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Aminopeptidase N in arterial hypertension

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Abstract Aminopeptidase N (APN) or CD13 is a conserved type II integral membrane zinc-dependent metalloprotease in the M1 family of ectoenzymes. APN is abundant in the kidneys and central nervous system. Identified substrates include Angiotensin III (Ang III); neuropeptides, including enkephalins and endorphins; and homones, including kallidan and somatostatin. It is developmentally expressed, a myelomonocytic marker for leukemias, and a receptor for coronovirus. There is evolving support for APN in the regulation of arterial blood pressure and the pathogenesis of hypertension. In rodent strains, intracerebraventricular (i.c.v.) infusions of APN reduces, while inhibitors of APN activity have a pressor effect on blood pressure. Dysregulation of central APN has been linked to the pathogenesis of hypertension in the spontaneously hypertensive rat. There is evidence that renal tubule APN inhibits Na flux and plays a mechanistic role in salt-adaptation. A functional polymorphism of the ANP gene has been identified in the Dahl salt-sensitive rat. Signaling by APN impacting on blood pressure is likely mediated by regulation of the metabolism of Ang III to Ang IV. Whether APN regulates arterial blood pressure in humans or is a therapeutic target for hypertension are subjects for future exploration.

Keywords Aminopeptidase N · Hypertension

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Aminopeptidase N (APN), which is also known as leucine aminopeptidase, alanyl aminopeptidase (AlaAP), aminopeptidase M, or CD13, is a conserved type II integral membrane zinc-dependent metalloprotease. This peptidase belongs to the M1 family of ectoenzymes [1–3]. Membrane-associated APN/CD13 is a non-covalently bonded homodimer with a mass of 160 kDa. Each subunit consists of 967 amino acids and contains a small (8–10 amino acid) N-terminal segment in the cytosol, a single transmembrane helical spanning domain, and an extracellular C-terminus (review [4]; Fig. 1). The APN protein is highly conserved in rabbit, rat, and human species with the differences occurring primarily in the stalk region immediately downstream from the transmembrane domain. APN contains a Zn^{2+}-binding HELAH motif. The protein is highly glycosylated in vivo, and evidence suggests that unique asparagine-linked sugar chains contain a bisecting *N*-acetylglucosamine residue [5, 6]. Hemoporphyrins and Zn^{2+} inhibit APN activity [7, 8]. A soluble form has been detected in both plasma and urine [9–11].

APN preferentially cleaves neutral amino acids from the amino terminus of oligopeptides. In biological systems, primary peptide substrates identified include neuropeptides such as Met- and Leu-enkephalins [12], neurokinin A, Met-lys-bradykinin, and endorphins [13–15]; hormones, including somatostatin [16], kallidin [17, 18], and collagen type IV [19]. In addition, APN metabolizes the heptapeptide angiotensin 2–8 (Ang III) to the hexapeptide angiotensin 3–8 (Ang IV) via cleavage of the N-terminal arginine, suggesting that this protease can regulate both systemic and tissue renin-angiotensin-aldosterone (RAS) signaling [16, 20, 21].

APN is widely expressed. In the brain, APN is most abundant in the meninges, choroid plexus, pineal gland, paraventricular nucleus, and pituitary gland, but has also

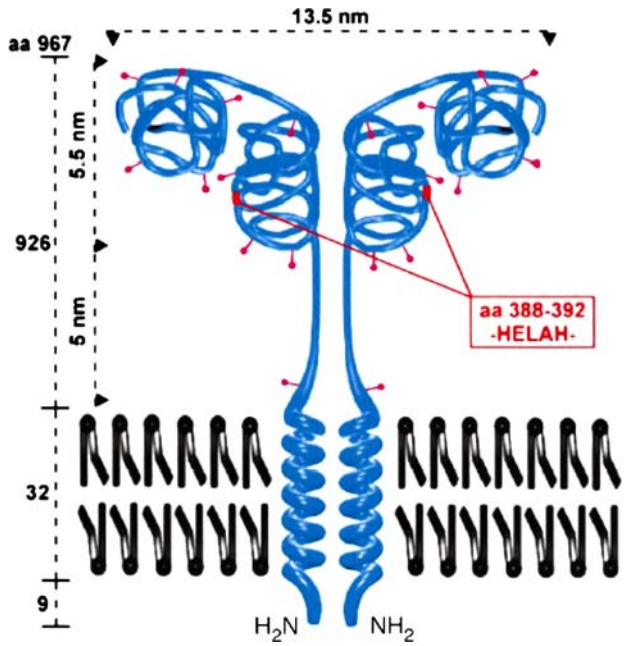

Fig. 1 Structure of aminopeptidase N. Type II homodimeric membrane with 160 kDa subunits, each with 10 glycosylation sites and 1 HELAH domain. The C-terminal domain is alleged to bind substrate and the N-terminal domain is the active center (reprinted from Ref. [31] and permission obtained from Elsevier)

been detected in the cortex, caudate-putamen, subthalamic nucleus, central periaqueductal gray, and thalamus as well as in the dorsal and ventral horn of the spinal cord, hippocampus, nucleus accumbens, substantia nigra, hypothalamus (dorsomedial and ventromedial nuclei), raphe nucleus, pontine nucleus, inferior olive, and in the granular layer of the cerebellum [22–27]. In the kidney, APN is concentrated in renal epithelial cells along the apical side of the brush border membranes, mesangial cells, and glomeruli [5, 28, 29].

APN/CD13 has been established as a myelomonocytic marker in leukemia typing [2, 19, 30, 31], as a receptor for human coronavirus 229E and cytomegalovirus [32–34], as a mediator of both inflammation and cell invasion (review [31]), and as a regulator of analgesia via metabolism of endorphins and enkephalins [35–38].

In this review, the role of APN in the regulation of blood pressure and the pathogenesis of hypertension is examined. Evidence for central and renal functions of APN is the focus.

Central APN in blood pressure regulation

A variety of studies in rats have provided evidence that central APN signaling regulates arterial blood pressure (review [39]). Intracerebraventricular (i.c.v.) infusions of the APN inhibitors bestatin and amistatin increase arterial blood pressure and are dipsogenic in normotensive (WKY and Sprague–Dawley) and hypertensive (SHR) rat strains [40–42]. In complementary studies, i.c.v. infusion of aminopeptidase M reduces arterial blood pressure in both the SHR and WKY rat strains [43, 44]. In microinfusion studies, APN placed explicitly into the paraventricular nucleus of the hypothalmous (PVN) decreases arterial blood pressure in both SHR and WKY rats, indicating that APN may specifically function to regulate blood pressure in the PVN [45, 46].

There is evidence that APN signaling in the brain is through the metabolism of Ang III to Ang IV. Ang III, versus Ang II, is the major active angiotensin metabolite in blood pressure control in the brain and i.c.v. infusion of Ang III causes a pressor response that is augmented by APN inhibitors bestatin and amistatin [47–52]. In further support of angiotensin metabolism mediating signaling by APN is the finding that (i) sarthran, a non-selective angiotensin antagonist blocks the pressor response to i.c.v. infusion of APN inhibitors [46, 51, 53] and (ii) the decrease in arterial blood pressure with i.c.v. infusion of aminopeptidase M in the SHR rats is prevented by an angiotensin type 1 receptor (AT1), as opposed to the angiotensin type 2 receptor (AT2) [54–56].

Other studies have indicated that the mechanism for central APN regulation of blood pressure may also involve secondary signaling independent of the metabolism of Ang III. Pretreatment with hexamethonium and/or an arginine vasopressin (AVP) receptor antagonist attenuates the hypotensive response to infusion of APN into the PVN in normotensive and hypertensive rat strains and i.v.c. administration of the APN inhibitor PC18 enhances vasopressin release, suggesting the involvement of signaling by vasopressin [45, 57]. The impact on arterial blood pressure of the metabolism of kinin (to bradykinin) and somatostatin by APN is not known.

A defect in receptor-mediated aminopeptidases has been postulated in the SHR rat [43, 55, 58]. Infusion of APN reduces arterial blood pressure in the SHR rat to a greater extent than in WKY rats [43]. There is reduced Ang II and Ang III metabolism, reflected in prolonged half-lives of Ang II and III and increased sensitivity to the i.c.v. injection of Ang II and Ang III, in the SHR versus WKY rat strains [59–62]. However, a reduction in metabolism of Ang III to Ang IV by APN does not fully account for the effect of APN infusion since similar decreases in central Ang II and Ang III levels have been observed in WKY and SHR rats following APN infusion even though blood pressure decreases to a greater extent in the SHR rat [43].

Renal APN in blood pressure regulation

There are several lines of evidence to suggest that renal APN regulates tubule salt handling, influences blood pressure, and plays a pathogenic role in hypertension.

First, renal tubule APN decreases basolateral Na/K ATPase, which is a preeminent Na^+ transporter in renal tubule epithelial cells, and therefore most likely regulates Na^+ flux [63]. We have linked regulation of Na/K ATPase to the production of Ang IV by APN [63]. Ang IV has been reported to promote natriuresis [64]. In the kidney, the primary Ang IV receptor (AT4) has been identified as an insulin-regulated membrane aminopeptidase (IRAP) [65, 66], and is present in human proximal and distal tubules, vascular smooth muscle, and endothelial cells [64, 67, 68]. Ang IV, via activation of AT4, stimulates mitogen-activated protein kinases (MAPKs), including p38 kinase and extracellular-signal-regulated-kinase (Erk)1/2; voltage-sensitive calcium channels; and tyrosine phosphorylation of focal adhesion kinase (FAK) and paxillin [67, 69–71]. Each of these regulates smooth muscle cell physiology and has been directly or indirectly related to blood pressure [72–75]. Infusion of Ang IV into the renal artery increases urinary Na^+ excretion and cortical blood flow [64, 76]. Ang IV inhibits transcellular Na^+ transport (measured by proximal tubule O_2 consumption rates) in rat proximal tubules [77] and Na^+ flux (reflected in increased Na^+ uptake) [67] in human kidney (HK-2) cells. We linked Ang IV/AT4 to regulation of basolateral Na/K ATPase by APN by showing that siRNA to AT4 blocked the decrease in basolateral Na/K ATPase activity and abundance associated with over-expression of APN in LLCPK-1 cells [63]. We speculate that reducing Ang III levels may also be a mechanism by which APN reduces arterial blood pressure and induces a natriuresis since Ang III, like Ang II, is a vasoconstrictor and pressor agent [78–80]. In support of APN reducing Ang III signaling is the finding that inhibitors of APN increased AT2-mediated natriuresis and sensitivity to Ang III in AT1 receptor-blocked mice [56]. Thus, there is the indication that APN may promote natriuresis both by increasing Ang IV/AT4 and reducing Ang III signaling.

Secondly, renal APN expression may be regulated by salt and reduced in hypertension. We have reported renal APN abundance and activity are increased by a high salt diet (8% vs. 0.8% NaCl) in salt-resistant rat strains, i.e., Dahl salt-resistant (SR) rats and Sprague–Dawley rats [81]. Consistent with increased APN activity is a reduction in renal Ang II/Ang III and increase in AT4 binding in SR rat strains in response to a salt-challenge [82, 83]. In contrast, renal APN does not increase in the Dahl salt-sensitive (SS) rat challenged with a high salt diet. Together, these results raise the possibility that renal APN regulates normal adaptation to high salt conditions and that dysregulation of APN contributes to salt sensitivity in the Dahl SS rat.

In a rodent nitric oxide inhibition model of hypertension, i.e., rats treated with *N*(omega)-nitro-l-arginine methyl ester (L-NAME), less renal, aortic, and serum APN activity than in controls has been reported [84, 85]. Although further studies are needed to determine the significance of APN in this model, a decrease in APN activity could be linked to the reported increase in the pressor response to Ang II by decreasing Ang III metabolism [86].

Thirdly, the APN gene maps to a reported quantitative trait locus (QTL) for hypertension in Dahl SS X Dahl SR rats on chromosome 1 and contains in the Dahl SS rat a functional single nucleotide polymorphism (SNP) four nucleotides upstream from a CCAAT/enhancer binding protein motif (CEBP-cis element; nucleotides −2256 to −2267) that is associated with CEBP binding and increased promotor activity of the 5′ flanking region [87]. Although this SNP is intriguing, transgenic and fine-mapping studies are needed to establish the relationship between the APN gene and hypertension in the Dahl rat.

Whether there are polymorphisms of the ANP gene in other models of genetic hypertension and, if so, whether they are linked to hypertension remains to be investigated. In the Milan Hypertensive (MHS) rat strain, greater renal cell membrane aminopeptidase M abundance than in the corresponding normotensive strain, i.e., Milan Normotensive (MNS), was identified in comparisons of 2D electrophoresis patterns of the two contrasting strains of rat [88]. However, Salardi et al. [88] showed that this is not genetically linked to hypertension in the MHS strain by generating recombinant strains in which the traits of hypertension and faster sodium transport do not cosegregate with the difference in expression or activity of APN.

APN and human arterial hypertension

Genetic association studies have raised the prospect that APN may be linked to human arterial hypertension. The human APN gene contains 20 exons and maps to chromosome 15q25–26 [89]. Thirty-three polymorphisms of adipocyte-derived APN have been identified from 48 unrelated Japanese individuals [90]. Two of the eight missense polymorphisms (Ile276Met and Lys528Arg) are reported to have a significant association with arterial blood pressure. Lys528Arg has been associated with essential and non-modulating hypertension [90, 91]. A reduction in the extent of regression of left ventricular hypertrophy following treatment with irbesartan, an AT1 receptor antagonist, has also been reported in patients with this polymorphism [92]. The Lys528Arg polymorphism has been shown to reduce enzymatic activity of APN by altering the tertiary structure and, in turn, reducing substrate binding Goto et al. [93]. Further analysis and examination of hypertensive subpopulations are needed to determine the significance of these mutations.

The future

As with most basic studies and animal work, the ultimate goal is to transition from experimental results to human disease. In vivo measurements of APN and angiotensin metabolites in human hypertensive patients are paramount. Increased urinary levels of APN have been linked to human renal transplant rejection [94] and renal cell carcinoma [95]; however, the significance of this increase is not clear with respect to blood pressure. Investigation of the role of aminopeptidases in general and of APN in human hypertension is needed.

The anti-hypertensive potential of inhibitors of both brain aspartyl aminopeptidase, which converts Ang I to Angiotensin 2–10 Aminopeptidase A (GluAP) and metabolizes Ang II to Ang III, have been evaluated previously [39]. Significant development of APN inhibitors as therapeutic agents to target a variety of malignancies and inflammatory diseases has occurred [2]. Studies to evaluate APN as a therapeutic target for arterial hypertension, specifically focusing on defined subsets, e.g., salt-sensitive patients and these agents, may provide insight. Because APN signals through the angiotensin metabolites, Ang III, Ang IV, and AT4, these also may be therapeutic agents and targets.

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