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**Kallikrein-kinin system as the dominant mechanism to counteract
hyperactive renin-angiotensin system**

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Abstract

The renin-angiotensin system (RAS) generates, maintains and makes worse hypertension and cardiovascular diseases (CVDs) through its biologically active component Angiotensin II (Ang II), that causes vasoconstriction, sodium retention and structural alterations of the heart and the arteries. A few endogenous vasodilators, kinins, natriuretic peptides and possibly angiotensin (1-7), exert opposite actions and may provide useful therapeutic agents. As endothelial autacoids, the kinins are potent vasodilators, active natriuretics and protectors of the endothelium. Indeed, the kallikrein-kinin system (KKS) is considered the dominant mechanism for counteracting the detrimental effects of the hyperactive RAS. The two systems, RAS and KKS are controlled by the angiotensin-converting enzyme (ACE) that generates Ang II and inactivates the kinins. Inhibitors of ACE can reduce the impact of Ang II and potentiate the kinins, thus contributing to restore the cardiovascular homeostasis. In the last 20 years, ACE-inhibitors (ACE-Is) have become the drugs of first choice for the treatments of the major CVDs. ACE-Is not only reduce blood pressure, as Sartans also do, but by protecting and potentiating the kinins, they can reduce morbidity and mortality and improve the quality of life for patients with CVDs. This paper provides a brief review of the literature on this topic.

Keywords: cardiovascular diseases, kinins, angiotensins, natriuretic peptides, angiotensin-converting enzyme inhibitors

1. Introduction

For years, the experts of CVDs have focussed on the detrimental roles of the RAS and have neglected the protective and beneficial functions of the KKS, with the exception of Ferrari (Ferrari 2005), Ruschitzka and Taddei (Ruschitzka and Taddei 2012), Regoli et al. (Regoli and Gobeil 2015, 2016; Regoli et al. 2012) and the excellent review of Taddei and Bortolotto (Taddei and Bortolotto 2016). The homeostasis and optimal functions of the cardiovascular system (CVS) are the result of the well-regulated balance between the RAS (vasoconstrictor, salt saving and cardiac/vascular hypertrophic) and the KKS (vasodilator, diuretic, natriuretic, and organ/vessel protective). Aging and the abuses of modern life (*e.g.*, sedentary life, unhealthy heating, smoking, and alcohol abuse) augment the impact of RAS and consequently the risks for the insurgence and the implantation of lesions that generate CVDs, namely hypertension, heart failure, myocardial infarction, coronary insufficiencies and the vascular pathology of diabetes.

The authors quoted above have described the beneficial roles of the KKS and in particular its unique and complex mechanism of action. Abundant literature has recently appeared on the vasodilatory action of Sartans, a class of drugs initially developed to block the major dangerous actions of Ang II (see reviews by Regoli et al. 2012; Regoli and Gobeil 2015). For some time, the vasodilatory and some other actions of Sartans were attributed to the intervention of AT₂ receptor (AT-2R), activated by the high concentration of Ang II brought about by the feedback stimulation of renin release produced by Sartans (Epstein et al. 2012). But, based on the experimental evidence collected so far, it is still a matter of debate whether the AT-2R stimulation during AT₁ receptor (AT-1R) blockade with Sartans is beneficial or even harmful to the CVS (te Riet et al. 2015).

In the meantime, a new concept has emerged, indicating that the RAS may act as a vasodilator through a second pathway that utilises ACE2-Ang (1-7)-MAS receptor (MAS-R) (Ferrario et al. 1997; Patel et al. 2016; Santos et al. 2013). An increasing amount of data *in vivo*, especially in the hypertensive rats, and *in vitro*, on isolated tissues of various species, as well as in preclinical pathological states *i.e.* CV experimental diseases, have been reported, but only in animals (Raffai et al. 2014; Santos 2014; Schindler et al. 2007).

In particular, literature on MAS-R has exploded, as demonstrated by two extensive reviews published in Pharmacological Reviews (Bader et al. 2014; Solinski et al. 2014). In these papers, the MAS-R has become a complex genetic entity, constituted by at least 5 different receptor subtypes, having diverse physiological and pathological implications and broad distribution in many tissues and organs (*e.g.*, the brain).

In front of such complexity, in the present paper, we will restrict ourselves to the analysis of the role of the ACE2 pathway as a vasodilator, using data obtained with the vascular native receptors studied with assays *in vivo* and *in vitro*, and recorded and characterized with the techniques of classical pharmacology.

2. RAS revisited

The functional roles of the RAS needs to be reconsidered, as indicated in Figure 1.

[To Editor: Please place Figure 1 here]

The existence of 2 axes, activated by 2 peptidases, ACE and ACE2, leads to the release of two bioactive peptides deriving from a common precursor the decapeptide Angiotensin I (Ang I; H₂-Asp-Arg-Val-Tyr-Ile-His-Pro-Phe-His-Leu-OH). These are the octapeptide Ang II (H₂-Asp-Arg-Val-Tyr-Ile-His-Pro-Phe-OH) and the heptapeptide Ang (1-7), which activate 2 different receptors, AT-1R by Ang II and the MAS-R by Ang (1-7). Ang II is the vasoconstrictor, pro-hypertensive agent responsible for all the well-known detrimental actions of the RAS. Ang (1-7) is the new vasodilator, expected to act as antihypertensive agent and to mediate the favourable protective actions of the MAS-R.

As shown in Figure 1, RAS activates two pathways, ACE/Ang II /AT-1R and ACE2 /Ang (1-7) /MAS-R, which exert two opposite actions. Here below, the 3 basic components of the ACE2 axis will be described and analysed for their potential impact to the maintenance of CV homeostasis as well as to the beneficial effects in the treatment of CVD.

3. Two opposite axes: ACE-Ang II-AT-1R and ACE2-Ang (1-7)-MAS-R.

ACE is a carboxydiptidase, which generates the vasoconstrictor Ang II and thus increases blood pressure, retains Na⁺ and H₂O and favours proliferation of cells in heart and vessels, thus acting as pro-hypertensive agent. ACE2 is a monocarboxypeptidase,

which generates the vasodilator Ang (1-7), and thus favours diuresis and natriuresis and acts as an anti-proliferative factor in heart and vessels. It is a potential anti-hypertensive factor. Not surprisingly with only 42% of sequence homology, ACE and ACE2 differ substantially. In contrast to ACE, which consists of two catalytic sites, ACE-2 contains only one active site. The catalytic sites of ACE are notoriously occupied and blocked by conventional ACE-Is, while these drugs do not affect ACE2 (Epstein et al. 2012; Patel et al. 2016). ACE is abundant in endothelia of organs targeted by CVDs and more generally in all organs, while ACE2 is present in the heart (cardiac myocytes, fibroblasts, endothelium) and especially in the kidney (renal vasculature), however, not co-localised with ACE (Epstein et al. 2012).

Therapeutic agents that inhibit the ACE axis have proven to be excellent drugs for the treatment of CVDs, while agents that activate the ACE2 axis are still in development, but may represent an attractive promise, according to some basic scientists and clinicians (Mendoza-Torres et al. 2015; Mollace et al. 2016; Parajuli et al. 2014; Patel et al. 2016; Romero et al. 2015). Among the options useful for potentiating the ACE2 axis, the first will be an agent that would increase the biological expression of the enzyme through gene therapy using lentiviral vectors, the second could be the administration of exogenous recombinant human form of ACE2, and the third, the use of a direct activator (*e.g.*, diminazene aceturate (DIZE)) of ACE2 (see comprehensive reviews by Mollace et al. 2016; Patel et al. 2016). However, these options receive little attention from the scientific community and especially by the pharmaceutical industries. It is obvious that such new therapeutic agents or approaches are, for the moment, far from giving the possibility of replacing the classical ACE-Is for the treatment of CVDs.

4. Ang (1-7): The agonist.

The heptapeptide Ang (1-7) is the result of a collaborative work between ACE, which generates Ang II and ACE2, which by removing the C-terminal Phe residue of Ang II changes the vasoconstrictor Ang II into the vasodilator Ang (1-7) (Figure 1). Ang (1-7) has been studied intensively in the last 25 years and important biological actions have been attributed to this heptapeptide (Romero et al. 2015; Santos 2014; te Riet et al. 2015).

It has been recommended for treating heart failure, shown to act as natriuretic, anti-thrombotic, anti-fibrotic and even able to ameliorate the metabolic syndrome-related vascular dysfunction (Santos 2014; te Riet et al. 2015). Because of its fragility, the circulating concentrations of Ang (1-7) are very low and difficult to measure, even in animals treated with Sartans or ACE-Is, which notoriously can increase the blood concentrations of Ang (1-7) up to 50 times (Ferrario et al. 1997), which, however, still are not probably sufficient to reach the concentrations needed to obtain measurable effects. It is interesting to note that much more modest increases of circulating plasma levels of Ang (1-7) were observed in hypertensive patients under treatment with ACE-Is (Luque et al. 1996) or Sartans (Campbell et al. 2005). Vasorelaxation responses to Ang (1-7) have been reported in tissues of several animal species and antihypertensive effects have been reported in spontaneously hypertensive rats (SHR) (see reviews by Patel et al. 2016; Schindler et al. 2007) while somewhat contradictory results have been obtained in man using the human forearm blood flow tests (Davie and McMurray 1999; Sasaki et al. 2001; Wilsdorf et al. 2001) (see section 5). The CV effects (via MAS-R) and potential therapeutic uses of the agonist Ang (1-7) have been explored at the preclinical level with agonist analogues resistant to degradation, as the non-peptide AVE0991 and the peptide CGEN856S, along with the selective MAS-R peptide antagonist A-779 (Mendoza-Torres et al. 2015; Mollace et al. 2016; Savergnini et al. 2010; te Riet et al. 2015) (see text below).

Let us first analyse the vasodilator agonist Ang (1-7) and eventually quote the nonapeptide bradykinin (BK; H₂-Arg-Pro-Pro-Gly-Phe-Ser-Pro-Phe-Arg-OH), the most-studied agonist of the KKS, for comparison. We will limit ourselves to data obtained on native vascular receptors, the genetics and molecular biology of the MAS-R being already extensively presented and discussed (Solinski et al. 2014).

The analysis of the work performed on the potentiation of BK by Ang (1-7) gives to us the opportunity to compare the two vasoactive agents both when tested alone and given together. This comparison, presented in Table 1, indicates that BK is a very potent hypotensive agent *in vivo* in the rat (Ferrario et al. 1997; Oliveira et al. 1999; Paula et al. 1995) as well as a potent *in vivo* arteriolar vasodilator of the mesentery bed (Santiago et

al. 1993; Oliveira et al. 1999; Osei et al. 1993). *In vitro*, BK is a potent relaxant of isolated arteries from different species (Brosnihan et al. 1996; Gorelik et al. 1998; Pörsti et al. 1994; Raffai et al. 2014; Tom et al. 2001). Conversely, Ang (1-7) is inactive or weakly active as hypotensive and antihypertensive agent in conscious normotensive and spontaneously hypertensive rats (Paula et al. 1995; Widdop et al. 1999) and is a very weak arteriolar vasodilator (Oliveira et al. 1999; Osei et al. 1993; Santiago et al. 1993) up to very high concentrations at least 100 times higher than BK. Moreover, Ang (1-7) has no effect on or decreased coronary blood flow at low- and high-dose treatment, respectively, in isolated *ex vivo* heart tissues perfused according to the Langendorff technique (Almeida et al. 2000; Neves et al. 1997), and is particularly weak as a relaxant of isolated arteries (Brosnihan et al. 1996; Gorelik et al. 1998; Pörsti et al. 1994; Raffai et al. 2014) and as a constrictor of veins (*e.g.*, rabbit jugular vein and human umbilical vein) (Gobeil et al. 2002) as compared to BK (Table 1).

[To Editor: Please place Table 1 here]

We know the efficiency of the KKS as antihypertensive and that of ACE-Is as first choice for the treatment of hypertension (James et al. 2013; Sindone et al. 2016) and of heart failure (Ponikowski et al. 2016). At this point, we are facing a question. Are the ACE2 axis and Ang (1-7) as an endogenous agent, suitable for replacing captopril and congeners? To answer this question, we have compared the two agents in a vascular tissue that has been used extensively in the pharmacology of kinins: the rabbit jugular vein, which responds to BK with a strong contraction mediated by B2R, which is completely blocked by HOE 140 (Gobeil et al. 2002; Regoli and Barabé 1980; Regoli et al. 1998). We have compared the potentiation of low concentration (1 nM) of BK evoked by captopril and by Ang (1-7) given at the different concentrations (Figure 2).

[To Editor: Please place Figure 2 here]

The difference of potency between the 2 agents is enormous (10 000 x) with Ang (1-7) being several times inferior to captopril (Gobeil et al. 2002). We have proposed the following interpretation to explain such results. The metabolism of BK into an inactive peptide can occur at the two homologous catalytic domains of ACE, termed N and C domains. Captopril and congeners are non-selective ACE-domain inhibitors while Ang (1-7) is considered a selective C-domain inhibitor (or substrate) of ACE. Thus, protection

of kinins can only be maximal when both the ACE C- and N-terminal domains are inhibited. The ensuing increased bioavailability of BK at the B2R compartment leads to a greater potency of BK in mediating contraction of the veins (Gobeil et al. 2002). Similar findings were obtained by Tom et al. who compared the potentiation effects of the ACE-Is ramiprilat, quinaprilat versus Ang (1-7) on BK-mediated vasodilation in intact arteries (e.g., porcine coronary arteries) (Tom et al. 2001; Tom et al. 2002). These latter results are in accordance with *ex vivo* ACE competition assays showing that Ang (1-7) inhibits human plasma and tissue (right atrium) ACE activity from healthy volunteers with IC₅₀ values 1 000 times superior to that of the classical ACE-I Lisinopril (Roks et al. 1999). From the above, we conclude that the replacement of ACE-Is by Ang (1-7) is far from becoming a viable therapeutic strategy. On the other hand, it is possible that ACE-Is may enhance the individual or combined effects of Ang (1-7) and BK, two potent endogenous ACE substrates (Chappell et al. 1998) acting both as an antihypertensive pathway (Ferrario et al. 2005; Iusuf et al. 2008).

5. MAS-R: The receptor

MAS-R is indicated as MRGPRD (MAS Related G protein-coupled receptor D) (Gembardt et al. 2008), MAS1 (Santos et al. 2003) and AT-2R (Walters et al. 2005). Since Ang (1-7) has very low affinity for AT-2R and AT-1R (Bosnyak et al. 2011; Rowe et al. 1995), we assume that the native vascular and cardiac functional site that is activated by Ang (1-7) and that mediates its actions and that we intend to characterise is the above described genetic entity, which is present in rats, primates and man (Gembardt et al. 2008; Santos et al. 2003).

As mentioned earlier, synthetic agonists (e.g., AVE 0991) and antagonists (e.g., A779) have been discovered for MAS-R (*vide supra*). The most important actions of MAS-R relating to the CV system are described in Figure 1.

The existence of a MAS-R in man is not sufficiently documented. Some contradictory results have been reported by Sasaki and co-workers (Sasaki et al. 2001) and Davie and Mac Murray (Davie and McMurray 1999) with the infusion of Ang (1-7) in human forearm of patients with CVDs. In addition, no vasodilating effect of Ang (1-7) at high dose rates was observed in forearm arteries of normotensive healthy subjects (Ueda et al.

2001; Wilsdorf et al. 2001). This is in marked contrast with the vasodilating and cardioprotective functions of BK that have been repeatedly demonstrated in patients with CVDs and healthy volunteers (Benjamin et al. 1989; Brown et al. 2000; Cockcroft et al. 1994; Cruden et al. 2011; Feher et al. 2013; Gunaruwan et al. 2009; Haefeli et al. 1997; Kuga et al. 1997; Leesar et al. 1999; Murphey et al. 2000; O'Kane et al. 1994; Prasad et al. 1999; Pretorius et al. 2008; Rosenbaum et al. 2002; Van Guilder et al. 2008; Wei et al. 2004).

To the best of our knowledge, only one study has identified and tested the human NATIVE MAS-R in isolated human vessels (Durand et al. 2016) quite to the contrary to B2R (Fulop et al. 2007; Gobeil et al. 1996; Gessi et al. 1997; Stork and Cocks 1994; Whalley et al. 1987). In that particular study, the group of Durand et al. has demonstrated that the MAS-R-mediated, endothelium dependent vasodilatory effects of Ang (1-7) in isolated human atrial and adipose microvessels are impaired in patients with coronary artery diseases compared to those without coronary artery diseases (Durand et al. 2016). This is in accord with some *in vitro* data on human MAS-R using cultured transfected cell lines (*e.g.*, Chinese hamster ovary (CHO) cells) and human aortic endothelial cells demonstrating that MAS-R can stimulate eNOS activation and NO production via Akt dependent pathway (Sampaio et al. 2007). It is unknown at this time whether the human endothelial MAS-R can produce and release other mediators, such as Prostacyclin (PGI₂) or the endothelium-derived hyperpolarizing factor (EDHF), contributing to relaxation of blood vessels, as reported for B2R (Fulop et al. 2007; Hammond et al. 2011; Honing et al. 2000; Kato et al. 1997; Knock and Poston 1996; Moyes et al. 2014; Nakashima et al. 1993; Whalley et al. 1987).

6. Mechanisms of action of the Ang (1-7)

To briefly recapitulate the information given above, the antihypertensive actions of Ang (1-7) can be demonstrated at least by four effects summarized below:

- 1- A weak intrinsic vasodilatory activity *in vitro*
- 2- A weak hypotensive/anti-hypertensive activity *in vivo*
- 3- A weak ACE-I like-activity and weak potentiator of BK actions

4- Ang (1-7) derives in large part from the conversion of Ang II. The reduction of the potent vasoconstrictor Ang II contributes to the decreasing of the peripheral vascular tension and the apparent vasodilatory effect of Ang (1-7).

Thus, the overall impact of Ang (1-7) is that of a weak modulator of the CVS in general and a weak counter-regulatory hormone of the hyperactive RAS.

7. The endogenous KKS: The dominant mechanism for negative regulation of RAS

Kinins are AUTACOIDS, namely locally-acting agents that are generated and released where and when they are needed. One of the kinin functions is in the case of inflammatory, traumatic lesions and in tissue and organ damages that follow all sorts of noxious stimuli. The role of the kinins is to activate the inflammatory reactions in order to contain and acutely limit the initial lesion and then prepare the reconstruction of the organ damaged.

The second major function of kinins is in the CVS and at the endothelial level where the kinins are predisposed to maintain optimal blood flow for the nutritional and metabolic needs of the organ (Mordi et al. 2016). This is obtained with a fine continuous monitoring that is regulated by a complex mechanism illustrated in Figure 3.

[To Editor: Please place Figure 3 here]

All components needed for the synthesis and the release of BK and Lys-BK, are present at the endothelium (Regoli and Gobeil 2015). The decapeptide Lys-BK is also a potent vasodilator in mammals (Regoli and Barabé 1980) and finds its receptor (the B2R) at the plasma membrane of the endothelial cells. The B2R, belonging to GPCR superfamily, mediates the formation and release of three biologically active agents, the NO, an unstable gas with a half-life of seconds, a prostanoid, Prostacyclin (PGI₂), and EDHF, another lipid mediator recently identified as the epoxyeicosatrienoic acids (EETs) (Fleming 2016). The NO is responsible for the vasodilatation sustained by cGMP. PGI₂ is at the same time a vasodilator with the half-life of 4-5 min and with a potent anti-thrombotic function by inhibiting the aggregation of thrombocytes. One of the favorable actions of PGI₂ is its ability to prevent the actions of thromboxane A₂, the most active

pro-aggregating agent. The other lipid, EDHF, opens the K^+ channels and hyperpolarises the vascular smooth muscle cell, thus reducing its excitability.

The complex mechanism of action of KKS endothelial autacoid as described in Figure 3 indicates the complexity and the efficiency of the BK-B2R system as a vasodilator. The endothelial B2R activates the generation of 3 potent agents, the NO, PGI_2 and EDHF. The 3 agents stimulate the formation of 3 different second messengers namely, cGMP, cAMP and K^+ , which are all inhibitors of vascular smooth muscle tonus through reduction of intracellular Ca^{2+} or induction of hyperpolarisation. Such a complex system confers to the kinin vasodilators rapidity, relative stability of action and actual flexibility to adapting to all necessary metabolic and functional needs of the tissues.

In this respect, the kinins are unique and, in our opinion, they represent the reference entity as the endogenous vasodilators. Actually, the KKS has been defined by Mordi et al. as the dominant mechanism for negative regulation of RAS (Mordi et al. 2016).

The presence of the above described system is instrumental not only for controlling the peripheral arterial resistance and blood pressure, but also for kinin mediated-natriuretic and cellular anti-proliferative actions in the heart and the vessels (Regoli and Gobeil 2015; Taddei and Bortolotto 2016).

8. Endogenous vasodilators as potential therapeutic targets for CVDs

An increasing number of new GPCR-acting vasodilators has been identified as a potential therapy for CVDs (*e.g.*, substance P, adrenomedullin, relaxin, Ang (1-7), the atrial natriuretic peptides (ANPs)) (Levick and Melendez 2016; Siryk-Bathgate et al. 2013). Among the endogenous vasodilators that can compete with or replace the kinins, two agents have recently attracted considerable attention from the scientific community: the Ang (1-7) as mentioned previously (Patel et al. 2016; Romero et al. 2015) and the ANPs (McKie et al. 2016; Volpe 2014; Volpe et al. 2016). As far as Ang (1-7) is concerned, we have demonstrated that it is a very weak vasodilator and poor inhibitor of ACE, thus cannot reach possible therapeutic applications in the clinic. As for ANP and congeners, recent promising data indicate that FDA-approved human recombinant versions of the peptides *e.g.* M-ANP Carperitide, BNP Nesiritide, CD-NP Cenderitide, can be used as a new pharmacological approach to manage CVDs, most notably heart failure (Volpe

2014). The continuous subcutaneous administration of stabilized NPs via patch pumps is envisioned to be feasible for chronic therapy of heart failure patients (McKie et al. 2016; Volpe et al. 2016). In this line of thoughts, the use of potent BK analogues resistant to enzymatic degradation in the lung by ACE, *e.g.* RMP-7 ([Hyp³, Thi⁵, (4-Me)Tyr⁸(Ψ-CH₂NH)-Arg⁹]-BK) (Borlongan and Emerich 2003; Emerich et al. 2001), B9972 (DArg-[Hyp³, Igl⁵,Oic⁷,Igl⁸]-BK) (Bawolak et al. 2007; Taraseviciene-Stewart et al. 2005) and NG291 ([Hyp³, Thi⁵, ^NChg⁷, Thi⁸]-BK) (Bélanger et al. 2009; Savard et al. 2013), should also be considered for subcutaneous applications.

The following section will briefly describe few aspects of the functions of NPs in the CV system. Natriuretic peptides are a group of vasodilators that may play a role in CVDs by promoting diuretic, natriuretic and anti-hypertensive actions in part through the inhibition of renin and aldosterone, the increase of glomerular filtration rate by dilatation of the renal afferent arteriole and the inhibition of vasopressin release and action on the renal tubules (Mollace et al. 2016; Volpe 2014; Volpe et al. 2016). Natriuretic peptides, however, show important differences from the KKS, at various levels. Firstly, ANP and congeners are hormones originating from various tissues (heart, brain, others tissues) to act on the kidneys, the peripheral vessels and the central and peripheral nervous systems. Unlike Kinins, NPs are not inactivated by the pulmonary ACE but rather metabolised by neutral endopeptidase (NEP, alias Neprilysin). ANP is synthesized in cardiac myocytes as a pre-prohormone of 151 amino acids (a.a.), which is cleaved to a pro-hormone of 126 a.a. in myocyte granules. ANP is secreted following atrial distension as a biological active peptide of 28 a.a. or from the kidney, as a peptide of 32 a.a. In healthy subject, the circulating plasma concentration is about 20 pM, which increases by 10 to 100 folds in heart failure patients and the increase is a reflection of the gravity of the disease. ANP is a fragile peptide rapidly inactivated such that its half-life in plasma is about 0.5 to 4 min (Volpe et al. 2016).

The two functional targets are the endothelium and the kidney nephron tubules and the collecting duct, where it reduces Na⁺ reabsorption by inhibiting an amiloride sensitive-cation channel. The receptor for ANP is in fact a plasma membrane guanylate cyclase, converting GTP to cGMP, in the vascular smooth muscle cells. Its activation requires

ATP as co-factor and is sufficient to produce smooth muscle relaxation and vasodilatation. In this way, ANP is a vasodilator that is independent of endothelium and consequently does not use the classical NO cascade described by the Nobel Prize recipient Furchgott (Furchgott 1999).

9. The saga ACE-Is / Angiotensin receptor blockers (ARBs, alias Sartans)

Modern therapies of CVDs and diabetes require block or inhibition of RAS. This is achieved by the use of ACE-Is or of ARBs. The two classes of drugs have gone through several phases of therapeutic utilisation during recent years.

1. At the beginning, ACE-Is + Sartans were recommended in order to obtain the block of RAS by inhibiting the formation of Ang II and the block of the AT-1R. Soon, it became evident that the combination did not have great advantage with respect to the use of each single agent (Epstein et al. 2012).
2. The two agents were then given separately on the assumption that they were equivalent. However ACE-Is showed side effects such as cough and angioedema.
3. Assuming that these side effects were due to BK, Sartans were used instead of ACE-Is, but soon became evident that Sartans have also the same side effects, however less frequently. In the meantime, Sartans showed some therapeutic limitations in CVDs with respect to ACE-Is (van Vark et al. 2012).
4. With the introduction of meta-analyses and of the three modern criteria required for a valuable evaluation of the therapies recommended for Hypertension and CVDs, namely reduction of blood pressure, of morbidity and of mortality, the advantages of ACE-Is with respect to Sartans became evident and the indications for the use of Sartans were contested (Regoli et al. 2012; Regoli and Gobeil 2015; Regoli and Gobeil 2016; Sindone et al. 2016; Taddei and Bortolotto 2016). Both the ACE-Is and the Sartans reduce blood pressure and the deleterious impact of Ang II but only the ACE-Is preserve and potentiate the kinins and show evident beneficial effects in reducing morbidity and mortality. This demonstrates the fundamental role of kinins in the therapy of CVDs.
5. Nowadays, Sartans are not used as first choice drugs and their use is limited only to replace ACE-Is in patients intolerant to this drug. This is discussed in the new 2016 guidelines for the treatment of heart failure produced by European Society of Cardiology,

which in our opinion is the most important and comprehensive document of this category (Ponikowski et al. 2016).

10. General conclusions

From the above, it emerges that despite the enormous amount of work accomplished and of published data on the new axis ACE2-Ang (1-7)-MAS-R, much work remains to be done to establish its importance and true value in human cardiovascular physiology and pathophysiology. In future studies in humans, MAS-R agonists should be tested not only on the vascular tone but also for their vasoprotective, anti-inflammatory, anti-fibrotic, anti-apoptotic, antioxidant and anti-thrombotic effects. The latter have been clinically documented for the BK-B2R and represent additional beneficial effects on the CVS and, most importantly, in the prevention of CVDs.

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Table 1. Comparison of biological activities of BK and Ang (1-7)

	BK	Ang (1-7)	Effects	References
<i>In Vivo</i>				
BP in conscious rats	+++	-	hypotension	(Paula et al. 1995) (Widdop et al. 1999)
Rat mesenteric arterioles	++	+	dilatation	(Oliveira et al. 1999)
Feline mesenteric bed	+++	+	dilatation	(Osei et al. 1993) (Santiago et al. 1993)
<i>In Vitro / Ex Vivo</i>				
Canine coronary arteries	+++	+	dilatation	(Brosnihan et al. 1996)
Porcine coronary arteries	+++	+ / -	dilatation	(Pörsti et al. 1994) (Gorelik et al. 1998) (Tom et al. 2001) (Raffai et al. 2014)
Rat heart	↑	- / ↓	coronary flow	(Neves et al. 1997) (Almeida et al. 2000)
Rabbit jugular veins*	+++	-	constriction	(Gobeil et al. 2002)
Human umbilical veins*	+++	-	constriction	(Gobeil F et al. 1996; unpublished data)

Qualitative assessment of peptide activity: +++, strong; ++, moderate; low, + and -, no activity. Abbreviation: BP; blood pressure.* Tissues devoid of endothelium.

Figure captions

Figure 1. Generation of Ang II and Ang (1-7) and their actions on the CVS.

Figure 2. Concentration-response curves of the two ACE-Is, captopril and Ang (1-7), on the potentiation of BK (1 nM)-mediated contraction of the isolated endothelium-denuded rabbit jugular vein. Modified from Gobeil et al. Studies on the angiotensin-converting enzyme and the kinin B2 receptor in the rabbit jugular vein: modulation of contractile response to bradykinin, Pages No. 151-161, © 2002, with permission from Canadian Science Publishing (NRC Research Press).

Figure 3. Formation of vasorelaxant products from endothelial cells in response to B2R stimulation. BK and Lys-BK promote the formation of vasodilatory agents. This results in the release of NO (from eNOS), PGI₂ (from PLA₂) and EDHF. Abbreviations: EDHF, endothelial hyperpolarizing factor; cGMP, cyclic guanosine monophosphate; cAMP, cyclic adenosine monophosphate; NO, nitric oxide; eNOS, endothelial nitric oxide synthase; sGC, soluble guanylyl cyclase; IP, prostacyclin receptor. Modified from Pharmacol. Ther. 135(1). Regoli, D., Plante, G.E., and Gobeil, F. Impact of kinins in the treatment of cardiovascular diseases, Pages No. 94-111, © 2012, with permission from Elsevier.

FIGURE 1

FIGURE 2

Figure 2

100x101mm (300 x 300 DPI)

FIGURE 3