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Review

Purinoceptors in renal microvessels: adenosine-activated and cytochrome P450 monooxygenase-derived arachidonate metabolites

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Abstract:
Cytochrome P450 (CYP)-dependent epoxyeicosatrienoic acids (EETs) dilate rat preglomerular microvessels (PGMVs) when adenosine 2A receptors ($A_{2A}R$) are stimulated. As high salt intake increases epoxygenase activity and adenosine levels, we hypothesized that renal adenosine responses would be greater in high salt-fed rats. We have obtained evidence supporting this hypothesis in rats fed a high salt diet for 7 days. Stimulation of adenosine receptors with 2-chloroadenosine in kidneys obtained from rats on high salt (4%) intake produced an increase in EET release that was several-fold greater than in kidneys of rats on normal salt (0.4% NaCl) diets, which was associated with a sharp decline in renovascular resistance. Under conditions of high salt intake, an associated upregulation of $A_{2A}R$ and 2C23 protein expression was observed. As EETs are renal vasodilator and natriuretic eicosanoids, the antipressor response to salt loading may operate through an $A_{2A}R$ – EET mechanism. These findings expand the role of adenosine-related mechanisms in protecting renal function.

Key words:
kidney, EETs, adenosine 2A receptors, salt

Abbreviations: AA – arachidonic acid, $A_{2A}R$ – adenosine 2A receptors, 2-CA – 2-chloroadenosine, CYP – cytochrome P450, DR – Dahl salt-resistant rat, DS – Dahl salt-sensitive rat, EETs – epoxyeicosatrienoic acids, NO – nitric oxide, P – purinoceptors, PGMVs – preglomerular microvessels, 20-HETE – 20-hydroxyeicosatetraenoic acid

Purinoceptors (P) are classified as either P_1 or P_2 according to their endogenous agonists: ATP (P_2) and its hydrolysis product, adenosine (P_1). They are found in the renal microcirculation in relative abundance [6, 12]. Purinoceptors in preglomerular microvessels (PGMVs) are linked to activation of cytochrome P450 monooxygenase-derived arachidonic acid (CYP-AA) metabolism: 1) ω-hydroxylase is coupled to the P_{2X} receptor with production of 20-hydroxyeicosatetraenoic acid (20-HETE) [16]; and 2) epoxygenases (mainly the 2C23 isoform) is coupled to the adenosine 2A receptor ($A_{2A}R$) with production of epoxyeicosatrienoic acids (EETs) [2]. A dynamic and antagonistic interaction between EETs and 20-HETE has been identified in PGMVs and is evident in their independent and opposing actions on autoregulation of renal blood flow [5]. Thus, the constrictor response of PGMVs to elevation of blood pressure (the signature of renal autoregulation), which maintains renal blood flow at a constant level, can be abolished by inhibiting

20-HETE production by ω-hydroxylase. The opposing renal vasodilator action of an EET to 20-HETE-mediated constriction of PGMVs was uncovered by inhibiting epoxygenases, which increased the vasoconstrictor response to 20-HETE [5].

P_1 receptors have been divided into four subtypes, of which the A_1 and A_{2A} subtypes present in the renal microcirculation exhibit opposing actions on microvascular reactivity: vasoconstriction in response to A_1 activation is related to inhibition of adenylate cyclase whereas A_{2A} activation, producing vasodilatation, is related to stimulating adenylate cyclase [11, 12]. Adenosine, acting through P_1 receptors, has been implicated in the renal functional responses to potentially catastrophic events; viz., hypoxia, ischemia and hemorrhage are associated with renal production of adenosine [14]. Recent studies also support a role for the $A_{2A}R$ as an essential component in renal mechanisms that respond to non-pathological challenges in addition to deleterious challenges to renal function. For example, activation of the $A_{2A}R$ epoxygenase pathway in response to salt loading, greatly enlarges the scope of this pathway; namely, the assignment of an antihypertensive function to adenosine via release of EETs.

Adenosine dilates rat PGMVs by releasing EETs *via* $A_{2A}R$ stimulation

Linkage of $A_{2A}R$ activation to EET production is in accord with the salutary/beneficial properties of both adenosine and EETs serving as endogenous anti-inflammatory agents [13]. Elimination of the antipressor action of EETs can explain the marked elevation of mean arterial pressure produced by deletion of the $A_{2A}R$ in knockout mice ($A_{2A}R$–/–) [8]. Further, as wild type mice ($A_{2A}R$+/+) respond to an $A_{2A}R$ agonist by a 30 mmHg drop in blood pressure, it is probable that endogenous adenosine acting through the $A_{2A}R$ exerts an EET-dependent antipressor action. Hypertension has also been produced by blockade of adenosine receptors [3] as well as by disrupting the $A_{2A}R$ gene [8]. These findings invite the development of novel approaches to the treatment of kidney disease and hypertension in terms of activation and augmentation of the $A_{2A}R$-epoxygenase system when the functional integrity of the kidney is challenged with elevation of blood pressure.

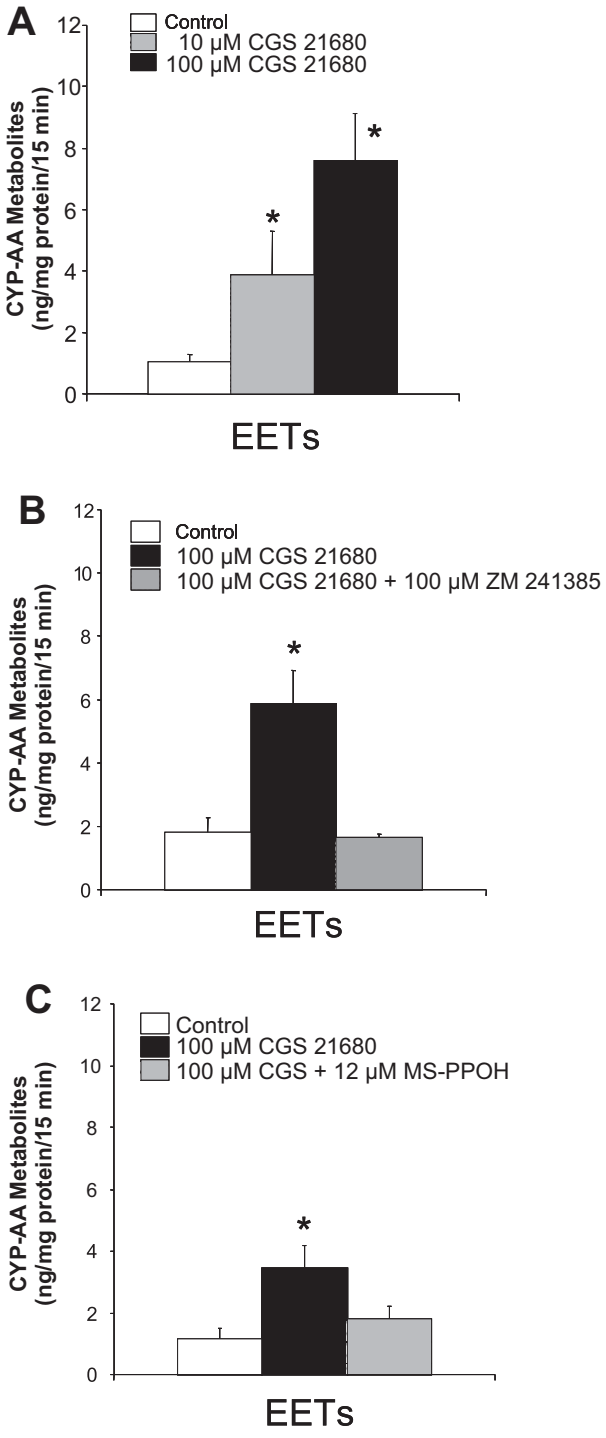

Fig. 1. Effect of A_{2A} R agonist on CYP-derived AA metabolites of PGMV. (**A**) The A_{2A} R agonist, CGS 21680, significantly increased EET levels of PGMV in a concentration-dependent manner, at 10 μM and 100 μM (*p < 0.05 *vs.* control; n = 6). CGS 21680 did not alter 20-HETE levels. (**B**, **C**) The stimulatory effect of CGS 21680 (100 μM) on EET levels of PGMV was abolished by a selective A_{2A} R antagonist, ZM 241385 (100 μM; * p < 0.05 *vs.* control; n = 6) and by an epoxygenase inhibitor, MS-PPOH (12 μM; * p < 0.05 *vs.* control; n = 4). Neither ZM 241385 nor MS-PPOH altered the levels of 20-HETE

Our most recent study [2] on vascular mechanisms served by EETs has uncovered a close relationship between stimulation of the $A_{2A}R$ and increasing epoxygenase activity (Fig. 1); 11,12-EET was identified as the likely candidate mediator of PGMV dilatation on activation of the $A_{2A}R$ by virtue of potency and activity independent of both nitric oxide (NO) and prostaglandins (Fig. 2). The specificity of this response was supported by failure of an A_1 receptor agonist to stimulate renal vascular EET formation in contrast to the concentration-dependent response of EET release to the $A_{2A}R$ agonist, CGS 21680. EET levels in PGMVs increased from basal levels of 1.06 to 3.86 and 7.57 ng mg^{-1} protein per 15 min in response to 10 and 100 μM CGS 21680. Antagonism of the $A_{2A}R$ as well as inhibition of epoxygenase activity prevented elevation of EET levels produced by CGS 21680. These findings are important for understanding adenosine acting as a "retaliatory metabolite", i.e. adenosine formation surges in response to injurious stimuli in order to limit tissue damage as, for example, ischemic injury to the kidney [14]. We submit that it is production of EETs by PGMVs in response to high adenosine levels that protects renal function; i.e. EETs mediate the protective effect of adenosine.

EETs are antihypertensive

The essential contribution of EETs to blood pressure regulation was established by Capdevila and colleagues in a landmark study: "a salt-inducible renal epoxygenase protects against hypertension" [1]. In rats maintained on a high salt diet, which by itself did not increase blood pressure, inhibition of epoxygenases with clotrimazole produced elevation of blood pressure; i.e. rendered the rat salt-sensitive [10]. The blood pressure of age-matched rats receiving standard laboratory Chow (0.4% sodium) was unaffected by epoxygenase inhibition with clotrimazole. CYP-2C23 has been identified in the rat kidney as: 1) the major 2C arachidonate epoxygenase and 2) the isoform of the 2C family that is subject to regulation by dietary salt [4]. Salt sensitivity, as defined by blood pressure elevation in response to a dietary salt load, therefore, can be reproduced by either gene deletion or inhibition of 2C23 epoxygenase activity.

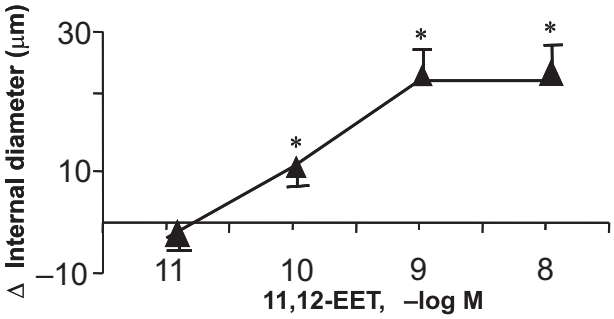

Fig. 2. Dilator responses to 11, 12-EET in rat pressurized arcuate arteries * $p < 0.05$ compared to baseline

Additional evidence supporting the decisive role of one or more EETs in moderating the pressor effect of salt loading was provided by demonstrating that the Dahl salt-sensitive rat (DS), a "model of genetic salt-dependent hypertension", was deficient in EETs [7]. DS rats responded to salt loading by an increase in blood pressure and failure to increase production of EETs. The Dahl salt-resistant (DR) rats, on the other hand, increased excretion of EETs in response to salt loading associated with maintenance of blood pressure at normotensive levels. These separate lines of evidence obtained from a rat model of genetic salt-dependent hypertension (DS) and hypertension induced by epoxygenase inhibition converge on a common antipressor mechanism, one mediated by renal CYP-derived EETs. These studies greatly enlarge the sphere of activity of renal EETs. Further, EETs possess antiinflammatory, thrombolytic and antiproliferative actions [13] that confer on EETs pleiotropic properties that enable them to serve in a variety of settings to protect/sustain renal function and to offset both pressor and other injurious challenges (hypoxic, immunologic, and inflammatory) to both the renal and systemic circulations.

At this juncture, having reviewed the support for an antipressor function for epoxides (EETs), it is appropriate to consider the potential role of an adenosine-dependent mechanism operating through stimulation of the $A_{2A}R$ that evokes EET production/release in response to salt loading. The demonstration of hypertension in mice lacking the $A_{2A}R$ [8] and the finding that renal adenosine receptors are affected by Na^+ intake [15], occasioned our testing the hypothesis that salt loading evokes a purinoceptor mechanism activated by adenosine with stimulation of the $A_{2A}R$ and release of EETs. We have obtained evidence support-

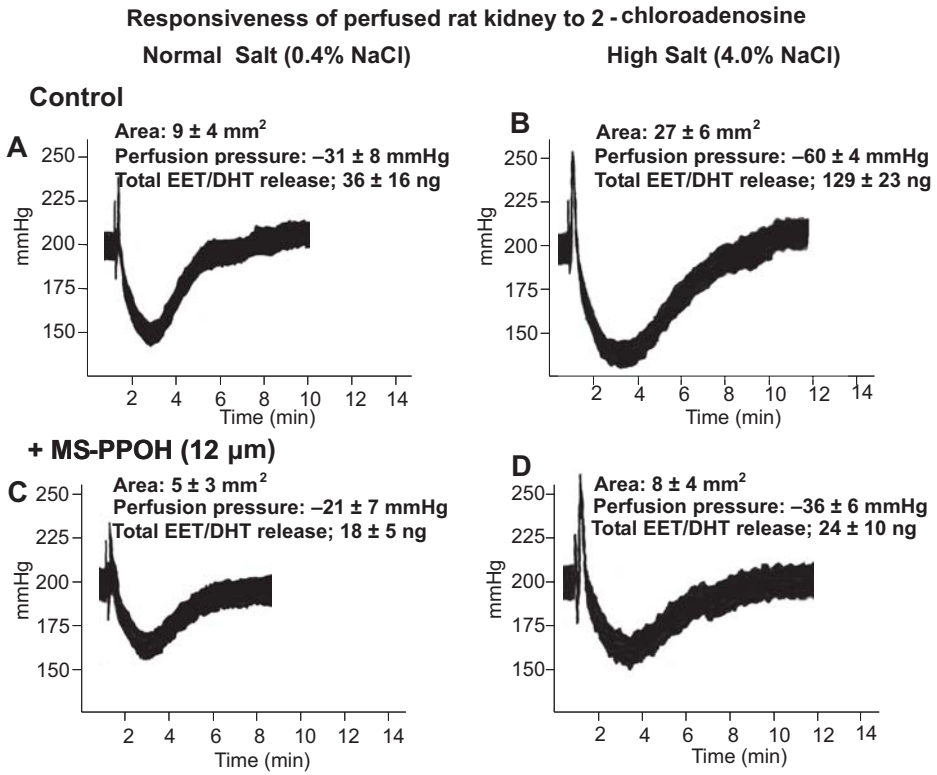

Fig. 3. Effect of salt loading (4.0% NaCl) on responses to 2-chloroadenosine in rat isolated, Krebs' perfused kidneys. Rats were fed a high salt intake for 7 days and the right kidney was isolated and perfused at 7 ml/min in the presence of phenylephrine, indomethacin and L-NAME. Perfusate was collected for measurement of EET/DHTs

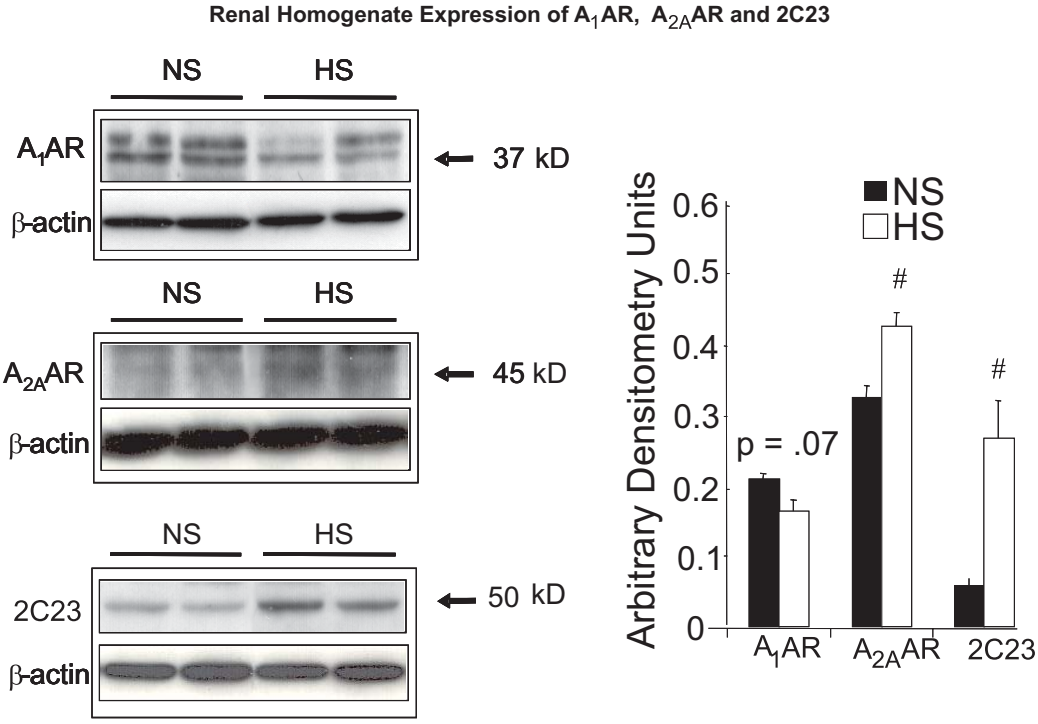

Fig. 4. Western blots of adenosine receptors and 2C23 expression under normal (NS) and high (HS) salt intake # $p < 0.05$

ing this hypothesis in rats fed a high salt diet for 7 days; that is, the antipressor response to salt loading operates through an $A_{2A}R$ – EET mechanism [9]. Kidneys were isolated and perfused with oxygenated Krebs' buffer at an elevated pressure of 200 mmHg (phenylephrine-induced). Stimulation of adenosine receptors with 2-chloroadenosine (2-CA) in kidneys obtained from rats on high salt (4%) intake produced an increase in EET release that was several-fold greater than in kidneys of rats on normal salt (0.4% NaCl) diets, which was associated with a sharp decline in renovascular resistance (Fig. 3). MS-PPOH, a selective epoxygenase inhibitor, diminished the renal efflux of EETs by 70% and blocked the enhanced vasodilator response produced by stimulating adenosine receptors. Under conditions of high salt intake, an associated upregulation of $A_{2A}R$ and 2C23 protein expression was observed (Fig. 4). These findings expand the role of adenosine-related mechanisms in protecting renal function [9].

Acknowledgments:
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