Current Topics

Aminopeptidases in Health and Disease

Role of Aminopeptidases in the Blood Pressure Regulation

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In addition to the neural and autoregulatory factors, blood pressure (BP) is regulated by humoral factors including vasoactive peptides. When evaluating the peptide actions, degradation by proteases should be also considered in addition to the generation of peptides and their receptors. This review describes the roles of aminopeptidase A, placental leucine aminopeptidase and kininase I, which are enzymes responsible for hydrolyzing angiotensin II (AngII), vasopressin (AVP) and bradykinin (BK), respectively, in BP regulation. Especially, we focus on the association of the proteases with preeclampsia, hypertensive disorder peculiar to pregnancy, since one of the representative organs that are rich in these proteases is placenta. Although the physiological roles of the placental proteases have not been fully understood, several lines of evidence suggest that the proteases are involved in the maintenance of pregnancy homeostasis including fetal and maternal BP regulation through the metabolism of bioactive peptides at the interface between mother and fetus.

Key words blood pressure; peptide hormones; placental protease; aminopeptidase; preeclampsia; hypertension

Blood pressure (BP) is defined as multiplication of cardiac output and vascular resistance. Although various peptides including angiotensin II (AngII), vasopressin (AVP) and bradykinin (BK) are known to affect cardiac output or peripheral vascular resistance, less attention has devoted to the peptide metabolism compared with the numerous studies on the generation of peptides and their receptors. However, since local concentrations of the peptides depend on the balance between synthesis and degradation, proteases also play a pivotal role in the BP regulation and pathogenesis of hypertension.

In this paper, among a number of proteases, we will review the involvement of aminopeptidase A (APA: EC 3.4.11.7), placental leucine aminopeptidase (P-LAP: EC 3.4.11.3) and kininase I in BP regulation, especially with focusing on their possible association with preeclampsia, disorder with hypertension peculiar to pregnancy.

RENIN-ANGIOTENSIN SYSTEM (RAS) AND APA

The role of the RAS in the maintenance of BP has been extensively investigated. Angiotensinogen is cleaved by renin to generate inactive decapeptide AngI, which is then converted to AngII by the angiotensin-converting enzyme (ACE). Subsequently, AngII is metabolized into AngIII by APA,^{1,2)} which is further converted to AngIV by other aminopeptidases including aminopeptidase N (APN: EC 3.4.11.2)³⁾ and P-LAP⁴⁾ (Fig. 1). Generally AngII is thought to be a principal effector peptide of the RAS, which induces vasoconstriction and increases sodium and water retention thereby leading to an increase in BP.

APA, which hydrolyzes N-terminal acidic amino acid such as aspartic and glutamic acid, is regarded as a principal and important candidate responsible for the conversion of AngII to AngIII. APA, therefore, would be deeply involved in regulating BP through AngII metabolism. The findings that sys-

temic administration of purified APA into spontaneous hypertensive rats (SHRs)⁵⁾ or AngII-infused hypertensive rats decreased their BP⁶⁾ and that APA inhibitor amastatin elevated BP in normotensive rats⁷⁾ support this notion. In addition, our study employing APA knockout mice has demonstrated more direct evidence of APA involvement of BP regulation.⁸⁾ The baseline systolic BP was significantly elevated in *APA*^{-/-} mice (136.3 ± 2.4 mmHg, $n=10$) compared with *APA*^{+/-} (117.4 ± 2.0 mmHg, $n=15$) and *APA*^{+/+} littermate mice (114.8 ± 2.0 mmHg, $n=9$) ($p<0.05$) (Fig. 2), suggesting that APA may lower BP through AngII metabolism. Moreover chronic infusion of AngII increased systolic BP to a greater extent in *APA*^{-/-} mice than *APA*^{+/+} mice. APA deficiency would increase the relative concentrations of functional AngII by protecting AngII from the degradation, leading to modest, but sustained BP elevation and hypersensitivity to AngII. Thus APA plays a critical and indispensable role in maintaining BP homeostasis. With respect to AngII metabolism, APA-deficient mice also suggest that other enzymes that can metabolize AngII *in vitro*, including neutral endopeptidase (EC 3.4.24.11),⁹⁾ prorylendopeptidase (EC

Fig. 1. Schematic Representation of the Relation between RAS and Proteases

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Fig. 2. Analysis of the Effects of APA Deficiency on Basal BP Level

The systolic BP of *APA*^{-/-}, *APA*^{+/-} and *APA*^{+/+} mice at 3-month of age were determined by a computed tail-cuff system. The average $\pm$ S.E.M. for each group is represented by an open circle and a bar. * $p < 0.05$.

3.4.21.26),¹⁰ and the recently-described angiotensin converting enzyme 2 (ACE2),¹¹ do not appear to compensate effectively for the loss of APA in BP regulation.

Recently another enzyme that may be involved in AngII metabolism has been identified. Adipocyte-derived leucine aminopeptidase (A-LAP) is a novel member of the M1 family of zinc-metalloproteases that shares high homology with P-LAP.¹² Based on the findings that A-LAP hydrolyzes AngII and kallidin and that A-LAP is expressed in the cortex of the human kidney, where tissue kallikrein is localized,¹³ A-LAP seems to play a role in the decrease of BP through the inactivation of AngII and/or the generation of BK that can induce hypotension by arterial vasodilation in the kidney. In support of this notion, the Lys528Arg polymorphism in the *ALAP* gene is reported to show significant association with essential hypertension.¹⁴ The estimated odds ratio for essential hypertension was 2.3 for presence of the Arg allele at codon 528, in comparison with presence of the Lys/Lys genotype. And further, treatment of AngII type I receptor (AT₁) antagonist irbesartan to the patients with the Arg/Arg genotype caused two-fold greater regression of left ventricular mass index than those with the Lys/Arg genotype.¹⁵ However, in contrast to this possible role of A-LAP as angiotensinase, a recent finding that A-LAP trims precursor peptides to MHC class I molecules¹⁶ may suggest that the chief role of A-LAP is intracellular function as a key component of the MHC class I antigen-presentation pathway.

However, rather unexpectedly, clinical study examining serum APA activity in humans demonstrated that hypertensive women have higher serum APA activity than age-matched normotensive women.¹⁷ On the relation between serum AngII concentrations and hypertension, no definitive results have been proposed until now. These findings may be interpreted by the speculation that tissue RAS plays a critical role in hypertension, which does not always parallel systemic RAS. Since AngII treatment increases APA expression *in vitro*,¹⁸ we could also speculate that activation of tissue RAS increases local AngII levels, which induces APA activity and elevate BP in hypertensives.

It should be noted that the biological activity of AngII and

AngIII, that is the function of APA, differs between in the peripheral tissues and central nervous system (CNS). Peripherally AngIII is less potent than AngII in enhancing local vasoconstriction, steroidogenesis, water and electrolyte absorption as well as elevating arterial BP. However, cerebroventricular infusion of purified APA to rats increased BP,¹⁹ suggesting that AngIII may be more effective peptide in CNS than AngII in elevating BP. In accordance with this finding, Llorens-Cortes and her group developed and cerebroventricularly administered APA specific inhibitor EC33 or APN inhibitor PC18 to rats.^{20,21} EC33 that prevents the generation of AngIII decreased BP and PC18 that prolongs half-life of AngIII increased BP, suggesting that AngIII plays a predominant role in the brain in increasing BP.

APA roles in the BP regulation and pathogenesis of preeclampsia, hypertensive disorder of pregnancy, are also intensely investigated. Since human fetuses produce equal or higher levels of various vasoactive peptides such as AngII and AVP compared with mothers along with their growth or in response to various stresses,²² placental proteases could metabolize excessive peptides derived from the fetus as a barrier between the mother and fetus. Indeed clinically, APA activity in maternal serum increases during normal pregnancy possibly to maintain fetomaternal homeostasis. Interestingly, in patients with preeclampsia APA activity shows biphasic changes.^{23,24} In mild preeclamptic patients, serum APA activity rather elevates above normal range, which may indicate the counteraction to the activated RAS; when preeclampsia gets worse, APA activity decreases, which may accelerate the increase in BP. In addition to the higher BP at basal state, preeclamptic patients are generally sensitive to the pressor effect of AngII contrary to the refractoriness in normal pregnant women. Hypersensitivity to exogenously infused AngII is also observed in APA-deficient mice,⁸ suggesting that APA may play a critical role in acquiring the refractory activity to AngII. Possible association of APA with hypertensive state and lack of refractoriness to AngII in preeclampsia is also investigated using SHR.²⁵ SHRs showed lower APA activity than control Wistar-Kyoto (WKY) rats in the kidney, a major APA production site, probably contributing to chronic hypertension of SHRs. Infused AngII increased BP in non-pregnant compared with pregnant WKY, while SHR showed little response to AngII whether pregnant or not. Renal APA activity significantly increased in pregnant than non-pregnant SHRs, while not apparently differ between pregnant and non-pregnant WKY rats.

PLACENTAL LEUCINE AMINOPEPTIDASE (P-LAP)

P-LAP is the only known membrane aminopeptidase that opens the N-terminal cystine ring structure of small peptides such as oxytocin and AVP.^{4,26} In addition, P-LAP is able to hydrolyze AngIII.⁴ Contrary to the previous belief that P-LAP is limited to placenta, northern blot analysis²⁷ and immunohistochemistry²⁸ have shown that P-LAP has a broad tissue distribution other than placenta. cDNA cloning of P-LAP has demonstrated that this enzyme is a homologue of rat insulin-regulated membrane aminopeptidase (IRAP), which is present in the glucose transporter isotype GLUT4 vesicles of rat adipocytes.²⁹ P-LAP, therefore, seems to play

a role in BP regulation *via* hydrolyzing AVP and AngIII in non-pregnant human as well as pregnant women. However currently, studies of P-LAP associated with BP regulation mainly focus on pregnant women.

In human placenta, P-LAP is predominantly expressed in differentiated trophoblasts,^{27,30)} which is regulated by AP-2 and Ikaros transcriptional factors.^{31–33)} Membrane-bound P-LAP is released into maternal blood flow *via* proteolytic cleavage by metalloproteases such as ADAMs, a disintegrin and metalloproteinases.^{34–36)} During normal pregnancy, P-LAP activity in maternal serum gradually increases to the maximum at near term,³⁰⁾ which may be associated with the maintenance of pregnancy homeostasis and the onset of labor pains. P-LAP activity is rather elevated in preeclamptic patients while preeclampsia is mild, but it sharply decreases when preeclampsia turns severe, which is the finding similar to APA.²³⁾ Since P-LAP substrate peptide AVP plays an important role in the fetal cardiovascular response to stress, P-LAP increase in maternal serum seems to counteract the increased fetal secretion of AVP at the interface between mother and fetus. To support this, P-LAP activity is inversely associated with the pulsatility index measured by a pulsed Doppler method, which reflects the constriction of placental vessels, in severe preeclamptic patients.³⁷⁾

KININASE I

In circulatory system, vasodilative peptides cooperatively work with vasoconstrictive peptides. BK is a naturally occurring vasoactive peptide that induces hypotension by arterial vasodilation and diminishing resistance of vessels through activating constitutively expressed B₂ receptors. Kininase I is a generic name for different carboxypeptidases that transform BK into des-Arg⁹-BK,^{38,39)} which no longer activate B₂ receptors. Plasma kininase I mainly consists of carboxyl peptidase N, which primarily yielded in human liver and secreted to the circulation. We purified kininase I from human placenta and showed its involvement in BK degradation.⁴⁰⁾ BK also contributes the regulation of fetal BP *via* vasodilation. Our study revealed no significant differences in the levels of kininase I between in umbilical arteries and veins, which was compatible with umbilical BK concentrations.⁴¹⁾ kininase I activity of fetal umbilical veins significantly decreased in the presence of acidemia without preeclampsia, which probably reduced BK concentrations in fetal circulation resulting in elevation of fetal BP. However, umbilical kininase I activity in patients complicated with preeclampsia was significantly higher than that without preeclampsia. In preeclampsia, the concentrations of BK might be decreased due to the increased degradation by kininase I in not only maternal blood but also fetal circulation.

CONCLUSIONS AND PERSPECTIVES

Figure 3 illustrates the role of placental proteases in regulating uteroplacental blood flow. Placental proteases play important roles in regulating fetal and maternal BP through controlling the concentrations of vasoactive peptides at the interface between mother and fetus.

The use of ACE inhibitors to preeclamptic patient, which could easily pass through placenta, is known to cause in-

Fig. 3. Effect of Placental Proteases on Uteroplacental Blood Flow

trauterine growth retardation (IUGR), severe disturbance of fetal renal dysfunction, and sometimes intrauterine fetal death, partly due to severe drug-induced fetal hypotension. Considering the mechanism for the maintenance of uteroplacental circulation, novel drugs that control the bioactivity of vasoactive peptides but do not go through placenta are promising. Placental proteases would serve as a novel therapeutic target for preeclampsia.

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