Institute of Experimental Morphology, Pathology and Anthropology with Museum
Bulgarian Anatomical Society

Acta morphologica et anthropologica, 20
Sofia • 2014

# Aminopeptidase A in different diseases: a minireview

*V. Petrova, M. Dimitrova, I. Ivanov**

*Institute of Experimental Morphology, Pathology and Anthropology with Museum, Bulgarian Academy of Sciences, Sofia, Bulgaria*
**Faculty of Biology, Sofia University "St. Kl. Ohridski"*

Aminopeptidase A is an ectoenzyme, widely expressed in mammals and humans. It performs important physiological functions. One of these functions is to play a role in the control of blood pressure by converting angiotensin II to angiotensin III in the brain. Aminopeptidase A is involved in development of preeclampsia during pregnancy. It is expressed in many malignant neoplastic lesions and can serve as a biomarker for neoangiogenesis. The aim of the present review is to summarize the existing data about aminopeptidase A and its role for different diseases.

*Key words:* Aminopeptidase A, renin-angiotensin system, tumor biomarker, neoangiogenesis

## Name and mammalian tissue distribution

Aminopeptidase A (APA, EC 3.4.11.7) was first identified in rat and guinea pig kidney sections where the enzyme catalyzes the hydrolysis of N-(α-L-glutamyl)-β-naphthylamide [7]. Subsequently, the enzyme was found to hydrolyze N-terminal aspartyl residues and was named Aminopeptidase A [7]. Since α-L-glytamyl derivatives are more efficiently hydrolyzed than are α-L-aspartyl derivatives, the enzyme is named glutamyl aminopeptidase, too. Different substrates had been used to determine the activity of Aminopeptidase A in preparations from different sources. That is why the enzyme has a lot of names – aspartate aminopeptidase, angiotensinase A, $Ca^{2+}$-activated glutamate aminopeptidase, membrane aminopeptidase II and the BP-1/6C3 antigen [22].

Tissue distribution of APA had been revealed by using immunohistochemistry [12]. In mammals, the highest APA levels had been detected in the intestinal brush border and kidney proximal tubules [27]. In the brain, APA is localized primarily in microvascular elements, the choroid plexus, and the ependymal lining [8]. The enzyme is expressed also in the capillary endothelium of all the studied organs.

## Structure and regulation

APA is a membrane-bound zinc metallopeptidase [5]. The enzyme cleaves specifically the N-terminal glutamyl or aspartyl residues from peptide substrates, such as angiotensin II and cholecystokinin-8 [25]. The pH optimum of APA depends on the source and the peptide substrate and varies in the interval 7.0-8.0 [22]. The enzyme is composed of a small N-terminal cytoplasmic domain (17 residues), a 22-residue transmembrane domain and a large extracellular C-terminal domain that contains the active site [30]. Its activity is modulated by calcium ions. In the molecule of APA $Ca^{2+}$-binding site is situated in the immediate vicinity to the catalytic $Zn^{2+}$ [33] to allow the correct orientation of the substrate in the enzyme active center. APA is sensitive to inhibition by metal chelating agents [4] and is completely inhibited by transitional metal ions such as $Zn^{2+}$, $Ni^{2+}$, $Cu^{2+}$, $Hg^{2+}$ and $Cd^{2+}$ [22]. EC33 [(S)-3-amino-4-mercapto-butyl sulfonic acid] is a specific and selective inhibitor of APA. In vivo experiments of APA inhibition are usually made using RB150 – a dimmer of EC33 generated by creating a disulfide bond [2]. In contrast to EC33, this substance is able to cross the blood brain barrier. In the brain, disulfide bridge is degraded by reductases to release two molecules EC33, effectively inhibiting APA.

## Aminopeptidase A in T and B cell development

APA is expressed on the pre-B and immature B cells [13]. The enzyme is also present on bone marrow-derived stromal cells and cortical epithelial cells of the thymus. A mouse model of BP-1 deficiency had been used to explore the physiologic role of APA in T- and B- cells maturation [13]. Those cells development appeared to be normal suggesting that APA is not essential for this process, possibly because the APA deficiency is compensated by other peptidases [22].

## Regulation of blood pressure

*APA as a therapeutic target for hypertension*

APA is present in several brain nuclei containing nerve terminals and AT1 receptors involved in blood pressure regulation [14]. Those data suggest that the enzyme is an integral component of the brain RAS in humans and rodents and plays a role in blood pressure regulation [16]. Many experiments show that in both central and peripheral RAS APA is responsible for the conversion of angiotensin II (AngII) to angiotensin III (AngIII). Both AngII and III possess a similar affinity to AT1 receptors [31]. AngII is a principal effector peptide of RAS, which induces vasoconstriction and increases sodium and water retention leading to an increase in blood pressure [16]. AngIII exerts a tonic stimulation and affects the control of blood pressure [23]. Whereas AngII and AngIII are believed to be of almost equivalent importance in the maintenance of central blood pressure, AngII is the most important peripheral agonist acting on AT1 receptors [18].

Overacting of RAS is responsible for the development of hypertension [23]. Thus, spontaneously hypertensive rats exhibit RAS hyperactivity and a significantly higher APA activity than normotensive rats, suggesting the enzyme contribution to increased blood pressure [34]. Intracerebroventricular (i.c.v.) injection of APA specific inhibitor EC33 in rats, leads to a decrease in blood pressure and activates the degradation of brain AngII by other peptidases (angiotensin-converting enzyme 2, endopeptidases or others) leading to a formation of peptides, inactive to AT1 receptors [9]. On the other hand, APA infusion by i.c.v. results in a significant increase in blood pressure [32]. Those ex-

periments confirm the leading role of AngIII and the enzyme generating it (APA) in the regulation of central blood pressure [23]. According to the above studies, APA which generates AngIII may be considered a potential therapeutic target for the treatment of hypertension. The enzyme specific inhibitors are currently tested with a view to a possible clinical use [14].

On the other hand, APA-deficient mice are known to develop a mild hypertension [18]. It has been speculated that the total absence of APA during fetal and adult life may induce compensatory mechanisms of yet unknown nature for blood pressure regulation resulting in a slight hypertensive effect [14].

## Role of APA in preeclampsia

The human fetus produces bioactive peptides such as oxytocin and vasopressin, as well as angiotensin II [21]. These peptides are highly uterotonic and vasoactive [17] and their secretion increases alongside with the fetus growth or under the action of stress factors. The peptides have low-molecular weight, so they can pass through the fetoplacental unit and affect the maternal organism [17]. It is believed that preeclampsia – a hypertensive disorder during pregnancy is caused by an overproduction of AngII in the fetus due to a failure in APA production and/or activation, since APA is the main enzyme responsible for degradation of AngII [16]. The high levels of AngII in fetus result in an increase in AngII concentration in maternal serum as well, and a subsequent raise in blood pressure. The experiments show that before and immediately after development of preeclampsia APA levels in maternal blood serum are substantially increased pointing out at a response to counter AngII increase [19]. The main goal of the treatment of preeclampsia is to decrease only maternal blood pressure without affecting the fetus. APA has a molecular weight of 109 kDa and does not cross the placental barrier [18]. In this respect, APA is an important candidate for the treatment of preeclampsia by its infusion in maternal organism.

## APA and local renin-angiotensin systems

The renin-angiotensin systems are two types – systemic and local [11]. The systemic RAS regulates blood pressure, electrolyte and fluid homeostasis. The local RASs play autocrine, paracrine and intracrine physiological roles. These local RASs have been found in a lot of organ systems such as pancreas, heart, kidney, vasculature and adipose tissue, nervous, reproductive and digestive systems [11]. APA is a part of RAS. In the pancreas, the local RAS plays an important role in regulating local blood flow, control the secretion of digestive enzymes, glucose – stimulated insulin release, etc. [11]. Studies show that the pancreatic RAS components are responsive to various stimuli, including hypoxia, pancreatitis, hyperglycaemia, diabetes mellitus type 2, and pancreatic cancer [11]. The role of APA in those pathological conditions remains to be evaluated in the future.

## Participation of aminopeptidase A in angiogenesis and tumorigenesis

Neovascularization consists of vasculogenesis and angiogenesis. Vasculogenesis is a process of formation of new capillaries from angioblasts and angiogenesis is a development of pre-existing vessels [24]. Angiogenesis is a result from a complex of interactions between vascular cells and cells from the surrounding environment [28]. Angio-

genic vasculature is a target for therapy in cancer [29]. Aminopeptidase A is expressed in blood vessels from several types of human tumors and is undetectable or barely detectable in normal vasculature [15]. Studies show that APA – deficient mice have a decreased neovascularization. Treatment of mice who have tumors with APA – inhibitors shows reduction of tumor growth [15].

Aminopeptidase A is expressed in neoplastic lesions of the uterine cervix and its expression is upregulated as the lesion progresses from cervical intraepithelial neoplasm toward invasive squamous cell carcinomas [26]. Studies show that APA may play a promoting role in neoplastic transformation and disease progression in cervical neoplasm [6]. Immunohistochemical studies show that APA is strongly expressed at the invasive front of the tumor lesions [26]. These findings support the fact that tumor–stromal interaction is essential for the expression of aminopeptidases, including APA in these types of tumors. In other kinds of tumors like Angiotensin II-mediated cervical cancer, overexpression of APA reduces the invasive potential[26]. That is so because Angiotensin II is not only a vasoconstrictor but it is also a growth factor that stimulates cell migration and invasiveness of some kinds of tumors[20].

APA is normally expressed in the brush-border membrane of renal tubules where it takes part in the luminal hydrolisis of polypeptides. Recent studies show that APA is over-expressed in clear cell renal cell carcinoma patients. However, the enzyme activity measured biochemically, is lower in comparison to the normal renal tissue [29]. This discrepancy could be explained either by inhibition of catalytic activity throughout the action of yet unknown cellular factors or by point mutation in the zinc binding motif of the protein. Obviously, APAis involved in pathogenesis of renal cancer, although the mechanism of this involvement remains to be elucidated in future studies [29].

Aminopeptidase A is expressed in human malignant gliomas and metastatic carcinomas in the brain [15]. The enzyme is overexpressed in perivascular cells and it is enzymatically active. APA may play a role in several functions such as secretion of growth factors, modulation of the extracellular matrix and regulation of vascular permeability [15].

The expression of aminopeptidase A has been detected in other kinds of tumors. Studies show that benign prostatic stroma exhibit no APA expression, but stromal cells surrounding prostatic carcinoma cells demonstrate an increased APA expression [3].

Fibroblasts are heterogeneous group of structural cells whose function is to produce all the precursors for extracellular matrix [1]. They take part in maintaining and repairing the normal tissue. They also synthesize and respond to a lot of cytokines and

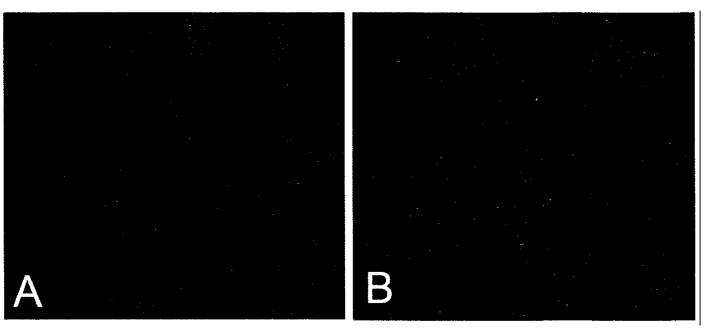


Fig. 1 Cytochemical demonstration of APA activity using a novel fluorescent method. Low enzyme activity in normal mouse fibroblasts (A); a substantially higher APA activity in mouse fibrosarcoma cells (B). 400x

mediators and are involved in the process of inflammation and healing [1]. Fibroblasts participate in tumorigenesis as they stimulate premalignant and malignant epithelial cells to proliferate and to form tumors in mice [10].

Recently, we examined the expression of APA in normal fibroblasts and in fibroblasts from mouse fibroblastoma using enzyme histochemistry. The results showed that in normal fibroblasts APA was weakly expressed but in fibroblasts from fibrosarcoma APA was visibly more active (Fig. 1).

In view of these preliminary results, it seems possible that the enzyme is involved in the regulation of malignant stromae fibroblasts. Since fibroblasts are the main cell type in all kinds of solid tumor stromae, it would be valuable to continue the above studies in order to elucidate the enzyme participation in the formation of tumor microenvironment.

In conclusion, APA activity is important for tumorigenesis. The enzyme role in different types of tumors deserves to be studied in order to establish its diagnostic and/or prognostic value.

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