

# Physiological Research Pre-Press Article

## **Purinoceptors, Renal Microvascular Function and Hypertension**

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**Short title:** Renal Autoregulation and Hypertension

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## Summary

Proper renal blood flow (RBF) and glomerular filtration rate (GFR) are critical for maintaining normal blood pressure, kidney function and water and electrolyte homeostasis. The renal microvasculature expresses a multitude of receptors mediating vasodilation and vasoconstriction, which can influence glomerular blood flow and capillary pressure. Despite this, RBF and GFR remain quite stable when arterial pressure fluctuates because of the autoregulatory mechanism. ATP and adenosine participate in autoregulatory control of RBF and GFR via activation of two different purinoceptor families (P1 and P2). Purinoceptors are widely expressed in renal microvascular and tubules. Emerging data show altered purinoceptor signaling in hypertension-associated kidney injury, diabetic nephropathy, sepsis and ischemia-reperfusion induced acute kidney injury and polycystic kidney disease. In this brief review, we highlight recent studies and new insights on purinoceptors regulating renal microvascular function and renal hemodynamics. We also address the mechanisms underlying renal microvascular injury and impaired renal autoregulation, focusing on purinoceptor signaling and hypertension-induced renal microvascular dysfunction. Interested readers are directed to several excellent and comprehensive reviews that recently covered the topics of renal autoregulation, and nucleotides in kidney function under physiological and pathophysiological conditions (Inscho 2009, Navar *et al.* 2008, Carlstrom *et al.* 2015, Vallon *et al.* 2020).

**Keywords:** ATP, renal autoregulation, inflammation, epithelial sodium channel, reactive oxygen species.

## Introduction

Kidneys constitute less than 1% of the body weight of healthy adults but receive a blood flow representing approximately 20% of cardiac output. This high renal blood flow (RBF) per unit organ weight emphasizes the key role for the renal microcirculation in regulating appropriate body fluid volume and composition and maintaining normal blood pressure (BP) (Hall 2015). The renal microcirculation represents a unique portal circulation with the glomerular tuft fed and drained by two resistance arterioles, the afferent and efferent arterioles. Resistance of these arterioles is regulated by neural, hormonal and autocrine/paracrine factors which are critical for stabilizing RBF and glomerular filtration rate (GFR) (Navar *et al.* 2008, Carlstrom *et al.* 2015).

Under normal conditions, glomerular hydrostatic pressure is maintained relatively constant via the mechanism of renal autoregulation. Renal autoregulation utilizes two primary mechanisms: a local myogenic response and tubuloglomerular feedback (TGF) (Inscho 2009, Navar *et al.* 2008, Carlstrom *et al.* 2015, Vallon *et al.* 2020). Both mechanisms influence afferent arteriole resistance to buffer fluctuations in arterial pressure to protect downstream glomeruli, thereby maintaining stable RBF and GFR. Studies also suggest involvement of a third and/or fourth mechanism to effect fine control of glomerular capillary pressure, but those “mechanisms” have not been defined (Just and Arendshorst 2003, Wang *et al.* 2007, Ren *et al.* 2007). For example, Just and Arendshorst reported that there was a slower rise of RVR following the myogenic response while TGF was inhibited with furosemide (Just and Arendshorst 2003). The authors postulated that this slow autoregulatory component, the third mechanism, is TGF-independent, but the nature of this extra component remains unclear. The fourth mechanism is referred to as the connecting tubule-glomerular feedback (CTGF) mechanism (Ren *et al.* 2007). Unlike the negative loop of the TGF response, the CTGF is a positive feedback mechanism by which increasing NaCl concentration in the connecting tubule dilates the attached afferent arteriole (Ren *et al.* 2007). Evidence indicates autoregulatory effectiveness declines in the early stages of human hypertension (Christensen *et al.* 1997, 1999, Schjoedt *et al.* 2009) as well as hypertensive animal models

(Inscho *et al.* 2004b, Feng *et al.* 2020). The impaired autoregulatory capacity increases the risk to hypertensive patients for developing chronic kidney disease (CKD).

### ***In vivo* and *in vitro* techniques used for renal autoregulatory studies**

Although the importance of renal autoregulation in kidney function is recognized, technical limitations make it difficult to assess autoregulation in human subjects. Only a few studies have measured GFR during manipulation of mean arterial pressure in hypertensive subjects (Christensen *et al.* 1997, 2003, Schjoedt *et al.* 2009). Therefore, most information on renal autoregulation is obtained using animal models. Several *in vivo* and *in vitro* techniques are used for assessing renal autoregulatory efficiency. At the whole kidney level, autoregulation of RBF and GFR is usually assessed in response to stepwise-decreases of renal perfusion pressure in anesthetized dogs, rabbits or rats (Navar 1978, Majid *et al.* 1999, Osmond and Inscho 2010, Fellner *et al.* 2015). Indirect evidence can be obtained using fiber-optic or needle probes to determine relative regional RBF measured by dual-channel laser Doppler flowmetry (Roman and Smits 1986). Time/frequency analysis is used to assess myogenic and TGF components in anesthetized animals (Just and Arendshorst 2003). The myogenic response operates at a frequency of 0.1–0.2 Hz and occurs at approximately 5 seconds after a rapid increase in arterial pressure while TGF operates at a frequency of 0.02–0.04 Hz and appears at approximately 25 seconds after a rapid increase in arterial pressure (Just and Arendshorst 2003). The hydronephrotic kidney is created by ligating the ureter for 6-8 weeks to eliminate TGF, thus isolating myogenic responses (Hayashi *et al.* 1989). Micropuncture, on the other hand, is useful for assessing TGF in anesthetized rats or mice by measuring proximal tubule stop-flow pressure ( $P_{SF}$ ; index of glomerular capillary pressure) during manipulation of tubular fluid composition or flow rate to the macula densa (Schnermann *et al.* 1970, Vallon *et al.* 2020). Several novel *in vitro* techniques have been developed to avoid the influences of circulating factors or sympathetic

nerve activity (Bencze *et al.* 2013) or to provide unique access to renal structures. For example, the isolated afferent arteriole with attached macula densa and distal tubule segment is used for investigating TGF signaling (Juncos *et al.* 1996, Bell *et al.* 2003, Peti-Peterdi 2006). Microdissected afferent arterioles are used to study myogenic reactivity (Lai *et al.* 2010, 2011). Since development of the *in vitro* blood-perfused juxtaglomerular nephron (JMN) technique (Casellas and Navar 1984), we and others applied this approach to study renal autoregulation and renal microvascular reactivity in a variety of rat and mouse models (Navar 1978, Inscho *et al.* 1990, 2004a, Carmines *et al.* 1992, Takenaka *et al.* 1994, Guan *et al.* 2009, Sorensen *et al.* 2012, Nagasawa and Imig 2013). With this technique, the kidney is carefully dissected to explore the inner cortical surface and the kidney is perfused with re-constituted blood to approximate *in vivo* conditions. Notably, afferent arterioles retain endogenous tone and myogenic and TGF responsiveness (Casellas *et al.* 1985, Moore and Casellas 1990, Takenaka *et al.* 1994, Guan *et al.* 2009). More recently, the Bidani group developed a new analytical method to assess renal autoregulation by concurrently collecting RBF data during BP fluctuations in conscious rats (Bidani *et al.* 2020).

## **Mechanisms of Renal Autoregulation**

### **Myogenic response**

Bayliss described the myogenic response more than 100 years ago (Bayliss 1902) and noted vasoconstriction or vasodilation in response to increases or decreases in transmural pressure, guarding against traumatic tissue injury from sudden arterial pressure changes. Myogenic responsiveness is inherent to vascular smooth muscle and independent of endothelium (Hill *et al.* 2006). Despite intensive research, the identity, or composition, of the mechanosensor that triggers myogenic responses is unclear, though there are several candidate structures, including epithelial sodium channel (ENaC)-like proteins, transient receptor potential ion

channels, and integrins. For more details interested readers can refer to excellent reviews (Hill *et al.* 2006, Drummond *et al.* 2008).

### ***Role of epithelial sodium channel (ENaC)-like proteins in the myogenic response***

Studies by the Drummond group implicate ENaC or ENaC-like proteins as mechanosensors regulating myogenic tone (Drummond *et al.* 2004, Jernigan and Drummond 2005, 2006). Using mouse isolated intrarenal arteries, Jernigan and Drummond revealed that myogenic reactivity was abolished by inhibiting degenerin/epithelial Na<sup>+</sup> channels (DEG/ENaC) with amiloride or benzamil, or was blunted by suppressing  $\beta$ -ENaC subunits using transfection of  $\beta$ -ENaC DN-cDNA or siRNA molecules (Jernigan and Drummond 2005, 2006). We applied the *in vitro* blood-perfused rat JMN preparation with papillectomy to eliminate TGF influences, and also found that amiloride or benzamil attenuated pressure-dependent vasoconstriction of rat afferent arterioles (Guan *et al.* 2009). Our observation was later confirmed using the same JMN preparation without papillectomy (Nagasawa and Imig 2013). While myogenic reactivity was blunted by pharmacological ENaC blockade, vasoconstrictor responses to membrane-depolarization (KCl),  $\beta$ ,  $\gamma$ -methylene ATP (P2X1 and P2X3 agonist) or 20-HETE were unaffected (Guan *et al.* 2009, Nagasawa and Imig 2013), supporting the idea that ENaC, or ENaC-like proteins, may form a mechanosensor-like complex transducing myogenic reactivity. Studies from hydronephrotic rat kidneys however, showed opposite results. Indeed, the myogenic response was unaffected by amiloride, and actually enhanced by benzamil (Wang *et al.* 2008).

ENaC protein and mRNA expression were detected in renal microvessels and freshly isolated preglomerular microvascular smooth muscle cells (MVSMC) from mice and rats (Jernigan and Drummond 2005, 2006, Guan *et al.* 2009). Rats express all three ENaC subunits ( $\alpha$ ,  $\beta$  and  $\gamma$ -ENaC) in preglomerular MVSMC (Guan *et al.* 2009) whereas mice only express  $\beta$ -ENaC and  $\gamma$ -ENaC (Jernigan and Drummond 2005, 2006). Importantly, mice with reduced  $\beta$ -ENaC expression showed impaired renal myogenic responsiveness, increased mean arterial pressure, and early

signs of inflammatory and vascular remodeling (Grifoni *et al.* 2010, Drummond *et al.* 2011). These studies strongly project ENaC or ENaC-like proteins as part of a mechanosensor complex in myogenic reactivity and support a pivotal role for impaired renal autoregulation contributing to kidney injury.

### ***Role of reactive oxygen species (ROS) in the myogenic response***

ROS are generated by univalent reduction of oxygen via a number of enzymatic reactions including nicotinamide adenine dinucleotide phosphate (NADPH) oxidase, xanthine oxidase, cyclo-oxygenase, lipoxygenase, mitochondrial electron transport or un-coupled nitric oxide synthase (NOS). ROS, mainly superoxide and hydrogen peroxide ( $H_2O_2$ ), were considered damaging molecules produced only during cellular injury, however, growing evidence suggests that ROS may also participate in normal physiological processes and contribute to renal autoregulation (Schnackenberg 2002, Carlstrom *et al.* 2015). Early studies using isolated-perfused mouse afferent arterioles showed blunted pressure-induced afferent arteriole vasoconstriction in the presence of ROS scavengers like Tempol or superoxide dismutase (SOD)-polyethylene glycol (PEG-SOD) (Lai *et al.* 2010, Lai *et al.* 2011). Interestingly, myogenic responsiveness was unaffected by PEG-catalase (Lai *et al.* 2011), suggesting that the myogenic contraction is enhanced by superoxide but not  $H_2O_2$ . Afferent arterioles exhibit NADPH oxidase (Wilcox 2003, Sharma *et al.* 2005) and xanthine oxidase activity, but xanthine oxidase activity is lower (Zou *et al.* 2001). Deletion of the NADPH oxidase subunit, p47<sup>phox</sup>, blunted myogenic reactivity, whereas it was not affected by knockout of endothelial NOS (eNOS) (Lai *et al.* 2012). This suggests that NADPH oxidase-dependent superoxide production modulates myogenic responses of afferent arterioles independent of eNOS.

### **Tubuloglomerular feedback (TGF)**



TGF is a unique renal mechanism where tubular and vascular functions are integrated in stabilizing RBF and GFR. TGF arises from the macula densa, a special plaque of cells in the distal convoluted tubule that senses changes in tubular fluid NaCl concentration and transmits signals to adjust afferent arteriole resistance (Schnermann 2015, Vallon *et al.* 2020). Although the messenger molecule that signals TGF-resistance adjustments remains uncertain, Bell and Peti-Peterdi provided convincing evidence that increases in tubular NaCl delivery to the macula densa cells triggers ATP release from basolateral membrane (Bell *et al.* 2003, 2009, Peti-Peterdi 2006). The released ATP either acts directly on afferent arterioles via P2X1 receptors (Bell *et al.* 2003, Inscho *et al.* 2003, 2004a, Inscho 2009) or is catabolized to adenosine which activates A<sub>1</sub> receptors to increase afferent arteriolar resistance (Just and Arendshorst 2007, Schnermann 2015, Vallon *et al.* 2020).

### **Purinoceptors and Renal Autoregulation**

Despite extensive research, identification of the signaling molecules responsible for renal autoregulatory control remains uncertain. Since the early 1980's, evidence implicates either adenosine, ATP or both as effectors of renal autoregulatory resistance adjustments via purinoceptor activation (Inscho 2009, Bell *et al.* 2009, Guan *et al.* 2014, Schnermann 2015, Vallon *et al.* 2020). Extracellular ATP and adenosine influence cellular function by interacting with two distinct purinoceptor families: P1 and P2 receptors (Burnstock *et al.* 2014, North 2016, von Kugelgen 2019). The P1 family includes four subtypes: A<sub>1</sub>, A<sub>2a</sub>, A<sub>2b</sub> and A<sub>3</sub> (Fredholm *et al.* 2000, Klotz 2000). A<sub>1</sub> and A<sub>3</sub> receptors are linked to G<sub>o</sub>/G<sub>i</sub> protein and inhibit adenylyl cyclase, hence decreasing cyclic AMP (cAMP, Figure 1) (Gerwins and Fredholm 1992). Conversely, A<sub>2a</sub> and A<sub>2b</sub> receptors are linked to G<sub>s</sub> protein and stimulate adenylyl cyclase activity to increase cAMP (Tucker and Linden 1993, Olah 1997). The P2 family is divided into two separate subfamilies: P2X and P2Y (Burnstock *et al.* 2014, von Kugelgen 2019). P2Y receptors include eight G protein-coupled receptors (P2Y<sub>1, 2, 4, 6, 11-14</sub>) whereas seven distinct P2X receptors function as non-

selective ion-channels (P2X1-7) (Burnstock *et al.* 2014, North 2016, von Kugelgen 2019). ATP is an endogenous ligand of P2 receptors and is rapidly hydrolyzed *in vivo* into ADP and AMP by widely expressed extracellular nucleotidases (Burnstock *et al.* 2014, North 2016). AMP is further catabolized by ecto-5'-nucleotidase (ecto-5'-NT) or CD73 (cluster of differentiation) to adenosine which acts on P1 receptors. Activation of membrane-bound P1 and P2 receptors by nucleosides and nucleotides is illustrated in Figure 1.

### **Purinoceptor expression in the renal microvasculature**

Both P1 and P2 receptors are widely expressed in the renal microvasculature and in different tubular segments (Schwiebert 2001, Burnstock *et al.* 2014, Menzies *et al.* 2015b). For the purpose of this review, we mainly focus on renal microvascular purinoceptor expression and their role in autoregulation. More details regarding P1 and P2 receptor expression in glomeruli and tubules can be found in several comprehensive reviews (Burnstock *et al.* 2014, Menzies *et al.* 2015b, Vallon *et al.* 2020). To date, Western blot, RT-PCR and immunohistochemistry studies demonstrated P2X1, P2X2, P2X4, P2X7, P2Y<sub>1</sub> expression and all four P1 receptor subtypes (A<sub>1</sub>, A<sub>2a</sub>, A<sub>2b</sub> and A<sub>3</sub>) by rat and mouse renal vasculature (Chan *et al.* 1998, Lewis and Evans 2001, Turner *et al.* 2003, Menzies *et al.* 2013, Lu *et al.* 2015). mRNA for A<sub>1</sub>, A<sub>2a</sub>, and A<sub>2b</sub> receptors was also present in rat outer medullary descending vasa recta (Kreisberg *et al.* 1997). Immunohistochemistry studies revealed that P2X1 receptors are heavily expressed in MVSMC along the preglomerular vasculature including arcuate, interlobular arteries, and afferent arterioles, but not efferent arterioles (Chan *et al.* 1998, Turner *et al.* 2003, Menzies *et al.* 2013). Lewis and Evans showed positive immunoreactivity for P2X1 and P2X7 in small and medium rat renal arteries, but negative staining for P2X2, P2X3 and P2X6 receptors (Lewis and Evans 2001). Later, Menzies *et al.* reported immune-positive staining for endothelial P2X4 and P2X7 receptors in rat preglomerular microvessels (Menzies *et al.* 2013). RT-PCR analysis demonstrated

expression of genes encoding for P2X1 and P2X4 receptors, but not for P2X2, P2X3, P2X5 and P2X7 in myocytes isolated from the rat renal vasculature (Harhun *et al.* 2010).

### **ATP and/or adenosine in mediating autoregulatory signaling**

Unlike the ATP-mediated vasorelaxation seen in other non-renal vascular beds, ATP vasoconstricts preglomerular microvessels via activation of both P2X and P2Y receptors (Inscho *et al.* 2003, 2004a, 2011, Franco *et al.* 2011, Guan *et al.* 2013, 2016, Gordienko *et al.* 2015). Using microdialysis in canine kidneys, Nishiyama *et al.* demonstrated a strong positive correlation between renal perfusion pressure and renal interstitial ATP levels but adenosine did not exhibit a similar correlation (Nishiyama *et al.* 2000, 2001b, 2006). Later, several studies by Bell and Peti-Peterdi using a single glomerulus with attached macula densa and ATP-sensitive biosensor cells provided convincing evidence that ATP was released directly from the basolateral membrane of macula densa cells in response to an increase in luminal NaCl concentration (Bell *et al.* 2003, 2009, Peti-Peterdi 2006). More recently Palygin and colleagues applied amperometric ATP sensitive microelectrodes embedded in kidneys to monitor ATP levels in real-time during manipulation of renal perfusion pressure in isolated-perfused rat kidneys or anesthetized rats (Palygin *et al.* 2015, 2017). They observed an immediate increase of cortical tissue ATP levels when perfusion pressure was increased, consistent with Nishiyama's observations in canine kidneys. Overall, these observations demonstrate that ATP is released in the kidney in response to increases in perfusion pressure, and support the hypothesis that ATP is a mediator of renal autoregulation.

Involvement of P2X1 receptors in renal autoregulation is further supported by pharmacological and/or genetic intervention studies (Inscho *et al.* 1996, 2003, 2004a). The earliest work by Inscho *et al.* using the blood-perfused rat or mouse JMN preparation showed that application of ATP, or the specific agonist for P2X1 and P2X3,  $\beta$ ,  $\gamma$ -methylene ATP, caused rapid and sustained

vasoconstriction of afferent arterioles in a concentration-dependent manner but had no detectable impact on efferent arteriole diameter (Inscho *et al.* 1991, 1992). Pressure-dependent vasoconstriction of afferent arterioles was blunted by application of the P2X1 antagonist, NF279 or by desensitization of P2X1 receptors by repeated superfusion of ATP (Inscho *et al.* 1996, 2003). Moreover, genetic deletion of P2X1 receptors attenuated pressure-dependent vasoconstriction of afferent arterioles in mice and this inhibition was unaltered by papillotomy or furosemide (Inscho *et al.* 2003), suggesting that P2X1 receptors participate in both myogenic and TGF responses. Furthermore, P2X1 receptor blockade with the non-selective antagonist (PPADS; pyridoxal phosphate-6-azo-benzene-2, 4-disulfonic acid) or a selective antagonist (IP5I; P1, P5-Di-inosine-5'-pentaphosphate pentasodium salt) also blunted whole kidney RBF autoregulation *in vivo* (Osmond and Inscho 2010). Taken together, activation of P2X1 receptors appears essential for autoregulatory responsiveness.

There is compelling evidence supporting adenosine as the mediator of TGF responses (Schnermann *et al.* 1990, Osswald *et al.* 1997, Ren *et al.* 2002, Thomson *et al.* 2000, Hashimoto *et al.* 2006, Li *et al.* 2012, Kim *et al.* 2015). Adenosine is an endogenous ligand for P1 receptors. Studies show that application of exogenous adenosine causes biphasic vascular responses at the whole kidney level or isolated arteriole level (Nishiyama *et al.* 2001a, Hansen *et al.* 2005, Lai *et al.* 2006, Lu *et al.* 2015). Adenosine vasoconstricts afferent arterioles at low concentrations but evokes vasodilation at higher concentrations (Nishiyama *et al.* 2001a, Hansen *et al.* 2005, Lai *et al.* 2006). This probably reflects activation of different renal microvascular adenosine receptor subtypes. For example, adenosine-mediated vasoconstriction was blunted by A<sub>1</sub> receptor inhibition or A<sub>1</sub> receptor gene deletion but was enhanced during A<sub>2</sub> receptor blockade (Nishiyama *et al.* 2001a, Hansen *et al.* 2005, Lai *et al.* 2006). Moreover, activation of A<sub>1</sub> receptors with the selective A<sub>1</sub> receptor agonist, N<sup>6</sup>-cyclohexyladenosine, reduced afferent arteriole diameter while A<sub>2</sub> receptor activation with CGS21680, increased afferent arteriole diameter, supporting

involvement of A<sub>1</sub> and A<sub>2</sub> receptors in regulating afferent arteriole reactivity. Unlike A<sub>1</sub> and A<sub>2</sub> receptors, activation of A<sub>3</sub> receptors had no significant effect on isolated mouse afferent arteriole diameter but dilated the afferent arterioles after precontraction with norepinephrine (Lu *et al.* 2015). Currently the role of A<sub>3</sub> receptors in renal microvascular function is unclear, but recent studies suggest that A<sub>3</sub> receptor inhibition protects against renal ischemia-reperfusion or ureteral obstruction-induced kidney injury (Lee and Emala 2000, Lee *et al.* 2013).

Although mRNA expression for the four adenosine receptor subtypes is detected in afferent arterioles, animal studies argue that only A<sub>1</sub> receptors are involved in the adenosine-mediated TGF response. Alternatively, A<sub>2</sub> and A<sub>3</sub> receptors might participate in modulating TGF responses. Micropuncture studies show that mouse TGF responses, measured by changes in stop-flow pressure, were absent during A<sub>1</sub> receptor blockade with 8-cyclopentyl-1,3-dipropylxanthine or A<sub>1</sub> receptor knockdown (Sun *et al.* 2001, Hashimoto *et al.* 2006), implicating adenosine as a mediator of TGF. This is supported by findings that vasoconstrictor responses of afferent arterioles evoked by increased tubular NaCl delivery to the macula densa was attenuated by selective A<sub>1</sub> receptor blockade with FK838 (Ren *et al.* 2002). Since ecto-5'-NT which degrades AMP to adenosine (Figure 1) has been detected in the juxtaglomerular apparatus region, experiments using ectonucleotidase gene deletion or mutation attenuated TGF responses (Castrop *et al.* 2004, Huang *et al.* 2006). Moreover, autoregulation of RBF and GFR was impaired in A<sub>1</sub> receptor deficient mice (Hashimoto *et al.* 2006), suggesting that this impairment of renal autoregulation may reflect an abolished TGF mechanism. Collectively, the data support adenosine as a mediator of TGF responses via activation of A<sub>1</sub> receptors.

### **Renal Autoregulation, Inflammation and Hypertension**

Typical features of hypertension-induced nephropathy include renal microvascular injury, remodeling, glomerulosclerosis, and/or interstitial fibrosis (Sommers *et al.* 1958). As discussed

above, the renal autoregulatory mechanism prevents transmission of high arterial pressure to glomeruli. In hypertension, loss of appropriate afferent arteriole resistance adjustments leaves glomeruli at risk for elevated glomerular capillary pressure, hyperfiltration, glomerulosclerosis and eventually nephron loss.

### **Renal autoregulation in hypertensive patients**

According to the National Center for Health Statistics-2011 report (Sebelius *et al.* 2011), approximately one-third of US adults suffer from hypertension. Meta-analysis revealed a strong correlation between the incidence of hypertension and CKD. Some hypertensive patients, particularly African-Americans are more susceptible to developing CKD than others (Palmer 2004, Kotchen *et al.* 2000) though the mechanisms contributing to kidney injury are poorly understood. Clinical studies indicate that impaired renal autoregulatory capacity in early stage hypertension might contribute to higher prevalence of hypertension-related chronic vascular disease in African-American individuals than white individuals (Palmer 2004, Kotchen *et al.* 2000). Human studies also indicate that impaired autoregulatory efficiency in hypertension can occur before structural changes in the renal vasculature (Palmer 2004, Christensen *et al.* 1999). However, to date there are few studies addressing renal hemodynamic changes in this population. Kotchen *et al.* reported that baseline RBF and GFR are higher in African-Americans than white counterparts (Kotchen *et al.* 2000). GFR was further increased in African-Americans after intravenous infusion of norepinephrine despite that RBF was unchanged. This demonstrates glomerular hyperfiltration and impaired GFR autoregulation in African-Americans. Additionally, individuals with impaired autoregulation are predisposed developing nephropathy in hypertensive subjects (Christensen *et al.* 1999). That work suggests that lack of efficient autoregulatory control renders glomeruli susceptible to hyperfiltration and hypertensive kidney injury putting the host at high risk for kidney failure, particularly in African-Americans with hypertension (Palmer 2004).

## **Renal autoregulation in hypertensive animals**

Work in recent decades shows that renal autoregulatory capability is impaired in hypertensive animal models including Ang II, Goldblatt (2K-1C), deoxycorticosterone acetate (DOCA)-salt, the 5/6 renal mass reduction, fawn-hooded rats and Dahl salt-sensitive (SS) rats (Ploth *et al.* 1981, Karlsten *et al.* 1997, Griffin *et al.* 2004, Burke *et al.* 2013, Guan *et al.* 2013, 2016). Impaired autoregulation contributes to hypertensive kidney injury. The molecular pathogenesis underlying hypertension-induced renal microvascular dysfunction is unclear. The study by Sharma *et al.* showed that inflammatory factors likely contribute to impaired renal autoregulation in diabetes (Sharma *et al.* 2005). Using the blood-perfused JMN preparation, pressure-dependent afferent arteriole vasoconstriction was blunted by an acute 15 min exposure to TGF- $\beta$ , but was preserved by simultaneous treatment with ROS scavengers (Tempol or apocynin). Moreover, apocynin blocked the TGF- $\beta$ -mediated increase in superoxide production in cultured renal MVSMC, suggesting that inflammatory factors could directly affect renal autoregulatory capacity by increasing superoxide accumulation.

Elegant studies from the Cowley group (Mori and Cowley 2004, Evans *et al.* 2017) provided compelling evidence that inflammation and renal injury is largely driven by elevation of arterial pressure in Ang II-infused hypertensive rats or Dahl SS rats fed a NaCl diet. An aortic clamp was applied between the renal arteries to control the downstream kidney perfusion pressure in the normotensive range while the upstream kidney received hypertensive pressure. After 14-days of treatment, the un-controlled kidney developed severe glomerular injury, kidney fibrosis and elevated TGF- $\beta$  and NF- $\kappa$ B deposition compared to the controlled kidney. We previously demonstrated that renal autoregulation was impaired in chronic Ang II-infused hypertensive rats as indicated by blunted pressure-dependent afferent arteriole vasoconstriction and the passive pressure/flow relationship (Guan *et al.* 2010, 2013, Inscho *et al.* 2011, Osmond *et al.* 2014). Co-treatment of Ang II-infused hypertensive rats with a nonspecific anti-inflammatory agent, pentosan

polysulphate (PPS) (Guan *et al.* 2010) or a more specific anti-inflammatory agent, mycophenolate mofetil (MMF) (Guan *et al.* 2013), which suppresses lymphocyte and macrophage infiltration, preserved pressure-dependent vasoconstriction of afferent arterioles (Figure 2). Consistent with *in vitro* findings, MMF-treated Ang II-infused rats also exhibited normal whole kidney autoregulation of RBF, reduced plasma TGF- $\beta$  levels and less glomerular injury despite similar hypertensive conditions in Ang II-infused control rats (Guan *et al.* 2013). This suggests that renal autoregulatory impairment can be at least partially attributed to inflammation. To distinguish if Ang II or hypertension triggers inflammatory cascades, we also examined the impact of PPS treatment in DOCA-salt hypertensive rats, a low-renin/low-Ang II model (Guan *et al.* 2016). Similar to findings in Ang II-infused rats, PPS treatment in DOCA-salt rats preserved pressure-dependent vasoconstriction (Figure 2) and reduced protein and MCP-1 excretion. Collectively, those studies suggest that hypertension triggers inflammatory cascades, leading to impaired renal autoregulatory efficiency and kidney injury involving MCP-1 and/or TGF- $\beta$  signaling pathways.

The Dahl SS rat is a well-characterized hypertensive model, which rapidly develops hypertension and glomerular injury upon high salt (HS) diet feeding. SS rats fed HS also exhibit impaired renal autoregulation (Roman 1986, Takenaka *et al.* 1992). ETS-1 is a transcription factor targeting the chemokine ligand 2 (CCL2) and mediates vascular inflammatory and fibrotic effects (Zhan *et al.* 2005). Feng *et al.* reported that SS rats with an inactivating mutation of one *Ets-1* allele (SS<sup>*Ets1*+/-</sup> rats) developed less severe hypertension in response to 4% HS feeding compared to normal SS rats (Feng *et al.* 2015). These studies were extended to assess afferent arteriole autoregulatory behavior in SS<sup>*Ets1*+/-</sup> rats fed HS (7 days). Autoregulatory behavior was determined at a time when BP had just begun to increase but was not statistically different from HS-treated SS rats (Feng *et al.* 2020). The SS rats receiving a low salt diet exhibited normal autoregulatory reactivity which was lost if the HS diet was given. Interestingly, renal autoregulation was preserved in SS<sup>*Ets1*+/-</sup> rats, even with HS feeding. SS<sup>*Ets1*+/-</sup> rats also showed less glomerular injury, fibrosis and reduced



mRNA expression of CCL2, TGF- $\beta$  and fibronectin in isolated renal microvessels compared to HS-treated SS rats. These observations are consistent with the findings of Elmarakby et al. showing that pharmacological inhibition of chemokine receptor 2b preserved pressure-induced afferent arteriole vasoconstriction in Ang II-infused hypertensive rats (Elmarakby *et al.* 2007). Overall, these studies reveal that haplo-insufficiency of ETS-1 preserves renal autoregulation in SS rats and that CCL2 in renal microvessels plays an important role in renal pathophysiological alterations in hypertension.

Currently, most methods used for assessing renal autoregulation employ isolated renal microvessels, isolated perfused kidneys, or anesthetized animals. To overcome the influence of anesthesia with isoflurane, and to assess renal autoregulation in a more dynamic state, Bidani et al developed a new analytical tool for assessing renal autoregulatory efficiency in conscious rats by calculating short-segment autoregulatory indices (SSARI) using concurrently collected BP and RBF measurements (Bidani *et al.* 2020). They determined renal autoregulatory efficiency in rats that underwent uninephrectomy plus  $\frac{3}{4}$  renal mass reduction (RK-NX). They found that RBF rapidly returned to baseline in 5-10 seconds in normal conscious control rats after ambient BP changes. Interestingly, RBF in conscious RK-NX rats also returned to baseline, even in rats receiving a calcium channel blocker. However, it took more time (~20 second) for RK-NX rats to restore RBF. In contrast, RK-NX rats displayed impaired renal autoregulation under anesthesia. The study suggests previously un-recognized mechanosensitive mechanism(s) which contribute to maintaining stable RBF and GFR in conscious rats can be suppressed by anesthesia. Together, these studies suggest that this slower response may significantly contribute to elevation of glomerular hydrostatic pressure, and hence glomerulosclerosis in hypertension.

### **Purinoceptors in hypertension-induced renal microvascular dysfunction**

As mentioned earlier, normal autoregulatory control requires P2X1 and/or A<sub>1</sub> receptor signaling (Inscho 2009, Bell *et al.* 2009, Guan *et al.* 2014, Schnermann 2015, Vallon *et al.* 2020). While purinoceptor signaling has recognized roles in regulating cardiovascular function, the pathophysiological implications in hypertension-induced renal microvascular dysfunction remain to be determined. Studies reveal a close relationship between hypertension and P2X signaling, especially P2X1, P2X4 and P2X7, in the development of hypertension-associated kidney injury (Inscho *et al.* 2011, Guan *et al.* 2014, 2016, Franco *et al.* 2011, 2017, Ji *et al.* 2012a, Menzies *et al.* 2015b). While renal autoregulation is impaired in Ang II-infused or DOCA-salt hypertensive rats, afferent arteriole responses to ATP or the selective P2X1, P2X3 receptor agonist  $\beta$ ,  $\gamma$ -methylene ATP were also attenuated (Figure 2) (Guan *et al.* 2010, 2013, Inscho *et al.* 2011). In contrast, afferent arteriole responses to the P1 receptor agonist, adenosine, or P2Y<sub>2</sub> agonist, UTP, were unchanged in both hypertensive models. PPS or MMF treatment preserved afferent arteriole responses to ATP and  $\beta$ ,  $\gamma$ -methylene ATP (Figure 2), suggesting that inflammation also impairs P2X1 receptor signaling but has no detectable impact on A<sub>1</sub> and P2Y<sub>2</sub> receptor activation in afferent arterioles of hypertensive rats (Inscho *et al.* 2011, Guan *et al.* 2016). These findings are consistent with the reduction in ATP- or  $\beta$ ,  $\gamma$ -methylene ATP-mediated intracellular Ca<sup>2+</sup> signaling in freshly isolated preglomerular MVSMC of Ang II-infused rats while responses to KCl remained normal (Inscho *et al.* 2011). These observations implicate impaired intracellular signaling pathways stimulated by P2X1 receptors in Ang II-hypertensive rats. Surprisingly, P2X1 receptor protein expression was similar in isolated preglomerular microvessels from control and Ang II rats (Inscho *et al.* 2011). The explanation for impaired P2X1 receptor activation with no change in P2X1 receptor protein expression is not understood. Possibly, blunted P2X1-mediated vasoconstriction could reflect elevation of interstitial ATP levels (Graciano *et al.* 2008), leading to P2X1 receptor desensitization. Micropuncture studies by Franco and colleagues revealed that chronic Ang II-infusion increased afferent and efferent arteriole resistance leading to elevation of glomerular pressure and reduction of single nephron GFR (Franco *et al.* 2011, 2017). Acute P2

receptor blockade with intra-aortic infusion of PPADS or the P2X1 receptor antagonist, NF449, restored afferent and efferent arteriolar resistance and single nephron GFR to normal in Ang II-infused rats without changes in mean arterial pressure (Franco *et al.* 2011, 2017). Unlike findings in our study (Inscho *et al.* 2011), Franco *et al.* found that Ang II-infused rats exhibited significant increases in P2X1 receptor protein and mRNA expression in cortical tissue. This increased expression could contribute to the increased renal vascular resistance reported.

Additionally, recent studies have shown that P2X7 receptor activation contributes to hypertension-associated kidney injury in rodents including Ang II-infused, Dahl SS, DOCA-salt, and ren-2 transgenic hypertensive rats or mice (Vonend *et al.* 2004, Ji *et al.* 2012a, 2012b, Menzies *et al.* 2015a, Franco *et al.* 2017). Since P2X7 receptor expression was detected in the endothelium of preglomerular arteries, the vascular smooth muscle layer of afferent arterioles and the vasa recta (Menzies *et al.* 2015a, 2015b), Menzies *et al.* proposed chronic Ang II-infusion promoted P2X7 receptor activation which contributed to renal microvascular dysfunction and reduction of medullary perfusion (Menzies *et al.* 2015a, 2015b). Indeed, the authors found that chronic, low-dose Ang II-infusion in F344 rats led to increased P2X7 receptor expression in the vascular smooth muscle of preglomerular arteries along with a significant reduction of medullary perfusion. This occurred despite mean BP rising by only ~15 mmHg and no detectable albuminuria. P2X7 receptor blockade with the specific antagonist AZ11657312 increased medullary blood flow, improved pressure-natriuresis and reduced glomerular macrophage infiltration via a NO-dependent mechanism (Menzies *et al.* 2015a), suggesting that activation of P2X7 receptor signaling by Ang II induces renal microvascular dysfunction and inflammation. This study is consistent with findings in Dahl SS rats fed an 8% HS diet demonstrating a role for P2X7 receptors in the development of hypertension and kidney injury (Ji *et al.* 2012a). Ji *et al.* showed that treatment with selective P2X7 receptor antagonists (BBG or A438079) not only decreased systolic BP but also mitigated kidney injury in Dahl SS rats. Furthermore, Franco *et al.* reported that P2X7

receptor blockade reduced afferent and efferent arteriolar resistance and restored single nephron GFR in Ang II-infused hypertensive rats, similar to P2X1 blockade (Franco *et al.* 2017). Together, those studies suggest that P2X7 receptor signaling is upregulated in hypertension. Inhibiting P2X7 receptor activation protects against hypertensive injury by normalizing renal microvascular resistance and hemodynamics.

In summary, clinical studies indicate a strong correlation between the incidence of hypertension and kidney injury/failure, particularly in African-American hypertensive patients. The underlying processes for hypertension-associated pathological renal changes may involve multiple factors such as genetics, severe hypertension, aging, sex, etc. Emerging clinical data indicate that renal autoregulatory impairment may play a critical role in some hypertensive patients who develop kidney injury and end-stage kidney disease more rapidly than others. Here, we also present evidence from animal studies demonstrating that activation of inflammatory cascades contribute to hypertension-induced renal microvascular dysfunction manifested as reduced pressure-mediated adjustments in afferent arteriole diameter and reduced responsiveness to P2X1 receptor activation. Compromised autoregulatory efficiency leads to glomerular hyperfiltration and promotes progression of glomerulosclerosis in hypertensive subjects. Furthermore, P2X7 receptor activation plays a key role in renal microvascular dysfunction and inflammation. Thus, it is vital to unravel the mechanisms underlying hypertension-mediated renal injury, and to develop effective, targeted, therapeutic interventions which could prevent, or delay, progressive renal decline.

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**Conflict of Interest**

No

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## Figure legends

### Figure 1. Signaling pathways for purinoceptor activation in vascular smooth muscle cells

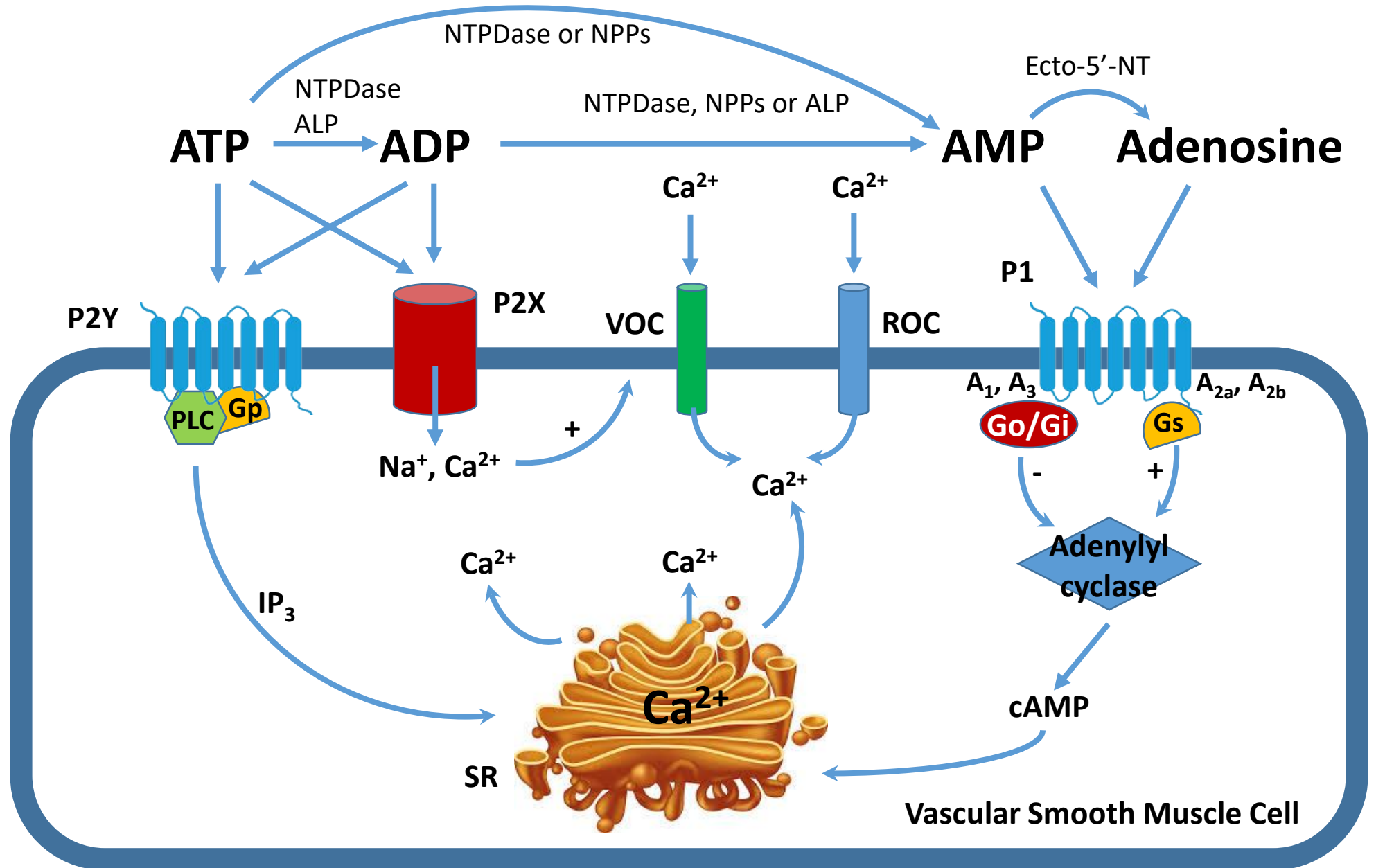
Illustration of the metabolic pathways for ATP to form ADP, AMP, and adenosine by ectonucleotidases. Adenosine and ATP activate P1 and P2 purinoceptors, respectively. The P1 receptor family is comprised of four subtypes: A<sub>1</sub>, A<sub>2a</sub>, A<sub>2b</sub> and A<sub>3</sub>. A<sub>1</sub> and A<sub>3</sub> receptors are linked to a G<sub>o</sub>/G<sub>i</sub> protein and inhibit adenylyl cyclase, thus decreasing cyclic AMP (cAMP). A<sub>2a</sub> and A<sub>2b</sub> receptors are linked to G<sub>s</sub> protein and stimulate adenylyl cyclase to increase cAMP. The P2 receptor family is divided to two distinct subfamilies: P2X and P2Y. P2X receptors function as non-selective ion-channels whereas P2Y receptors are G protein-coupled receptors. Ecto-5'-NT: ecto-5'-nucleotidases; VOC: voltage-operated calcium channel; ROC: receptor-operated calcium channels; NTPDase: nucleoside triphosphate diphosphohydrolase; NPPs, nucleotide pyrophosphatase/phosphodiesterase; ALP: alkaline phosphatase; IP3: inositol 1, 4, 5-trisphosphate.

### Figure 2. Afferent arteriole responses to changes in renal perfusion pressure (RPP) and to P2X1 receptor activation in hypertensive rats

**A:** Renal autoregulation was assessed by afferent arteriole responses to alterations in RPP in control (squares), Ang II-infused hypertensive rats alone (60 ng/min, circles) and treated with mycophenolate mofetil (Ang II+MMF, 30 mg/kg.day, triangles). **B:** Afferent arteriole autoregulatory responses assessed in rats with uninephrectomy (UNx, squares), DOCA-salt hypertension (circles, a low-renin/low-Ang II model) and DOCA<sub>salt</sub> hypertension with pentosan polysulphate treatment (DOCS<sub>salt</sub>+PPS, 100 mg/kg. day, triangles). **C:** Afferent arteriole responses to increasing concentrations of  $\beta$ ,  $\gamma$ -methylene ATP (P2X1 and P2X3 receptor agonist) in control (squares), Ang II-infused alone (circles) and Ang II+MMF (triangles) rats. **D:**

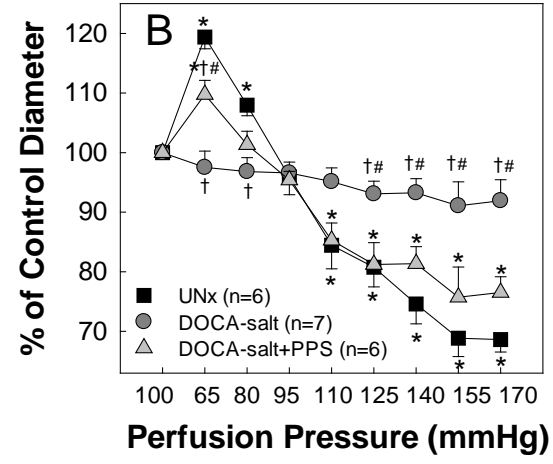
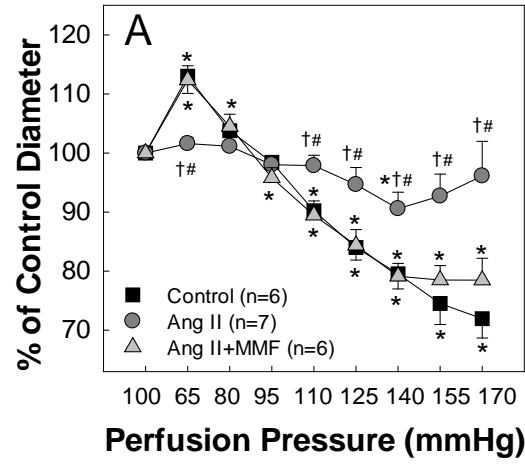
Afferent arteriole responses to increasing concentrations of  $\beta$ ,  $\gamma$ -methylene ATP in UNx (squares), DOCA-salt alone (circles) and DOCA-salt+PPS (triangles) rats. Both hypertensive models exhibit impaired renal autoregulation, indicated by the blunted pressure-dependent afferent arteriole vasoconstriction and also associated with reduced  $\beta$ ,  $\gamma$ -methylene ATP reactivity. Anti-inflammatory treatment with MMF or PPS preserved renal autoregulation and vasoconstriction to  $\beta$ ,  $\gamma$ -methylene ATP in hypertensive rats without changes in systolic blood pressure (Guan *et al.* 2013, 2016). Data are normalized as a percent of the control diameter at 100 mmHg. Values are expressed as mean  $\pm$  SEM. \* $P$ <0.05 vs. control diameter in the same group; † $P$ <0.05 vs. control or UNx at the same RPP; #  $P$ <0.05 vs. Ang II+MMF or DOCA-salt+PPS at the same RPP.

Figure 1.



**Figure 2.**

**Renal autoregulatory behavior**



**P2X1 receptor activation**

