Current Hypertension Reports (2019) 21: 50
https://doi.org/10.1007/s11906-019-0957-4

ANTIHYPERTENSIVE AGENTS: MECHANISMS OF DRUG ACTION (ME ERNST, SECTION EDITOR)

Orally Active Aminopeptidase A Inhibitor Prodrugs: Current State and Future Directions

Mathilde Keck[1,2] · Reda Hmazzou[1] · Catherine Llorens-Cortes[1]

Published online: 21 May 2019

Abstract

Purpose of Review To review the data supporting the use of aminopeptidase A (APA) inhibitor prodrugs as centrally acting antihypertensive agents.

Recent Findings Brain renin–angiotensin system (RAS) hyperactivity has been implicated in the development and maintenance of hypertension. Angiotensin III, generated by APA, one of the main effector peptides of the brain RAS, exerts a tonic stimulatory control over blood pressure in hypertensive rats. This identified brain APA as a potential therapeutic target for the treatment of hypertension, leading to the development of RB150/firibastat, an orally active prodrug of the specific and selective APA inhibitor, EC33. When given orally, RB150/firibastat crosses the gastrointestinal and blood–brain barriers, enters the brain, and generates two active molecules of EC33 which inhibit brain APA activity, blocking brain angiotensin III formation, and decrease blood pressure for several hours in hypertensive rats.

Summary Orally active APA inhibitor prodrugs, by blocking brain RAS activity, represent promising novel strategy for treating hypertension.

Keywords Aminopeptidase A inhibitor · Brain renin–angiotensin system · Hypertension

Introduction

Hypertension affects one third of the adult population and is one of the most important risk factors of cardiovascular diseases [1]. Several pharmacological treatments are available for effective blood pressure (BP) control [2]. Moreover, monotherapy for hypertension treatment is ineffective in more than 50% of all cases and most hypertensive patients require two or more antihypertensive drugs [3–5]. However, the overall incidence of resistant hypertension to at least three antihypertensive drugs (including a diuretic) has been estimated at 15% of the hypertensive population in the USA [6], highlighting the need to develop new classes of antihypertensive agents acting on new targets, with diversified modes of action, to improve BP control.

Several studies have implicated brain renin–angiotensin system (RAS) hyperactivity in the development and maintenance of hypertension [7–10]. All known components of the systemic RAS, including the precursor and enzymes required for the production and metabolism of angiotensin peptides and specific angiotensin II type 1 and type 2 receptors (AT_1R and AT_2R), have been identified in the brain [11]. Among the effector peptides of the brain RAS, angiotensin II (AngII) and angiotensin III (AngIII) display similar affinities for AT_1Rs and AT_2Rs. By acting on AT_1R, both peptides equally increase BP and arginine-vasopressin (AVP) release [12]. We previously reported that the brain aminopeptidase A (APA, EC 3.4.11.7), a membrane-bound zinc metalloprotease,

This article is part of the Topical Collection on *Antihypertensive Agents: Mechanisms of Drug Action*

✉ Catherine Llorens-Cortes
c.llorens-cortes@college-de-france.fr

[1] Laboratory of Central Neuropeptides in the Regulation of Body Fluid Homeostasis and Cardiovascular Functions, Collège de France, Center for Interdisciplinary Research in Biology (CIRB), INSERM U1050/CNRS UMR 7241, 11 Place Marcelin Berthelot, 75005 Paris, France

[2] Quantum Genomics, 33 rue Marbeuf, 75008 Paris, France

generates AngIII from AngII and that aminopeptidase N (APN, EC 3.4.11.2), another zinc metalloprotease, metabolizes AngIII into angiotensin IV (AngIV) [13].

Identification of the In Vivo Metabolic Pathways of Brain AngII and AngIII

Among the enzymes potentially able to hydrolyze AngII and AngIII, two membrane-bound zinc metallopeptidases [14–16], aminopeptidase A (APA: EC3.4.11.7) and aminopeptidase N (APN: EC 3.4.11.2), are particularly good candidates for this function. It is well established that, in vitro, purified APA hydrolyzes the N-terminal Asp of AngII to generate AngIII (reviewed in [17]), whereas purified APN hydrolyzes the N-terminal Arg of AngIII to generate AngIV [18]. Specific and selective APA and APN inhibitors were designed to determine whether APA and/or APN hydrolyze in vivo brain AngII and/or AngIII: the specific and selective APA inhibitor, EC33 (3S)-3-amino-4-sulfanyl-butane-1-sulfonic acid, in which the carboxylate of the side chain of the glutamate thiol is replaced by a sulfonate [15], and the APN inhibitor, PC18 (2-amino-4-methylsulfonyl butane thiol) [14]. Studies in vitro with purified APA and APN showed that EC33 inhibited APA almost 100 times more strongly than it inhibited APN, whereas PC18 inhibited APN 2150 times more strongly than it inhibited APA.

These compounds were used in vivo by Zini et al. [13] and Reaux et al. [18] to study the metabolism of brain angiotensins. Conscious mice were injected intracerebroventricularly with radiolabeled angiotensins in the presence or absence of EC33 or PC18, and the kinetics of the appearance and disappearance of radiolabeled AngIII in the hypothalamus were evaluated. EC33 completely blocked the formation of [^{3}H]AngIII, whereas PC18 increased the half-life of [^{3}H]AngIII 3.9-fold. These studies provided the demonstration that APA is involved in vivo in the formation of brain AngIII from AngII, whereas APN metabolizes AngIII into AngIV (Fig. 1).

Brain AngIII in the Control of Blood Pressure

Either AngII or AngIII injected centrally causes dose-dependent pressor responses [11, 19–21] by three mechanisms: (i) an increase in sympathetic nerve activity, (ii)

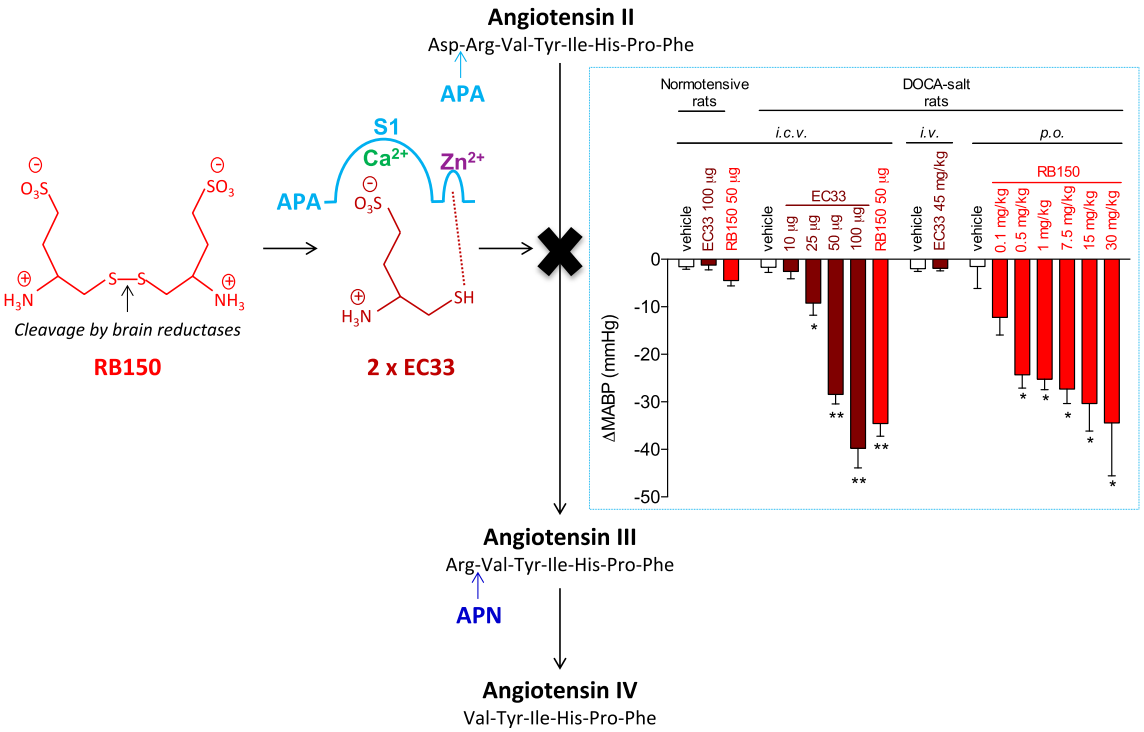

Fig. 1 Metabolic pathways of angiotensin II and angiotensin III in the brain involving the zinc metalloproteases, aminopeptidase A (APA), and aminopeptidase N (APN), and effects of intracerebroventricular (i.c.v.) or intravenous (i.v.) or per os (p.o.) administration of the APA inhibitor EC33 and its prodrug RB150 on mean arterial blood pressure (MABP) in normotensive and hypertensive DOCA–salt rats. We observed that i.c.v. injection of EC33 and RB150 significantly decrease MABP in hypertensive DOCA–salt rats. This a central but not a peripheral effect since, even at a high dose of 45 mg/kg, the APA inhibitor EC33 injected by i.v. route does not modify MABP. However, the APA inhibitor prodrug RB150 given by oral route crosses the intestinal, hepatic, and blood–brain barriers; enters the brain; and generates two active molecules of EC33 which inhibit brain APA activity, resulting in a dose-dependent decrease in MABP. No decrease in MABP was observed in normotensive rats after i.c.v. injection of EC33 or RB150, suggesting EC33 is an antihypertensive agent and not a hypotensive agent

synaptic inhibition of the baroreflex in the nucleus of the tractus solitarius, and (iii) a release of AVP into the bloodstream [21]. Several studies have tried to determine the respective roles of AngII and AngIII in the central control of BP [22–26]. However, these results do not differentiate between AngII and AngIII as the possible effector peptide responsible for the increase in BP.

To provide new insight into this issue, we investigated the effects of AngII and AngIII, injected i.c.v., on BP in hypertensive rats in the absence and presence of APA or APN inhibitors, EC33 and PC18 respectively. Two experimental models of hypertension were used: the SHR, a genetic model of hypertension sensitive to systemic RAS blockers, and the DOCA–salt rat, a salt- and volume-dependent but renin-independent (low plasma renin levels) model of hypertension resistant to systemic RAS blockers. We demonstrated that the i.c.v. injection of EC33 leads to an immediate and total inhibition of brain APA activity, thus blocking in the brain the conversion of AngII to AngIII and inducing an antihypertensive effect in these two experimental models of hypertension, SHR [17] and DOCA–salt rats [16]. In contrast, a high intravenous dose of EC33 did not modify BP of hypertensive rats, demonstrating that the i.c.v. EC33-induced decrease in BP is not due to a systemic effect [16]. Consistent with these data, other groups showed that, despite the high molecular mass of APA and APN (approximately 120–130 kDa for the monomer), the i.c.v. infusion of APA produces a significant increase in BP [26], whereas the i.c.v. infusion of APN in SHR rats decreases BP [27]. The pressor effect probably results from a higher level of production of brain AngIII, whereas the hypotensive effect might be related to an increase in AngIII metabolism.

Brain APA, the enzyme responsible for generating brain AngIII, therefore constitutes a promising target for hypertension treatment, justifying the development of potent and selective APA inhibitors as centrally acting antihypertensive agents.

Orally Active Aminopeptidase A Inhibitor Prodrugs as Centrally Acting Agents for the Treatment of Hypertension in Animal Experimental Models

For APA inhibitors to be used as central antihypertensive agents, they must be able to cross the blood–brain barrier (BBB) and inhibit the activity of brain APA after their oral administration. The central bioavailability of thiol inhibitors of zinc metallopeptidases, such as neutral endopeptidase 24.11 (NEP) and APN, has been shown to be enhanced by designing prodrugs in which the compound is dimerized by the formation of a disulfide bridge [28]. We applied a similar approach to increase the bioavailability of the APA inhibitor EC33 by dimerization involving a disulfide bond, to generate the prodrug RB150 (4,4-dithio{bis[(3S)-3-aminobutyl sulfonic acid]}) [16, 29]. Orally administered RB150 crosses the intestinal, hepatic, and blood–brain barriers, entering the brain, where the disulfide bridge is immediately cleaved by brain reductases to generate two active molecules of EC33, which inhibit brain APA activity, block the formation of brain AngIII, and decrease BP and AVP release in alert hypertensive rats [16, 30–32]. The RB150-induced BP decrease began 2 h after administration, was maximal between 5 and 9 h, remained after 15 h but not significantly, and disappeared after 24 h [30] (Fig. 1). This effect is due to decrease in AVP release into the bloodstream, a reduction in sympathetic tone, and an improvement in baroreflex function [16, 30–32].

However, the high dose of orally active RB150 required to decrease BP in SHR and DOCA–salt rats [30, 32] led us to develop new more potent and selective APA inhibitor prodrugs with greater bioavailability for inhibiting brain APA activity. We recently reported the development of a novel central acting APA inhibitor prodrug obtained by disulfide bridge-mediated dimerization of NI929 ((3S,4S)-3-amino-4-mercapto-6-phenyl-hexane-1-sulfonic acid), a non-peptidic APA inhibitor that is 10 times more potent than EC33 at inhibiting recombinant mouse APA activity in vitro (K_i value of 30 nM) [29]. We showed the high potency of oral NI956/QGC006 treatment for normalizing brain APA hyperactivity and BP for 10 h after a single administration, decreasing plasma AVP levels, and increasing diuresis and natriuresis, without affecting plasma sodium and potassium concentrations, at a dose one tenth of that required for RB150, in an experimental salt-dependent model of hypertension [33••] (Table 1). Therefore, NI956/QGC006 is a "best-in-class" central acting APA inhibitor prodrug, belonging to the same drug class as RB150, supporting the development of treatments targeting brain APA for hypertension treatment.

In conclusion, these studies demonstrated that orally active APA inhibitor prodrugs could act as novel antihypertensive agents.

Orally Active Brain-Penetrating Aminopeptidase A Inhibitor Prodrugs in Clinical Trials

RB150, renamed firibastat by the WHO, was selected for clinical development. In a first-in-human study, the safety, tolerability, pharmacokinetics, and pharmacodynamic effects of single ascending doses of RB150/firibastat (phase Ia) were determined in humans [35]. Single oral administration of RB150/firibastat up to 2000 mg in healthy volunteers was shown to be safe and well tolerated [35]. As in animal experiments, in normotensive subjects, RB150/firibastat had no effect on BP and HR, either on the systemic RAS parameters or on plasma copeptin concentrations, a marker of AVP release. These results support further evaluation of multiple oral doses of RB150/

Table 1 In vitro and in vivo characteristics of the two aminopeptidase A inhibitor prodrugs, RB150/firibastat and NI956/QGC006, in normotensive WKY and hypertensive DOCA–salt rats

In vitro parameters		RB150/firibastat	NI956/QGC006 [33]
Mode of binding to APA active site		S1 subsite [34]	S1 and S1' subsites
Inhibitory potency (K_i) on APA		$2.0 \pm 0.2 \times 10^{-7}$ M [16]	$3.1 \pm 0.5 \times 10^{-8}$ M
In vivo parameters (change)		RB150/firibastat (50 mg/kg) [30]	NI956/QGC006 (4 mg/kg) [33]
Brain APA activity	DOCA–salt rat	− 34% 3.5 h after treatment	− 38% 4 h after treatment
MABP	DOCA–salt rat	− 49 ± 10 mmHg 5 h after treatment	− 44 ± 13 mmHg 5 h after treatment
	WKY rat	No change	No change
Plasma AVP levels	DOCA–salt rat	− 32% 3.5 h after treatment	− 41% 4 h after treatment
	WKY rat	No change	No change
Diuresis	DOCA–salt rat	+ 146% 5 h after treatment	+ 86% 5 h after treatment
	WKY rat	No change	No change
Natriuresis	DOCA–salt rat	+ 61% 5 h after treatment	+ 93% 5 h after treatment
	WKY rat	No change	No change
Plasma sodium and potassium concentrations	DOCA–salt rat	No change	No change
	WKY rat	No change	No change

firibastat in human volunteers. In a second clinical study (phase Ib), the safety and the tolerability of a multiple oral doses up to at least 2 × 750 mg/day for 7 days of RB150/firibastat were confirmed in healthy adult subjects (F. Balavoine, M. Azizi, D. Bergerot, N. De Mota, R. Patouret, B. P. Roques, and C. Llorens-Cortes, unpublished work).

Then, a pilot multicenter double-blind randomized placebo-controlled crossover pharmacodynamic study (phase IIa) was conducted to evaluate the BP and the hormonal effects of RB150/firibastat, in patients with hypertension [36••]. After a 2-week run-in period, 34 patients (with daytime ambulatory BP of at least 135/85 mmHg and less than 170/105 mmHg) were randomly assigned to receive either RB150/firibastat (250 mg b.i.d. for 1 week uptitrated to 500 mg b.i.d. for 3 weeks) and then placebo for 4 weeks each or vice versa, with a 2-week washout period on placebo. The results of this pharmacodynamic study showed that brain APA inhibition with a 4-week RB150/firibastat treatment, in patients with mild hypertension, tended to decrease daytime ambulatory and office systolic BP relative to placebo [36••]. The more the basal daytime ambulatory systolic BP is elevated, the more the RB150/firibastat-induced systolic BP decrease is majored. This is in agreement with the observation that, in experimental models of hypertension, RB150/firibastat acted as an antihypertensive agent and not as a hypotensive agent, knowing that this compound decreased BP in hypertensive but not in normotensive rats [30–32]. Importantly, there were no major safety events. In addition, RB150/firibastat did not modify the activity of the systemic RAS, in patients with hypertension.

These results were subsequently used to guide the design of a large clinical trial phase IIb in the USA called NEW-HOPE (ClinicalTrials.gov Identifier: NCT03198793) [37]. Two hundred and fifty-six overweight hypertensive patients were recruited, including a high proportion of African-American and Hispanic patients which showed a significant BP-lowering efficacy and a safe tolerability profile. They received an 8-week treatment with RB150/firibastat: 250 mg b.i.d. orally for 2 weeks and then 500 mg b.i.d for 6 weeks. Safe tolerability and efficacy of RB150/firibastat in lowering BP was proven in all subgroup analyses, including age, sex, ethnic origin, and weight [38], supporting the development of RB150/firibastat for hypertension in patients for whom optimal BP control is difficult to obtain with currently available antihypertensive treatments.

Potential New Directions for Orally Active Aminopeptidase A Inhibitor Prodrugs

There is evidence that the brain RAS plays a key role in the progression of heart failure (HF) after myocardial infarction (MI). In fact, the hyperactivity of the brain RAS post-MI leads to an increase in sympathetic neuron activity and AVP release, contributing to left ventricular (LV) remodeling and dysfunction [39–41]. Central and systemic AT_1R blockade with losartan prevents sympathetic hyperactivity and markedly

attenuates cardiac fibrosis and lowers LV end-diastolic pressure (LVEDP) post-MI [42, 43]. However, only central infusion of losartan improves LV systolic function, relative to systemic infusion [42, 43], suggesting that inhibition of brain APA may counteracts LV dilation and dysfunction post-MI. Since RB150 treatment blocks brain RAS hyperactivity, Huang et al. evaluate the effects of such a treatment on the development of HF post-MI [44]. Continuous i.c.v. infusion of RB150 for 4 weeks after MI in rats attenuates sympathetic hyperactivity, normalizes brain APA hyperactivity, improves baroreflex function, and prevents cardiac dysfunction [44]. With a view to the potential clinical use of RB150 for HF treatment, we recently investigated the effects of brain APA inhibition by chronic oral treatment with RB150 for 4 weeks, comparing the effects on LV remodeling of this treatment and systemic ACE inhibition by enalapril in mice post-MI [45•]. Our data suggest that chronic oral RB150 treatment in mice post-MI normalizes brain APA hyperactivity, thereby normalizing brain RAS and sympathetic hyperactivity, whilst preventing cardiac dysfunction and attenuating cardiac hypertrophy and fibrosis [45•]. Moreover, RB150 treatment was as effective as enalapril treatment [45•]. Similar data were obtained in rats post-MI orally treated with RB150 for 4 weeks after MI [46]. In conclusion, orally active APA inhibitor prodrugs may, therefore, constitute a potential new class of therapeutic agents for the treatment of post-MI HF. A phase IIb clinical trial in Europe and the USA, called QUORUM, sponsored by Quantum Genomics, aimed to evaluate the efficacy and safety of RB150/firibastat compared to ramipril in HF patients after acute MI [47]. This study should constitute a proof-of-concept in a disease needing new therapeutic approaches to reduce the morbidity and mortality of post-MI HF.

Hyperactivity of RAS is associated with the pathogenesis of Alzheimer's disease believed to be mediated by the activation of AT_1Rs by AngII and/or AngIII since both peptides have the same affinity for AT_1Rs [48]. The group of J. Scott Miners measured in human postmortem mid-frontal cortex in a cohort of Alzheimer's disease patients and age-matched non-demented controls, AngII and AngIII levels, and APA and APN expression [49]. They found that both brain AngII and AngIII levels were significantly higher in Alzheimer's disease patients compared to age-matched controls and that brain AngIII variations were strongly associated with those of amyloid beta peptide and tau levels, the core pathological components of Alzheimer's disease. The authors proposed that the increase in brain AngIII levels was due to an increase in the conversion of brain AngII in AngIII by APA together with a reduction in the metabolism of brain AngIII in AngIV by APN [49]. These data suggest that elevated brain AngIII levels could contribute to the pathogenesis of Alzheimer's disease. Therefore, blocking brain AngIII formation with an orally active APA inhibitor prodrug in animal models of Alzheimer's disease could prevent progression of the disease.

Conclusion

Development of selective and specific APA inhibitors and their prodrugs have led to the demonstration that brain AngIII, generated by APA, is a major effector peptide of the brain RAS, exerting central tonic stimulatory control over BP in hypertensive rats, and that the inhibition of brain APA leads to a large and sustained decrease in BP via inhibition of systemic AVP release, sympathetic neuron activity, and improvement of the baroreflex function in conscious hypertensive animals. A large clinical trial phase IIb in overweight hypertensive patients of whom 50% were African- or Hispanic Americans, showed a significant BP-lowering efficacy and a safe tolerability profile of 8-week treatment with RB150/firibastat, underlining the adequacy between pre-clinical and clinical studies. Therefore, the focus on drugs causing effective blockade of brain APA constitutes new directions for BP control.

Acknowledgments We thank all of our collaborators for their contributions to work on the aminopeptidase A inhibitors, especially Professors B. Roques, M.-C. Fournie-Zaluski, N. Inguimbert, and Doctor E. Chauvel, who designed and synthesized the specific and selective aminopeptidase A and aminopeptidase N inhibitors.

Funding Our own work described in the present paper was supported from the *Agence Nationale de la Recherche* ("BIOTECS," RBIB CLINAPAI and "LabCom CARDIOBAPAI"), the *Institut National de la Santé et de la Recherche Médicale* (INSERM), the *Collège de France*, the *Fondation pour la Recherche Médicale*, and the Quantum Genomics Company.

Compliance with Ethics Guidelines

Conflict of Interest MK is a full-time employee of Quantum Genomics SA. No conflicts of interest, financial or otherwise, are declared by the other co-authors.

Human and Animal Rights and Informed Consent All human and animal studies have been approved by the appropriate Ethics Committee and have been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki and its later amendments.

All persons gave their informed consent prior to their inclusion in the study.

References

Papers of particular interest, published recently, have been highlighted as:
- • Of importance
- •• Of major importance

1. Benjamin EJ, Blaha MJ, Chiuve SE, Cushman M, Das SR, Deo R, et al. Heart disease and stroke statistics—2017 update: a report from the American Heart Association. Circulation. 2017;135:e146–603.
2. Mancia G, Fagard R, Narkiewicz K, Redon J, Zanchetti A, Böhm M, et al. 2013 ESH/ESC guidelines for the management of arterial

hypertension: the Task Force for the Management of Arterial Hypertension of the European Society of Hypertension (ESH) and of the European Society of Cardiology (ESC). Eur Heart J. 2013;34:2159–219.
3. Düsing R. Optimizing blood pressure control through the use of fixed combinations. Vasc Health Risk Manag. 2010;6:321–5.
4. Gradman AH, Parisé H, Lefebvre P, Falvey H, Lafeuille M-H, Duh MS. Initial combination therapy reduces the risk of cardiovascular events in hypertensive patients: a matched cohort study. Hypertension. 2013;61:309–18.
5. Jamerson K, Weber MA, Bakris GL, Dahlöf B, Pitt B, Shi V, et al. Benazepril plus amlodipine or hydrochlorothiazide for hypertension in high-risk patients. N Engl J Med. 2008;359:2417–28.
6. Pimenta E, Calhoun DA. Resistant hypertension: incidence, prevalence, and prognosis. Circulation. 2012;125:1594–6.
7. Zini S, Masdehors P, Lenkei Z, Fournie-Zaluski MC, Roques BP, Corvol P, et al. Aminopeptidase A: distribution in rat brain nuclei and increased activity in spontaneously hypertensive rats. Neuroscience. 1997;78:1187–93.
8. Huang BS, Leenen FH. Brain renin-angiotensin system and ouabain-induced sympathetic hyperactivity and hypertension in Wistar rats. Hypertension. 1999;34:107–12.
9. Wright JW, Tamura-Myers E, Wilson WL, Roques BP, Llorens-Cortes C, Speth RC, et al. Conversion of brain angiotensin II to angiotensin III is critical for pressor response in rats. Am J Physiol Regul Integr Comp Physiol. 2003 [cited 2017 Nov 7];284:R725–33. Available from: http://ajpregu.physiology.org.gate2.inist.fr/content/284/3/R725.
10. Campos LA, Bader M, Baltatu OC. Brain renin-angiotensin system in hypertension, cardiac hypertrophy, and heart failure. Front Physiol. 2011;2:115.
11. Saavedra JM. Brain and pituitary angiotensin. Endocr Rev. 1992;13:329–80.
12. Wright JW, Harding JW. Brain angiotensin receptor subtypes in the control of physiological and behavioral responses. Neurosci Biobehav Rev. 1994;18:21–53.
13. Zini S, Fournie-Zaluski MC, Chauvel E, Roques BP, Corvol P, Llorens-Cortes C. Identification of metabolic pathways of brain angiotensin II and III using specific aminopeptidase inhibitors: predominant role of angiotensin III in the control of vasopressin release. Proc Natl Acad Sci U S A. 1996;93:11968–73.
14. Fournié-Zaluski MC, Coric P, Turcaud S, Bruetschy L, Lucas E, Noble F, et al. Potent and systemically active aminopeptidase N inhibitors designed from active-site investigation. J Med Chem. 1992;35:1259–66.
15. Chauvel EN, Llorens-Cortès C, Coric P, Wilk S, Roques BP, Fournié-Zaluski MC. Differential inhibition of aminopeptidase A and aminopeptidase N by new beta-amino thiols. J Med Chem. 1994;37:2950–7.
16. Fournie-Zaluski M-C, Fassot C, Valentin B, Djordjijevic D, Reaux-Le Goazigo A, Corvol P, et al. Brain renin-angiotensin system blockade by systemically active aminopeptidase A inhibitors: a potential treatment of salt-dependent hypertension. Proc Natl Acad Sci U S A. 2004;101:7775–80.
17. Reaux A, Fournie-Zaluski MC, David C, Zini S, Roques BP, Corvol P, et al. Aminopeptidase A inhibitors as potential central antihypertensive agents. Proc Natl Acad Sci U S A. 1999;96:13415–20.
18. Réaux A, de Mota N, Zini S, Cadel S, Fournié-Zaluski MC, Roques BP, et al. PC18, a specific aminopeptidase N inhibitor, induces vasopressin release by increasing the half-life of brain angiotensin III. Neuroendocrinology. 1999;69:370–6.
19. Wright JW, Morseth SL, Abhold RH, Harding JW. Pressor action and dipsogenicity induced by angiotensin II and III in rats. Am J Phys. 1985;249:R514–21.
20. Abhold RH, Sullivan MJ, Wright JW, Harding JW. Binding, degradation and pressor activity of angiotensins II and III after aminopeptidase inhibition with amastatin and bestatin. J Pharmacol Exp Ther. 1987;242:957–62.
21. Phillips MI. Functions of angiotensin in the central nervous system. Annu Rev Physiol. 1987;49:413–35.
22. Felix D, Schlegel W. Angiotensin receptive neurones in the subfornical organ. Structure-activity relations. Brain Res. 1978;149:107–16.
23. Harding JW, Felix D. Angiotensin-sensitive neurons in the rat paraventricular nucleus: relative potencies of angiotensin II and angiotensin III. Brain Res. 1987;410:130–4.
24. Harding JW, Felix D. The effects of the aminopeptidase inhibitors amastatin and bestatin on angiotensin-evoked neuronal activity in rat brain. Brain Res. 1987;424:299–304.
25. Batt CM, Klein EW, Harding JW, Wright JW. Pressor responses to amastatin, bestatin and Plummer's inhibitors are suppressed by pretreatment with the angiotensin receptor antagonist sarthran. Brain Res Bull. 1988;21:731–5.
26. Wright JW, Mizutani S, Murray CE, Amir HZ, Harding JW. Aminopeptidase-induced elevations and reductions in blood pressure in the spontaneously hypertensive rat. J Hypertens. 1990;8:969–74.
27. Wright JW, Jensen LL, Cushing LL, Harding JW. Leucine aminopeptidase M-induced reductions in blood pressure in spontaneously hypertensive rats. Hypertension. 1989;13:910–5.
28. Fournié-Zaluski MC, Coric P, Turcaud S, Lucas E, Noble F, Maldonado R, et al. "Mixed inhibitor-prodrug" as a new approach toward systemically active inhibitors of enkephalin-degrading enzymes. J Med Chem. 1992;35:2473–81.
29. Inguimbert N, Coric P, Dhotel H, Bonnard E, Llorens-Cortes C, Mota N, et al. Synthesis and in vitro activities of new non-peptidic APA inhibitors. J Pept Res. 2005;65:175–88.
30. Marc Y, Hmazzou R, Balavoine F, Flahault A, Llorens-Cortes C. Central antihypertensive effects of chronic treatment with RB150: an orally active aminopeptidase a inhibitor in deoxycorticosterone acetate-salt rats. J Hypertens. 2018;36:641–50.
31. Bodineau L, Frugière A, Marc Y, Inguimbert N, Fassot C, Balavoine F, et al. Orally active aminopeptidase A inhibitors reduce blood pressure: a new strategy for treating hypertension. Hypertension. 2008;51:1318–25.
32. Marc Y, Gao J, Balavoine F, Michaud A, Roques BP, Llorens-Cortes C. Central antihypertensive effects of orally active aminopeptidase A inhibitors in spontaneously hypertensive rats. Hypertension. 2012;60:411–8.
33.•• Mathilde K, Hugo DA, Delphine C, Nicolas I, Adrien F, Fabrice B, et al. NI956/QGC006, a potent orally active, brain-penetrating aminopeptidase A inhibitor for treating hypertension. Hypertension . [cited 2019 Apr 15];0:HYPERTENSIONAHA.118.12499. Available from: https://www.ahajournals.org/doi/10.1161/HYPERTENSIONAHA.118.12499. **This original paper reports the development of a best-in-class aminopeptidase A inhibitor prodrug which could constitutes a new approach for the treatment of hypertension.**
34. Couvineau P, de AH, Maigret B, Llorens-Cortes C, Iturrioz X. Involvement of arginine 878 together with Ca^{2+} in mouse aminopeptidase A substrate specificity for N-terminal acidic amino-acid residues. PLoS One. 2017;12(9):e0184237.
35. Balavoine F, Azizi M, Bergerot D, De Mota N, Patouret R, Roques BP, et al. Randomised, double-blind, placebo-controlled, dose-escalating phase I study of QGC001, a centrally acting aminopeptidase a inhibitor prodrug. Clin Pharmacokinet. 2014;53:385–95.
36.•• Azizi M, Courand P-Y, Denolle T, Delsart P, Zhygalina V, Amar L, et al. A pilot double-blind randomized placebo-controlled crossover pharmacodynamic study of the centrally active aminopeptidase A inhibitor, firibastat, in hypertension. J Hypertens. 2019. **This**

original paper reports the clinical evaluation in hypertensive patients of firibastat as the first in-class of new antihypertensive drugs targeting the brain renin–angiotensin system.

37. Late-breaking science abstracts from the American Heart Association's Scientific Sessions 2018 and late-breaking abstracts in Resuscitation Science From the Resuscitation Science Symposium 2018. Circulation. 2018;138:e751–82.
38. Ferdinand Keith C, Balavoine Fabrice, Besse Bruno, Black Henry R., Desbrandes Stephanie, Dittrich Howard C., et al. Efficacy and Safety of Firibastat, a First-in-Class Brain Aminopeptidase A Inhibitor, in Hypertensive Overweight Patients of Multiple Ethnic Origins A Phase 2, Open-Label, Multicenter, Dose-Titrating Study. Circulation [Internet]. [cited 2019 Apr 30];0. Available from: https://www.ahajournals.org/doi/10.1161/CIRCULATIONAHA.119.040070.
39. Zhang Z-H, Francis J, Weiss RM, Felder RB. The renin-angiotensin-aldosterone system excites hypothalamic paraventricular nucleus neurons in heart failure. Am J Physiol Heart Circ Physiol. 2002;283:H423–33.
40. Wang H, Huang BS, Ganten D, Leenen FHHL. Prevention of sympathetic and cardiac dysfunction after myocardial infarction in transgenic rats deficient in brain angiotensinogen. Circ Res. 2004;94:843–9.
41. Leenen FHH. Brain mechanisms contributing to sympathetic hyperactivity and heart failure. Circ Res. 2007;101:221–3.
42. Huang BS, Ahmad M, Tan J, Leenen FHH. Sympathetic hyperactivity and cardiac dysfunction post-MI: different impact of specific CNS versus general AT1 receptor blockade. J Mol Cell Cardiol. 2007;43:479–86.
43. Huang BS, Ahmad M, Tan J, Leenen FHH. Chronic central versus systemic blockade of AT(1) receptors and cardiac dysfunction in rats post-myocardial infarction. Am J Physiol Heart Circ Physiol. 2009;297:H968–75.
44. Huang BS, Ahmad M, White RA, Marc Y, Llorens-Cortes C, Leenen FHH. Inhibition of brain angiotensin III attenuates sympathetic hyperactivity and cardiac dysfunction in rats post-myocardial infarction. Cardiovasc Res. 2013;97:424–31.
45.• Boitard SE, Marc Y, Keck M, Mougenot N, Agbulut O, Balavoine F, et al. Brain renin-angiotensin system blockade with orally active aminopeptidase A inhibitor prevents cardiac dysfunction after myocardial infarction in mice. J Mol Cell Cardiol. 2019;127:215–22. **This paper provides new insight on the use of brain-penetrating aminopeptidase A inhibitor prodrugs as potential therapeutic agents for the treatment of heart failure post-myocardial infarction**.
46. Leenen FHH, Ahmad M, Marc Y, Llorens-Cortes C. Specific inhibition of brain angiotensin III formation as a new strategy for prevention of heart failure after myocardial infarction. J Cardiovasc Pharmacol. 2019;73:82–91.
47. Press releases | Quantum genomics. [cited 2019 Mar 28]. Available from: http://www.quantum-genomics.com/www/actus/communiques-de-presse/.
48. Kehoe PG. The renin-angiotensin-aldosterone system and Alzheimer's disease? J Renin-Angiotensin-Aldosterone Syst. 2003;4:80–93.
49. Kehoe PG, Hibbs E, Palmer LE, Miners JS. Angiotensin-III is increased in Alzheimer's disease in association with amyloid-β and tau pathology. J Alzheimers Dis. 2017;58:203–14.