The kallikrein-kinin and the renin-angiotensin systems have a multilayered interaction

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Schmaier, Alvin H. The kallikrein-kinin and the renin-angiotensin systems have a multilayered interaction. Am J Physiol Regul Integr Comp Physiol 285: R1–R13, 2003; 10.1152/ajpregu.00535.2002.—Understanding the physiological role of the plasma kallikrein-kinin system (KKS) has been hampered by not knowing how the proteins of this proteolytic system, when assembled in the intravascular compartment, become activated under physiological conditions. Recent studies indicate that the enzyme prolylcarboxypeptidase, an ANG II inactivating enzyme, is a prekallikrein activator. The ability of prolylcarboxypeptidase to act in the KKS and the renin-angiotensin system (RAS) indicates a novel interaction between these two systems. This interaction, along with the roles of angiotensin converting enzyme, cross talk between bradykinin and angiotensin-(1–7) action, and the opposite effects of activation of the ANG II receptors 1 and 2 support a hypothesis that the plasma KKS counterbalances the RAS. This review examines the interaction and cross talk between these two protein systems. This analysis suggests that there is a multilayered interaction between these two systems that are important for a wide array of physiological functions.

bradykinin; angiotensin; prolylcarboxypeptidase; angiotensin converting enzyme; angiotensin receptors; bradykinin receptors; ACE; ACE2; kininogen; prorenin

THE MECHANISM FOR ACTIVATION of the plasma kallikrein-kinin system (KKS) has been elusive. Although it is well known that the plasma KKS becomes activated when exposed to a negatively charged surface, hence its name the “contact system,” a comprehensive physiological, negatively charged surface has never been discovered. We observe that when the proteins of the plasma KKS assemble on endothelial cells or their matrix on a multiprotein receptor complex, the zymogen plasma prekallikrein (PK) becomes activated to plasma kallikrein (81, 90, 91). Our efforts to identify an endothelial cell-associated plasma PK activator recognized that the enzyme prolylcarboxypeptidase (lysosomal carboxypeptidase, angiotensinase C, PRCP, PCP) has this property (88, 118). Inasmuch as PRCP had only been previously proposed as a degrading enzyme of ANG II, the recognition that it also functions as a PK activator indicates a new interaction between KKS and the renin-angiotensin system (RAS) (99, 116). This interaction, along with the many other communications between these two systems, has led us to formulate a new hypothesis for the physiological activity of the plasma KKS. The plasma KKS serves as the physiological counterbalance to the RAS (116). The purpose of this review is to describe the intimacy and profundity of the interaction between these two systems. These places of interaction serve as foci to examine this hypothesis in the future in both in vitro and in vivo models.

INTERACTIONS BETWEEN THE PLASMA KKS AND RAS

Figure 1 presents a schema on the interactions between the KKS and the RAS. The assembly of high molecular weight kininogen (HK) and PK on endothelial cells results in PRCP activation of PK to plasma kallikrein (118, 119). Plasma kallikrein has several substrates in these systems. It autodigests HK or, at lower affinity, low molecular weight kininogen (LK), to liberate bradykinin (BK). The residual, cleaved HK (HKa) participates in its many activities such as cysteine protease inhibition, anti-angiogenesis, and anti-proliferation of cell growth (23, 63, 51). Alternatively, BK, HK, LK, and tissue kallikrein are proangiogenic (Table 1) (24, 39). Not shown on Fig. 1, plasma kallikrein also converts factor XII to its activated forms and favorably activates single-chain urokinase activation to two-chain urokinase (90, 107). Plasma and tissue kallikreins have also been recognized to be one of the activators of prorenin to renin, an older observation whose physiological significance is questioned (Fig. 1). Renin, an aspartyl protease, activates angio-
tensinogen to ANG I. Angiotensin converting enzyme (ACE) has the bifunctional activity of being one of the degrading peptidases (kininase II) of BK and converting the inactive 10-amino acid ANG I to the biologically active 8-amino acid peptide ANG II [ANG-(1–8)]. ACE is another regulatory juncture point between these two systems (Fig. 1). Liberated BK stimulates vasodilation, nitric oxide (NO) formation, tissue plasminogen activator (tPA) liberation, prostacyclin formation, and superoxide formation like BK (30), it induces NO and prostacyclin formation, tissue plasminogen activator (tPA) liberation, prostacyclin formation, and superoxide formation like BK (30), it induces superoxide and NO formation, platelet activation (55). ANG II counterbalances some of the activities of BK. Although ANG II can stimulate superoxide and NO formation like BK (30), it induces local vasoconstriction and contributes to elevation of blood pressure. ANG II also directly stimulates tissue factor production and plasminogen activator inhibitor 1 release (97, 138). PRCP degrades ANG II to form angiotensin-(1–7) along with ACE2 and, possibly, neutral endopeptidase 24.11 (45, 139). Previously angiotensin-(1–7) was believed to be an inactive breakdown product of ANG II, but it too has been recognized to have biologic activities that result in vasodilation and blood pressure lowering (134). Last, stimulation of the BK B2 receptor (BKB2R) and ANG II receptor result in vasodilation and NO and prostacyclin formation, whereas stimulation of the ANG I receptor results in vasoconstriction and blood pressure elevation (Fig. 1). Thus there appears to be many interaction points and a number of counterbalancing influences of each of these systems on the other in health and inflammatory diseases (10).

It is the intent of this review to examine each of these interactions in more detail and ascertain their relative importance as determined by in vitro and in vivo stud-

ies. In particular, the role of ACE in activating ANG I and inactivating BK will be discussed. The contribution of plasma kallikrein to prorenin activation will be reexamined in light of a physiological mechanism for PK activation. The summing biologic effects of BK and angiotensin-(1–7) will be studied. The role of PRCP in ANG II degradation and plasma PK activation will be presented. The modifying influence of angiotensin receptors 1 and 2 on these systems also will be reviewed. Finally the counterbalancing effect of each of these systems on thrombosis, fibrinolysis, and angiogenesis will be introduced.

ACE

The first recognized important link between the plasma KKS and RAS was the discovery by Erdös and colleagues (46, 143, 146, 147) that kininase II, a major BK degrading enzyme liberating the Phe8,Arg9-dipeptide, was ACE. BK is the preferred substrate of ACE over ANG I with a K_m of 0.18 µM and a k_cat/K_m ratio ~20 times higher (45, 69). Recently, a homologue of ACE, angiotensin converting enzyme 2 (ACE2), has been recognized (8, 26, 35, 131). ACE2 has a different substrate specificity than ACE (Fig. 2). ACE2 is a carboxypeptidase mainly located in the heart, kidney, and testis. It degrades ANG I by removing the COOH-terminal lysine, making the peptide angiotensin-(1–9), which has been reported to enhance arachidonic acid release by BK and resensitize the BKB2R (8, 84). Alternatively, ACE degrades ANG I by proteolyzing it at the penultimate phenylalanine to produce ANG II [angiotensin-(1–8)] (Fig. 2). ACE2 is 100-fold kinetically better degrading enzyme of ANG II to angiotensin-(1–7) than prolylcarboxypeptidase (K_m of inactivation of 2 vs. 200 µM) (99, 139). Thus, in those tissues where ACE2 is present, it is the preferred angiotensinase. The two converting enzymes also have different specificities to BK. ACE2 does not degrade BK, but degrades des-Arg9-BK at its carboxy terminal amino acid (35) (Fig. 2). The implications of these findings are not completely understood at this time. BK is the preferred agonist for the constitutively present BKB2R

Table I Angiogenic activity of proteins and peptides of the kallikrein-kinin and renin-angiotensin systems

<table>
<thead>
<tr>
<th>Proteins</th>
<th>Anti-Angiogenic Activity</th>
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<tr>
<td>HK (HKA)</td>
<td>Domain 5 of HK</td>
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<tr>
<td>LK</td>
<td>Angiotensinogen</td>
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<tr>
<td>Tissue kallikrein</td>
<td>Cleaved angiotensinogen</td>
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<tr>
<td>BKB2 receptor</td>
<td>Des(angiotensin I)angiotensinogen</td>
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<tr>
<td>BKB1 receptor</td>
<td>Angiotensin 1 receptor</td>
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<td>ACE</td>
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<td>Peptides</td>
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<tr>
<td>Angiotensin II</td>
<td>HOE-140</td>
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<tr>
<td>Bradykinin</td>
<td>Des-Arg9-Leu4-BK</td>
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<tr>
<td>Kallidin</td>
<td>HK peptide-(479–498)</td>
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HK, high molecular weight kininogen; LK, low molecular weight kininogen; HKA, cleaved HK; ACE, angiotensin converting enzyme.

Fig. 1. Interaction of the plasma kallikrein/kinin system (KKS) with the renin-angiotensin system (RAS). HK, high molecular weight kininogen; PK, prekallikrein; PRCP, prolylcarboxypeptidase; HKa, plasma kallikrein-cleaved, high molecular weight kininogen free of bradykinin; ACE, ANG I converting enzyme; ACE2, angiotensin converting enzyme 2; tPA, tissue plasminogen activator; PAI-1, plasminogen activator inhibitor 1; PGI2, prostaglandin I2 or prostacyclin.

R2 INVITED REVIEW

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and it usually lowers blood pressure. Des-Arg^9-BK is the preferred agonist for the BK B_1 receptor (BKB1R), which can become quickly expressed in inflammatory states, resulting in elevation of blood pressure (95). Thus different converting enzymes may modulate the degradation of the different forms of BK that have similar biologic activity under different stresses. The modulation of the expression of BK and des-Arg^9-BK by the two converting enzymes may be another level of interaction between the KKS and the RAS.

The influence of ACE and possibly ACE2 on BK metabolism has more profound effects than just modulating blood pressure. The effects of ACE on BK metabolism have been recognized by the influence of ACE inhibitors (ACEI) on a number of biologic processes. ACEI have been shown in a large clinical trial to result in a 25% reduction in death from cardiovascular disease, 20% reduction in myocardial infarction, 30% reduction in stroke, 22% reduction in heart failure, and 16% reduction in complications related to diabetes (130). Although there is no consensus of opinion as to the mechanism of protection from arterial thrombosis, it may be due to an elevation of BK as result of reduced metabolism. ACEI also may reduce the risk for thrombosis by decreasing ANG II formation, thus lowering tissue factor and plasminogen activator inhibitor 1 production. BK elevation after ACEI treatment improves left ventricular diastolic dysfunction by modifying NO release (50). In the endothelial cell NO synthase knockout mouse, there is a significant decrease in the cardioprotective effects of ACEI (76). The ability of ACEI to improve insulin resistance in diabetic mice is mediated by BK and NO, because HOE140, a BKB2R antagonist, and l-NAME, an NO synthase inhibitor, block the enhancement of glucose uptake the agent (120). Last, the mechanism by which ACEI reduces the progression of various fibrotic renal diseases in animal models may be mediated by BK (115). In the BKB2R^−/− mouse or in mice treated with the BKB2 antagonist HOE140, there is increased interstitial fibrosis and decreased overall plasminogen activator and metalloproteinase-2 enzymatic activity (115). This information, although supporting the hypothesis that ACE mediates much of its actions through elevation of BK, has to be interpreted with caution, because there may be strain differences in the mice used. BKB2R^−/− mice in a J129 background carry two renin genes, one of which is not regulated by sodium. These animals develop hypertension, cardiac hypertrophy, and reparative fibrosis that is reversed by lifelong angiotensin 1 receptor blockade (38, 79). This phenotype may be less in BKB2R^−/− mice backcrossed into a stable BL6 background that carries one renin gene.

In addition to influencing BK degradation, ACEI influences BK activity. ACEI increases BK-related effects by an interaction with the BKB2R itself that increases the intrinsic activity of unoccupied BKB2R molecules (58, 59). Alternatively, it has been proposed that ACEI interfere with the sequestration of the BKB2R in cell membranes (7). More recent evidence indicates that the increase in the concentration of ACE by ACEI augments activation of the BKB2R by BK (85). In addition to an effect on the BKB2R, ACEI directly activate human BKB1Rs in the absence of ACE and the BKB1R agonist, des-Arg^{10,14}Lys^{1}-BK (66). Enalaprilat or ramiprilat, but not lisinopril, binds and activates the zinc binding motif, H^{198}EXXH^{199}, on the BKB1R to stimulate calcium mobilization (66). Last, chronic ACEI administration is associated with functional vascular and renal BKB1R, but not BKB2R, induction (83). These latter data suggest that ACEI also influence the inflammatory systems that regulate BKB1R expression. The full implications of this up-regulation of the BKB1R are not completely known at this time.

**PRORENIN ACTIVATION**

Over 20 years ago, both plasma and tissue kallikreins had been proposed as activators of prorenin (Fig. 1). Sealey et al. (113) presented a hypothesis that plasma kallikrein is an activator of plasma prorenin. The basis of this hypothesis was that prorenin activation in plasma could occur after cryoinactivation of plasma protease inhibitors and contact activation of plasma both, promoting PK activation. Investigations by Derkx et al. (33) indicated that after acid activation of plasma, a technique that inactivates the plasma serpins, plasma prorenin is activated and the level of plasma increased by 10.220.33.1 on June 29, 2017 http://ajpregu.physiology.org/ Downloaded from
have increased renin mRNA (133). The meaning of these may be an activator of prorenin. Plasma kallikrein activation of prorenin can occur at neutral pH, although activation must be used to eliminate the activity of the kallikrein neutralizing inhibitors (32). Thus it is possible that the kallikrein activation mechanism is an artifact. However, it was noted that a total kininogen-deficient patient had reduced elevation of plasma renin when assuming the upright position after salt loading (142). These data support the notion that plasma kallikrein may participate in prorenin expression. Because prolylcarboxypeptidase, an endothelial cell membrane-associated PK activator, has been identified, plasma prorenin may be activated under these circumstances. This question should be examined again.

Tissue kallikreins have also been proposed as prorenin converting enzymes, although there is little information on how tissue prokallikrein is activated to tissue kallikrein. Porcine pancreatic kallikrein activates prorenin at an alkaline pH of 8.2 (64). Mouse submandibular glandular tissue kallikrein activates mouse prorenin (74). Mouse tissue kallikreins mK1, mK9, mK13, and mK22 also have been shown to be prorenin activators (73). Human hK1 activates human prorenin (34). Although plasma and tissue kallikrein along with cathepsin B and PC5 have been shown to be activators of prorenin, it is still unsettled as to which of these enzymes or any other is the major responsible prohormone convertase (6, 94).

Last, tissue kallikrein knockout mice (KLK1−/−) have reduced renal renin mRNA compared with wild-type mice, but the BKB2R−/− mice have increased renin mRNA (133). The meaning of these data is presently unknown. Tissue kallikrein could promote gene expressions of renin; plasma kallikrein may be an activator of prorenin.

INTERACTION BETWEEN ANGIOTENSIN-(1–7) AND BK

The recognition that angiotensin-(1–7) and BK share the RAS with the KKS and itself (109, 134). Angiotensin-(1–7) is produced by ACE2 (Km = 2 μM) or prolylcarboxypeptidase (Km = 200 μM) degradation of ANG II (99, 139) (Fig. 3). Neprolysin (endopeptidase 24.11) and thimet oligopeptidase (endopeptidase 24.15) also can produce angiotensin-(1–7) from the breakdown of ANG I (45, 47, 51) (Fig. 3). All of these enzymes are directly or indirectly involved in BK metabolism. There may be other angiotensinasases as well, because ACE2 is only found in the heart, kidney, and testis, and angiotensin-(1–7) is found ubiquitously throughout the vasculature. Once formed, angiotensin-(1–7) exerts its effects by binding to the angiotensin receptor 1 in some cases to antagonize ANG II and, in other cases, to the angiotensin receptor 2 (60, 110) (Fig. 2). Angiotensin-(1–7) may also have its own receptor (47, 67, 75) (Fig. 2). Angiotensin-(1–7) also is degraded by ACE (Fig. 3).

The interactions between angiotensin-(1–7) and BK have been best studied in the kidney (111). In essence, there are two kinds of interactions between BK and angiotensin-(1–7): potentiation of BK by angiotensin-(1–7) and mediation of the vascular activity of angiotensin-(1–7) by BK (111). Angiotensin-(1–7) potentiates the hypotensive effect and vasodilation action of BK in the normotensive or hypertensive rat and in rat mesenteric vessels, respectively. Evidence that angiotensin-(1–7) action is mediated by BK is provided by the observation that HOE140 blocks some angiotensin-(1–7)-mediated activity (111). Angiotensin-(1–7) by stimulating the angiotensin 2 receptor may stimulate BK release (136).

Angiotensin-(1–7) has been described as the most pletotropic metabolite of ANG I, manifesting actions often the opposite of those described for ANG II (47). It dilates canine coronary arteries through kinins and NO (11). Angiotensin-(1–7) augments BK by locally acting as a synergistic modulator of kinin-induced vasodilation by inhibiting ACE and releasing NO (103). These investigations in isolated aortic rings were confirmed by animal studies in rats. Angiotensin-(1–7) decreases blood pressure in the rat, and this effect is mediated by the BKB2R and is unaffected by angiotensin receptor 1 and 2 antagonists (1). Angiotensin-(1–7) is both a substrate and inhibitor of ACE (29). It potentiates arachidonic acid release by an ACE-resistant BK analag acting on BKB2Rs (29). Vasodilation and NO formation induced by angiotensin-(1–7) result from indirect potentiation of BK as an agonist of the BKB2R (29). Angiotensin-(1–7) along with angiotensin-(1–9) also may potentiate the effects of BK by inducing cross talk between ACE and the BKB2R (84). Because ACE inhibitors block desensitization of the BKB2R, angiotensin-(1–7) functions as an ACE inhibitor blocking the ACE COOH domain (29, 132). In doing so, angiotensin-(1–7) acts synergistically with NH2 domain-specific ACE inhibitors (29, 132). Both angiotensin-(1–9) and angiotensin-(1–7) potentiate BK’s action on the BKB2R to elevate arachidonic acid and NO release to occur at lower concentrations (0.01–0.1 μM) than the IC50 (1.2 μM) for ACE inhibition (29, 68). This finding indicates that angiotensin-(1–7) potentiates BK by another mechanism independent of ACE inhibition. ACE
inhibition results in reduced ANG II vasoconstriction and increased angiotensin-(1–7) in plasma and tissue, resulting in vasodilation (108). In human internal mammary arteries, contractions induced by ANG I and II and a non-ACE-specific substrate, Pro11,D-Ala12-ANG I, are antagonized by angiotensin-(1–7) (108). Topical application of BK or angiotensin-(1–7) induces vasodilation in exposed rat mesenteric vessels, and this phenomenon is abolished by the BKB2R antagonist HOE140 or the angiotensin-(1–7) antagonist A-779 (100). This result suggests that each of these biologically active peptides is mediating this activity through its own receptor system. This assessment is especially important for angiotensin-(1–7), because its own receptor has yet to be identified. The potentiation of BK-induced vasodilation by angiotensin-(1–7) is a receptor-mediated phenomenon that is dependent on cyclooxygenase-related products and NO release (100). Angiotensin-(1–7) significantly increases formation of cGMP and N^G-nitro-L-arginine methyl ester (L-NAME), the NO synthase inhibitor, and a selective soluble guanylate cyclase inhibitor blocks the angiotensin-(1–7)-induced relaxations in canine middle cerebral arteries (48). Finally, angiotensin-(1–7) causesafferent rabbit arteriole dilatation and this effect is mediated by NO and not cyclooxygenase products, suggesting a role for kinins (106). Thus angiotensin-(1–7) influences BK by inhibiting ACE, stimulating the BKB2R, and possibly stimulating its own receptor that may cross talk with the BK receptors (see below).

However, there are some data in animals and humans suggesting that the KKS does not counterbalance the RAS. Widdop et al. (141) found that angiotensin-(1–7) failed to enhance the hypotensive effects of BK in the spontaneously hypertensive (SHR) and Wister-Kyoto rats. Furthermore, angiotensin-(1–7) infusion for 7 days has a variable effect of blood pressure in SHR (141). Angiotensin-(1–7) infusion in the forearm of patients with heart failure treated with an ACE inhibitor did not lower blood pressure nor potentiate the vasodilating effects of BK (28). This latter study should not be considered definitive because the model is in a limited population of patients heavily pretreated with medication. However, these animal and human studies question the importance of angiotensin-(1–7) as a clinically significant vasodilator. More animal and human models are needed to clarify the physiological role of angiotensin-(1–7).

PRCP

The enzyme PRCP (lysosomal Pro-X carboxypeptidase, Pro-X carboxypeptidase, pep tidyl prolylamin o acid hydrolase, angiotensinase C) has been recognized as an ANG II degrading enzyme (99, 144). PRCP is a carboxypeptidase discovered by Erdös and Yang (144) when it was noted that a pig kidney extract unexpectedly cleaved des-Arg⁹-BK at the Pro⁷,Phe⁸-OH bond. The enzyme has an optimal pH for activity ~5, but, at pH 7, it retains 20–50% of its maximal activity with physiological substrates. PRCP is a serine protease inhibitable by diisopropyl fluorophosphate and PMSF (118). It is also inhibited by antipain, leupeptin, corn trypsin inhibitor, and high concentrations of mercuric chloride. EDTA, o-phenanthroline, iodoacetic acid, io doacetamide or benzamidine does not inhibit this enzyme (118). Its physiological inhibitor is not known. PRCP is purified from lysosomal fractions from kidney homogenates and human umbilical vein endothelial cells (98, 99, 118, 144, 145). Inasmuch as angiotensin-(1–7) is found circulating in plasma and ACE2’s location is limited to certain organs, there must be a pool of PRCP expressed on the external membrane of unperturbed endothelial cells (109). This notion was recently confirmed by the identification of PRCP on the external membrane of cultured human umbilical vein endothelial cells and cell matrix by various techniques (88, 118). Furthermore, recent preliminary evidence indicates that the constitutive presence of PRCP activity and antigen on cultured endothelial cells is blocked by treatment of the cells with an antisense oligonucleotide (119).

In addition to being a degrading enzyme of ANG II, PRCP has recently been recognized as a plasma PK activator that is independent of factor XIIa (118). The $K_m$ of PK activation by PRCP is 7–17 nM. These data suggest that PRCP is a better PK activator than ANG II degrading enzyme. PRCP is the first endothelial cell PK activator that has been specifically identified (90, 118). Recently, heat shock protein 90 has been proposed as an endothelial cell activator of PK (70, 71). Because this protein, which is not an enzyme, was affinity purified on a corn trypsin inhibitor affinity column, it may have contained trace quantities of PRCP sufficient to activate PK (70, 71). PRCP activity is neutralized by serine protease inhibitors and antibodies to this protein. It is also present on endothelial cell matrix, another site where PK assemblies to become activated (88, 91). We can postulate that the expression of PRCP results in ANG II degradation with the elimination of its sodium retention ability and vasoconstrictive activity to make angiotensin-(1–7), a vasodilator. Furthermore, the ability of PRCP to activate PK should result in BK liberation (Fig. 1). Thus we can speculate that the sum of PRCP activity is angiotensin-(1–7) formation and BK release, resulting in increased NO formation, vasodilatation, and lowering of blood pressure. These hypotheses need to be examined in the PRCP knockout mouse. Recent investigations with the C1 inhibitor (C1 INH) knockout mouse suggest that plasma kallikrein is constitutively present in the intravascular compartment to generate BK (54). The C1 INH KO mouse has persistent paw edema that is blocked by C1 INH infusion, HOE140 infusion, or mating of the C1 INH KO mouse with the BKB2R−/− mouse (54). Because C1 INH is one of the major plasma protease inhibitors of plasma kallikrein, not tissue kallikrein, formed plasma kallikrein must be constitutively present to proteolyze kininogens and liberate BK to mediate the formation of the angioedema. This animal model is consistent with studies in tissue culture indicating that the assembly
of HK and PK on endothelial cells results in immediate PK activation by PRCP (88, 90, 118, 119). These data also suggest that in the intravascular compartment, constitutive activation of plasma PK contributes to BK formation. Although no PK knockout mouse has been available to know for certain, the present data suggest that plasma kallikrein activation is an intravascular kininogenase for the constitutive expression of BK.

This information on plasma PK has to be contrasted with good evidence for tissue kallikrein (KLK1) formation in the intravascular compartment. First, the physiological activator(s) of tissue PK is not known. Second, in cultured endothelial cells, tissue kallikrein mRNA, synthesis, antigen, and activity are noted (30). Furthermore, ANG II stimulation results in increased kallidin and BK production, suggesting that there was increased KLK1 expression. ANG II elevation may result in augmentation of vascular kinins through increased expression of tissue kallikrein. Last, ANG II stimulated vasodilation of venous rings from umbilical cords and this activity is attenuated by the KLK1 inhibitor CH694 (30). Recent studies in tissue kallikrein (KLK1) knockout mice also indicate that these animals are unable to generate significant levels of kinins in most tissues and develop cardiovascular abnormalities early in adulthood despite normal blood pressure (87). These animals also have low kinin-generating activity in isolated tissues that is important for local organ development (87). Tissue kallikrein itself appears to be important for organ development and angiogenesis (see below). Thus the Meneton et al. (87) report indicates the importance of tissue kallikrein in BK formation in tissues and the development of the cardiovascular system. When the plasma PK knockout is created, the relative importance of the two BK-generating enzymes on modulation of intravascular BK and cardiovascular activity will need to be examined.

**ANGIOTENSIN RECEPTORS 1 AND 2**

The receptors for ANG II have been implicated in the cross talk between the RAS and KKS. The many layers of interaction between the KKS and RAS are shown in the communication of ANG II with its receptors. One could argue that the fine tuning between these systems contributes to the homeostasis of blood pressure control and other biologic processes. Evidence for the interaction between the RAS and KKS at the level of the angiotensin receptor was first recognized by the finding that the RAS stimulates renal BK production and cGMP formation through the ANG II receptor 2 (AT2) (125). Inhibition of renin, not the angiotensin 1 receptor, decreases renal BK levels during salt depletion (125). This investigation introduces the notion that stimulation of AT2 receptor releases BK and NO. In 1997, Liu and coworkers (76) recognized that in experimental heart failure, ACE inhibitors and ANG II receptor 1 (AT1) blockade have cardioprotective effects that are due to direct effects on the heart or secondary hemodynamics mediated by BK derived from activation of the AT2 receptor. The ability of ANG II to directly stimulate renal BK production was confirmed in conscious rats, and this effect is blocked by an AT2 receptor antagonist, but not by an AT1 receptor antagonist, losartan (123). Vasodilation produced by stimulation of AT2 in stroke-prone hypertensive rats is produced by BK and NO increasing aortic cGMP (52). Similar findings were made in AT2 receptor transgenic mice that overexpress the AT2 receptor in vascular smooth muscle (136). Furthermore, infusion of ANG II into AT2 receptor transgenic mice abolishes the AT1 receptor pressor effect, which is blocked by the BKB2R antagonist HOE140 and NO synthase inhibitor l-NAME (136). Moreover, removal of endothelium eliminated these AT2 receptor-mediated effects. Thus AT2 receptor-dependent vasodilation under flow conditions is mediated by activation of the vascular kallikrein/kinin system with the elaboration of BK (72). How the AT2 receptor system leads to increased BK formation is not known. It may be mediated by increased tissue kallikrein expression (30). Alternatively, it is not known whether ANG II influences PRCP, PK, or HK expression.

Although ANG II can stimulate the AT2 receptor to release NO, and indirectly BK, there is additional evidence that there also is an interaction between AT1 receptor and the BKB2R on a more fundamental level. The AT1 and BKB2Rs form stable heterodimers causing increased activation of Goαq and Goαq-proteins (3, 4). Heterodimerization also results in a change in the endocytic pathways of both receptors. Heterodimerization between the AT1 and BKB2R occurs in platelets and omental vessels in preeclamptic women (3). This interaction results in a four- to fivefold increase in protein levels of the BKB2R (3). Last, the AT2 receptor binds AT1 receptors to form additional heterodimers to antagonize AT1 receptor function (2). These studies indicate regulatory interactions between the two systems at the level of their receptor proteins.

The clinical importance of the interactions between AT receptor system and the KKS has been examined in a number of studies. Young BKB2R null mice 10–12 wk old in a 129/SvEv background do not have a cardiac phenotype under normal physiological conditions (148). When these animals are aged over 12 mo, they develop hypertension and an increase in left ventricular growth marked by chamber dilatation, elevation of left ventricular end-diastolic pressure, and reparative fibrosis (38, 80). This latter mouse phenotype is prevented by treatment with ACE inhibitors or AT1 receptor antagonists (79, 148). In isolated rat hearts, inhibition of the BKB2R with HOE140 increases myocardial ischemia/reperfusion injury, and inhibition of the AT1 receptor with losartan reduces it (112). AT1 receptor knockout mice have an activated KKS that ameliorates the severity of renal vascular disease (135). In the developing kidney, there is much cross talk between the RAS and KKS. High salt loads during gestation suppress the fetal RAS and provoke abnormal renal development in the BKB2 knockout mouse backcrossed into stable C57BL/6J background (36). Furthermore,
BK stimulates renin gene expression, renal kallikrein is regulated by a negative feedback loop mediated by the BKB2R, and angiotensinogen, ACE, and AT1 are not BK-targeted genes (150). In rats, AT2 receptor stimulation induces a systemic vasodilator response mediated by BK and NO that counterbalances the vasoconstrictor action of ANG II at the AT1 receptor (19). This latter information suggests that AT1 receptor blockade potentiates the cardiovascular effects of ACE inhibitors in the heart and kidney (124). ANG II infusion in the BKB2R knockout animals in a stable C57BL/6J background vs. control induces hypertension, suggesting that the KKS selectively buffers the vasoconstrictor activity of ANG II (21). Alternatively, ANG II infusion in rats in the presence of an AT1 vasoconstrictor action of ANG II (21). Alternatively, ANG II infusion in the BKB2R knockout animals in a stable C57BL/6J background vs. control induces hypertension, suggesting that the KKS selectively buffers the vasoconstrictor activity of ANG II (21). Alternatively, ANG II infusion in rats in the presence of an AT1 antagonist elicits vascular relaxation that was blocked by a BKB2R antagonist and was reduced in kininogen-deficient rats (72). These animal data suggest that in the presence of increased ANG II with stimulation of the AT2 receptor, BK production is secondarily produced (72, 137). The mechanisms that modulate ANG II action on AT1 vs. AT2 receptors are not completely known.

INTERACTIONS BETWEEN THE KKS AND RAS WITH THROMBOSIS, FIBRINOLYSIS, AND ANGIOGENESIS

Although the RAS and KKS are recognized as important modulators of vascular biology, blood pressure regulation, and vascular inflammation (10), they have also been examined for their influence on thrombosis, fibrinolysis, and angiogenesis. Therapeutic manipulations of the RAS and KKS appear to result in risk alteration for arterial thrombosis. Treatment with ACE inhibitors or AT1 receptor antagonists results in an ~15–20% decrease in risk for myocardial infarction and stroke (27, 130). Furthermore, ACE inhibitor treatment before thrombolytic therapy reduces an early increase in plasma plasminogen activator inhibitor 1 (PAI-1) levels in acute myocardial infarction (140). The experimental basis for these clinical results will be reviewed.

THE RAS IS PROTHROMBOTIC

The RAS has been proposed as a prothrombotic system (18). ANG II was recognized to increase plasminogen activator inhibitor-1 (PAI-1) mRNA, antigen, and activity levels from cultured astrocytes and endothelial cells in culture (97, 101, 138). ANG II also increases tissue factor mRNA and activity without affecting tPA or tissue factor protease inhibitor in rat aortic endothelial cells (97). Atrial natriuretic peptide suppresses ANG II-induced expression of tissue factor and PAI-1 mRNA in cultured rat aortic endothelial cells (149). The vasodilating peptide adrenomedullin blocks ANG II upregulation of tissue factor and PAI-1 mRNA in cultured rat endothelial cells (128). In cultured vascular smooth muscle cells, activation of MEK/ERK and Rho-kinase pathways contribute to angiotensin-induced elevation of PAI-1 mRNA (129). In cultured human monocytes, ANG II increases tissue factor mRNA and antigen, but not PAI-1 (92). An ACE inhibitor (captopril) and ANG II receptor 1 antagonist (candesartan) decrease tissue factor levels in these cells. The levels of PAI-1 protein are also reduced by captopril, but this effect is blocked by a BKB2R antagonist (92).

Some support for the above findings in cultured cells has been seen by in vivo studies. Angiotensin infusion in Sprague-Dawley rats induces PAI-1 mRNA in all tissues, and that effect is blocked by an ANG II receptor 1 antagonist (93). However, it is not known if this rise in PAI-1 levels increases the risk for thrombosis. Salt depletion in normal individuals on an ACE inhibitor significantly decreases the 24-h PAI-1 inhibitor activity and antigen levels (13). An ACE inhibitor may reduce the incidence of thrombotic events by reducing peak morning PAI-1 inhibitor levels. However, when the effects of an ACE inhibitor were compared with an AT1 receptor antagonist in 25 normotensive individuals, ACE inhibitor treatment, but not the AT1 receptor antagonist treatment, lowered PAI-1 inhibitor activity and antigen levels (12). In contrast, the AT1 receptor antagonist reduces plasma tPA antigen levels, but the ACE inhibitor does not (12). Alternatively, when studied in 20 insulin-resistant hypertensive individuals, an ACE inhibitor or an AT1 receptor antagonist significantly reduced plasma PAI-1 antigen; however, the ACE inhibitor had a longer duration of effect (16). These studies are conflicting and indicate that these drugs may have different effects depending on the well-being of the subject. Also, ANG II may elevate PAI-1 levels by mechanisms independent of the AT1 receptor and it may downregulate expression of tPA. These studies also point to the complex mechanism by which ACE inhibitors and AT1 receptor antagonists protect from thrombosis in large clinical trials. Investigations in rats suggest that the antithrombotic effects of ACE inhibitors and AT1 receptor antagonists may be mediated by angiotensin-(1–7) (75). Angiotensin-(1–7) infusion reduced thrombus weight and this effect was blocked by an angiotensin-(1–7) antagonist (A-779), an AT1 receptor antagonist (EXP 3174), but not by an AT2 receptor antagonist. Furthermore, the antithrombotic effects of captopril or losartan were attenuated by A-779 or 1-NAME and indomethacin, a prostacyclin inhibitor (75). However, caution must be exercised overall on broadcasting the antithrombotic effects of ACE1 or AT1 receptor antagonists. Recent studies indicate that a thiazide-type diuretic was superior to ACE1 or AT1 receptor antagonism in preventing cardiovascular complications, indicating that the antithrombotic effects of antihypertensives may be by another mechanism than inhibition of the RAS (5). Animal studies are needed to determine if stimulation of the RAS induces thrombosis.

THE KKS IS PROFIBRINOLYTIC AND ANTITHROMBOTIC

As an alternative to the RAS, the KKS has been recognized to influence fibrinolysis since its original
characterization. Factor XII deficiency was characterized over 40 years ago to have defective fibrinolysis (96). To date there have not been good animal models to convincingly show that the plasma KKS is involved in thrombosis. Plasma kallikrein, factor XIIa, and factor XIa have the ability to activate plasminogen to plasmin, albeit much less efficiently than tissue-type plasminogen activator and two-chain urokinase plasminogen activator (22, 53, 82). However, plasma kallikrein has been shown to be a kinetically favorable activator of single-chain urokinase (65). Activation of plasma PK when bound to HK on endothelial cells results in kinetically favorable single-chain urokinase formation with subsequent plasmin formation, suggesting that this pathway is the preferred route for plasmin formation in vivo (90).

In addition to stimulating fibrinolysis by participating in single-chain urokinase activation, kallikrein-induced BK liberation influences fibrinolysis by stimulating tPA release from venous endothelium (15, 17, 126). The mechanism by which BK liberates tPA from human vasculature is mediated through the BKB2R and is independent of NO and prostacyclin liberation also induced by BK (14). BK stimulation of cyclooxygenase 2 (COX2) with the vasodilator prostacyclin liberation also contributes to the anticoagulant state (49, 62, 105). Stimulation of COX2 in mice also results in increased renal medullary blood flow, increased urine flow, and reduced pressor effects of ANG II, contributing to the antithrombotic state (105).

In addition to the profibrinolytic activity of the plasma KKS, there is evidence to suggest that this system is antithrombotic. HK and LK were found to inhibit thrombin-induced platelet aggregation (86, 104). In total kininogen-deficient plasma, there is a lower threshold for gamma thrombin-induced platelet activation than in normal plasma (104). The HK-deficient rat also has a lower threshold for thrombosis than a normal rat (25). The thrombin inhibitory region on kininogens was found to be minimally contained in the ACE breakdown product of BK, BK-(1–5), Arg-Pro-Pro-Gly-Phe (RPPGF) (55). High concentrations of RPPGF prevent coronary thrombosis in the dog using the Lucchesi model of electrolytic injury and in the Folk's model of cyclic flow variations (56, 57). It is presently unclear whether there is a sufficient physiological elevation of the ACE breakdown product of BK (RPPGF) to contribute to the constitutive anticoagulant nature of the intravascular compartment. However, recent preliminary results suggest that it may be possible. The BKB2R−/− mouse in a J129/B6 background has delayed time to arterial thrombosis using the Rose Bengal model of carotid artery thrombosis (127). Infusion of RPPGF or HOE140 into control J129/B6 mice delays the time to arterial thrombosis in these mice. These animal data conflict with in vitro studies and in vivo data indicating that BK administration is a potent liberator of tPA release (15, 17, 126). Further investigations are necessary to understand the physiological sum of the anticoagulant, antithrombotic mechanism(s) of the plasma KKS.

THE INTERACTION OF THE PLASMA KKS AND RAS IN ANGIogenesis

The influence of the KKS and RAS on angiogenesis has recently been appreciated (Table 1). It has been known for some time that BK may have some proangiogenic effects. BK stimulates a neovascular response in implanted sponges in the rat and new vessel formation is blocked by [Leu8]des-Arg9-BK (63). More recently, intact HK and LK also have been shown to be proangiogenic (24). A monoclonal antibody directed to HK's domain 5 blocks HK-induced angiogenesis probably by interfering with kallikrein cleavage of the HK to liberate BK (24, 117). Alternatively, plasma kallikrein-cleaved HK (HKα) (kininostatin), recombinant domain 5 of HK, and isolated peptides from the domain 5 region inhibit angiogenesis and cell proliferation and growth (23, 151).

Upregulating the KKS or interfering with the RAS influences angiogenesis. The interaction between BK and ANG II appears to be essential for normal cardiac development. BKB2R−/− mice treated from birth with an AT1 receptor antagonist have reduced left ventricular mass, chamber volume, wall thickness, and myocardial fibrosis than animals left untreated (37, 78). These data suggest that ANG II is intimately involved in the cardiac development.

The components of the RAS are intimately involved in reparative angiogenesis. In limb ischemia models, the AT1 receptor is involved in and necessary for reparative angiogenesis (43). AT1 receptor blockade or ACE inhibition delays postischemic reparative processes (43). However, proangiogenic activity is not unique to ANG II. ACE inhibitors, HOE140, or the absence of the BKB2R results in reduced intimal hyperplasia produced by interrupted carotid blood flow in mice (41). These data, along with evidence that ACE inhibitors increased vessel density and capillary number in a model of surgically induced hindlimb ischemia in wild-type but not BKB2R−/− mice indicate that the BKB2R mediates the proangiogenic effect of these drugs (121). Furthermore, a low-dose combination of an ACE inhibitor and the diuretic indapamide induces neovascularization in ischemic rat hindlimbs (122). Gene transfer of tissue kallikrein increased urinary kinins, cGMP, and cAMP and had a protective effect on neointima formation (40). This protective effect was not seen when gene transfer was performed in the BKB2R−/− mouse (40). In other investigations, the SHR was also found to have impaired reparative angiogenesis (42). Gene transfer of the human tissue kallikrein gene augmented capillary density and restored the physiological angiogenic response needed for wound healing in this animal (39).

In addition to ANG II and the BKB2R, other components of the KKS and RAS contribute to angiogenesis. The BKB1R also participates in angiogenic reparative processes. In cells in culture, stimulation of the BKB1R initiates endothelial cell proliferation and survival; antagonism of this receptor results in apoptosis (44). In a murine model of limb ischemia, interference with
BKB1R signaling inhibits the native angiogenic response to ischemia. The BKB1R knockout animals are susceptible to limb necrosis after limb ischemia (44). Alternatively, local delivery of a BKB1R agonist enhances collateral vascular growth and accelerated perfusion recovery (44). Last, angiotensinogen and its cleaved derivatives interfere with angiogenesis (20). Because angiotensinogen is a serpin and antithrombin has a strong antiangiogenic activity, investigations showed that angiotensinogen and its derivatives [reactive loop cleaved form, and des(ANG I) angiotensinogen] have antiangiogenic activity (20). In sum, these data suggest that breakdown products of protein components of the KKS and RAS are antiangiogenic, whereas intact proteins and defined physiologically biologically active peptides are proangiogenic (Table 1).

**SUMMARY**

This review indicates that the plasma KKS and RAS are thoroughly intertwined throughout the cardiovascular system. Activation of one system probably results in a counteractivation of some aspect of the other proteolytic system to maintain physiological homeostasis. In the information reviewed, the interaction of both pathways at the whole protein level and the successive enzymatic breakdown products of their biologically active peptides is multilayered and profound. Presently, a few critical juncture points in these systems have been recognized as drug targets. More drug targets for the treatment of cardiovascular disease related to these two systems can be developed by better understanding of the interactions of these systems.

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