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 (61, 62, 102, 126). BK also results in 
lowering of blood pressure. BK and its ACE break- 
down product BK-(1–5) inhibit thrombin-induced 
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 angio- 
tensin-(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 contribu-
tion 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 an-
giotensin 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-dipep-
tide, 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 BK I by proteolyzing it 
at the penultimate phenylalanine to produce ANG II 
[angiotensin-(1–8)] (Fig. 2). ACE2 is 100-fold kineti-
cally better degrading enzyme of ANG II to angioten-
sin-(1–7) than prolylcarboxypeptidase (K_m of inactiva-
tion of 2 vs. 200 µM) (99, 139). Thus, in those tissues 
where ACE2 is present, it is the preferred angiotensi-
nase. The two converting enzymes also have different 
substrate specificities for 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

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<tr>
<th>Peptides</th>
<th>Pro-Angiogenic Activity</th>
<th>Anti-Angiogenic Activity</th>
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<tr>
<td>Angiotensin II</td>
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<td>Bradykinin</td>
<td>HOE-140</td>
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<tr>
<td>Kallidin</td>
<td>Des-Arg8-Leu⁴-BK</td>
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<td></td>
<td>HK peptide-(479–498)</td>
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<tr>
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<td>Domain 5 of HK</td>
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<tr>
<td>Tissue kallikrein</td>
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<tr>
<td>BKB1 receptor</td>
<td>Des[angiotensin-I]angiotensinogen</td>
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<td>Angiotensin 1 receptor</td>
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<td>ACE</td>
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HK, high molecular weight kininogen; LK, low molecular weight 
angiotensinogen; 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, plas-
minogen activator inhibitor 1; PGI2, prostaglandin I2 or prostacyclin.

HK, high molecular weight kininogen; LK, low molecular weight 
kininogen; HKa, cleaved HK; ACE, angiotensin converting enzyme.
and it usually lowers blood pressure. Des-Arg<sup>9</sup>-BK is the preferred agonist for the BK B<sub>1</sub> receptor (BKB<sub>1</sub>R), 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<sup>9</sup>-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 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 BKB1R in the absence of ACE and the BKB1R agonist, des-Arg<sup>10</sup>Lys<sup>1</sup>-BK (66). Enalaprilat or ramiprilat, but not lisinopril, binds and activates the zinc binding motif, H<sup>195</sup>EXXH<sup>199</sup>, 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.

**PRORENNIN 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, a technique that inactivates the plasma serpins, plasma prorenin is activated and the level of plasma plasminogen activator inhibitor 1 is increased by 10.220.33.5 on May 1, 2017 http://ajpregu.physiology.org/ Downloaded from
Angiotensin I

ACE

Angiotensin II

ACE2, PrPC

Angiotensin 1-9

ACE2, Cathipain A

Angiotensin 1-7

ACR

Angiotensin 1-5

The interactions between angiotensin-(1–7) and the KKS 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 pleotropic 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 analog 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 BKBR 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\textsuperscript{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) causes afferent 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 BKBR, 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, peptidyl prolylaminoo 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-Arg\textsuperscript{9}-BK at the Pro\textsuperscript{7}, Phe\textsuperscript{8}-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 atipain, leupeptin, corn trypsin inhibitor, and high concentrations of mercuric chloride. EDTA, o-phenanthroline, iodoacetic acid, iodoacetamide 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 an 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 assembles 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, vasodilation, 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 BKBR\textsuperscript{−/−} 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) forma-
tion in the intravascular compartment. First, the phys-
iological activator(s) of tissue PK is not known. Second,
in cultured endothelial cells, tissue kallikrein mRNA,
synthesis, antigen, and activity are noted (30). Fur-
thermore, ANG II stimulation results in increased kal-
idin and BK production, suggesting that there was
increased KLK1 expression. ANG II elevation may result
in augmentation of vascular kinins through in-
creased 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 kal-
ikrein (KLK1) knockout mice also indicate that these
animals are unable to generate significant levels of
kinins in most tissues and develop cardiovascular ab-
normalities early in adulthood despite normal blood
pressure (87). These animals also have low kinin-gen-
erating 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 exam-
inied.

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 con-
trol and other biologic processes. Evidence for the in-
teraction 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 recep-
tor, 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 exper-
imental heart failure, ACE inhibitors and ANG II re-
ceptor 1 (AT1) blockade have cardioprotective effects
that are due to direct effects on the heart or secondary
hemodynamics mediated by BK derived from activa-
tion 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, not by an AT1 receptor antag-
onist, losartan (123). Vasodilation produced by stimu-
lation of AT2 in stroke-prone hypertensive rats is pro-
duced 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 condi-
tions is mediated by activation of the vascular kal-
ikrein/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 in-
creased tissue kallikrein expression (30). Alterna-
atively, 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 caus-
ing increased activation of Goq- and Goqα-proteins (3, 4).
Heterodimerization also results in a change in the
endocytic pathways of both receptors. Heterodimeriza-
tion 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 ventricu-
lar growth marked by chamber dilatation, elevation of
left ventricular end-diastolic pressure, and reparative
fibrosis (38, 80). This latter mouse phenotype is pre-
vented by treatment with ACE inhibitors or AT1 recep-
tor antagonists (79, 148). In isolated rat hearts, inhi-
bition of the BKB2R with HOE140 increases myocard-
ial ischemia/reperfusion injury, and inhibition of the
AT1 receptor with losartan reduces it (112). AT1 recep-
tor knockout mice have an activated KKS that amelio-
rates 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 blockage 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 at the AT1 receptor (21). This latter information suggests that AT1 receptor antagonism 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 N,N-diisopropyl-L-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 ANTIITHROMBOTIC

As an alternative to the RAS, the KKS has been recognized to influence fibrinolysis since its original

AJP-Regul Integr Comp Physiol • VOL 285 • JULY 2003 • www.aipregu.org
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 plasmin 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 Folt’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 [Leu]des-Arg²-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 (HKa) (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 physiological 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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REFERENCES


