP2C23, responsible for increased EET synthesis in HS-fed rats was unresponsive to HS feeding in Dahl SS rats; 3) expression of the A_{2A} R linked to epoxygenase activity was also unresponsive to HS intake in the Dahl SS rat.

7. Inhibition of the A_{2A} R-EET pathway renders Dahl salt-resistant rats (SR) hypertensive

Single nucleotide polymorphisms in the CYP2J2 epoxygenase gene have been associated with a hypertensive phenotype in humans (95). The inability to upregulate CYP2C epoxygenases in response to salt-loading has been associated with the development of salt-sensitive hypertension (60;82) and inhibition of the epoxygenase pathway with MS-PPOH, has been reported to increase blood pressure in pregnant rats (96). Although adenosine has traditionally been implicated in the renal functional responses to pathological events such as ischemia and inflammation (41;42), its role in the adaptation of the kidney to enhance salt excretion has only recently become appreciated (44;71). It has been reported that treatment of Wistar rats with 1,3-dipropyl-8-sulphophenylxanthine (DPSPX), a non-selective adenosine receptor antagonist, results in hypertension (97). Furthermore, blood pressure was

elevated in transgenic A_{2A} R knockout mice on a NS diet (30), suggesting that A_{2A} R activation can serve in mechanisms that contribute to the basal regulation of blood pressure.

In Dahl SR rats, salt-loading augmented renovascular responses to an adenosine analog, an effect associated with upregulation of the protein expression of the CYP2C23 and CYP2C11, as well as A_{2A} R, changes that were not observed in Dahl SS rats (92). Therefore, we examined the effect of *in vivo* inhibition of the A_{2A} R-epoxygenase pathway on the adaptive natriuretic response to salt-loading in Dahl SR rats, both at the level of epoxygenase inhibition and A_{2A} R antagonism. In agreement with Makita *et al.* (60), we saw an increase in blood pressure in salt-loaded Dahl SR rats treated with an epoxygenase inhibitor. In the study by Makita *et al.*, clotrimazole, a non-selective inhibitor of CYP epoxygenases was used, whereas we used MS-PPOH, an inhibitor selective for CYP epoxygenases. In addition, in our study we examined the first 3 days of HS intake (*i.e.* the early, adaptive natriuretic response to salt-loading) after epoxygenase inhibition, whereas Makita *et al.* examined the effect of epoxygenase inhibition after rats were maintained for 6 weeks on a HS diet. We observed a similar increase in blood pressure in salt-loaded Dahl SR rats treated with a selective A_{2A} R antagonist, ZM 241385. *In vivo* administration of ZM 241385 to SHR attenuated the hypotensive response produced by exogenous adenosine (98). Moreover, oral administration of a selective A_{2A} R adenosine agonist elicited a sustained hypotensive response in SHR (99).

The increase in blood pressure in response to salt-loading seen in both MS-PPOH- and ZM 241385-treated Dahl SR rats was associated with a more positive sodium balance (as assessed by the daily difference between sodium intake and urinary sodium excretion), compared with vehicle-treated Dahl SR rats, during the first two days of salt-loading. By the third day of HS intake however, no differences in sodium balance were detected among the three groups, indicating that either *in vivo* epoxygenase inhibition or antagonism of A_{2A} R results in a delay in the natriuretic response to salt-loading. On Day 3 of HS intake, MS-PPOH- and ZM-241385-treated rats, plasma Na^+ levels were significantly increased (163.3 ± 1.2 and 158.1 ± 4.5 mEq/L, respectively) compared with vehicle-treated rats (142.1 ± 1 mEq/L), reflecting a diminished natriuretic capacity. Such increases in plasma Na^+ concentration are not without precedent, as previous studies have shown similar changes in rats rendered salt-sensitive by uninephrectomy and DOCA (100). To our knowledge, our observations provide the first evidence for a role of the A_{2A} R-EET pathway in the early, adaptive natriuretic response to salt-loading, as we have been able to render Dahl SR rats salt-sensitive, by either *in vivo* epoxygenase inhibition or A_{2A} R antagonism.

Compared with vehicle-treated Dahl SR rats, in Dahl SR rats treated with MS-PPOH showed a tendency for urinary K^+ excretion to be reduced, as reflected by the positive difference between K^+ intake and urinary K^+ excretion, irrespective of salt diet. These results are in agreement with those of Sun *et. al.* (101), who showed that EETs activate large-conductance calcium-activated K^+ (BK_{Ca}) channels and flow-stimulated K^+ secretion in the cortical collecting duct, thereby regulating K^+ secretion. Moreover, in that study, epoxygenase inhibition abolished K^+ secretion mediated by BK_{Ca} and renal outer medullary K^+ channels.

Dahl SR rats treated with either a CYP epoxygenase inhibitor or antagonist of A_{2A} R were unable to increase plasma levels of EETs and DHTs to the same extent as vehicle-treated Dahl SR rats, in response to salt-loading (Fig. 7). In fact, in Dahl SR rats treated with an A_{2A} R antagonist, the concentration of plasma EETs and DHTs declined to levels below the basal levels on NS diet. Renal levels of EETs and DHTs were also significantly lower with either CYP epoxygenase inhibition or A_{2A}R antagonism, thus further supporting a role for

the A_{2A} R-EET pathway in the adaptive natriuretic response to salt-loading in normotensive, Dahl SR rats.

8. Concluding Remarks

The stimulation of adenosine levels with salt loading and downregulation of A_1 R, with increased adenylyl cyclase activity via A_{2A} R stimulation, may play an important role in the adaptation of the kidney to enhance salt excretion. Indeed, there is evidence linking changes in A_{2A} R-coupled activity to blood pressure regulation: *1*) blood pressure is elevated in transgenic A_{2A} R knockout mice and *2*) A_{2A} R wild-type mice exhibit a decrease in blood pressure in response to an A_{2A} R agonist. The central finding of this study is that the renal response to adenosine is exaggerated in rats fed a HS diet, presumably via A_{2A}R activation and subsequent increased production of EETs. Unlike Dahl SR rats, Dahl SS rats exhibit an inability to upregulate the A_{2A} R-EET pathway with salt loading. With inhibition of the A_{2A} R or epoxygenase pathway, Dahl SR rats exhibited a positive sodium balance, reflecting a diminished natriuretic capacity and became hypertensive. These studies support a role for the A_{2A} R -EET pathway in the adaptive natriuretic response to modulate blood pressure during salt loading. As salt-sensitivity is an important characteristic of a subgroup of humans with essential hypertension and other forms of salt-dependent hypertension that occurs in African-Americans, diabetics, and the aged (102), identification of potential targets for the management of salt-sensitive hypertension may be of therapeutic benefit. The adenosine-A_{2A} R-EET pathway may be an important therapeutic target for managing salt-sensitive hypertension.

Highlights

- Epoxyeicosatrienoic acids (EETs) are renal vasodilator and natriuretic eicosanoids.
- Adenosine-activated renovascular dilatation is mediated via A2A receptor (R) which is linked to increasing EET synthesis.
- The A2AR-EET pathway is upregulated in normotensive rats and contributes to the adaptive response to high salt intake.
- Failure to upregulate the A2AR-EET pathway may contribute to the development of salt-sensitive hypertension

Acknowledgments

This review is dedicated to my dear brother, John Carroll, who died on July 4th, 2011. I wish to thank Monica K. Cheng, Anabel B. Doumad, Elvira L. Liclican and Jing Li for their significant contribution to these studies. In addition, I wish to thank J.R. (Camille) Falck for providing MS-PPOH and John C. McGiff for his constant advice. This work was supported by grants from the National Institutes of Health DK69687, HL-25394, and GM31278 and from the Pharmaceutical Research and Manufacturers of America Foundation.

References

1. Blaustein MP, Leenen FH, Chen L, Golovina VA, Hamlyn JM, Pallone TL, Van Huysse JW, Zhang J, Wier WG. How NaCl raises blood pressure: A new paradigm for the pathogenesis of salt-dependent hypertension. Am J Physiol Heart Circ Physiol. 2011
2. He FJ, Jenner KH, Macgregor GA. WASH-world action on salt and health. Kidney Int. 2010; 78(8): 745–753. [PubMed: 20720531]
3. Cowley AW Jr. Genetic and nongenetic determinants of salt sensitivity and blood pressure. Am J Clin Nutr. 1997; 65(2 Suppl):587S–593S. [PubMed: 9022553]

4. Burnstock G. Purinergic receptors in the heart. Circ Res. 1980; 46(6 Pt 2):I175–I182. [PubMed: 6247089]
5. Olah ME, Stiles GL. The role of receptor structure in determining adenosine receptor activity. Pharmacol Ther. 2000; 85(2):55–75. [PubMed: 10722120]
6. Vallon V, Muhlbauer B, Osswald H. Adenosine and kidney function. Physiol Rev. 2006; 86(3):901–940. [PubMed: 16816141]
7. Jackson EK, Zhu C, Tofovic SP. Expression of adenosine receptors in the preglomerular microcirculation. Am J Physiol Renal Physiol. 2002; 283(1):F41–F51. [PubMed: 12060585]
8. Yamaguchi S, Umemura S, Tamura K, Iwamoto T, Nyui N, Ishigami T, Ishii M. Adenosine A1 receptor mRNA in microdissected rat nephron segments. Hypertension. 1995; 26(6 Pt 2):1181–1185. [PubMed: 7498992]
9. Vitzthum H, Weiss B, Bachleitner W, Kramer BK, Kurtz A. Gene expression of adenosine receptors along the nephron. Kidney Int. 2004; 65(4):1180–1190. [PubMed: 15086457]
10. Smith JA, Sivaprasadarao A, Munsey TS, Bowmer CJ, Yates MS. Immunolocalisation of adenosine A(1) receptors in the rat kidney. Biochem Pharmacol. 2001; 61(2):237–244. [PubMed: 11163338]
11. Pawelczyk T, Grden M, Rzepko R, Sakowicz M, Szutowicz A. Region-specific alterations of adenosine receptors expression level in kidney of diabetic rat. Am J Pathol. 2005; 167(2):315–325. [PubMed: 16049319]
12. Silldorff EP, Pallone TL. Adenosine signaling in outer medullary descending vasa recta. Am J Physiol Regul Integr Comp Physiol. 2001; 280(3):R854–R861. [PubMed: 11171666]
13. Weaver DR, Reppert SM. Adenosine receptor gene expression in rat kidney. Am J Physiol. 1992; 263(6 Pt 2):F991–F995. [PubMed: 1481890]
14. Al-Mashhadi RH, Skott O, Vanhoutte PM, Hansen PB. Activation of A(2) adenosine receptors dilates cortical efferent arterioles in mouse. Kidney Int. 2009; 75(8):793–799. [PubMed: 19165174]
15. Ren Y, Garvin JL, Liu R, Carretero OA. Possible mechanism of efferent arteriole (Ef-Art) tubuloglomerular feedback. Kidney Int. 2007; 71(9):861–866. [PubMed: 17342182]
16. Singh P, Thomson SC. Renal homeostasis and tubuloglomerular feedback. Curr Opin Nephrol Hypertens. 2010; 19(1):59–64. [PubMed: 19864948]
17. Zou AP, Wu F, Li PL, Cowley AW Jr. Effect of chronic salt loading on adenosine metabolism and receptor expression in renal cortex and medulla in rats. Hypertension. 1999; 33(1 Pt 2):511–516. [PubMed: 9931157]
18. Guyton AC, Coleman TG. Quantitative analysis of the pathophysiology of hypertension. Circ Res. 1969; 24(5 Suppl):1–19. [PubMed: 4306217]
19. Nishiyama A, Navar LG. ATP mediates tubuloglomerular feedback. Am J Physiol Regul Integr Comp Physiol. 2002; 283(1):R273–R275. [PubMed: 12069954]
20. Spielman WS, Arend LJ. Adenosine receptors and signaling in the kidney. Hypertension. 1991; 17(2):117–130. [PubMed: 1991645]
21. Holz FG, Steinhausen M. Renovascular effects of adenosine receptor agonists. Ren Physiol. 1987; 10(5):272–282. [PubMed: 3454474]
22. Brown R, Ollerstam A, Johansson B, Skott O, Gebre-Medhin S, Fredholm B, Persson AE. Abolished tubuloglomerular feedback and increased plasma renin in adenosine A1 receptor-deficient mice. Am J Physiol Regul Integr Comp Physiol. 2001; 281(5):R1362–R1367. [PubMed: 11641103]
23. Schnermann J, Levine DZ. Paracrine factors in tubuloglomerular feedback: adenosine, ATP, and nitric oxide. Annu Rev Physiol. 2003; 65:501–529. Epub;%2002 May 1.:501–529. [PubMed: 12208992]
24. Hansen PB, Hashimoto S, Oppermann M, Huang Y, Briggs JP, Schnermann J. Vasoconstrictor and vasodilator effects of adenosine in the mouse kidney due to preferential activation of A1 or A2 adenosine receptors. J Pharmacol Exp Ther. 2005; 315(3):1150–1157. [PubMed: 16120812]
25. Sun D, Samuelson LC, Yang T, Huang Y, Paliege A, Saunders T, Briggs J, Schnermann J. Mediation of tubuloglomerular feedback by adenosine: evidence from mice lacking adenosine 1 receptors. Proc Natl Acad Sci U S A. 2001; 98(17):9983–9988. [PubMed: 11504952]

26. Fredholm BB, Ijzerman AP, Jacobson KA, Klotz KN, Linden J. International Union of Pharmacology. XXV. Nomenclature and classification of adenosine receptors. Pharmacol Rev. 2001; 53(4):527–552. [PubMed: 11734617]
27. Navar LG, Inscho EW, Majid SA, Imig JD, Harrison-Bernard LM, Mitchell KD. Paracrine regulation of the renal microcirculation. Physiol Rev. 1996; 76(2):425–536. [PubMed: 8618962]
28. Carroll MA, Doumad AB, Li J, Cheng MK, Falck JR, McGiff JC. Adenosine2A receptor vasodilation of rat preglomerular microvessels is mediated by EETs that activate the cAMP/PKA pathway. Am J Physiol Renal Physiol. 2006; 291(1):F155–F161. [PubMed: 16478979]
29. Levens N, Beil M, Jarvis M. Renal actions of a new adenosine agonist, CGS 21680A selective for the A2 receptor. J Pharmacol Exp Ther. 1991; 257(3):1005–1012. [PubMed: 2046017]
30. Ledent C, Vaugeois JM, Schiffmann SN, Pedrazzini T, El Yacoubi M, Vanderhaeghen JJ, Costentin J, Heath JK, Vassart G, Parmentier M. Aggressiveness, hypoalgesia and high blood pressure in mice lacking the adenosine A2a receptor. Nature. 1997; 388(6643):674–678. [PubMed: 9262401]
31. Zou AP, Nithipatikom K, Li PL, Cowley AW Jr. Role of renal medullary adenosine in the control of blood flow and sodium excretion. Am J Physiol. 1999; 276(3 Pt 2):R790–R798. [PubMed: 10070140]
32. Beach RE, Good DW. Effects of adenosine on ion transport in rat medullary thick ascending limb. Am J Physiol. 1992; 263(3 Pt 2):F482–F487. [PubMed: 1415577]
33. Wei Y, Sun P, Wang Z, Yang B, Carroll MA, Wang WH. Adenosine inhibits ENaC via cytochrome P-450 epoxygenase-dependent metabolites of arachidonic acid. Am J Physiol Renal Physiol. 2006; 290(5):F1163–F1168. [PubMed: 16234312]
34. Carlstrom M, Wilcox CS, Welch WJ. Adenosine A2A receptor activation attenuates tubuloglomerular feedback responses by stimulation of endothelial nitric oxide synthase. Am J Physiol Renal Physiol. 2011; 300(2):F457–F464. [PubMed: 21106859]
35. Feng MG, Navar LG. Adenosine A2 receptor activation attenuates afferent arteriolar autoregulation during adenosine receptor saturation in rats. Hypertension. 2007; 50(4):744–749. [PubMed: 17664389]
36. Carlstrom M, Wilcox CS, Welch WJ. Adenosine A(2) receptors modulate tubuloglomerular feedback. Am J Physiol Renal Physiol. 2010; 299(2):F412–F417. [PubMed: 20519378]
37. Martin PL, Potts AA. The endothelium of the rat renal artery plays an obligatory role in A2 adenosine receptor-mediated relaxation induced by 5'-N-ethylcarboxamidoadenosine and N6-cyclopentyladenosine. J Pharmacol Exp Ther. 1994; 270(3):893–899. [PubMed: 7932201]
38. Feng MG, Navar LG. Afferent arteriolar vasodilator effect of adenosine predominantly involves adenosine A2B receptor activation. Am J Physiol Renal Physiol. 2010; 299(2):F310–F315. [PubMed: 20462966]
39. Di SF, Cerull R, Babich V, Casavola V, Helmle-Roth C, Burckhardt G. Short- and long-term A3 adenosine receptor activation inhibits the Na+/H+ exchanger NHE3 activity and expression in opossum kidney cells. J Cell Physiol. 2008; 216(1):221–233. [PubMed: 18286509]
40. Lee HT, Ota-Setlik A, Xu H, D'Agati VD, Jacobson MA, Emala CW. A3 adenosine receptor knockout mice are protected against ischemia- and myoglobinuria-induced renal failure. Am J Physiol Renal Physiol. 2003; 284(2):F267–F273. [PubMed: 12388399]
41. Miller WL, Thomas RA, Berne RM, Rubio R. Adenosine production in the ischemic kidney. Circ Res. 1978; 43(3):390–397. [PubMed: 679422]
42. Okusa MD. A(2A) adenosine receptor: a novel therapeutic target in renal disease. Am J Physiol Renal Physiol. 2002; 282(1):F10–F18. [PubMed: 11739107]
43. Siragy HM, Linden J. Sodium intake markedly alters renal interstitial fluid adenosine. Hypertension. 1996; 27(3 Pt 1):404–407. [PubMed: 8698445]
44. Lorenz JN, Weihprecht H, He XR, Skott O, Briggs JP, Schnermann J. Effects of adenosine and angiotensin on macula densa-stimulated renin secretion. Am J Physiol. 1993; 265(2 Pt 2):F187–F194. [PubMed: 8368332]
45. Williams JM, Murphy S, Burke M, Roman RJ. 20-hydroxyeicosatetraeonic acid: a new target for the treatment of hypertension. J Cardiovasc Pharmacol. 2010; 56(4):336–344. [PubMed: 20930591]

46. Spector AA. Arachidonic acid cytochrome P450 epoxygenase pathway. J Lipid Res. 2009; 50(Suppl):S52–S56. [PubMed: 18952572]
47. McGiff JC, Quilley J. 20-hydroxyeicosatetraenoic acid and epoxyeicosatrienoic acids and blood pressure. Curr Opin Nephrol Hypertens. 2001; 10(2):231–237. [PubMed: 11224699]
48. Campbell WB, Fleming I. Epoxyeicosatrienoic acids and endothelium-dependent responses. Pflugers Arch. 2010; 459(6):881–895. [PubMed: 20224870]
49. Pfister SL, Gauthier KM, Campbell WB. Vascular pharmacology of epoxyeicosatrienoic acids. Adv Pharmacol. 2010; 60:27–59. [PubMed: 21081214]
50. Sudhahar V, Shaw S, Imig JD. Epoxyeicosatrienoic acid analogs and vascular function. Curr Med Chem. 2010; 17(12):1181–1190. [PubMed: 20158473]
51. Bellien J, Joannides R, Richard V, Thuillez C. Modulation of cytochrome-derived epoxyeicosatrienoic acids pathway: a promising pharmacological approach to prevent endothelial dysfunction in cardiovascular diseases? Pharmacol Ther. 2011; 131(1):1–17. [PubMed: 21514320]
52. Node K, Ruan XL, Dai J, Yang SX, Graham L, Zeldin DC, Liao JK. Activation of Galpha s mediates induction of tissue-type plasminogen activator gene transcription by epoxyeicosatrienoic acids. J Biol Chem. 2001; 276(19):15983–15989. [PubMed: 11279071]
53. Potente M, Michaelis UR, Fisslthaler B, Busse R, Fleming I. Cytochrome P450 2C9-induced endothelial cell proliferation involves induction of mitogen-activated protein (MAP) kinase phosphatase-1, inhibition of the c-Jun N-terminal kinase, and up-regulation of cyclin D1. J Biol Chem. 2002; 277(18):15671–15676. [PubMed: 11867622]
54. Carroll MA, Balazy M, Margiotta P, Huang DD, Falck JR, McGiff JC. Cytochrome P-450-dependent HETEs: profile of biological activity and stimulation by vasoactive peptides. Am J Physiol. 1996; 271(4 Pt 2):R863–R869. [PubMed: 8897975]
55. Croft KD, McGiff JC, Sanchez-Mendoza A, Carroll MA. Angiotensin II releases 20-HETE from rat renal microvessels. Am J Physiol Renal Physiol. 2000; 279(3):F544–F551. [PubMed: 10966934]
56. Hercule HC, Oyekan AO. Cytochrome P450 omega/omega-1 hydroxylase-derived eicosanoids contribute to endothelin(A) and endothelin(B) receptor-mediated vasoconstriction to endothelin-1 in the rat preglomerular arteriole. J Pharmacol Exp Ther. 2000; 292(3):1153–1160. [PubMed: 10688635]
57. Fulton D, McGiff JC, Quilley J. Role of phospholipase C and phospholipase A2 in the nitric oxide-independent vasodilator effect of bradykinin in the rat perfused heart. J Pharmacol Exp Ther. 1996; 278(2):518–526. [PubMed: 8768699]
58. Imig JD, Falck JR, Wei S, Capdevila JH. Epoxygenase metabolites contribute to nitric oxide-independent afferent arteriolar vasodilation in response to bradykinin. J Vasc Res. 2001; 38(3): 247–255. [PubMed: 11399897]
59. Ito O, Roman RJ. Role of 20-HETE in elevating chloride transport in the thick ascending limb of Dahl SS/Jr rats. Hypertension. 1999; 33(1 Pt 2):419–423. [PubMed: 9931140]
60. Makita K, Takahashi K, Karara A, Jacobson HR, Falck JR, Capdevila JH. Experimental and/or genetically controlled alterations of the renal microsomal cytochrome P450 epoxygenase induce hypertension in rats fed a high salt diet. J Clin Invest. 1994; 94(6):2414–2420. [PubMed: 7989598]
61. Yu Z, Xu F, Huse LM, Morisseau C, Draper AJ, Newman JW, Parker C, Graham L, Engler MM, Hammock BD, Zeldin DC, Kroetz DL. Soluble epoxide hydrolase regulates hydrolysis of vasoactive epoxyeicosatrienoic acids. Circ Res. 2000; 87(11):992–998. [PubMed: 11090543]
62. Capdevila JH, Wei S, Yan J, Karara A, Jacobson HR, Falck JR, Guengerich FP, DuBois RN. Cytochrome P-450 arachidonic acid epoxygenase. Regulatory control of the renal epoxygenase by dietary salt loading. J Biol Chem. 1992; 267(30):21720–21726. [PubMed: 1400482]
63. Oyekan AO, Youseff T, Fulton D, Quilley J, McGiff JC. Renal cytochrome P450 omega-hydroxylase and epoxygenase activity are differentially modified by nitric oxide and sodium chloride. J Clin Invest. 1999; 104(8):1131–1137. [PubMed: 10525052]
64. Imig JD, Falck JR, Inscho EW. Contribution of cytochrome P450 epoxygenase and hydroxylase pathways to afferent arteriolar autoregulatory responsiveness. Br J Pharmacol. 1999; 127(6):1399–1405. [PubMed: 10455289]

65. Imig JD, Navar LG, Roman RJ, Reddy KK, Falck JR. Actions of epoxygenase metabolites on the preglomerular vasculature. J Am Soc Nephrol. 1996; 7(11):2364–2370. [PubMed: 8959626]
66. McGiff JC, Carroll MA. Cytochrome P450-dependent arachidonate metabolites, renal function and blood pressure regulation. Adv Prostaglandin Thromboxane Leukot Res. 1991; 21B:675–682. [PubMed: 1847570]
67. Romero MF, Madhun ZT, Hopfer U, Douglas JG. An epoxygenase metabolite of arachidonic acid 5,6 epoxy- eicosatrienoic acid mediates angiotensin-induced natriuresis in proximal tubular epithelium. Adv Prostaglandin Thromboxane Leukot Res. 1991; 21A:205–208. [PubMed: 1847766]
68. Staudinger R, Escalante B, Schwartzman ML, Abraham NG. Effects of epoxyeicosatrienoic acids on 86Rb uptake in renal epithelial cells. J Cell Physiol. 1994; 160(1):69–74. [PubMed: 8021300]
69. Liclican EL, Doumad AB, Wang J, Li J, Falck JR, Stier CT Jr, Carroll MA. Inhibition of the adenosine2A receptor-epoxyeicosatrienoic acid pathway renders Dahl salt-resistant rats hypertensive. Hypertension. 2009; 54(6):1284–1290. [PubMed: 19822802]
70. Cheng MK, Doumad AB, Jiang H, Falck JR, McGiff JC, Carroll MA. Epoxyeicosatrienoic acids mediate adenosine-induced vasodilation in rat preglomerular microvessels (PGMV) via A2A receptors. Br J Pharmacol. 2004; 141(3):441–448. [PubMed: 14718251]
71. Liclican EL, McGiff JC, Pedraza PL, Ferreri NR, Falck JR, Carroll MA. Exaggerated response to adenosine in kidneys from high salt-fed rats: role of epoxyeicosatrienoic acids. Am J Physiol Renal Physiol. 2005; 289(2):F386–F392. [PubMed: 15814528]
72. Lin CH, Lo WC, Hsiao M, Tseng CJ. Interaction of carbon monoxide and adenosine in the nucleus tractus solitarii of rats. Hypertension. 2003; 42(3):380–385. [PubMed: 12913065]
73. Spector AA, Fang X, Snyder GD, Weintraub NL. Epoxyeicosatrienoic acids (EETs): metabolism and biochemical function. Prog Lipid Res. 2004; 43(1):55–90. [PubMed: 14636671]
74. Zeldin DC. Epoxygenase pathways of arachidonic acid metabolism. J Biol Chem. 2001; 276(39): 36059–36062. [PubMed: 11451964]
75. Zeldin DC, Kobayashi J, Falck JR, Winder BS, Hammock BD, Snapper JR, Capdevila JH. Regio- and enantiofacial selectivity of epoxyeicosatrienoic acid hydration by cytosolic epoxide hydrolase. J Biol Chem. 1993; 268(9):6402–6407. [PubMed: 8454612]
76. Zeldin DC, Wei S, Falck JR, Hammock BD, Snapper JR, Capdevila JH. Metabolism of epoxyeicosatrienoic acids by cytosolic epoxide hydrolase: substrate structural determinants of asymmetric catalysis. Arch Biochem Biophys. 1995; 316(1):443–451. [PubMed: 7840649]
77. Nayeem MA, Poloyac SM, Falck JR, Zeldin DC, Ledent C, Ponnoth DS, Ansari HR, Mustafa SJ. Role of CYP epoxygenases in A2A AR-mediated relaxation using A2A AR-null and wild-type mice. Am J Physiol Heart Circ Physiol. 2008; 295(5):H2068–H2078. [PubMed: 18805895]
78. Nayeem MA, Ponnoth DS, Boegehold MA, Zeldin DC, Falck JR, Mustafa SJ. High-salt diet enhances mouse aortic relaxation through adenosine A2A receptor via CYP epoxygenases. Am J Physiol Regul Integr Comp Physiol. 2009; 296(3):R567–R574. [PubMed: 19109366]
79. Holla VR, Makita K, Zaphiropoulos PG, Capdevila JH. The kidney cytochrome P-450 2C23 arachidonic acid epoxygenase is upregulated during dietary salt loading. J Clin Invest. 1999; 104(6):751–760. [PubMed: 10491410]
80. Ma J, Qu W, Scarborough PE, Tomer KB, Moomaw CR, Maronpot R, Davis LS, Breyer MD, Zeldin DC. Molecular cloning, enzymatic characterization, developmental expression, and cellular localization of a mouse cytochrome P450 highly expressed in kidney. J Biol Chem. 1999; 274(25): 17777–17788. [PubMed: 10364221]
81. Zeldin DC, DuBois RN, Falck JR, Capdevila JH. Molecular cloning, expression and characterization of an endogenous human cytochrome P450 arachidonic acid epoxygenase isoform. Arch Biochem Biophys. 1995; 322(1):76–86. [PubMed: 7574697]
82. Zhao X, Pollock DM, Inscho EW, Zeldin DC, Imig JD. Decreased renal cytochrome P450 2C enzymes and impaired vasodilation are associated with angiotensin salt-sensitive hypertension. Hypertension. 2003; 41(3 Pt 2):709–714. [PubMed: 12623984]
83. Rapp JP, Dene H. Development and characteristics of inbred strains of Dahl salt-sensitive and salt-resistant rats. Hypertension. 1985; 7(3 Pt 1):340–349. [PubMed: 3997219]

84. Roman RJ, Kaldunski M. Pressure natriuresis and cortical and papillary blood flow in inbred Dahl rats. Am J Physiol. 1991; 261(3 Pt 2):R595–R602. [PubMed: 1887948]
85. Kunert MP, Drenjancevic-Peric I, Dwinell MR, Lombard JH, Cowley AW Jr, Greene AS, Kwitek AE, Jacob HJ. Consomic strategies to localize genomic regions related to vascular reactivity in the Dahl salt-sensitive rat. Physiol Genomics. 2006; 26(3):218–225. [PubMed: 16772359]
86. Ma YH, Schwartzman ML, Roman RJ. Altered renal P-450 metabolism of arachidonic acid in Dahl salt-sensitive rats. Am J Physiol. 1994; 267(2 Pt 2):R579–R589. [PubMed: 8067471]
87. Kelly RA, Pfeffer JM, Mitch WE, Smith TW. Plasma nonesterified fatty acids in the Dahl rat. Response to salt loading. Hypertension. 1987; 10(2):198–203. [PubMed: 3610294]
88. Kuczeriszka M, Olszynski KH, Gasiorowska A, Sadowski J, Kompanowska-Jezierska E. Interaction of nitric oxide and the cytochrome P-450 system on blood pressure and renal function in the rat: dependence on sodium intake. Acta Physiol (Oxf). 2011; 201(4):493–502. [PubMed: 21073660]
89. Cheng J, Ou JS, Singh H, Falck JR, Narsimhaswamy D, Pritchard KA Jr, Schwartzman ML. 20-hydroxyeicosatetraenoic acid causes endothelial dysfunction via eNOS uncoupling. Am J Physiol Heart Circ Physiol. 2008; 294(2):H1018–H1026. [PubMed: 18156192]
90. Larsen BT, Gutterman DD, Sato A, Toyama K, Campbell WB, Zeldin DC, Manthati VL, Falck JR, Miura H. Hydrogen peroxide inhibits cytochrome p450 epoxygenases: interaction between two endothelium-derived hyperpolarizing factors. Circ Res. 2008; 102(1):59–67. [PubMed: 17975109]
91. Liu X, Li C, Falck JR, Roman RJ, Harder DR, Koehler RC. Interaction of nitric oxide, 20-HETE, and EETs during functional hyperemia in whisker barrel cortex. Am J Physiol Heart Circ Physiol. 2008; 295(2):H619–H631. [PubMed: 18502903]
92. Liclican EL, McGiff JC, Falck JR, Carroll MA. Failure to upregulate the adenosine2A receptor-epoxyeicosatrienoic acid pathway contributes to the development of hypertension in Dahl salt-sensitive rats. Am J Physiol Renal Physiol. 2008; 295(6):F1696–F1704. [PubMed: 18829737]
93. Yamada K, Goto A, Ishii M, Yoshioka M, Sugimoto T. Plasma adenosine concentrations are elevated in Dahl salt-sensitive rats. Experientia. 1995; 51(3):227–229. [PubMed: 7698285]
94. Casati C, Monopoli A, Dionisotti S, Zocchi C, Bonizzoni E, Ongini E. Repeated administration of selective adenosine A1 and A2 receptor agonists in the spontaneously hypertensive rat: tolerance develops to A1-mediated hemodynamic effects. J Pharmacol Exp Ther. 1994; 268(3):1506–1511. [PubMed: 8138961]
95. King LM, Gainer JV, David GL, Dai D, Goldstein JA, Brown NJ, Zeldin DC. Single nucleotide polymorphisms in the CYP2J2 and CYP2C8 genes and the risk of hypertension. Pharmacogenet Genomics. 2005; 15(1):7–13. [PubMed: 15864120]
96. Huang H, Chang HH, Xu Y, Reddy DS, Du J, Zhou Y, Dong Z, Falck JR, Wang MH. Epoxyeicosatrienoic Acid inhibition alters renal hemodynamics during pregnancy. Exp Biol Med (Maywood). 2006; 231(11):1744–1752. [PubMed: 17138762]
97. Guimaraes S, Albino-Teixeira A. Hypertension due to chronic blockade of P1-purinoceptors. J Auton Pharmacol. 1996; 16(6):367–370. [PubMed: 9131418]
98. Poucher SM, Keddie JR, Brooks R, Shaw GR, McKillop D. Pharmacodynamics of ZM 241385, a potent A2a adenosine receptor antagonist, after enteric administration in rat, cat and dog. J Pharm Pharmacol. 1996; 48(6):601–606. [PubMed: 8832494]
99. Yagil Y, Miyamoto M. The hypotensive effect of an oral adenosine analog with selectivity for the A2 receptor in the spontaneously hypertensive rat. Am J Hypertens. 1995; 8(5 Pt 1):509–515. [PubMed: 7662228]
100. Kunes J, Zicha J, Jelinek J. The role of chloride in deoxycorticosterone hypertension: selective sodium loading by diet or drinking fluid. Physiol Res. 2004; 53(2):149–154. [PubMed: 15046550]
101. Sun P, Liu W, Lin DH, Yue P, Kemp R, Satlin LM, Wang WH. Epoxyeicosatrienoic Acid Activates BK Channels in the Cortical Collecting Duct. J Am Soc Nephrol. 2008
102. Weinberger MH, Fineberg NS, Fineberg SE, Weinberger M. Salt sensitivity, pulse pressure, and death in normal and hypertensive humans. Hypertension. 2001; 37(2 Part 2):429–432. [PubMed: 11230313]

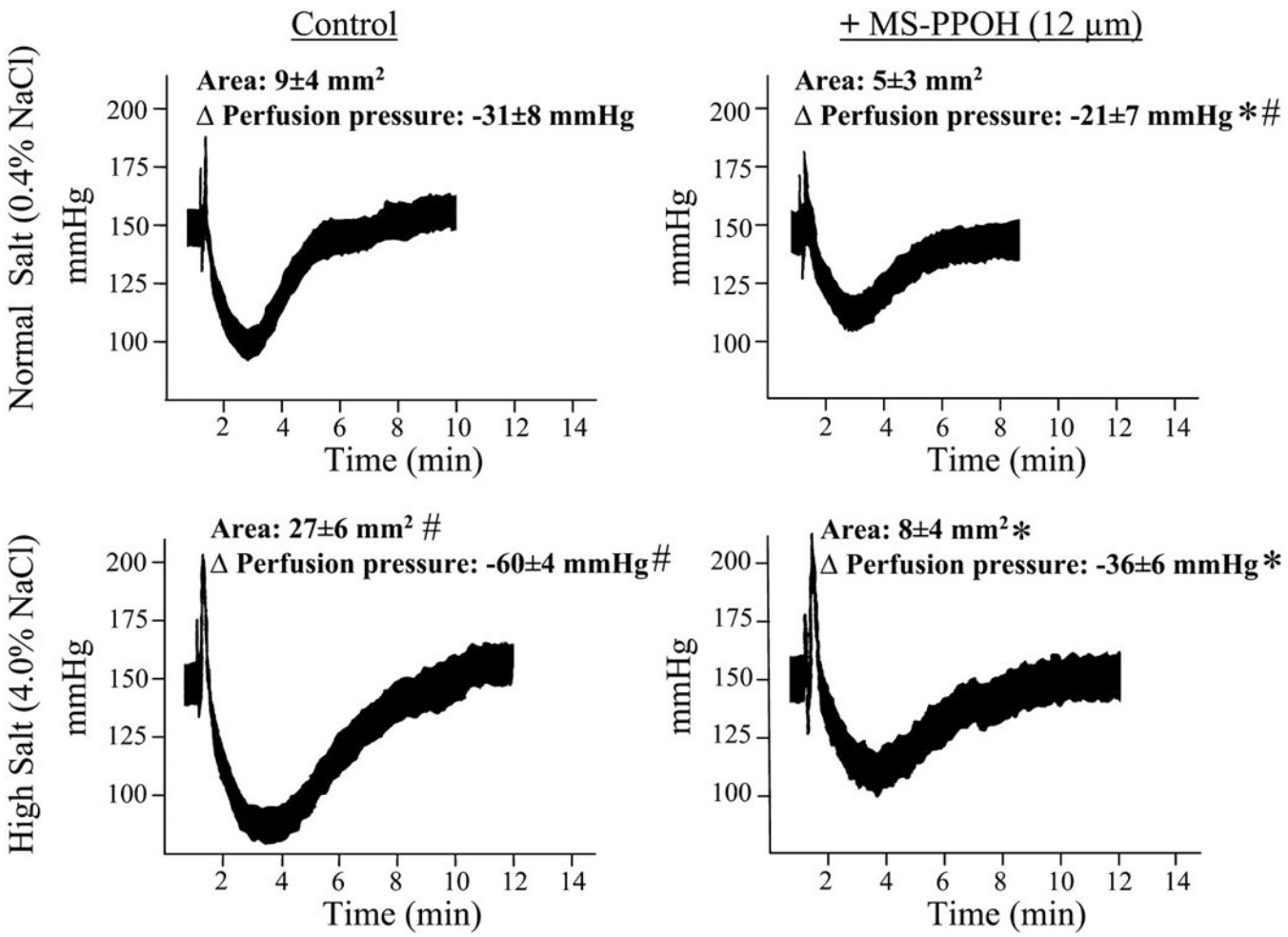

Figure 1. Responsiveness of isolated, perfused rat kidneys to 10 μg 2-chloroadenosine (2-CA)

Changes in perfusion pressure and area of response to a bolus injection of 10 μg 2-CA were compared in kidneys obtained from normal salt (NS)-fed rats vs. high salt (HS)-fed rats for 7 days in the absence and presence of MS-PPOH (12 μM). Data expressed as means ± SE; n = 4. *P < 0.05 vs. control (i.e., NS vs. NS + MS-PPOH or HS vs. HS + MS-PPOH). #P < 0.05 NS vs. HS. *#P < 0.05 HS vs. NS + MS-PPOH.

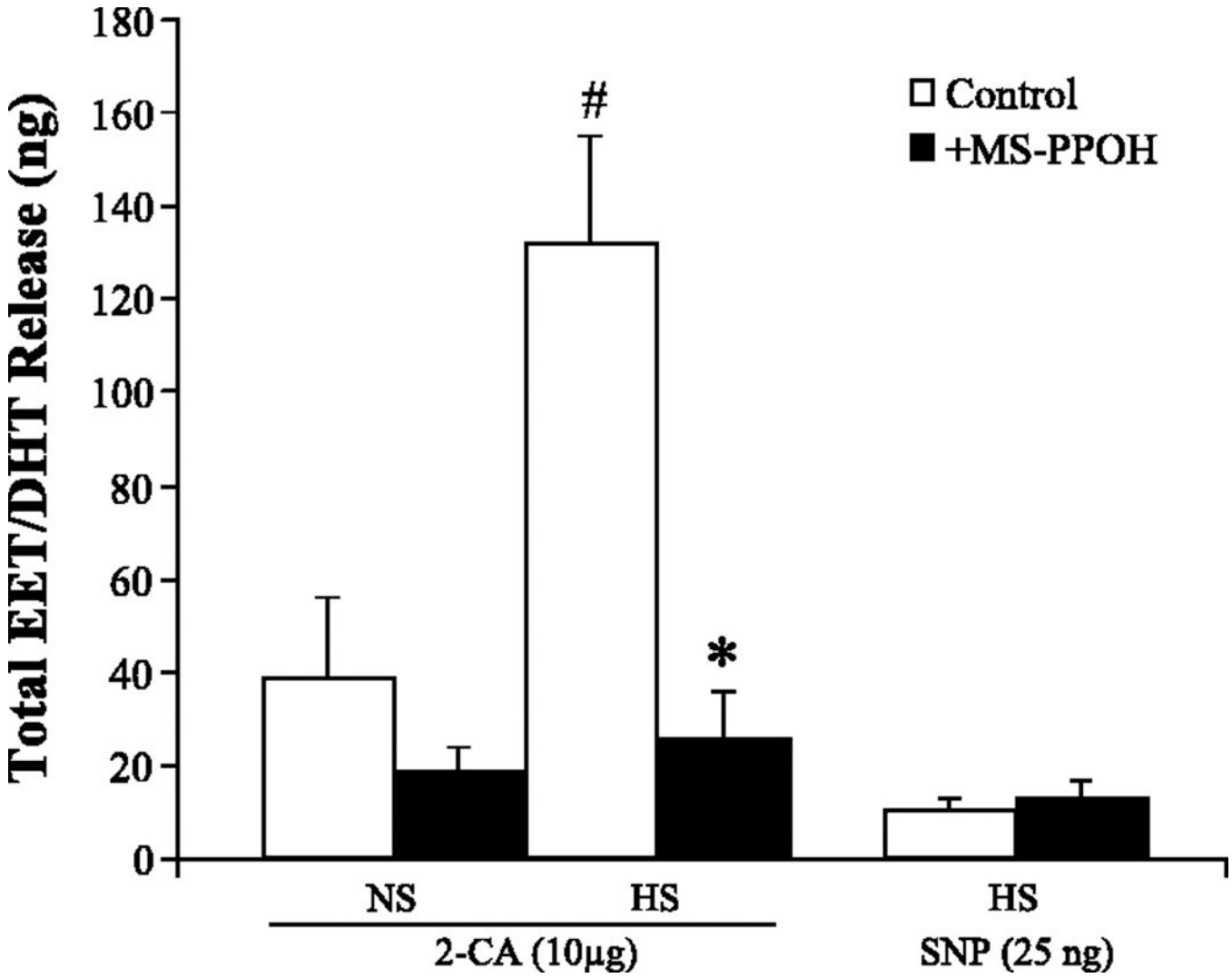

Figure 2. Renal release of CYP epoxygenase metabolites in response to 10 µg 2-CA

Release of total EETs + DHTs in response to 10 µg 2-CA was compared in NS- and HS-fed rats in the absence and presence of MS-PPOH. Total EET + DHT release in response to sodium nitroprusside (SNP; 25 ng) in HS-fed rats was also measured. Data are expressed as means ± SE; $n = 4–5$. *$P < 0.05$ vs. control (i.e., HS vs. HS + MS-PPOH). #$P < 0.05$ NS vs. HS.

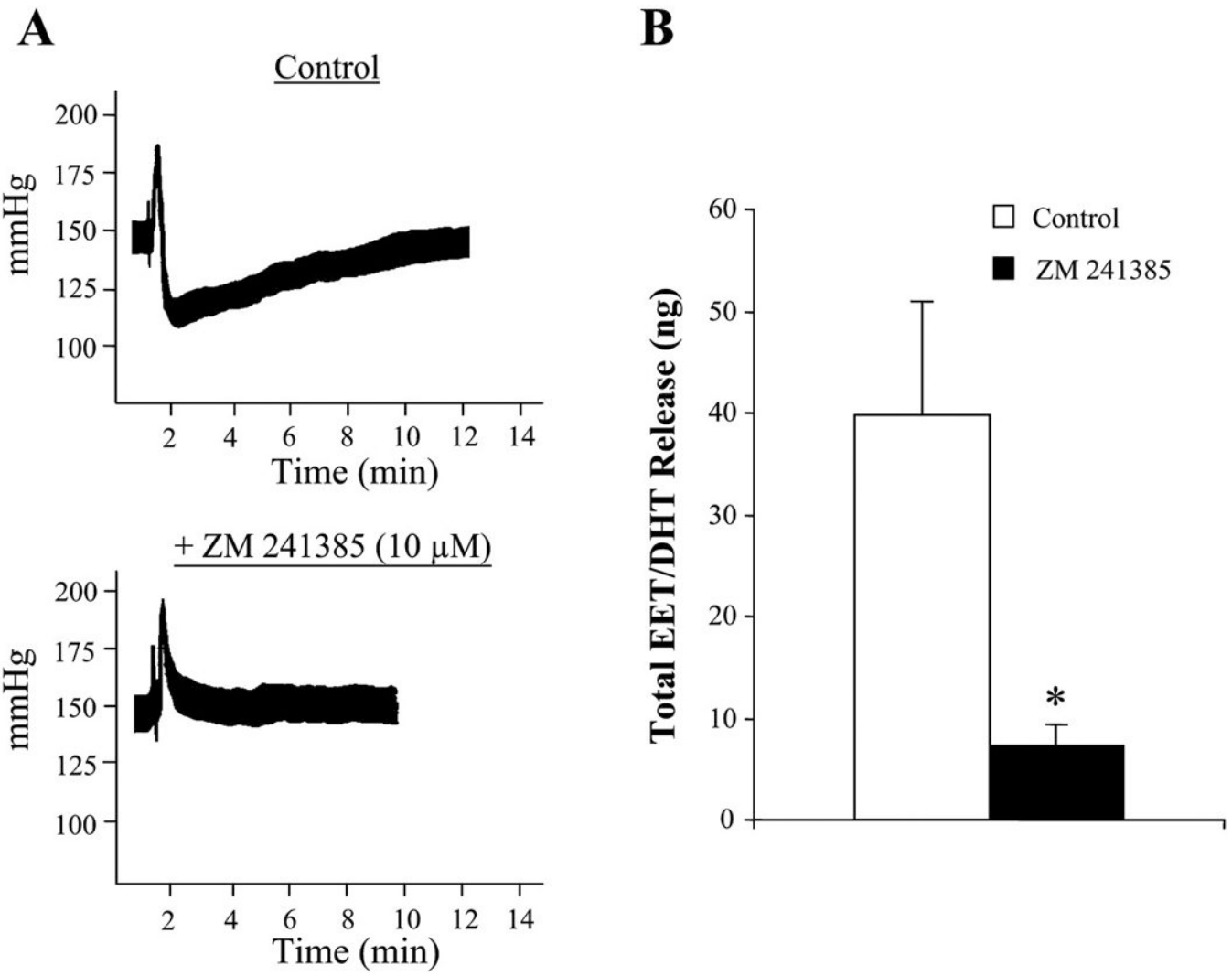

Figure 3. Effect of A_{2A} R inhibition on the responsiveness of isolated, perfused rat kidneys to 10 μg 2-CA under NS intake

A: vascular response to 10 μg 2-CA was assessed before and after A_{2A}R blockade by ZM-241385 (10 μM). ***B***: total release of EET + DHT in response to 10 μg 2-CA was compared in the absence and presence of ZM-241385. Data are expressed as means ± SE; $n = 3$. *$P < 0.05$ vs. control.

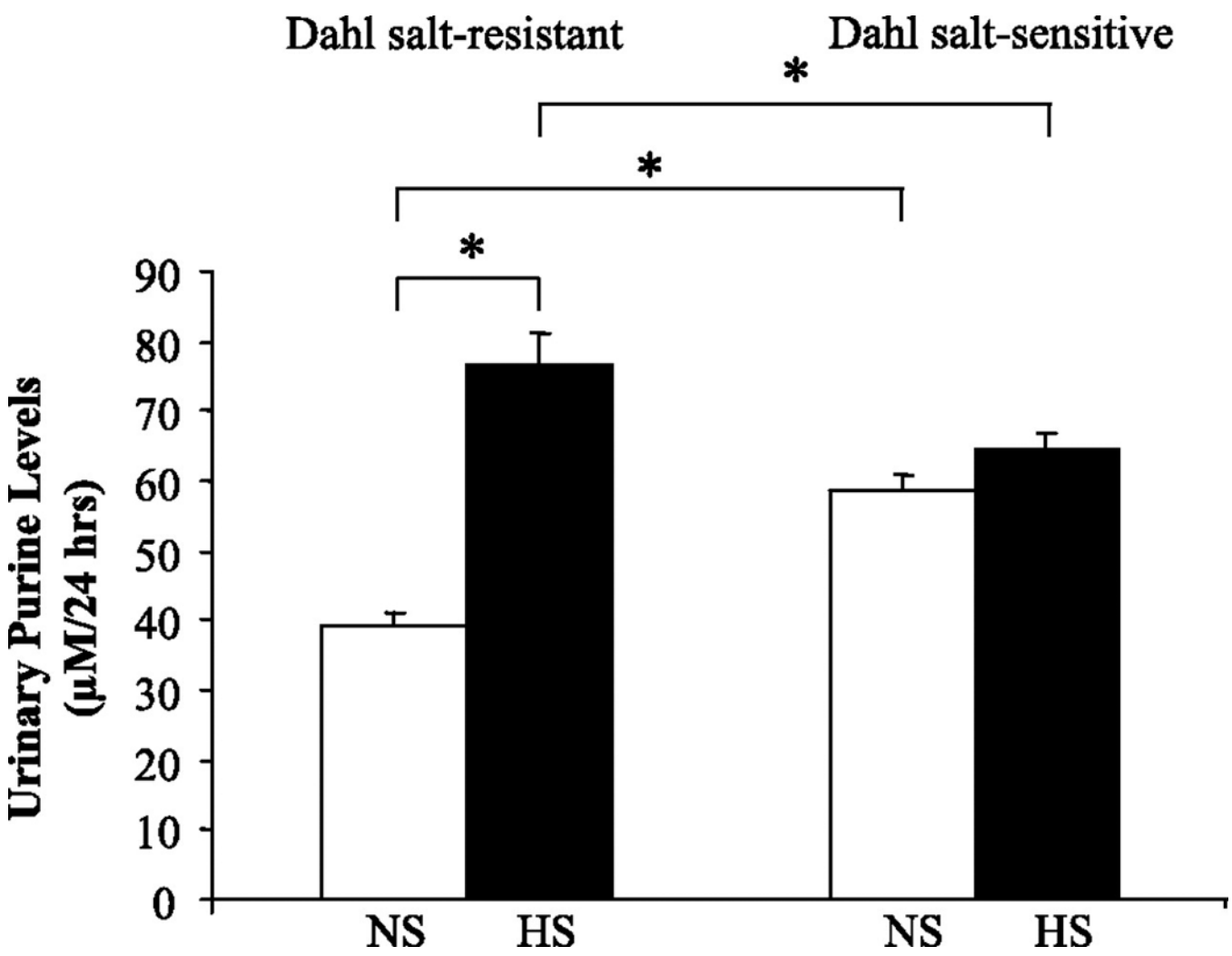

Figure 4. Urinary purine levels after 7 days of HS diet in Dahl SR and SS rats

Levels of urinary purines (i.e., adenosine, hypoxanthine, xanthine, and inosine) were measured in Dahl SR and SS rats on NS diet and after 7 days of HS diet. Data are means ± SE; $n = 5–7$. $*P < 0.05$.

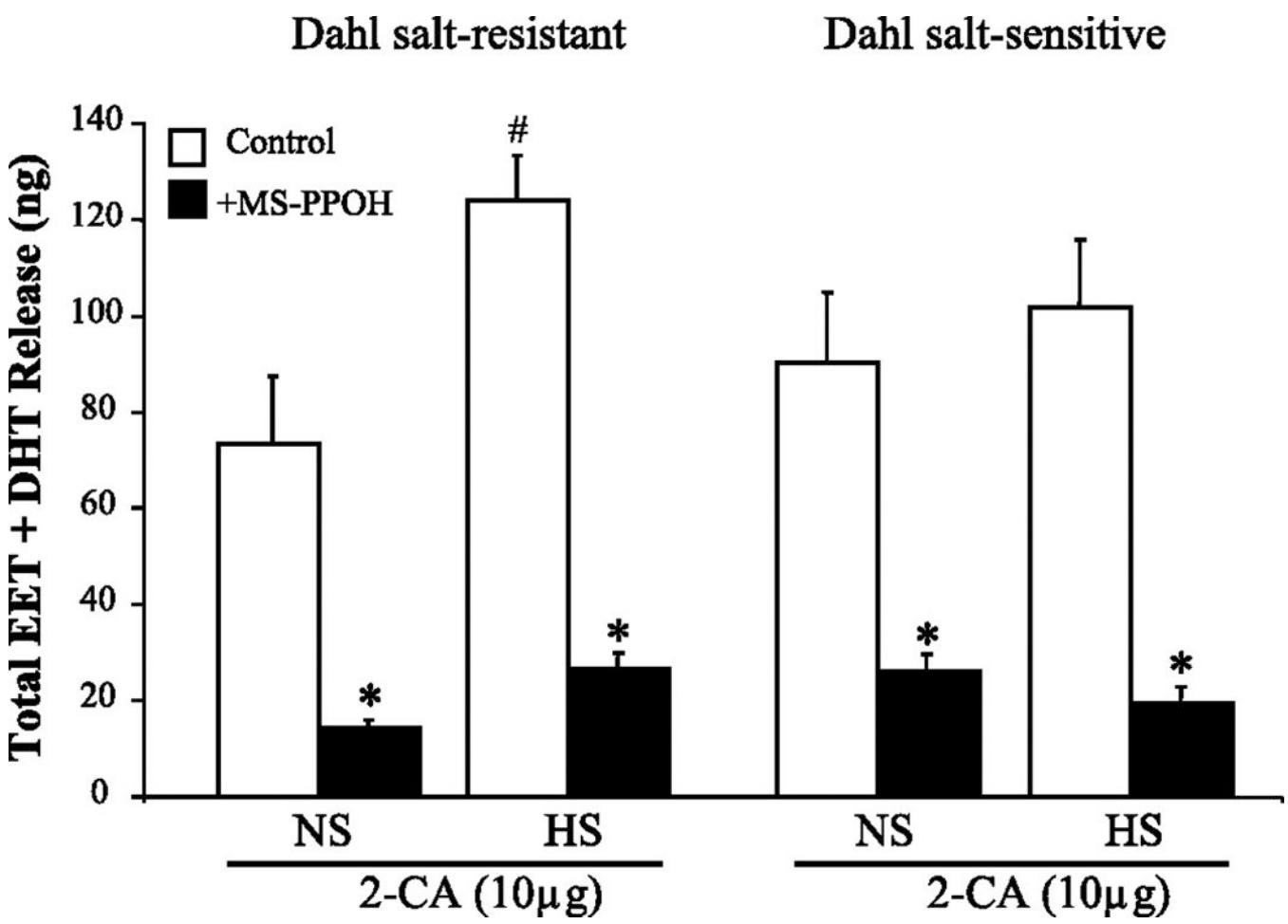

Figure 5. Renal release of cytochrome *P*-450 (CYP) epoxygenase metabolites in response to 2-CA in Dahl SR and SS rats

Data are means ± SE; $n = 4–5$. *$P < 0.05$ vs. control (i.e., HS vs. HS + MS-PPOH). #$P <$ 0.05 NS vs. HS.

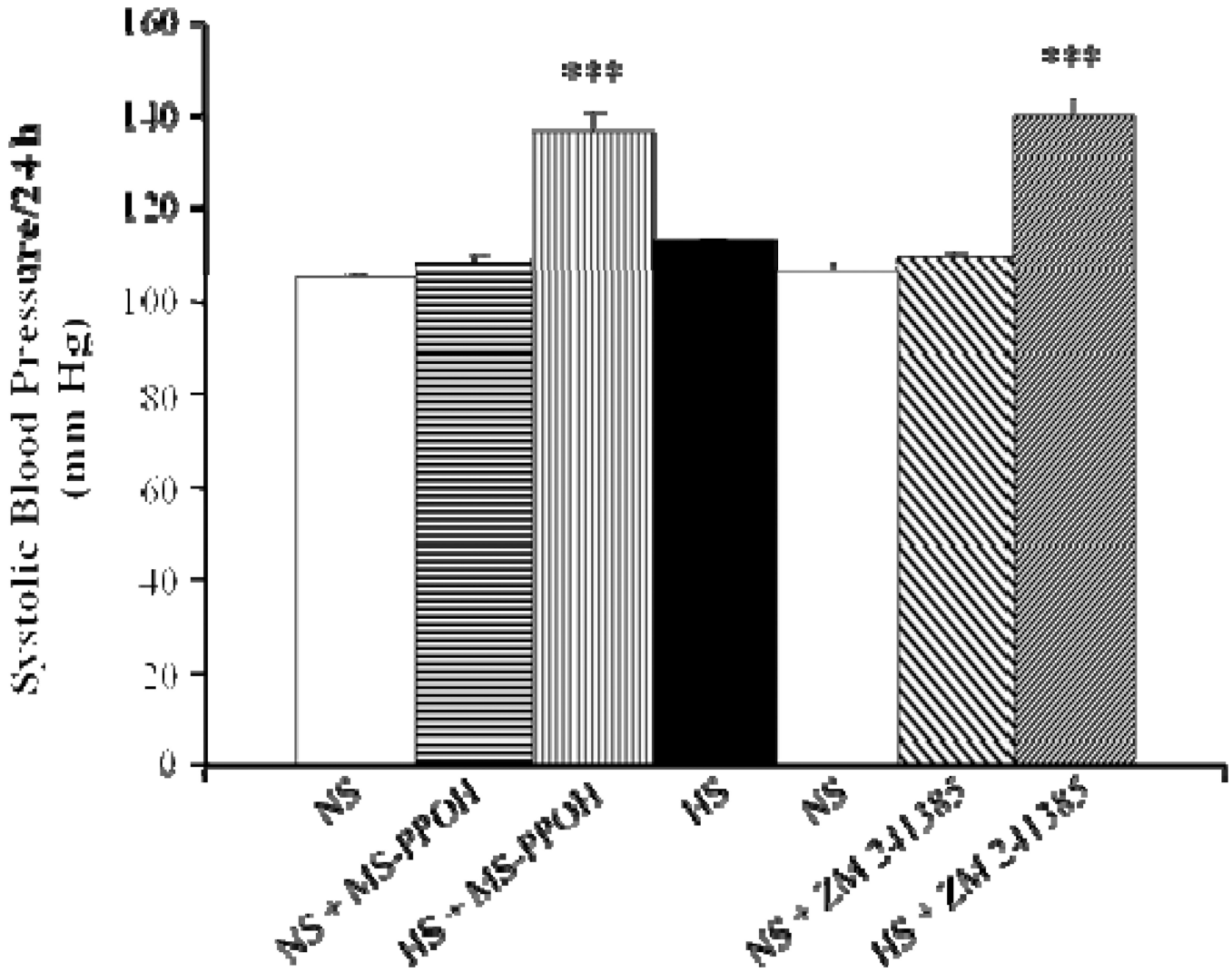

Figure 6. Effect of epoxygenase inhibition or A_{2A} R antagonism on systolic BP of Dahl SR rats after 3 days treatment

Dahl SR rats on NS diet were pretreated with the epoxygenase inhibitor, MS-PPOH (20 mg/Kg/day), for 3 days prior to switching rats to a HS (2% saline drinking water) intake. After 3 days of HS intake with MS-PPOH treatment, MS-PPOH was withdrawn while rats remained on HS intake for 3 more days. Rats were then switched to NS diet for 7 days. The selective A_{2A} R antagonist, ZM 241385 (5 mg/Kg/day), was then given for 3 days prior to switching rats back to HS intake. ZM 241385 treatment continued during 3 days of HS intake. Data are expressed as means ± SEM; n = 6–7; *** $p<0.001$ vs. NS.

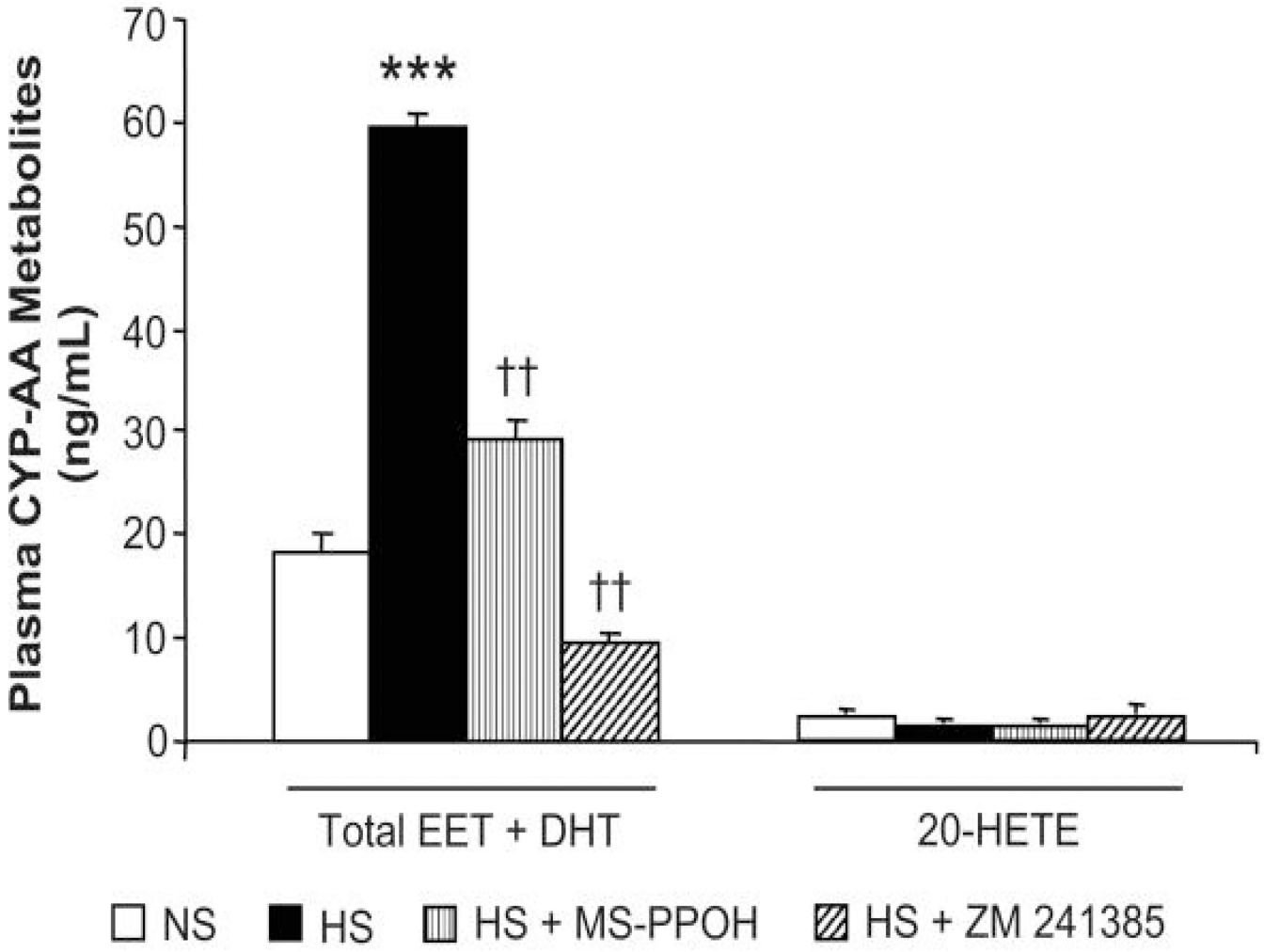

Figure 7. Effect of epoxygenase inhibition or A_{2A} R antagonism on plasma levels of CYP-AA metabolites in Dahl SR rats

Plasma levels of CYP-AA metabolites were measured rats during NS (0.4% NaCl) diet, and after 3 days of HS (2% saline drinking water) intake or 3 days of HS intake with either epoxygenase inhibition (HS + MS-PPOH; 20 mg/Kg/day) or A_{2A} R antagonism (HS + ZM 241385; 5 mg/Kg/day). Data are expressed as means ± SEM; n = 4–6; *** $p<0.001$ vs. NS, †† $p<0.001$ vs. HS.