

## New insights into the importance of aminopeptidase A in hypertension

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**Abstract** The renin-angiotensin system (RAS) plays an important role in the maintenance of normal blood pressure and the etiology of hypertension; however, minimal attention has been paid to the degradation of the effector peptide, angiotensin II (AngII). Since aminopeptidase A (APA)-deficient mice develop hypertension APA appears to be an essential enzyme in the control of blood pressure via degradation of AngII. The robust hypertension seen in the spontaneously hypertensive rat (SHR) is due to activation of the RAS, and an accompanying decrease in kidney APA. Changes in APA have also been measured during the activation of the RAS in the Goldblatt hypertension model and Dahl salt-sensitive (DSS) rat. The DSS rat shows an elevation in renal APA activity at the onset of

hypertension suggesting a protective role against elevations in circulating AngII, followed by decreased APA activity with advancing hypertension. Changes seen in human maternal serum APA activity during preeclampsia are similar to changes measured in renal APA in the DSS rat model. APA activity is higher than during normal pregnancy at the onset of preeclampsia, and with advancing preeclampsia (severe preeclampsia) declines below that seen during normal pregnancy. Serum APA activity is also increased during hormone replacement therapy (HRT), perhaps in reaction to elevated levels of AngII. Thus, it appears important to consider the relationship among activation of the RAS, circulating levels of AngII, and the availability of APA in hypertensive disorders.

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### Abbreviations

ACE	Angiotensin-converting enzyme
ACE2	Angiotensin-converting enzyme 2
APA	Aminopeptidase A
APN	Aminopeptidase N
AP	Area postrema
ARB	AT <sub>1</sub> receptor blocker
AT <sub>1</sub> R	AT <sub>1</sub> receptor
AT <sub>2</sub> R	AT <sub>2</sub> receptor
AngI	Angiotensin I
AngII	Angiotensin II
AngIII	Angiotensin III
AngIV	Angiotensin IV
CVOs	Circumventricular organs
DSR	Dahl Salt-resistant

DSS	Dahl Salt-sensitive
HF	Acute heart failure
HRT	Hormone replacement Therapy
icv	Intracerebroventricular
NTS	Nucleus of the solitary tract
OVLT	Organum vasculosum of the lamina terminalis
PVN	Paraventricular nucleus
RAS	Renin-angiotensin system
SFO	Subfornical organ
SHR	Spontaneously hypertensive rat
SON	Supraoptic nucleus
WKY	Wistar-Kyoto
2K1C	Two-kidney one-clip

## Introduction

According to the United States Center for Disease Control hypertension affects approximately 1 in 4 adults in the United States and heart disease is the leading cause of death (28.5% in 2002). In addition, preeclampsia occurs in approximately 5% of pregnant women, and is the major cause of maternal morbidity and mortality worldwide with an estimated 50,000 eclamptic deaths per year [1]. Despite the magnitude of these health related problems, and substantial advances in understanding their pathophysiology, insight concerning the biochemical mechanisms underlying essential hypertension and preeclampsia–eclampsia remains incomplete. This review focuses on the contribution of the renin-angiotensin system (RAS) to hypertension and preeclampsia, and especially the role of aminopeptidase A (APA) in the maintenance of normal blood pressure and the development of hypertension. A key component of this process is the availability of sufficient levels of APA to facilitate conversion of the octapeptide angiotensin II

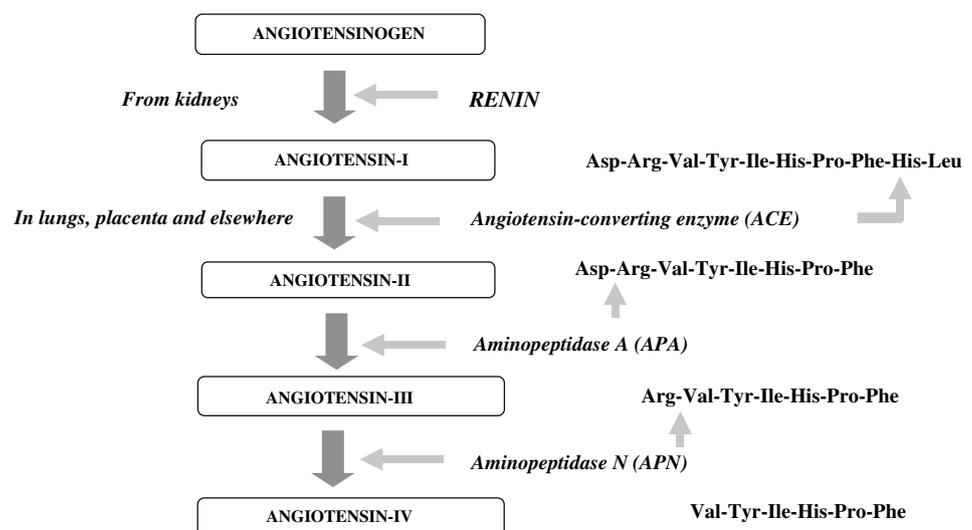
(AngII), to the heptapeptide angiotensin III (AngIII). Much is known concerning the role of APA under both normotensive and hypertensive states, however gaps in our knowledge exist and will be noted.

This review begins with a description of how angiotensin ligands are formed and the receptors they activate, followed by the importance of the brain RAS in the maintenance of systemic blood pressure. We then discuss the role of APA in several animal models of hypertension and also acute heart failure (HF), and conclude with sections concerning changes in APA during pregnancy and preeclampsia, and hypertension during menopause and hormone replacement therapy (HRT).

## Formation of angiotensin ligands and their receptors

The RAS regulates several classic physiologies including blood pressure, sodium and water balance, and pituitary gland hormones. These processes are primarily mediated by the AT<sub>1</sub> receptor subtype (AT<sub>1</sub>R; reviewed in [2–7]). A second subtype, the AT<sub>2</sub> receptor (AT<sub>2</sub>R) is also involved in the mediation of blood pressure, renal function, and vascular growth [3, 8, 9]. AngII has been considered the end product of the peripheral RAS and acts as an agonist at these two receptor subtypes. Angiotensinogen serves as a precursor protein to all angiotensin peptides (Fig. 1). The decapeptide, angiotensin I (AngI), is formed by the protease renin (EC3.4.23.15) acting upon the N-terminal of angiotensinogen. In turn AngI is a substrate for angiotensin-converting enzyme (ACE: EC3.4.15.1), a zinc metalloprotease that hydrolyzes the carboxyl terminal dipeptide histidine-leucine (His-Leu) to form AngII [10]. AngII is converted to AngIII by APA that cleaves the aspartic acid (Asp) residue at the N-terminal [11–14].

**Fig. 1** The renin-angiotensin cascade. Angiotensinogen is cleaved by renin to form angiotensin I. Angiotensin I is converted to AngII by angiotensin-converting enzyme. The N-terminal aspartic acid is cleaved from AngII to form AngIII, and the N-terminal arginine is cleaved from AngIII to form AngIV



**Table 1** Characteristics of the angiotensin receptor subtypes

Characteristics	AT <sub>1</sub> R	AT <sub>2</sub> R
Affinity	AngII>AngIII>AngI	AngIII>AngII
Sensitivity to SH reagents	Inactivation	Enhancement
Selective antagonists	CGP46027, DuP753, DuP532, EXP3174, L158809	CGP42112A PD123177
	GR117289, SK/F108566	PD121981
	SC51316, UP269-6, LR-B/081	PD123319 PD124125
Coupling to G-protein	Yes	Yes
Signal transduction	↑Ca <sup>2+</sup> , ↑P3, DAG Src, JAK/STAT ↓Adenylyl cyclase ↑Prostaglandins PL-A, -C, -D	↓cGMP/↑cGMP PL-A <sub>2</sub> , NO ↑Prostaglandins
Structure	359 Amino acids 7 Transmembrane Domains	363 Amino acids 7 Transmembrane Domains
Molecular size	41–42 kDa	40–41 kDa
Physiologies	Blood pressure Thirst Body water balance Cyclicity of reproductive Hormones and behaviors Sympathetic activation ACTH release Memory/cognition	Blood pressure Thirst Renal function Vascular growth PAI-expression

Adapted from Wright and Harding [7, 15]

Membrane alanyl aminopeptidase N (APN: EC3.4.11.2) cleaves arginine (Arg) at the N-terminal of AngIII to form the hexapeptide, angiotensin IV (AngIV) [15].

The AT<sub>1</sub>R subtype is G-protein linked with signaling via phospholipase-C and calcium (Table 1). Thus, the angiotensin ligand binds to the AT<sub>1</sub>R and induces a conformational change in the receptor protein, thus activating G-proteins that mediate signal transduction. This transduction includes several plasma membrane mechanisms including phospholipase-C, phospholipase-A<sub>2</sub>, and phospholipase-D-adenylyl cyclase, plus L-type and T-type voltage sensitive calcium channels [3, 16]. The AT<sub>1</sub>R (now designated AT<sub>1A</sub>) is also coupled to intracellular signaling cascades that regulate gene transcription and expression of proteins that mediate cellular proliferation and growth in many target tissues. The AT<sub>1B</sub> subtype is approximately 92–95% homologous with the amino acid sequence of the AT<sub>1A</sub> subtype [9, 17]. Out of these two isoforms the AT<sub>1A</sub> subtype appears to be responsible for the classic functions associated with the RAS (reviewed in [6, 18]). The AT<sub>2</sub>R

subtype also evidences a 7-transmembrane domain characteristic of G-protein coupled receptors but shows only about 32–34% amino acid sequence identity with the AT<sub>1A</sub> receptor. The AT<sub>2</sub>R subtype appears to be maximally sensitive to AngIII, but AngII also serves as a ligand at this subtype.

### Brain angiotensins, APA, and blood pressure

Intracerebroventricular (icv) infusions of AngII produce reliable pressor responses by acting at the AT<sub>1</sub>R located in circumventricular organs (CVOs), specifically the subfornical organ (SFO), the organum vasculosum of the lamina terminalis (OVLT), and the area postrema (AP). Each of these CVOs directly or indirectly projects to the paraventricular (PVN) and supraoptic (SON) nuclei of the hypothalamus to induce vasopressin release that acts as a potent vasoconstrictor (reviewed in [19, 20]). It has also been shown that microinjections of AngII into the SFO, OVLT, and PVN elicit elevations in blood pressure (reviewed in [20]). The pressor response induced by increases in circulating AngII appears to be mediated, primarily by the SFO and AP.

AngII activates AT<sub>1</sub>R in the medulla that also contribute to the control of blood pressure. Target structures include the nucleus of the solitary tract (NTS), AP, and anterior ventrolateral medulla [21]. In particular, the AP appears to detect blood-borne AngII; while AngII activation of the NTS influences the baroreceptor reflex. Thus, circulating levels of AngII impact the baroreceptor reflex by a pathway from the AP to the NTS [22, 23]. In addition, AngII activation of the AT<sub>1</sub>R subtype in the anterior ventral medulla increases blood pressure by activation of the sympathetic nervous system, tachycardia, and catecholamine released from the adrenal medulla [22, 24–27].

Zini and colleagues [28] have developed selective inhibitors of APA and APN. The APA inhibitor 3-amino-4-thio-butyl-sulfonate (EC33) has been shown to increase the half-life of AngII by 2.6-fold as measured in hypothalamic homogenates, and completely blocks the formation of AngIII. An APN inhibitor 2-amino-pentane-1,5-dithiol (EC27) increased the half-life of AngIII by 2.3-fold. When AngII was icv injected in mice plasma vasopressin levels were increased 2-fold. The co-application of EC33 with AngII inhibited this AngII-induced vasopressin response in a dose-dependent fashion. In contrast, icv injection of EC27 increased plasma vasopressin levels in a dose-dependent fashion. This increase in vasopressin release following the application of EC27 could be blocked by the accompanying injection of the nonspecific angiotensin receptor antagonist saralasin (Sar<sup>1</sup>, Ale<sup>8</sup>-AngII). These results suggest that central angiotensin-induced vasopressin

release is dependent upon the conversion of AngII to AngIII, and therefore AngIII may be the main effective peptide in the brain with respect to the mediation of vasopressin release. Consistent with these findings, Song and colleagues [29] developed an antiserum with anticatalytic activity against APA. When icv infused it reduced both drinking and blood pressure responses to the subsequent icv delivery of AngII by 73 and 59%, respectively. This same antiserum had no effect on icv-infused AngIII-induced drinking and blood pressure responses.

Our laboratory has utilized icv-infused EC33 and PC18 (an APN inhibitor with similar structure to EC27) followed by the metabolically stable analogs [D-Asp<sup>1</sup>]AngII or [D-Arg<sup>1</sup>] AngIII to determine the relative contributions by AngII and AngIII to pressor responses in rats [30]. Intracerebroventricular pretreatment with EC33 blocked the pressor activity induced by the subsequent infusion of [D-Asp<sup>1</sup>]AngII; while EC33 had no effect on the pressor response induced by the subsequent infusion of [D-Arg<sup>1</sup>]AngIII. In contrast, pretreatment infusion with PC18 extended the duration of the [D-Asp<sup>1</sup>]AngII pressor effect by approximately 2–3 times and the duration of the [D-Arg<sup>1</sup>]AngIII effect by approximately 10–15 times. Pretreatment with the specific AT<sub>1</sub>R antagonist losartan blocked the pressor responses induced by the infusion of both analogs indicating they act via the AT<sub>1</sub>R subtype. These results suggest that the brain AT<sub>1</sub>R might be designed to preferentially bind AngIII, or an AngIII-like compound, in mediating blood pressure maintenance. Recent attempts to enhance the bioavailability and potency of EC33 have focused on the synthesis of non-peptidic inhibitors that interact at the S1 and S'1 subsites of aminopeptidase A [31].

Given the above results, it would appear that AngII and AngIII are at least of equivalent importance in the maintenance of blood pressure. However, the *in vivo* half-life of AngIII in circulation is significantly shorter than that of AngII [32], making AngII the most important peripheral agonist acting at the AT<sub>1</sub>R subtype.

### Angiotensin II, renal disease and hypertension

Nephropathy contributes to both the development and severity of hypertension; while hypertension accelerates kidney disease and multiple cardiovascular risk factors [33]. Autoregulatory mechanisms normally protect the microvasculature from temporary elevations in systemic blood pressure. However, if these mechanisms are compromised as in diabetes, arterial hypertension produces glomerulopathy. Early on this failure results in afferent arteriolar vasodilation accompanied by pressure-induced vasoconstriction [34]. With progression of renal disease

hypertension predisposes proteinuria and progressive renal failure [35]. AngII plays an important role in this etiology by inducing both systemic and glomerular hypertension [34], and producing intrarenal vasoconstriction. Thus, decreased renal blood flow leads to ischemic-induced injuries, resulting in further renin release and the promotion of proteinuria that ultimately contributes to tubular injury [36].

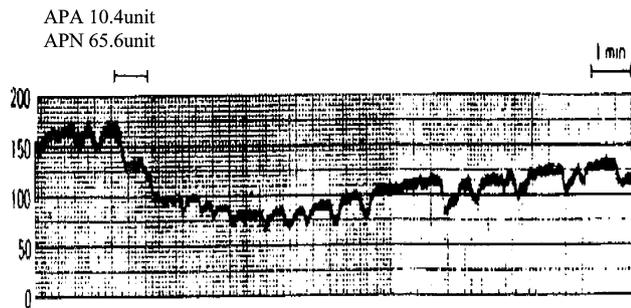
The AngII can also facilitate kidney disease via non-hemodynamic factors including stimulating the release of inflammatory mechanisms, oxidative stress mediators, and growth factors that encourage extracellular matrix accumulation and cell hypertrophy [34]. Taken together these factors contribute to accelerated glomerulosclerosis, glomerular capillary hypertension, and hyperfiltration.

### Changed APA levels in animal models of hypertension

As described above, the removal of C-terminal His-Leu from AngI by ACE to form AngII, and the conversion of AngII to AngIII due to the removal of the N-terminal Asp by APA, are critical steps in determining the availability and survival rate of circulating AngII. In fact, this hydrolysis of AngII to AngIII mediated by APA might serve as a rate-limiting step in AngII degradation [12, 37]. For these reasons the level of APA activity appears to be of major importance in hypertensive patients and in animal models of hypertension will be the focus of the remainder of this review.

### Spontaneously hypertensive rats (SHR)

The SHR model resembles human essential hypertension in several ways. In agreement with essential hypertension the SHR evidences normal or decreased plasma renin activity and initially was considered to possess a renin-independent etiology. However, treatment with an ACE inhibitor, or an AT<sub>1</sub>R blocker (ARB), normalized blood pressure suggesting a role for the RAS in this model [38, 39]. Administration of APA plus APN (purified from human placenta) lowered AngII-induced blood pressure in rats [40]; while purified APA decreased SHR blood pressure in a dose-dependent fashion ([41]; Fig. 2). Thus, an increase in APA activity may play a protective role in hypertension; whereas impairment of APA activity may contribute to the development of hypertension in the SHR by permitting increased levels of circulating AngII. Given that the kidney influences systemic blood pressure via its release of renin, and APA is highly expressed within the kidney [42], it appeared appropriate to measure APA activity in the renal



**Fig. 2** Representative example of the effects of partially purified APA from human placenta on blood pressure in an SHR when intravenously infused over a 1 min duration. Mean arterial base level blood pressure was approximately 165 mmHg with a maximum decrease to approximately 80 mmHg 4 min following treatment

cortex of SHRs as compared with that of normotensive Wistar-Kyoto rats (WKY; [43]). Renal APA activity in the SHR (12 weeks of age) was significantly lower than measured in WKY rats, supporting the notion that a reduced concentration of renal APA may facilitate the development of hypertension in this model. Along these lines, AngII intravascularly infused via osmotic pump during days 15–20 of gestation provoked significantly greater blood pressure increases in nonpregnant than pregnant SHR and WKY rats [43]. Renal APA activity was greater in pregnant than nonpregnant SHRs. In addition, renal APA activity was significantly lower in SHRs at 12 weeks than measured in WKYs. In contrast, Healy and Song [38] have reported major increases in renal APA activity in SHRs as compared with WKY rats at 4, 8, and 16 weeks of age.

Our laboratory has succeeded in developing techniques to mass produce human recombinant APA using a baculo virus system [44]. Bolus intravenous injection of this APA at a low dose significantly decreased systolic blood pressure in SHRs for 36 h (Ishii et al. submitted). The effective dose of APA was about one tenth that of the ARB, Candesartan. Since the difference in molecular weights of APA (109 kDa) and Candesartan (440 Da) is large, APA was calculated to possess a 2,300-fold advantage in efficacy over Candesartan. Therefore APA could be an appropriate treatment for hypertensive emergencies including: hypertensive encephalopathy, apoplexy, acute dissection of aorta, and acute heart failure (HF).

Future studies must resolve differences in findings by evaluating the role of circulating levels of AngII and APA in SHR and WKY rats, especially from the viewpoint of determining rate of angiotensin synthesis and degradation. Given the majority opinion, reduced plasma APA activity would be expected to accompany the well-developed hypertension in SHRs as compared with WKY rats.

### Goldblatt hypertension model

The Goldblatt preparation involves placement of a renal arterial clip on one kidney. This results in elevated plasma renin activity and an increase in blood pressure mediated by facilitated conversion of AngI to AngII [45]. Circulating levels of renin and AngII are transiently elevated during the initial acute stage, subside during the intermediate stage, and then once again increase and remain elevated during the chronic maintenance stage. Song and Healy [38] have reported that although the level of APA in glomeruli was increased, there was APA decreases in kidney cortical membranes in both clipped and non-clipped kidneys at 4 weeks. Prieto and colleagues [46] have shown that clipped and non-clipped kidneys evidence reductions in APA expression in cortical homogenates. They speculated that this reduction might contribute to the maintenance of hypertension in this model by facilitating the tubular effects of AngII on sodium and water reabsorption. These investigators [47] also examined the influence of two kidney-one clip hypertension on the metabolism of AngIII to AngIV by aminopeptidase M (AlaAP) and AngI to Angiotensin(2–10) by aspartyl aminopeptidase (AspAP) in the renal cortex and medulla of rats. Cortical AlaAP levels were elevated in the nonclipped kidneys of the hypertensive rats, as compared with the clipped kidneys and normotensive rats, suggesting increased formation of AngIV. The authors interpreted these results to indicate a potential role for AlaAP in the etiology of renovascular hypertension.

Wolf et al. [48] measured a biphasic pattern of glomerular APA expression in this model. One week after clipping both APA enzyme activity, and its mRNA expression, were elevated in the clipped kidney as compared with the contralateral kidney, or sham-operated controls. In contrast, 4 weeks after clipping APA was up-regulated in the contralateral kidney, whereas expression in the clipped kidney decreased to baselevel. These investigators also reported that isolated glomeruli from the groups with increased APA expression metabolized AngII to a greater extent. Recently, Bivol and colleagues [45] have noted that in the established stage of the Goldblatt hypertensive model the vascular response to AngII injection in the non-clipped kidney was substantially reduced despite normal vascular levels of AT<sub>1</sub>R expression. This could be due to increased APA levels as reported by Wolf et al. [48, 49].

Our laboratory has reported increased APA activity due to the addition of AngII to a primary culture of normal placental trophoblast [50]. If APA expression is induced by AngII as suggested by these studies, target cells responsible for the up-regulation of APA must be capable of sensing increased AngII concentrations in the local

micro-environment. This sensing mechanism likely involves the AT<sub>1</sub>R subtype given that treatment with an ARB abolishes APA expression under a condition of stimulated RAS. It is also clear that AngII facilitates the proliferation of trophoblastic cells via the AT<sub>1</sub>R, and this process is linked to the protein kinase C/MAPK-dependent signaling pathway. It is noteworthy that APA is up-regulated during AngII-induced cell proliferation [51].

The Goldblatt hypertension model offers valuable insights into the interactions of AngII, the AT<sub>1</sub>R, and APA during renin-induced hypertension. Future studies must sort out the precise interrelationship among these mediators as hypertension is established and maintained over time.

#### APA-deficient mouse model

Lin and colleagues [52] developed an APA/BP-1 deficient mouse model in order to investigate the contribution of APA to the immune system. They found APA-deficient mice to maintain a normal immune response. However, homozygous APA mutant mice evidenced elevated systolic blood pressure when compared with heterozygous mutant and wild-type litter mate mice (Fig. 3; [53]). When APA-deficient mice were challenged with AngII infusion they presented an exaggerated blood pressure response (Fig. 4). However, the histological appearance of kidney and heart tissues were not different from control mice. Moreover, urine volume, osmolality and electrolyte content were normal. This sustained blood pressure response by the APA-deficient mouse model is presumably due to elevated levels of AngII, however, this remains to be confirmed.

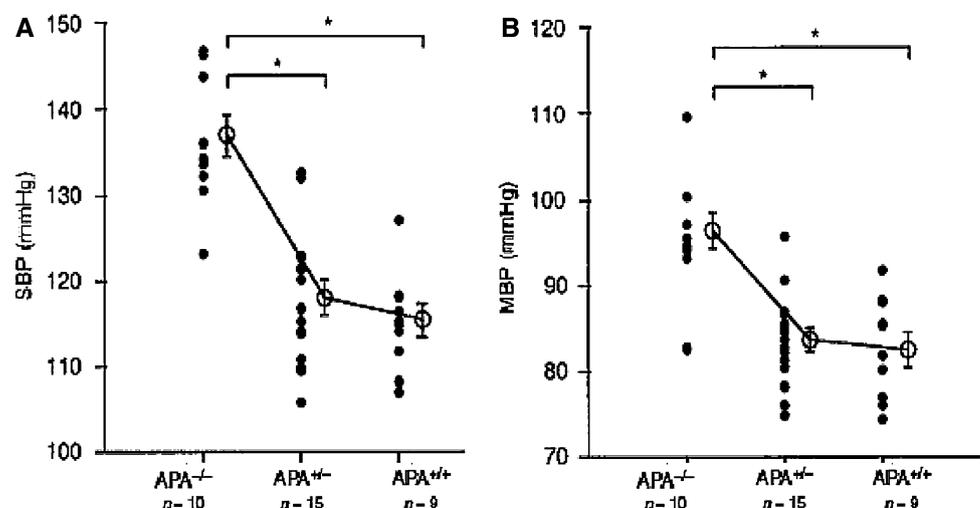
While APA deficiency leads to increased blood pressure, mice deficient in renin, angiotensinogen, ACE, and AT<sub>1</sub>Rs evidence reduced blood pressure [54]. This finding is consistent with the notion that reduced RAS activity should

result in reduced blood pressure. Further studies by this group utilizing angiotensinogen, ACE, and AT<sub>1</sub>R-deficient mice noted abnormal changes in kidney and heart development primarily due to a loss of AngII stimulation of the AT<sub>1</sub>R subtype, suggesting that stimulation of AT<sub>1</sub>R by AngII is essential for normal development of these organs. APA deficient mice retain the AngII/AT<sub>1</sub>R pathway, thus accounting for the absence of heart and kidney damage in this model. Both urine volume and osmolality were not reduced, but rather elevated, thus excluding the possibility that APA-deficient mice suffer decreased glomerular flow rate.

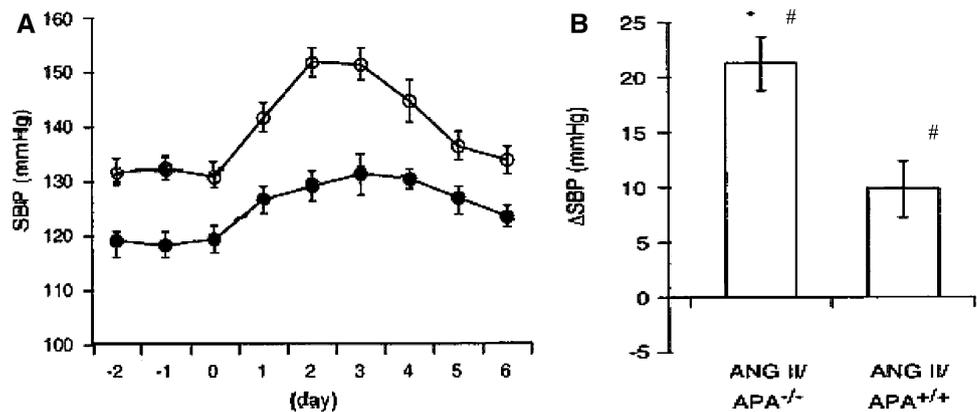
It has been proposed that the AT<sub>2</sub>R subtype opposes the actions of the AT<sub>1</sub>R subtype with respect to blood pressure and cellular proliferation. Therefore, AT<sub>2</sub>R knockout mice are invaluable in testing this hypothesis [55]. These mice are similar to APA-deficient mice in presenting increased blood pressure, hypersensitivity to AngII, and normal kidney and heart development. Thus, the AT<sub>2</sub>R subtype may be acting to oppose at least some of the blood pressure elevating effects of AngII acting at the AT<sub>1</sub>R subtype; however further evaluation of this model is necessary.

The absence of cardiac hypertrophy in AT<sub>2</sub>R deficient mice has been interpreted to suggest that hypertensive cardiac hypertrophy might result via the AT<sub>2</sub>R subtype [56]. Interestingly, increased circulating levels of AngII suppressed AT<sub>2</sub>R binding to a greater extent than AT<sub>1</sub>R binding. Given these results it may be that APA-deficient mice have reduced AT<sub>2</sub>R expression that results in blood pressure elevations without cardiac hypertrophy. In this way AngII stimulation of the AT<sub>1</sub>R in APA-deficient mice could elevate blood pressure, but not cause renal and cardiac abnormalities via upregulation of transforming growth factor- $\beta$ . Kagami et al. [57] have reported AngII-induced mesangial cell synthesis of matrix proteins and these effects were mediated by AngII induction of TGF- $\beta$

**Fig. 3** Analysis of the effects of APA deficiency on blood pressure levels. (A) Systolic and (B) mean arterial blood pressure of APA<sup>-/-</sup>, APA<sup>+/-</sup> and APA<sup>+/+</sup> mice at 3 months of age were determined by tail-cuff measurement. The mean  $\pm$  SEM for each group is represented by an open circle and a bar. Each group consisted of 9–15 animals. \**P* < 0.05



**Fig. 4** Effects of chronic AngII infusion on systolic blood pressure. **(A)** Daily changes in systolic blood pressure accompanying an AngII infusion in *APA*<sup>-/-</sup> (○) or *APA*<sup>+/+</sup> (●) mice. The osmotic pumps were implanted on day 0. **(B)** Increases in systolic blood pressure from base level on day 0 to the levels on day 2 in *APA*<sup>-/-</sup> and *APA*<sup>+/+</sup> mice. Values are mean ± SEM. \**P* < 0.05



expression. An enhanced systolic blood pressure response by infusion of AngII in *APA*-deficient mice is consistent with the biochemical property of *APA*-induced metabolism of AngII.

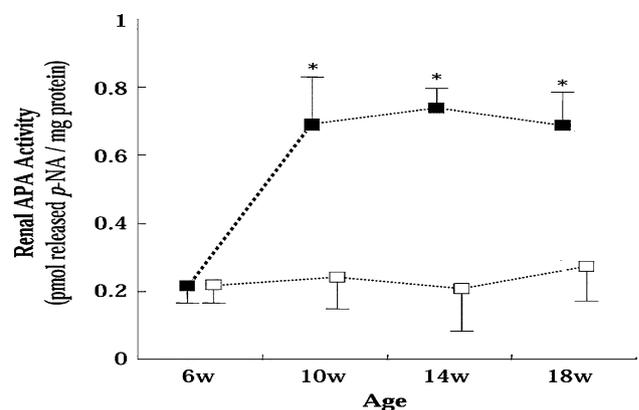
Taken together, these results suggest that although other enzymes [endopeptidase (EC3.4.24.11), prolylendopeptidase (EC3.4.21.26) and angiotensin converting enzyme 2 (ACE2)] are capable of metabolizing AngII in vitro, they do not appear to compensate for the loss of *APA* in vivo [53]. Although the cleavage of C-terminal phenylalanine from AngII by ACE2 to generate the vasodilator Angiotensin1–7, can influence both blood pressure and AngII concentrations, endogenous ACE2 activity is incapable of reversing the hypertensive effect of *APA*-deficiency. Compensatory increases in the activity of other *APA*-like aminopeptidases is unlikely given that *APA*-deficient mice evidence negligible *APA*-like activity [53]. Thus, the *APA*-deficient mouse is a particularly valuable model that deserves further research attention.

#### Dahl salt-sensitive rat

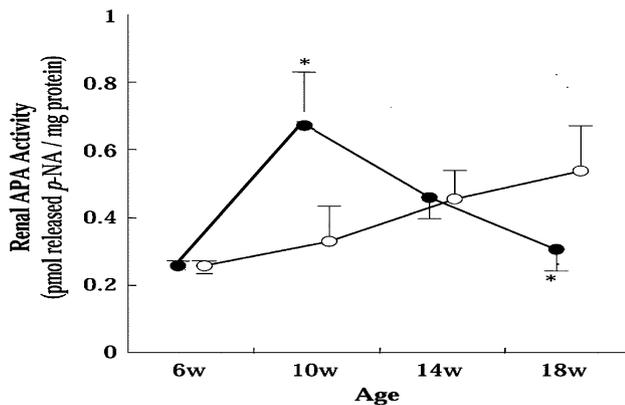
An understanding of the mechanisms underlying salt-induced hypertension in the Dahl salt-sensitive rat (DSS) is of considerable interest given the important role of sodium in hypertensive disorders [58]. Our laboratory has measured changes in renal *APA* activity and histological changes in the glomerulus of both DSS and Dahl salt-resistant (DSR) rats [59]. When low-(0.3%) or high-(8%) salt diets were administered to DSR and DSS rats, only high-salt DSS rats revealed systolic blood pressure increases that reached approximately 150 mm Hg by week 10, peaking at just under 250 mm Hg by week 18. Several mechanisms have been suggested to underlie this response by DSS rats including sympathetic arousal, deficiency in nitric oxide, increased plasma endothelin-1, an overactive RAS [59]. Although there is no evidence that the peripheral RAS is activated, the local renal RAS is stimulated in DSS

rats suggesting a significant contribution by the RAS to the hypertension seen in this model [58]. Renal *APA* activity is elevated 3-fold at week 10 in high-salt DSR rats, similar to that seen in high-salt DSS rats. Thereafter it remained at this level (Fig. 5). This increase in renal *APA* may serve to metabolize AngII, thus protecting the high-salt DSR rat from hypertension in response to high salt intake. On the other hand, *APA* activity in low-salt DSR rats was unchanged. DSR rats on high-salt intake revealed lowered renal AngII levels at week 10 ( $305 \pm 15$  fmol/g protein versus  $212 \pm 16$  fmol/mg protein) and they remained at this level accompanied by an increase in *APA* activity. Thus, it is reasonable to speculate that the decreases in AngII levels measured in DSR rats were due to increased degradation of AngII by *APA*, thus preventing elevations in blood pressure.

Although *APA* activity in high-salt DSS rats increased 3-fold by week 10, it subsequently fell back and assumed the level of week 6, by week 18 (Fig. 6). This increase in renal *APA* at week 10 may serve to degrade AngII and temporarily protect this model. However, AngII levels in high-salt DSS rats at week 10 did not change despite



**Fig. 5** Age-related changes in renal *APA* activity in DSR rats. DSR rats were fed an 8%-salt diet (closed squares) or a 0.3%-salt diet (open squares). Values are mean ± SD (*n* = 6 at each point). \**P* < 0.05



**Fig. 6** Age-related changes in renal APA activity in DSS rats. DSS rats were fed an 8%-salt diet (closed circles) or a 0.3%-salt diet (open circles). Values are mean  $\pm$  SD ( $n = 6$  at each point). \* $P < 0.05$

increased APA activity. Since it is known that a high-salt diet increases intra-renal angiotensinogen levels in DSS rats, elevations in AngII levels would be anticipated [58]. Presumably these increases in AngII exceed the degradative capacity of APA and hypertension results. High-salt intake promotes glomerulosclerosis in the DSS rat with increased mesangial matrix including mild fibrosis or hyper-cellularity at week 10. Expansion of glomerular size and hyalinization are observed with advancing weeks. Severe tubulointerstitial damage with tubular dilation and arteriosclerosis with thrombi were also observed at week 18. There are a number of reports indicating increased glomerulosclerosis with advancing hypertension in DSS rats. The inhibition of RAS activity by ACE inhibitors or ARBs is known to reduce glomerular injury irrespective of the antihypertensive effects in DSS rats, suggesting that local AngII plays a significant role in the pathogenesis of glomerulosclerosis [60]. The AngII produces glomerulosclerosis through various mechanisms including enhanced cellular proliferation and over expression of chemotactic factors and cell adhesion molecules. The low-salt DSS rat showed no histopathological renal injury.

Our laboratory has found the immunostaining of glomerular APA in high-salt DSS rats to be enhanced at week 10, accompanied by mild damage due to fibrosis and hypercellularity. With advancing weeks APA staining decreased in the severely injured glomeruli and finally disappeared. Thus, sclerotic glomeruli progressively lose the ability to express APA. We also noted hyalinization of glomeruli in DSS rats over time that could have contributed to this failure to produce APA. The degree of APA immunostaining in the glomeruli, or elsewhere in low-salt DSS rats, did not change over weeks. As previously mentioned Kobori and colleagues [58] showed that high-salt diet increased intrarenal angiotensinogen levels in DSS rats. Taken together these reports suggest that over time

this model suffers increased glomerular damage primarily due to progressive elevations in AngII levels that occur as the capacity to synthesize APA diminishes. Although this scenario appears to be unusually simple as an explanation it is a reasonable initial hypothesis that will hopefully encourage the additional investigations necessary to validate, modify, or reject this notion.

#### APA levels during pregnancy and pre-eclampsia

Feto-placental circulation and clearance of AngII by placental APA appears to be an important factor in the maintenance of fetal and maternal blood pressures [61]. As the volume of the feto-placental unit increases with advancing gestation the tonus of feto-placental circulation increases its influence upon maternal blood pressure. Broughton-Pipkin and Symonds [62] compared AngII levels in umbilical arterial and venous blood, and maternal venous blood during normal pregnancy and preeclampsia. They showed that the level of AngII in umbilical venous blood is higher than that in umbilical arterial blood in both normal pregnancy and preeclampsia, and the levels in both umbilical venous and maternal venous blood in cases of severe preeclampsia were also much higher than those of normal pregnancy. The gradient of AngII between umbilical venous and arterial blood indicates the involvement of placental ACE in AngII production in the feto-placental unit. They measured even greater differences between umbilical venous and arterial blood in cases of preeclampsia, suggesting increased angiotensin synthesis presumably due to a combination of fetal stress and decreased angiotensin degradation by placental APA.

The observation that maternal serum ACE activity does not change in preeclampsia as compared with normal pregnancy further supports an important role for APA during preeclampsia [61]. Of note, maternal serum APA activity in early preeclampsia patients (mild preeclampsia) were higher than those seen in normal pregnancy patients [63]. However, as the severity of preeclampsia increased, APA activity decreased, ultimately becoming lower than that measured during normal pregnancy. It is likely that increased APA activity in maternal sera before, and immediately after, the appearance of preeclampsia is in response to increased production of AngII from the fetus, thus representing a response to counter such increases. If the APA levels of maternal serum reflect the production rate in the placenta, then the decreased serum APA levels noted during severe preeclampsia could signify placental damage due to angiotensin-induced vasoconstriction. Thus, the trophoblastic degeneration seen during severe preeclampsia may be due to a failure in APA production. In this way normal levels of APA activity could act to protect

the mother; while the drop in APA activity during preeclampsia permits increased AngII levels in the fetoplacental circulation that can leak into maternal circulation resulting in hypertension.

While pregnancy in humans is normally characterized by a relative refractoriness to the hypertensive response associated with exogenously infused AngII, AngII sensitivity in the preeclampsia patient becomes higher reaching a level equivalent to, or greater than, that of non-pregnant women [64]. This phenomenon has been extensively studied as a possible cause of preeclampsia, however, it remains controversial. In contrast, pregnant SHR evidence no refractoriness to the hypertensive response of exogenously infused AngII (Fig. 7; [43]). Recently, Saito and colleagues [65] established the importance of the AT<sub>1</sub>R-mediated pathway in the etiology of preeclampsia by the combined use of transgenic and knockout mice. This further illustrates the importance of fetal AngII in the pathophysiology of preeclampsia.

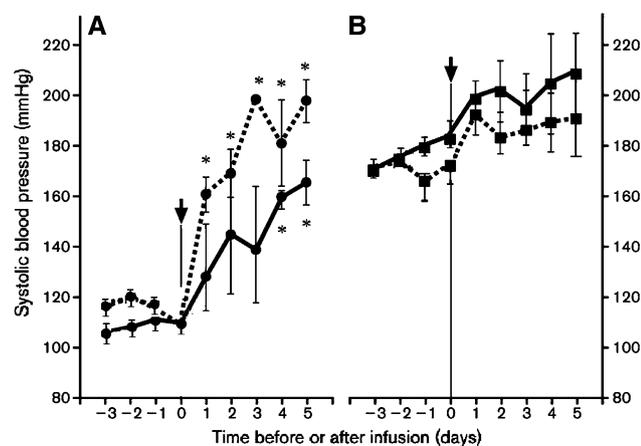
It is well established that treatment with ACE inhibitors, and/or an ARB, can be lethal to the fetus during human pregnancy given that molecules with molecular weights less than 5–8 kDa can pass through the fetoplacental barrier. The mechanism of action appears to be the transfer of these drugs from the mother to the fetus resulting in profound hypotension in the fetoplacental circulation, thus demonstrating a vital and necessary role for AngII in the fetoplacental unit [66]. At present there is no adequate treatment for severe preeclampsia short of terminating the pregnancy. The ultimate goal concerning the control of

preeclampsia is to focus treatment on maternal hypertension without influencing fetal blood pressure, and to avoid the fetal hypertension that occurs due to incremental AngII levels resulting from utero-placental hypoperfusion. Since APA has a molecular weight of 109 kDa and does not cross the placenta it may be a candidate for the treatment of preeclampsia. Along these lines, the intravenous administration of human recombinant APA decreased blood pressure in SHR at a dose similar to that conventionally used with sodium nitrate (unpublished data), supporting the argument that APA, or an APA-like compound, may be efficacious as an antihypertensive agent.

### APA levels with menopause and hormone replacement therapy (HRT)

It appears that serum APA activity in men and women significantly increases with aging [67], as does metabolic clearance rates for AngII [68]. Even higher elevations in serum APA have been reported if the woman is elderly, hypertensive, or receiving HRT [69]. Since plasma AngII levels appear to be unchanged [70], or reduced [71], with age in healthy women it could be that the elevated APA levels seen with hypertension are in response to increases in circulating AngII and represent an attempt to offset the resulting rise in blood pressure. This notion is supported by the observation that plasma AngII levels decline over time in hypertensive patients [71].

Hormone replacement therapy has a beneficial effect on the elevated blood pressure noted in postmenopausal women by enhancing APA activity and reducing circulating AngII levels. HRT has also been shown to increase angiotensinogen and decrease renin and ACE activity [69]. Hinojosa-Laborde et al. [72] have shown that adult female normotensive DSS rats maintained on a low-salt diet become hypertensive after ovariectomy. Thus, the ovariectomized DSS rat shows promise as a useful model of postmenopausal hypertension. These investigators also reported that increased RAS activity contributed to the development of hypertension, and estrogen protects against this process. Thus, HRT enhanced APA activity might contribute to the observed reduction in circulating AngII levels. This hypothesis is in need of further testing.



**Fig. 7** Changes in maternal systolic blood pressure in response to infusion of AngII in (A) WKY rats, and (B) SHRs. AngII (200 ng/kg per min) was infused from day 15 of gestation in pregnant animals (solid lines) and in non-pregnant animals (dotted lines) at the same weeks (arrows). AngII increased systolic blood pressure more rapidly and markedly in non-pregnant than in pregnant WKY rats. Pregnant SHRs showed a loss of refractoriness to the hypertensive effect of exogenously infused AngII ( $n = 10$  animals per group) \* $P < 0.05$

### Possible role of APA in heart failure

Acute heart failure (HF) is one of the most common diseases encountered in medical practice, leading to high morbidity and mortality. However, studies of this syndrome have failed to produce a comprehensive understanding of its pathogenesis and hence appropriate

treatment. There is a need for appropriate animal models that develop HF based on the same cardiovascular dysfunctions seen in humans. Such models will greatly facilitate our understanding of disease progression and provide insights into appropriate treatment. Inoko et al. [73] reported a progression to HF due to severe renal dysfunction in DSS rats placed on an 8% NaCl diet for 6 weeks. Findings from our laboratory utilizing DSS rats suggest the likely involvement of APA as a mechanism of hypertension. Given that APA is a very potent hypotensive agent in SHR, and is in fact comparable to nitroprusside in this regard (Ishii et al. submitted), [74], APA holds promise as a treatment for HF.

### Conclusions

Since the renin-angiotensin system is known to be an important factor in hypertension, research attention has focused on the production of AngII and its interaction with the AT<sub>1</sub> and AT<sub>2</sub> receptor subtypes. Although important, emphasis on this approach ignores the interrelationship between AngII degradation and APA activity in the maintenance of normal and dysfunctional blood pressure.

It is well known that the fetus produces bioactive peptides such as angiotensin, vasopressin and oxytocin, that are highly vasoactive and uterotonic, respectively. Secretions of these peptides by the fetus increase in parallel with fetal growth and in response to stressors such as hypoxia. Therefore, the fetoplacental unit functions as a local RAS system in the pregnant woman. The degradation of these peptides in the placenta is necessary in order to maintain normal homeostasis by both the fetus and mother. This homeostasis appears to be dependent upon balancing AngII levels with the degradative capacity of APA.

Animal studies utilizing APA deficient mice, SHR, Goldblatt hypertension and DSS rats support the pivotal role of APA as the primary degradative enzyme of AngII. It is conceivable that the leakage of fetal-derived AngII into maternal circulation causes preeclampsia. It is known that serum APA activity is increased with hormone replacement therapy, and may counteract increases in AngII thus preventing blood pressure elevations. Given these considerations it appears prudent to monitor APA activity in preeclampsia and hypertensive patients in which elevated levels of AngII are present and a deficiency in APA activity is suspected.

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