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Portal hypertensive response to kinin

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ABSTRACT

Portal hypertension is the most common complication of chronic liver diseases, such as cirrhosis. The increased intrahepatic vascular resistance seen in hepatic disease is due to changes in cellular architecture and active contraction of stellate cells. In this article, we review the historical aspects of the kallikrein-kinin system, the role of bradykinin in the development of disease, and our main findings regarding the role of this nonapeptide in normal and experimental models of hepatic injury using the isolated rat liver perfusion model (mono and bivascular) and isolated liver cells. We demonstrated that: 1) the increase in intrahepatic vascular resistance induced by bradykinin is mediated by B2 receptors, involving sinusoidal endothelial and stellate cells, and is preserved in the presence of inflammation, fibrosis, and cirrhosis; 2) the hepatic arterial hypertensive response to bradykinin is calcium-independent and mediated by eicosanoids; 3) bradykinin does not have vasodilating effect on the pre-constricted perfused rat liver; and, 4) after exertion of its hypertensive effect, bradykinin is degraded by angiotensin converting enzyme. In conclusion, the hypertensive response to BK is mediated by the B2 receptor in normal and pathological situations. The B1 receptor is expressed more strongly in regenerating and cirrhotic livers, and its role is currently under investigation.

Key words: bradykinin, hepatic metabolism, portal hypertension, bradykinin receptors.

INTRODUCTION

The first description of isolated liver perfusion in the scientific literature was recorded in 1855 by Claude Bernard, when he was studying hepatic carbohydrate metabolism:

> *"J'ai choisi un chien adulte, vigoureux et bien portant, qui depuis plusiers jours était nourri exclusivement avec de la viande, et je l'ai sacrifié par la section du bulbe rachidien, sept heures aprés un repas copieux de tripes. Aussitôt l'abdomen fut ouvert, le foie fut enlevé en évitant de blesser son tissu, et cet organe encore tout chaud et avant que le sang êut le temps de se coaguler dans ses vaisseaux, fut soumis à un lavage à l'eau froide par la veine porte. Pour cela, je pris un tube de gutta-percha, long de 1 mètre environ et portant à ses deux extrémités des ajustages en cuivre. Le tube étant préalablement rempli d'eau, une de ses extrémités fut solidement fixée au robinet de la fontaine du laboratoire de médicine du Collége de France. En ouvrant le robinet,*

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l'eau traversa le foie avec une grande rapidité, car la force du courrant d'eau était capable, ainsi que cela fut mesuré, de soulever une colonne de mercure à 127 centimètres de hauteur (Bernard 1855)."

In 1948, Maurício Rocha e Silva, Wilson T. Beraldo, and Gastão Rosenfeld, working in the Biological Institute of São Paulo, described a substance that, when generated in the plasma by *Bothrops jararaca* venom or trypsin, showed an important arterial hypotensive action in cats and rabbits and stimulated guinea pig intestinal and uterine musculature contraction. They named the molecule bradykinin (BK, from Greek *brady*, slow and *kinesia*, movement) indicating a substance that produced a slow movement of the gut (Rocha e Silva et al. 1949). Their report follows: "*In 1948, Beraldo, Rosenfeld, and I were interested to discover whether the venom of the* B. jararaca *liberated histamine from dog's liver as trypsin had been shown to do. The liver was perfused with defibrinated blood through the portal vein and the perfusates collected in the inferior vena cava just above the diaphragm. Since dog's blood practically does not contain histamine or any substance stimulating the guinea-pig gut, the perfusates were assayed directly upon the gut after the muscle had been made insensitive to the venom by desensitization. As soon as the venom was injected in the perfusing cannula, the perfusates acquired enormous activity upon the guinea-pig ileum*." (Rocha e Silva 1955).

The primary structure of bradykinin (Arg-Pro-Pro-Gly-Phe-Ser-Pro-Phe-Arg) was elucidated at the National Institute for Medical Research, Mill Hill, London (Elliott et al. 1960) and it was only in 1991 that the first kinin receptor cDNA was cloned (McEachern et al. 1991). In 1965, Ferreira found that the *Bothrops jararaca* venom contained a factor that potentiated several pharmacological actions of BK *in vivo* and *in vitro*. This factor was the basis for the development of the first generation of potent pharmaceuticals (captopril) for the treatment of arterial hypertension.

Angiotensin (hypertensin) was first described in 1939 in Argentina by Muñoz et al., and angiotensin-converting enzyme (ACE) was later characterized from horse plasma (Skeggs et al. 1956). The tissue conversion of angiotensin I (AI) to angiotensin II (AII) was demonstrated in Leal Prado's Laboratory at Escola Paulista de Medicina (Carlini et al. 1958) and this pioneering work was recognized later in an important review (Vane 1974).

In 1976, we demonstrated that BK and angiotensins I and II produced a portal hypertensive response in the liver, and that the liver efficiently inactivates BK (90% from an initial dose of 19μM) and converts AI to AII. We also verified that doses ranging from 0.17 to 0.34 nmols of AII were approximately 90% inactivated during a single passage through the liver (Borges et al. 1976). Later we verified that AI did not induce, per se, a portal hypertensive response, but its action was a result of its conversion to AII and subsequent action on the AT1 receptor, primarily within the periportal zone of the liver (Carvalho et al. 2007).

This review will summarize the hepatic response to bradykinin in normal and experimental models of liver disease.

Characteristics of the Kinin System and its Role in Diseases

The kinin system (KS) comprises several proteins and peptides, including precursors (kininogens), kinin-liberating enzymes (pro-kallikrein), biologically active kinins, and kinin-metabolizing enzymes. The kininogens (high molecular weight kininogen, HMWK, or low molecular weight kininogen, LMWK) are multifunctional glycoproteins synthesized by the liver (Borges and Gordon 1976) and other tissues (Figueroa et al. 1992); kininogens contain the kinin sequence in its mid portion and are the only precursors of the kinin peptides. Tissue and plasma kallikreins are serine proteases that differ from one another in their biochemical and functional characteristics (Bhoola et al. 1992). Kininogens and tissue kallikreins are expressed in many different tissues, whereas plasma kallikrein is predominantly produced by the liver. Plasma prokallikrein is a single chain glycoprotein synthesized and secreted by the liver; its cleavage by factor XIIa or its fragments generates plasma kallikrein. Its proteolytic activity is regulated by plasma inhibitors and hepatic clearance (Borges et al. 1981, 1986, Kouyoumdjian et al. 1989). Plasma kallikrein is primarily cleared by the liver (Borges et al. 1985); clear-

ance is mediated by a galectin in a calcium-independent pathway (Nagaoka et al. 2003). The hepatic removal of tissue kallikreins (pig pancreatic and horse urinary) from circulation involves receptor-mediated endocytosis by C-type lectins, which were characterized as mannosyl- and galactosyl-specific, respectively (Kouyoumdjian et al. 1989).

In humans, plasma kallikrein forms BK from HMWK, whereas tissue kallikreins form kallidin (Lys-BK) from HMWK and LMWK. By contrast, both plasma and tissue kallikrein generate BK in rodents (Bhoola et al. 1992, Campbell 2001). The cleavage of Lys-BK by aminopeptidase generates BK, and cleavage by carboxypeptidase generates des-Arg^9-BK and des-Arg^{9}Lys-BK. Several enzymes are responsible for inactivation and metabolism of BK; for a long period of time, inactivation was attributed almost exclusively to angiotensin-converting enzyme (ACE) from the lung, although the liver plays an important role in BK inactivation (Prado et al. 1975). Hepatic modulation of KS, as demonstrated by the isolated and exanguinated perfused rat liver model, is shown in Figure 1.

In the rat liver, the kininase metalloendopeptidase EC 3.4.24.15 predominates over ACE and prolylendopeptidase (Molina et al. 1996). However, our evidence suggests that ACE, and not EC 3.4.24.15, is the main kininase involved in BK degradation by the liver (Gioli-Pereira et al. 2005). Hepatic EC 3.4.24.15 cleaves BK at the Phe^5-Ser^6 residue, releasing two peptides; the BK 1-5 fragment has a protective role against the deleterious effects of lipopolysaccharide in rats (Morinelli et al. 2001).

The KS has paracrine activity that is the release of BK from HMWK. Once released, BK has a potent effect on vascular tone: it relaxes the arterial vasculature, causing hypotension, and increases portal vascular tone, causing isolated hypertension in this area (Borges et al. 1976).

Bradykinin acts on two types of receptors: B1 (inducible) and B2 (constitutive; see Table I). Both mediate the same action, and their differentiation was based initially on a comparison of the potency of agonists and antagonists in several vascular preparations (Regoli et al. 1994). The B_1 receptor (B_1R) responds more efficiently to the octapeptide des-Arg^9-BK than to BK itself, whereas the B_2 receptor (B_2R) responds more efficiently to BK than to des-Arg^9-BK. Antagonists of B_1R and B_2Rs are both available: des-Arg^9[Leu^8]-bradykinin and icatibant, a synthetic peptidomimetic (HOE-140), respectively (Hall 1997).

In addition to vascular effects, BK evokes the cardinal signs of inflammation (pain, swelling, redness, and heat) and is therefore characterized as a chemical mediator of the inflammatory response (Rocha e Silva 1964). BK acts near the top of the inflammatory cascade via interactions with B_1R and B_2R on target cell membranes. Activation of BK receptors is involved in the pathogenesis of multiple conditions (Table II).

B_2R appears to be involved in the acute inflammatory response, whereas B_1R has a role in chronic inflammatory processes (Perkins and Kelly 1993, Blais et al. 2000, Leeb-Lundberg et al. 2005, Costa-Neto et al. 2008). B_1R may also play a significant role in the development of inflammatory diseases, particularly those with an immune etiology such as diabetes, asthma, rheumatoid arthritis, and multiple sclerosis (Couture et al. 2001). In streptozotocin-induced diabetes, B_2R is overexpressed in peripheral tissues (Couture et al. 2001, Leeb-Lundberg et al. 2005) and, interestingly, the progression of diabetes mellitus was prevented by [Leu^8]desArg^9-BK (B_1R antagonist), but not by icatibant (B_2R antagonist) (Zuccollo et al. 1999).

There is direct and indirect evidence for the role of kinins in inflammatory airway diseases. Inhalation of BK or Lys-BK, but not des-Arg^9-BK, causes bronchospasms in asthmatic person. Bronchoconstriction induced by BK was inhibited by HOE-140 (B_2R antagonist), which confirms the involvement of B_2R in BK-induced bronchospasm (Abraham et al. 2006).

In multiple sclerosis and septic shock, B_1R has a protective role, while in other conditions it produces adverse effects, such as pain and inflammation (Calixto et al. 2004, Gabra et al. 2003). Although BK is capable of initiating the cascade of cytokine release that mediates hyperalgesic responses, antagonists of B_1R and B_2R fail to inhibit this response (Ferreira et al. 1993, Poole et al. 1999). In cardiovascular and renal diseases, these actions are mediated primarily by nitric oxide (NO) and prostaglandins.

Activation of the KS could prove beneficial due to

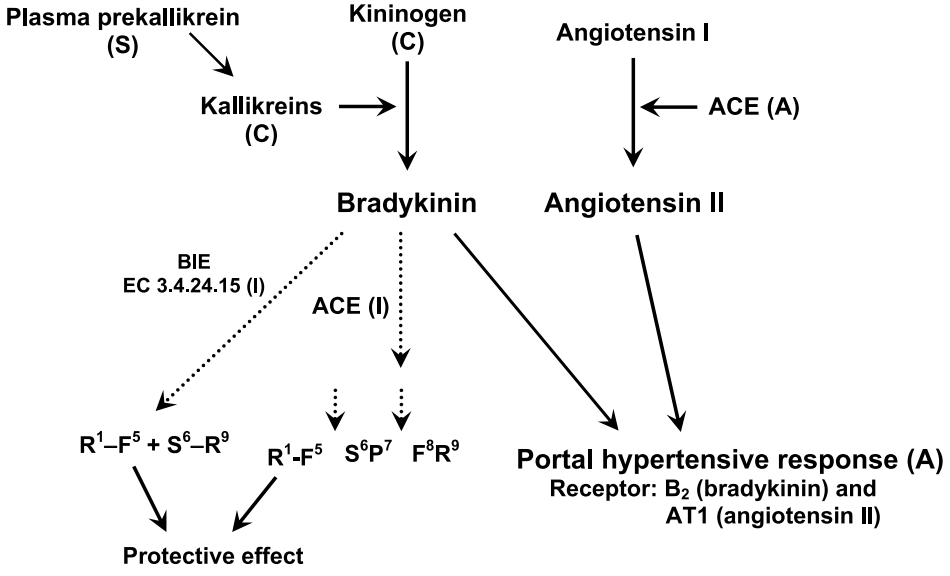

Fig. 1 – The liver and vasoactive peptides: bradykinin and angiotensin II. Legend: ACE: angiotensin converting enzyme; BIE: bradykinin inactivating enzyme; S: synthesis (Borges and Gordon 1976, Borges et al. 1981); C: clearance (Borges et al. 1985); I: inactivation (Kouyoumdjian et al. 1984, Molina et al. 2000); A: action (Borges et al. 1976, Loureiro-Silva et al. 2001, Carvalho et al. 2007).

TABLE I
Vasoactive peptides: action and reaction.

A) Systemic				
Peptide	Effect		Receptor	Antagonist
	Arterial	Venous		
Bradykinin/Lys-BK	Vasodilatation	Venoconstriction	B_2	HOE-140
Des-Arg^9-(Lys)-BK			B_1	des-Arg^9[Leu^8]BK
Angiotensin II	Hypertension	Hypertension	AT_1	Losartan, irbersartan
B) Hepatic				
Peptide	Effect		Receptor	Antagonist
	Arterial	Venous		
Bradykinin/Lys-BK	Hypertensive response	Hypertensive response	B_2	HOE-140
Des-Arg^9-(Lys)-BK	No response	No response	B_1	—
Angiotensin II				
Portal vein infusion	No response	Hypertensive response	AT1	Losartan
Hepatic artery infusion	Hypertensive response	Hypertensive response		

its effects on angiogenesis, cardiac regeneration, and coronary blood flow; the KS also possesses anti-thrombotic, anti-inflammatory, and anti-apoptotic effects. It has been demonstrated that B_2R is important in reducing the size of muscle infarction (Westerman et al. 2008).

KS activation is also related to basic pathological processes such as ischemia (Blais et al. 2000). In hypoxic situations, AII-induced cardiac angiogenesis is mediated by increased levels of BK and B_2R activation (Munk et al. 2007). In the ischemic heart, des-Arg^9-BK and BK promote protective effects, via B_1R and B_2R, that are due to the involvement of other mediators such as NO, prostanoid, and endothelium-derived hyperpolarizing factor (EDHF) (Lagneux et al. 2003).

In renal transplantation, the incidence of primary dysfunction and poor initial graft function appears to de-

TABLE II
Bradykinin receptors and some pathophysiological functions.

Disease	Effect	Receptor	RAS conection
Inflammation[1,2]	Pro-inflammatory	B1	—
Pain[3,4]	Hyperalgesia	B_1 and B_2	
Infection (sepsis)[7]	KS activation: BK increase	B2	—
	Vascular leakage and vasodilation	B1	—
	Arterial vasodilatation		
Metabolism			
Diabetes[3]	Prevention of progression of insulin-dependent diabetes	B1	—
Respiratory system[3] (asthma and rhinitis)	Bronchospasm in asthmatic subject (not in normal subjects)	B2	—
	Allergic inflammation	B1	
Cardio-vascular[3]	Hypertrophy	B_2	ACE inhibitors
	Cardiopathy	B_2	
Neurological diseases[3]	Increase blood-brain barrier permeability	B_2	—
	Decrease intracranial pressure		
Alzheimer's[5]	Improvement of cognitive deficits	B_1 / B_2	
Epilepsy[6]	Deleterious and protective effects	B_1 / B_2	
Liver			
Thrombosis[9]	Anti-thrombotic (B_2R knockout mice)	B2	Compensatory over-expression of AT_2 receptor
Fibrosis[10]	Attenuates fibrosis / hepatocellular damage	B_1 / B_2	
Other diseases[3]			
Arthritis	B_2R increase	B_2	
Pre-eclampsia	B_2R increase in platelets	B_2	AT_1 receptor dimerization
Angiogenesis[11]	Endotelial cell proliferation	B_2	AII/AT_2
Arterial hypertension[4]	Vasodilatation – Anti-hypertensive	B_2	ACE inhibition
Neoplasia[3,8]	Tumor growth	B_1 / B_2	—
	Angiogenesis stimulation		

RAS: renin-angiotensin system. [1]Leeb-Lundberg et al. 2005; [2]Blais et al. 2000; [3]Ferreira et al. 1993; [4] Poole et al. 1999; [5]Prediger et al. 2008; [6]Perosa et al. 2007; [7]Imamura et al. 2006; [8]Costa-Neto et al. 2008; [9]Schmaier 2008; [10]Sancho-Bru et al. 2007; [11]Westermann et al. 2008.

pend on the length of cold storage and has thus been ascribed to injury from harvesting, cold storage, and warm reperfusion after blood vessel reconnection; *i.e.*, cold ischemia-warm reperfusion injury (IRI). Kakoki et al. (2007) showed that both B_1R and B_2R protect the kidney from damage caused by IRI, including reducing DNA damage, apoptosis, morphological and functional kidney changes, and mortality. Recently, Wang et al. (2008) showed that B_1R antagonism promoted down regulation of pro-inflammatory and upregulation of anti-inflammatory molecules, representing a new therapeutic strategy for the prevention and treatment of renal IRI.

Souza et al. (2004) demonstrated an increase in tissue kallikrein activity and activation of B_2R after intestinal IRI, demonstrating the role of B_2R in the development of inflammation and tissue loss. Therefore, B_2R antagonists may be useful adjunct therapy for the treatment of the severe inflammatory injuries that follow ischemia and reperfusion of the superior mesenteric artery. B_2R, but not B_1R, also protects against cardiac IRI by inhibit-

ing apoptosis and limiting ventricular remodeling (Yin et al. 2007). The role of kinins in hepatic IRI is now under investigation in our laboratory.

Although the KS has been related to thrombotic risk, it was observed that B_2R knock-out mice were protected against arterial thrombosis, and that overexpression of the AT_2 receptor had a compensatory effect (Schmaier 2008).

KS, via B_2R activation, is associated with suppression of reactive oxygen species production and consequent prevention of renal tissue damage (Chao et al. 2007). The antifibrotic effect of B_2R within the kidney was also demonstrated by Schanstra and co-workers (2002), supporting the potential role of BK in the antifibrotic effects of ACE inhibitors. Recently, in the unilateral ureteral obstruction model of renal fibrosis, B_1R has been found to be overexpressed, and delayed treatment with an orally active nonpeptide B_1R antagonist which blocked macrophage infiltration, leading to reversal of renal fibrosis (Klein et al. 2009).

The participation of kinin receptors during sepsis remains unclear. It is well known that B_1R is induced and overexpressed during tissue injury, following inflammatory processes, and after LPS administration.

Liver and the kinin system

The liver has a very sophisticated vascular system that plays an active role in the maintenance of hepatic function. As a key organ in the control of the internal milieu, the liver relies on appropriate tissue perfusion to control the composition of the blood that drains into the systemic circulation. Excluding the hepatic artery and its branches, which are responsible for only 20% to 40% of the blood entering the liver, the intra-hepatic circulation is maintained at very low pressure in physiological conditions. The vascular tone in portal venules, sinusoids, and hepatic venules is actively controlled by several relaxing and contracting substances, including BK (Borges et al. 1976, Loureiro-Silva et al. 1999, 2001, Gioli-Pereira et al. 2005, Nagaoka et al. 2006).

BK plays an important role in the control of vascular tone in physiological and pathological conditions. Both a widespread distribution throughout the vascular system and its very short half-life in circulation (Pellacani et al. 1992) indicate that BK participates in vascular tone control in an autocrine/paracrine way. However, since it can be detected in the circulation, an endocrine mechanism of action may also be involved, particularly in pathologic conditions such as sepsis (Weipert et al. 1988). Interestingly, BK has a dual role in the vascular tone control: it is a potent endothelium-dependent vasodilator but can also cause vasoconstriction by acting directly on vascular smooth muscle cells.

The vasodilatory effect of BK depends on endothelial cells that, after stimulation, release at least 3 compounds: nitric oxide, prostacyclin, and EDHF (Busse et al. 1994, Blatter et al. 1995, Nakashima et al. 1993). Briefly, activation of B_2R located on endothelial cells initiates a sequence of events that results in the production of nitric oxide and prostacyclin. These two compounds migrate to adjacent smooth muscle cells and, by activating guanylate and adenylate cyclases, increase the intra-cellular concentration of the second messengers cGMP and cAMP, respectively (Holzmann et al. 1980, Waldman and Murad 1987). These cyclic nucleotides activate different mechanisms that reduce intracellular calcium concentration and, ultimately, cause smooth muscle cell relaxation. The vasodilatory effect of EDHF was originally attributed to the activation of smooth muscle potassium channels. More recent evidence suggests that EDHF release from endothelial cells occurs following the opening of potassium channels in stimulated endothelial cells (Busse et al. 2002). Although several putative candidates have been proposed, the identity of EDHF remains uncertain (Villar et al. 2008). In humans, the vasodilatory effect of BK has been demonstrated in both arteries and veins (Bönner et al. 1992).

Most of the compounds and pathways that mediate this BK effect are expressed in the liver and are likely to participate in intrahepatic vascular tone control. This is most likely to be true of intra-hepatic vessels that are structurally similar to the vessels where the BK vasodilatory effect has been demonstrated, i.e., the portal vein, hepatic artery, and their branches (Loureiro-Silva et al. 1999).

The vasoconstrictive effect of BK is caused by the direct action of this peptide on vascular smooth muscle. It is endothelium-independent and can, in fact, be blunted by the presence of endothelial tissue. Although

most of studies have shown that this BK effect is mediated by B_2R, the participation of B_1R has also been demonstrated (Felipe et al. 2007). It seems clear that the pivotal event in BK-induced constriction of the vascular smooth muscle cell is an increase in intracellular Ca^{2+} concentration, which is primarily due to increased Ca^{2+} influx from the extracellular space (Bkaily et al. 1997, Dong et al. 2005, Eguchi et al. 1997). Using a rabbit saphenous vein preparation, Eguchi et al. (1997) demonstrated the involvement of Ca^{2+} release from intracellular thapsigargin-sensitive storages sites and a G-protein-mediated increase in the Ca^{2+} sensitivity of the contractile apparatus. Most of the BK vasoconstriction effect observed in different preparations has been shown to be mediated by the release of eicosanoids (Wong et al. 1977, Barabé et al. 1979, Gioli-Pereira et al. 2005).

To study the portal hypertensive response (PHR) to BK, we used the isolated rat liver perfusion (IRLP) model in both monovascular (via portal vein) and bivascular (via portal vein and hepatic artery) conditions, in a hemoglobin-free medium enriched with oxygen. The IRLP is an elegant experimental model, since the liver integrity is maintained and changes in the intrahepatic vascular tone can be easily detected by measuring the perfusion pressure; perfusion pressure can be monitored by the connection of a vertical, open, graduated (cm) column to the portal cannula between the peristaltic pump and the liver in the monovascular perfusion (Borges et al. 1976). In the bivascular perfusion technique, the portal perfusion pressure is measured as described, and the hepatic artery pressure is measured by the pressure transducer. Using these experimental models, we have studied the intrahepatic vascular response to several vasoactive compounds, including BK, des-Arg^9-BK, AI, and AII, in both normal and pathologic conditions. Additionally, we have carried out *in vitro* studies using isolated liver cells. Our findings are summarized in the following paragraphs.

In recirculating monovascular liver perfusion, BK induces a PHR that is mediated by B_2R and modulated by the NO system in normal livers as well as in livers affected by inflammation, fibrosis, and cirrhosis (Loureiro-Silva et al. 2001, Nagaoka et al. 2006). During liver regeneration, the PHR to BK is present, although it is decreased from day 2 until 7 when compared to days 0 and 1 (Teixeira et al. 1999). The PHR was abolished in the presence of 6μM icatibant and, interestingly, when this B_2R antagonist was used at higher concentration ($> 120\mu$M), portal pressure continuously increased until this antagonist was removed from the circuit (Gioli-Pereira et al. 2005).

Molar excess of captopril (ACE inhibitor) and JA-2 (EC 3.4.24.15 specific inhibitor) did not modify the PHR to BK. However, ACE, but not EC 3.4.24.15 inhibitor, increased the BK half-life in the liver perfusion model. On the other hand, HOE-140 inhibited the hypertensive action of BK but had no effect on its hydrolysis (Gioli-Pereira et al. 2005).

The hypertensive effects on the hepatic microcirculation were also studied in a bivascular rat liver perfusion, performed in the anterograde mode. When BK was injected into the arterial bed, it produced a double hemodynamic effect, including both hepatic arterial and portal hypertensive responses. The arterial hypertensive response was calcium-independent and mediated by eicosanoids; the PHR, occurring through a distinct pathway, was not inhibited by naproxen. On the other hand, BK injected into the portal vein produced a portal, but not arterial, hypertensive response. This response was calcium-dependent and was not affected by the presence of naproxen (Gioli-Pereira et al. 2005).

The hemodynamic effects were associated with metabolic effects when BK was injected via the arterial route; these effects included a decrease in oxygen consumption and increase in glucose release. The metabolic effects on oxygen consumption (in the absence of Ca^{2+}) and on glucose release were not altered by naproxen. When BK reaches the liver by the venous route, the hemodynamic effect was also associated with the metabolic effects of decreased oxygen consumption and increased glucose release. The effect on oxygen consumption was calcium-dependent and was not affected by naproxen. The effect on glucose release was neither calcium-dependent nor affected by naproxen.

The hypertensive effect of BK on sinusoidal cells of the periportal region is followed by ACE hydrolysis of the peptide, primarily in the perivenous region. Therefore, we suggested that ACE is the main kininase involved in hepatic BK degradation (Gioli-Pereira et al. 2005).

Using the indicator dilution technique to study distribution volumes and transport phenomena in the liver, we observed that, in the steady state, BK is predominantly distributed in the extracellular space. The mean hepatic extraction of BK (200 nmol) was 16 nmol (8%), which is compatible with the fact that BK is rapidly hydrolyzed by ACE after its action on sinusoidal B_2R in the periportal region (Gioli-Pereira et al. 2005).

Due to their anatomic location and ability to contract or relax in response to vasoactive mediators, endothelins, AII or TXA_2, and NO or CO, respectively, stellate cells seem to be involved in the regulation of portal flow (Rockey 2003). Nevertheless, Kupffer cells and endothelial cells also contain filaments, tubules, and proteins that confer upon these cells the ability to regulate sinusoidal diameter (McCuskey and Reilly 1993). Using isolated liver cells, we demonstrated that sinusoidal cells (quiescent stellate and the fraction containing Kupffer and endothelial cells), but not hepatocytes, respond to BK, causing a basal extracellular acidification (microphysiometer model). This response to BK was specific because it was blocked by HOE-140, a B_2R antagonist (Gioli-Pereira et al. 2005).

As discussed before, B_1R is generally absent from normal tissue, but its expression can be induced in a variety of cells following an inflammatory stimulus. We extensively pursued the effect of des-Arg^9-BK on the intrahepatic vascular response. Among the experimental models that we have studied were: lipopolysaccharide (classical model of induction of B_1R), acute phase inflammation (turpentine oil injection), chronic inflammation (*i.p.* CCl_4 injection up to 56 days), fibrosis (*i.p.* porcine serum injection), cirrhosis (bile duct ligation, BDL), and partial hepatectomy (70% resection). des-Arg^9-BK or des-Arg^9-Lys-BK did not elicit change in the portal pressure in any of these models. In a methoxamine pre-constricted liver, the hypertensive effect of BK was maintained, but no intrahepatic vascular response to B_1R agonists was observed in normal, LPS-treated, or BDL rats (Nagaoka et al. 2006). On isolated cells, we also observed that des-Arg^9-BK did not elicit a response in hepatocyte or sinusoidal hepatic cells (Gioli-Pereira et al. 2005).

Interestingly, in spite of the absence of intrahepatic vascular response, B_1R agonists were metabolized by the liver of normal and LPS-treated rats at the same rate, suggesting an efficient clearance machinery for these kinins (Nagaoka et al. 2006).

The induction of B_1R was confirmed by Western blot in some experimental groups which were studied, such as BDL and partial hepatectomy (Nagaoka et al. 2006). In the CCl_4-ip model, there was a slight band at all different times of treatment (10, 21, and 56 days). Interestingly, in the BDL group, B_1R expression increased in relation to the time of progression of cirrhosis, suggesting a possible role of B_1R in the development of fibrosis; this hypothesis is now under investigation. In the partial hepatectomy group, B_1R expression was observed on days 1 and 2 and disappeared on day 7, suggesting a role in tissue remodeling (Nagaoka et al. 2006).

These results were confirmed by Sancho-Bru and co-workers (2007), who showed that rat normal liver expresses both B_1R and B_2R and, in addition, cirrhotic livers (CCl_4 induction) have an overexpression of these receptors. Continuous BK infusion had a hepato-protective and anti-apoptotic role, via reduction in hepatocellular injury and attenuation of the progression of experimental fibrosis. They suggested that the anti-fibrotic effect of BK infusion was probably associated with the decrease of activated hepatic stellate cells and reduction of collagen and TGF-β1 expression (Sancho-Bru et al. 2007).

In summary, BK induces an increase in the intrahepatic vascular resistance that is mediated by B_2R and preserved in the presence of inflammation, fibrosis, and cirrhosis. The hepatic arterial hypertensive response to BK is calcium-independent and mediated by eicosanoids, and BK does not have a vasodilatation effect on the pre-constricted perfused rat liver. Following its hypertensive effect, BK is degraded by ACE. In cirrhosis and hepatic regeneration, B_1R is overexpressed, suggesting a role in fibrogenesis and the tissue remodeling that now is under investigation.

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RESUMO

Hipertensão portal é a complicação mais comum das doenças crônicas do fígado, tais como cirrose. A resistência intravascular aumentada observada na doença hepática é devida a alterações na arquitetura celular e contração ativa das células estreladas. Neste trabalho revisamos aspectos históricos do estudo do sistema calicreína-cinina e os resultados de nossos estudos do papel deste nonapeptídeo no controle do tono vascular intra-hepático em condições normais e modelos experimentais de agressão hepática usando a perfusão de fígado isolado de rato (mono e bivascular) e células hepáticas isoladas. Nós demonstramos que: 1) o aumento da resistência vascular intra-hepática induzido pela bradicinina é mediado por receptores B_2, envolve a participação de células endoteliais sinusoidais e células estreladas e não é alterada pela presença de inflamação, fibrose ou cirrose; 2) a resposta hipertensiva induzida pela bradicinina no sistema arterial hepático é cálcio-independente e mediada por eicosanóides; 3) bradicinina não tem efeito dilatador na circulação intra-hepática; 4) após exercer efeito vasoconstritor intra-hepático, a bradicinina é degradada pela enzima conversora de angiotensina. Em conclusão, a resposta hipertensiva à bradicinina é mediada pelo receptor B_2 em condições normais e patológicas. Receptor B_1 é expresso mais fortemente nos fígados em regeneração e cirróticos e seu papel está sob investigação.

Palavras-chave: bradicinina, metabolismo hepático, hipertensão porta, receptores de bradicinina.

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