Published in final edited form as:
Kidney Int. 2009 May ; 75(10): 1019–1030. doi:10.1038/ki.2008.647.

The kallikrein–kinin system in health and in diseases of the kidney

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Abstract

Since kallikrein was discovered as a vasodilatory substance in human urine, the kallikrein–kinin system (KKS) has been considered to play a physiological role in controlling blood pressure. Gene targeting experiments in mice in which the KKS has been inactivated to varying degrees have, however, questioned this role, because basal blood pressures are not altered. Rather, these experiments have shown that the KKS has a different and important role in preventing changes associated with normal senescence in mice, and in reducing the nephropathy and accelerated senescence-associated phenotypes induced in mice by diabetes. Other experiments have shown that the KKS suppresses mitochondrial respiration, partly by nitric oxide and prostaglandins, and that this suppression may be a key to understanding how the KKS influences senescence-related diseases. Here we review the logical progression and experimental data leading to these conclusions, and discuss their relevance to human conditions.

Keywords

ACE inhibitors; aging; bradykinin; DNA damage; electron transport chain; oxidative stress

BACKGROUND

Angiotensin-I-converting enzyme and bradykinin

The angiotensin-I-converting enzyme (ACE, also known as kininase II) is a carboxydipeptidase that removes two amino acids from the carboxyl terminus of the inactive peptide angiotensin I and converts it into the active blood pressure-raising peptide, angiotensin II. ACE also converts the active blood pressure-lowering kinins, bradykinin (1–9) and kallidin (1–10), into inactive bradykinin (1–7) and kallidin (1–8) (Figure 1a). ACE has a 30 times lower K_m and 10 times higher k_{cat} for the kinins than for angiotensin I.

A very common insertion/deletion (I/D) polymorphism of the *ACE* gene is associated with different relative plasma levels of the enzyme, ranging from about 0.75–1.0 to 1.25 in I/I, I/D, and D/D individuals. The *ACE* I/D polymorphism in humans does not significantly affect blood pressure,[3] nor does a modest genetically induced decrease in expression of the *Ace* gene (to 0.5 × normal) or a modest increase (to 1.5 × normal) affect blood pressure in mice.[4] Nevertheless, the two human alleles are associated with different risks for developing a wide

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DISCLOSURE

The authors declared no competing interests.

constellation of diseases, including diabetic nephropathy,[5] breast cancer,[6–8] prostate cancer,[9,10] gastric cancer,[11,12] Alzheimer's disease,[13] Parkinson's disease, [14] congestive heart failure, [15] myocardial infarction,[16] stroke, [17] and retinal macular degeneration[18]. In all these many senescence-associated human disorders, it is the D allele with its higher levels of ACE that confers the increased risk.

A clear example of this association is provided by the demonstration that the D allele is an independent risk factor for both the onset and progression of nephropathy in type I diabetic patients.[5] This association led to experiments in which diabetes was induced with streptozotocin (STZ) in mice having different genetically determined levels of ACE.[19] These experiments showed that, when diabetic, mice having the higher levels of the enzyme (about 1.5 × normal) developed significantly more urinary albumin excretion than their siblings with normal or reduced (0.5 × normal) Levels of ACE. These experiments consequently established a causative link between genetically increased levels of ACE and the nephropathy induced by type I diabetes. Yet earlier experiments with varying degrees of ACE inhibition,[20] and computer simulations of the effects of genetically altering the levels of ACE,[21] had shown that such modest changes in ACE levels have little effect on the levels of its products (including the active peptide angiotensin II), although they change the levels of its substrates (including the active peptides bradykinin (1–9) and kallidin (1–10)). This consideration led to the inference that decreases in the level of the active ACE substrate bradykinin probably mediate the harmful effects of the *ACE* D allele.

The kinins

The kinins, bradykinin and kallidin in humans or bradykinin and the kallidin-like peptide in rodents, are generated from kininogens by kallikreins (Figure 1a). Humans have one kininogen gene; rodents have two closely linked kininogen genes.[22] Kallidin can be converted into bradykinin by a plasma aminopeptidase. All the kinins are strong agonists of the bradykinin 2 receptor (B2R, Bdkrb2), although less so of the B1 receptor (B1R, Bdkrb1). Kininase I (carboxypeptidase-N) and carboxypeptidase-M remove arginine from the carboxyl terminus of the kinins and generate their des-Arg derivatives, which are agonists mainly of B1R. Kininase II (a synonym for ACE),[23] neprilysin (endopeptidase 24.11),[23] and endothelin-converting enzyme[24] all remove two amino acids (Phe and Arg) from the carboxyl terminus of the kinins, and inactivate them.

Bradykinin B1 and B2 receptors

In mammals, as indicated above, two bradykinin receptors have been identified: B1R and B2R, both of which are G protein-coupled receptors with seven transmembrane domains. Mice deficient in both B1R and B2R have no contractile response to bradykinin in isolated smooth muscle tissues, suggesting that there are no other major receptors for bradykinin, at least in smooth muscle cells.[25] The B2R protein is constitutively expressed in most tissues. Vascular endothelial cells express B2R abundantly, where it is functionally linked to activation of endothelial nitric oxide (NO) synthase (eNOS, Nos3). Expression of B1R is minimal under normal circumstances, but is induced by inflammation,[26] diabetes,[27] ischemia/reperfusion injury,[28] and by the absence of B2R.[29] B2R mRNA is expressed in all segments of the kidney under physiological conditions, and lipopolysac-charide (LPS) increases this expression. In contrast, no B1R mRNA levels can be detected in any segments of the kidney under physiological conditions, although treatment with LPS induces the expression of B1R mRNA in all renal segments except the outer medullary collecting ducts.[30]

The transcriptional regulation of the two receptor genes differ, although the intracellular signals that follow stimulation of B1R and B2R are quite similar (Figure 1b).[31,32]

Stimulation of bradykinin receptors by kinins elevates $[Ca^{++}]i$ by activation of phosphatidylinositol (PI)-specific phospholipase C (PI-PLC) in Gq-protein-dependent and Gq-protein-independent ways. Allosteric activation of PI-PLCβ isoforms by Gq/11 proteins and direct tyrosine phosphorylation of PI-PLCγ isoforms by bradykinin receptors[33,34] both play an important role in the bradykinin-induced changes in $[Ca^{++}]i$ (Figure 1b).

Stimulation of either B1R or B2R increases eNOS activity and prostacyclin synthesis in endothelial cells, at least partly by elevating intracellular calcium levels ($[Ca^{++}]i$).[35,36] And it has recently been shown that bradykinin, kallidin, and kallidin-like peptide are equipotent at eliciting Ca^{++}-transients by B2R in humans and rodents.[37] As the concentration of kallidin or kallidin-like peptide is much higher than that of bradykinin in humans[38] and rats,[39] kallidin and kallidin-like peptide rather than bradykinin may be the major endogenous agonists of the KKS.

The NO release in response to agonist binding by B1R is slow in onset, but desensitization of B1R does not occur.[40] In contrast, the NO response of the B2R is rapid[40] and agonist-occupied phosphorylated B2R[41] is internalized into endo-somes in a β-arrestin 2- and clathrin-dependent manner[42,43]. This uncouples B2R from the G proteins and desensitizes the second messenger-mediated signal.[42] Interestingly, eNOS also reversibly translocates from the cell membrane into the cell cytosol following B2R stimulation or administration of a calcium ionophore.[44] Although the kinins promote B2R endocytosis, they delay B1R endocytosis, which is clathrin-dependent but β-arrestin 2-independent.[43]

In transgenic mice, over expression of B2R results in hypotension,[45] but overexpression of B1R does not change blood pressure.[46] In contrast, the absence of either B2R orB1R does not change blood pressure,[47,48] nor does lack of both bradykinin B1R and B2R, which abolishes most ofbradykinin signaling[25,49]. Furthermore, mice lacking tissue kallikrein and kininogen-deficient Brown Norway Katholiek rats have normal blood pressure.[50,51] These observations suggest that the KKS plays only a minor physiological role in regulating chronic blood pressure in mammals, even though transient decreases in blood pressure are caused by administration of kallikrein or the kinins.

Bradykinin and nitric oxide (NO)

The absence of B2R decreases the urinary excretion of stable metabolites of NO (NO_2^- and NO_3^-),[52] and lack of both B1Rand B2R reduces fasting plasma NO_2^-/NO_3^- concentration.[49]

Stimulation of the bradykinin receptors by the kinins elevates $[Ca^{++}]i$ and activates the Ca^{++}-dependent iso-forms of the NOS (eNOS and neuronal NOS).[53,54] Bradykinin through its receptors also leads sequentially to activation of PI3-kinase, phosphorylation of Akt, and phosphorylation of eNOS, which sensitizes it to $[Ca^{++}]i$.[55] Furthermore, B2R forms a complex with eNOS from which the active enzyme is released following receptor activation.[56] Bradykinin also increases the association of heat-shock protein 90 with eNOS, which is required for NO formation by eNOS.[57]

The expression of inducible NOS (the Ca^{++}-independent isoform of the NOS) is also increased by bradykinin through both B1R[58], and B2R.[59] The bradykinin-induced expression of inducible NOS is dependent on intranuclear calcium and Akt signaling in rat hepatocytes.[59]

Lipopolysaccharide causes hypotension in normal rats, which is diminished by a B2R antagonist[60]. Furthermore, kininogen-deficient rats and mice lacking both B1R and B2R are resistant to LPS-induced septic shock.[25,60] Inducible NOS mRNA levels are increased by

LPS in wildtype, but in mice lacking both B1R and B2R this increase is diminished. [25] Thus, bradykinin plays a role in the development of septic shock, in part by inducible NOS.

Together these various studies show that the KKS is important in controlling NO production through all the isoforms of NOS. The NO so formed has the potential of acting on the cells that produced it or on neighboring cells.

Bradykinin and the prostaglandins

Most of the nonsteroidal anti-inflammatory drugs, including aspirin, indomethacin, ibuprofen, and the more isoform-specific cyclooxygenase inhibitors, exert their anti-inflammatory effects by inhibiting the formation of arachidonic acid metabolites, including the prostaglandins (PGs). Bradykinin acts through its receptors in at least three ways to increase production of PGs. First, it leads to the Ca^{++}-dependent phosphorylation and translocation into the cell membrane of cytosolic phospholipase A2.[61,62] Second, bradykinin stimulates membrane-associated Ca^{++}-independent phospholipase A2.[63] Both the Ca^{++}-dependent and -independent A2 phospholipases liberate arachidonic acid from membrane phospholipids. Third, bradykinin leads to the induction of cyclooxygenase-2,[64–66] which converts arachidonic acid into PGs. The bradykinin-stimulated formation of PGE2 is inhibited by pertussis toxin,[67] suggesting that this response is mediated by the Gi protein that is associated with the bradykinin receptors.[68] The PGs formed following stimulation of the bradykinin receptors, acting through PG receptors, mediate some of the effects of the kinins on vascular tone and on mitochondrial respiration. When kinins are injected in supra-physiological amounts, they cause inflammation, pain, and increased vascular permeability,[69] at least partly through the PGs. KKS antagonists are therefore effective for suppressing excessive inflammatory responses.[70]

Bradykinin and other second messengers

The endothelium-dependent vasodilatory effect of bradykinin is not completely abolished by the simultaneous administration of NOS inhibitors and cyclooxygenase inhibitors. However, this unexplained vasodilation is inhibited by blockers of Ca^{++}-activated K^+-channels or high external $[K^+]$, suggesting the presence of an endothelium-derived hyperpolarizing factor.[71] Epoxyeicosatrienoic acids, P450 epoxygenase metabolites of arachidonic acid, are most likely candidates for the endothelium-derived hyperpolarizing factor.[72]

The C-terminal part of B2R interacts with the protein-tyrosine phosphatase SH2 domain-containing phosphatase-2 (SHP-2), and activates it in rat mesangial cells.[73] As the antimitogenic effect of bradykinin is abolished by transfection of dominant-negative SHP-2 in mesangial cells,[73] it is possible that bradykinin, acting through SHP-2, plays a role in controlling the mesangial expansion that occurs in many glomerular diseases.

Bradykinin, oxidative stress, and senescence

Mitochondrial oxidative respiration is a much more efficient source of energy than anaerobic glycolysis. However, oxidative metabolism generates reactive oxygen species, which can have deleterious effects. Several studies have shown that NO reversibly suppresses mitochondrial oxidative metabolism,[74,75] in part by inhibiting cytochrome c oxidase, a key enzyme in electron transport chain.[76,77] Recent studies have also shown that cAMP decreases mitochondrial respiration by activating NADH-ubiquinone oxidoreductase activity of complex I and by inhibiting cytochrome *c* oxidase.[78,79] As bradykinin, acting through B1R and B2R, stimulates eNOS activity in vascular endothelial cells and increases cAMP levels in kidney epithelial cells, it is not surprising that the KKS can influence the level of oxidative stress. For example, when bradykinin is administered to rats that were made hyperglycemic with STZ, it reduces their oxidative stress phenotype, as judged by

hydrogen peroxide and malondialdehyde levels.[80] Furthermore, because the binding of B2R to eNOS, referred to above, is through the oxygenase domain of the enzyme, the ability of eNOS to catalyze uncoupled NADPH oxygenation is blocked by B2R,[81] suggesting that B2R can affect the generation of reactive oxygen species by eNOS even without agonist stimulation.

In the kidney, proximal tubular epithelial cells are densely packed with mitochondria, which supply the energy for the active transport of sodium ions by Na^+/K^+ ATPase. These cells are among the most oxygen-using cells of the body, and are therefore at risk for oxidative damage. It is consequently again not surprising that several indicators of oxidative damage are increased in these and other high oxygen-using cells when the KKS is impaired by the absence of B2R. Figure 2a illustrates this by showing that lack of B2R greatly enhanced the accumulation in diabetic mice of lipofuscin-like intracellular inclusions (an indicator of mitochondrial damage) in renal proximal tubules.[82]

Lipofuscins are electron dense substances contained in autophagolysosomes derived from damaged organelles (Figure 2b). They are a manifestation of senescence as well as of oxidative stress.[83] Mice lacking the senescence marker protein-30 have systemic premature senescence, and lipofuscin accumulation is observed in their proximal tubular cells preceded by senescence-associated β-galactosidase activity,[84] an established hallmark of aging.[85] We have also observed that the deposition of lipofuscin in the proximal tubule cells of B2R-null Akita mice aged 12 months is associated with the presence of senescence-associated β-galactosidase activity (unpublished observation).

Point mutations and deletions in mitochondrial DNA, which are also known reflections of aging,[86,87] were increased in the kidney by the absence of B2R even in non-diabetic mice, although additively more so with diabetes[82] (Figure 2b and c).

Transforming growth factor β1 enhances autophagy,[88] and its expression is increased in aged cells[89] and in a number of fibrogenic kidney diseases.[90] We have found that the absence of B2R and/or presence of diabetes enhances the renal expression of transforming growth factor β1.[82] Thus, the increase in transforming growth factor β1 expression may play a causative role in lipofuscin accumulation in the kidney of B2R-null Akita mice.

EXPERIMENTAL STUDIES ON KKS IN RENAL DISEASES

Diabetic nephropathy

We have described above how genetically increased ACE levels, which do not change blood pressure or angiotensin II levels,[4] nevertheless enhance urinary excretion of albumin in mice with diabetes induced by STZ.[19] In the reverse direction, ACE inhibition is protective in many models of diabetic nephropathy.[91–94] That this protection is mediated in part by the KKS is strongly suggested by experiments showing that the beneficial effects of ACE inhibitors were attenuated by a B2R antagonist in rats that were made diabetic with STZ,[91,92] in obese Zucker diabetic fatty rats,[93] and in C57BLKS db/db mice.[94]

We have found that albuminuria, glomerular sclerosis, interstitial fibrosis, lipofuscin accumulation in proximal tubules, and lifespan shortening in Akita diabetic mice are enhanced by the absence of B2R.[82,95] In agreement with these observations, adeno-associated virus-mediated expression of the human tissue kallikrein has been shown to mitigate nephropathy induced by STZ and high-fat diet as assessed by urinary albumin excretion, histological changes, creatinine clearance, and urinary osmolarity.[96] However, Tan *et al.*[97] have reported that deletion of B2R protects against the albuminuria and histological changes which develop in diabetic nephropathy induced by STZ. These opposite

results may be due to the differences in the strains of mice used and/ or in the method of induction of diabetes.

The importance of NO in relation to experimental diabetic nephropathy is well documented. Thus, L-arginine, the substrate of the NO synthases, reduces the proteinuria that develops in STZ-induced diabetic rats.[98] Furthermore, L-NAME, an NOS inhibitor, aggravates the proteinuria and histological changes that occur in the diabetic nephropathy of Otsuka Long-Evans Tokushima Fatty rats.[99] eNOS deficiency also accelerates the severity of diabetic nephropathy in C57BLKS/J db/db mice,[100] lepr(db/db) mice,[101] and STZ-treated C57BL/6 mice.[102] As bradykinin induces eNOS activity,[40] these observations bear directly on how the KKS exerts its protective effects.

Cicaprost, a PGI2 analog, also attenuates the progression of diabetic renal injury in STZ-treated rats,[103] suggesting that the beneficial effects of the KKS also involve the PG arm of the system.

Hypertensive glomerulosclerosis and other chronic fibrogenic kidney diseases

In Dahl salt-sensitive rats, an animal model of salt-sensitive hypertension, an ACE inhibitor reduced the proteinuria, urinary excretion of *N*-acetyl-β-D-glucosaminidase (an indicator of damages in proximal tubules), and renal fibrotic changes caused by high salt. The protective effects of an angiotensin II type 1 receptor blocker (ARB) were, however, much smaller than those of the ACE inhibitor, despite the same reduction of blood pressure.[104] In Ren-2 transgenic rats, an ACE inhibitor or a vasopeptidase inhibitor significantly reduced tubulointerstitial fibrosis, and this reduction was annulled by a B2R antagonist.[105] Long-term infusion of rat urinary kallikrein[106] or bradykinin[107] into Dahl salt-sensitive rats attenuated their urinary protein excretion and glomerulosclerosis, despite no changes in blood pressure. The beneficial effect of infused kallikrein was attenuated by a B2R antagonist.[108] Adenovirus-mediated human tissue kallikrein gene delivery caused a similar favorable effect in both Dahl salt-sensitive rats and Goldblatt hypertensive rats, again without affecting blood pressure.[109,110]

In rats with passive Heymann nephritis, an experimental model of human membranous nephropathy, an ACE inhibitor exerted antiproteinuric action, which was prevented by a B2R antagonist.[111]

Alport syndrome is a hereditary cause of endstage renal disease (ESRD) due to defects in type IV collagen genes. In mice lacking the COL4A3 gene, an animal model of Alport syndrome, an ACE inhibitor reduced the proteinuria and renal interstitial fibrosis.[112] In subsequent experiments, it was shown that the ACE inhibitor extended the lifespan of the COL4A3-null mice by 111%, whereas an ARB resulted in only a 38% prolongation of the lifespan,[113] emphasizing the importance of non-angiotensin II mechanisms in helping survival of these animals.

Unilateral ureteral obstruction in rodents is a well-established non-immune inflammatory experimental model that results in tubulointerstitial fibrosis in the obstructed kidney. ACE inhibitors,[114] and to a lesser extent ARBs,[115] prevent the progression of tubulointerstitial fibrosis in this unilateral ureteral obstruction models. Co-treatment of the animals with an ACE inhibitor and a NOS inhibitor reversed the beneficial effect of the ACE inhibitor in the obstructed kidney.[116] Unilateral ureteral obstruction-induced fibrosis is more severe in B2R-null mice than wild-type mice, and less severe in the human tissue kallikrein-transgenic rats than in control Sprague Dawley rats.[117] These several experiments together provide a strong case for the importance of the KKS in limiting the tubulointerstitial fibrosis caused by unilateral ureteral obstruction.

Aminoglycoside-induced renal injury

Aminoglycoside antibiotics have been used primarily to repress infection by aerobic Gram-negative bacteria. They are, however, notorious for their ototoxicity and nephrotoxi-city, although how they cause the proximal tubular cell necrosis that constitutes their renal effects is largely unknown. However, in this context it is intriguing that the administration of tissue kallikrein protein[118] or tissue kallikrein-expressing adenovirus[119] are both protective against gentamicin-induced renal injury in rats. NAD(P)H oxidase activity and superoxide production in the kidney are elevated by gentamicin treatment.[120] Administration of tissue kallikrein partially reverses these increases,[118] indicating that enhancing KKS activity can reduce gentamicin-induced renal injury.

Other studies have shown that the NOS substrate L-arginine prevents gentamicin-induced tubular damage in rats,[121] whereas the NOS inhibitor NG-nitro-L-arginine-methyl ester increases the damage.[121,122] PGI2 overexpression in renal tubular cells also prevented apoptosis induced by gentamicin, and decreased the generation of reactive oxygen species induced by the drug.[123] Thus, the protective effect of tissue kallikrein against gentamicin-induced renal injury is probably mediated by its ability to increase both NO and PGI2.

Ischemia-reperfusion injury

The angiotensin-I-converting enzyme inhibitors markedly and consistently reduce the tissue injury that occurs in ischemic acute renal failure (iARF), including the tubular necrosis, loss of endothelium-dependent vasorelaxation, and excretory dysfunction.[124,125] In contrast, it is still debatable whether ARBs have any beneficial effects on the tissue damage caused by iARF.[126,127] There is agreement, however, that ACE inhibitors are more effective than ARBs in protecting against ischemia-reperfusion injury.[127–130] Furthermore, a B2R antagonist and NOS blocker markedly attenuate the protective effects of the ACE inhibitors.[128,129] These observations support the concept that, in many contexts, ACE inhibitors are beneficial more by inhibiting the inactivation of the kinins than by suppressing angiotensin II formation.

Recent studies are beginning to provide a more detailed explanation for this protection. Thus, it is now known that ischemia-reperfusion injuries are associated with mitochondrial Ca^{2+} overload consequent to a burst of reactive oxygen species, which together trigger the opening of mitochondrial permeability transition pores leading to cell apoptosis.[131] This opening of mitochondrial pores is suppressed by bradykinin.[132] As NO, a second messenger of both bradykinin receptors, reversibly suppresses mitochondrial oxidative metabolism,[74,75] it is possible that the KKS can reduce or prevent the burst of reactive oxygen species.

In contrast to the consensus that ACE inhibitors have beneficial effects on ischemia-reperfusion injury, in part mediated by the KKS, there are conflicting reports on the effects of administered bradykinin on iARF. Thus, exogenous supplementation of bradykinin has been reported to aggravate iARF,[133] yet suppression of the endogenous KKS[49] is detrimental to functional recovery after iARF. These findings suggest that physiological levels of bradykinin produced endogenously, but not the presumably higher levels achieved with exogenously administered bradykinin, play a beneficial role in iARF. In agreement with this interpretation, adenovirus-mediated gene transfer of tissue kallikrein has been shown to protect against ischemic stroke in rats.[134] Similarly, transgenic expression of tissue kallikrein in mice attenuates ischemic cardiac damage,[135] whereas knocking out tissue kallikrein aggravates the damage.[28]

We have found that lack of B2R alone or lack of both receptors, B1R and B2R, aggravates the renal morphological and functional damages and mortality following iARF.[49] Furthermore, the absence of both B1R and B2R had more detrimental effects than

deficiency in B2R only, showing that both B1R and B2R are protective in iARF.[49] As NO donors attenuate[136] and NOS inhibitors aggravate tissue damage in iARF,[137,138] endothelium-derived NO is most likely to be one of the mediators of the beneficial effect of bradykinin. In addition, earlier studies have shown that prostaglandins E1, E2, and I2[139–143] are protective in mitigating renal injury caused by iARF, suggesting that phospholipase A2-derived products are involved in bradykinin-induced protection following iARF.

Autosomal dominant polycystic kidney disease

Autosomal dominant polycystic kidney disease (ADPKD) accounts for 10% of cases of ESRD. In the heterozygous cystic Han:Sprague-Dawley-cy rat, which is an animal model of ADPKD, urinary kallikrein and bradykinin levels are increased compared with age-matched controls.[144] ACE inhibition significantly reduces the albuminuria and prevents the enlargement of kidney size and decline in glomerular filtration rate, independently of its effects on blood pressure,[145–148] suggesting that the KKS has a protective role in this ADPKD model. However, it has been reported that a B2R antagonist significantly reduced proteinuria and albu-minuria in this model,[148] and in human ADPKD patients, the KKS system is not activated.[144]

Chronic renal failure

Irrespective of their etiology, most renal diseases eventually lead to a reduction in the number of nephrons and to insufficiency of renal excretory function and chronic renal failure (CRF). The rate of decline in creatinine clearance accelerates as the absolute value of creatinine clearance is reduced, suggesting overload on the remaining nephrons (‘hyperfiltration theory’).[149] In the 5/6 nephrectomy animal model of CRF, ACE inhibitors,[118,150] adenovirus virus-mediated tissue kallikrein gene delivery,[151] or adeno-associated virus delivery,[152] all decelerate the decline in renal function. However, whether a B2R antagonist abrogates the beneficial efficacy of ACE inhibitors in this 5/6 renal mass reduction model is debatable.[118,150] Nevertheless, as renal pathology and mortality in this model are both exaggerated by eNOS deficiency,[153] it is most likely that changes in eNOS activity are partly responsible for the beneficial effect of KKS in CRF.

RELEVANCE OF THE KKS TO RENAL DISEASES IN HUMANS

Studies of mice in which genes have been knocked out have proved invaluable in determining the role of many genes in mammals. However, the common human diseases, such as diabetes and hypertension, do not appear to be prevalent because of the complete absence of any gene function. Rather they appear to be due to a variety of combinations of genetic differences that individually have relatively small quantitative effects.[154] Furthermore, humans are genetically more hetero-genous than inbred experimental animals. For these reasons, it has been proved difficult to identify genetic differences in humans that influence these common multifactorial conditions. Nevertheless, polymorphisms in several KKS-related genes have been associated with the risks of developing a number of renal problems. Thus, the insertion/deletion *ACE* polymorphism has been clearly shown to influence diabetic nephropathy.[5] And a polymorphism in the human B2R gene has been correlated with altered urinary albumin/creatinine values in diabetic patients.[155] Similarly, a polymorphism in intron 4 of the *Nos3* gene has been associated with an increased risk for nephropathy in patients with either type 1[156] or type 2 diabetes.[157] Other reports have shown that the *ACE* D/D genotype is a risk factor for progression to CRF in IgA nephropathy.[158,159] ADPKD patients with the *ACE* D/D genotype have a 5–10 years earlier onset of ESRD than those with the I/I genotype.[160–162] The progression of IgA nephropathy and ADPKD into ESRD are both influenced by the *Nos3* polymorphism.[163] In humans, polymorphisms in most of the genes in the KKS have also been associated with the

progression of CRF into ESRD, including *ACE*,[164,165] *Bdkrb1*,[166,167] *Bdkrb2*,[167,168] and *Nos3*.[169,170]

THERAPEUTIC IMPLICATIONS

Of the presently available drugs, the ACE inhibitors are most effective in enhancing the KKS, and many of their benefits are independent of blood pressure lowering. Besides retarding the decline in renal function, the KKS may be of particular relevance in the processes of angiogenesis and cardiac regeneration, following myocardial infarction.[171] The recently developed vasopeptidase inhibitors that inhibit ACE, neprilysin and/or endothelin-converting enzyme, all of which degrade the kinins, may also prove to be beneficial, when they become available for clinical use.[172] Yet the ACE inhibitors are not suitable for use in pregnancy because of teratogeni-city, nor are they recommended in patients with CRF who are not under dialysis therapy, because they are susceptible to cardiotoxic hyperkalemia.[173] Additionally, some individuals have to discontinue their usage because of coughing. At present, no drugs are available that enhance KKS specifically without affecting the renin–angiotensin–aldoster-one system.

In principle, KKS-specific antagonists and agonists could both be useful, although for different purposes. KKS antagonists could be used for acute life-threatening inflammatory conditions including septic shock,[174] asthma,[175] acute pancreatitis,[176] and attacks in patients with hereditary angioedema.[177] However, because the KKS is important for retaining renal blood flow and suppressing oxidative stress by NO and the PGs, long-term usage of KKS antagonists is most likely to be undesirable. KKS-specific agonists (represented currently almost exclusively by ACE inhibitors) have, in contrast, proved effective in long-term usage for the treatment of senescence-associated renal diseases. Two of the adverse effects of ACE inhibitors, teratogenicity and cardiotoxic hyperkalemia, might be avoidable by developing bradykinin receptor-specific agonists, which would probably have minimal effects on the renin–angiotensin–aldosterone system. If the receptor-specific agonists proved not to have the adverse effects of the ACE inhibitors, they could be useful for the treatment of diabetes and/or fibrogenic renal diseases in pregnant women, and in non-dialysed patients with advanced CRF.

CONCLUSIONS

The KKS affects a variety of physiological and pathophysiological functions in mammals including pain, inflammation, vascular permeability, oxidative stress, and calcium homeostasis.[178,179] Of the currently available agents that affect the KKS, the ACE inhibitors are most important. Thus, although ACE was initially discovered as a component of renin–angiotensin–aldosterone system, it has a greater affinity for bradykinin than for angiotensin I. This accounts for the fact that ACE inhibitors and the *ACE* insertion polymorphism have beneficial effects on a number of fibrogenic kidney diseases independently of changes in blood pressure and angiotensin II levels. The importance of the KKS in renoprotection is now well established. Thus, many studies have shown that the KKS inhibits the development and progression of a variety of kidney diseases and senescence partly by NO and PGs, both of which shift metabolism away from mitochondrial respiration towards glycolysis. An interesting possibility is that KKS-specific drugs could be used to alter the balance between oxidative and non-oxidative metabolism, thereby providing a new way of decreasing oxidative stress.

Acknowledgments

This study was supported by Career Development Award (no. 2-2006-108) from Juvenile Diabetes Research Foundation International to M.K. and National Institutes of Health grants HL49277, HL70523, and HL71266 to O.S. We are grateful to Dr J. Charles Jennette for his critical reading of our manuscript.

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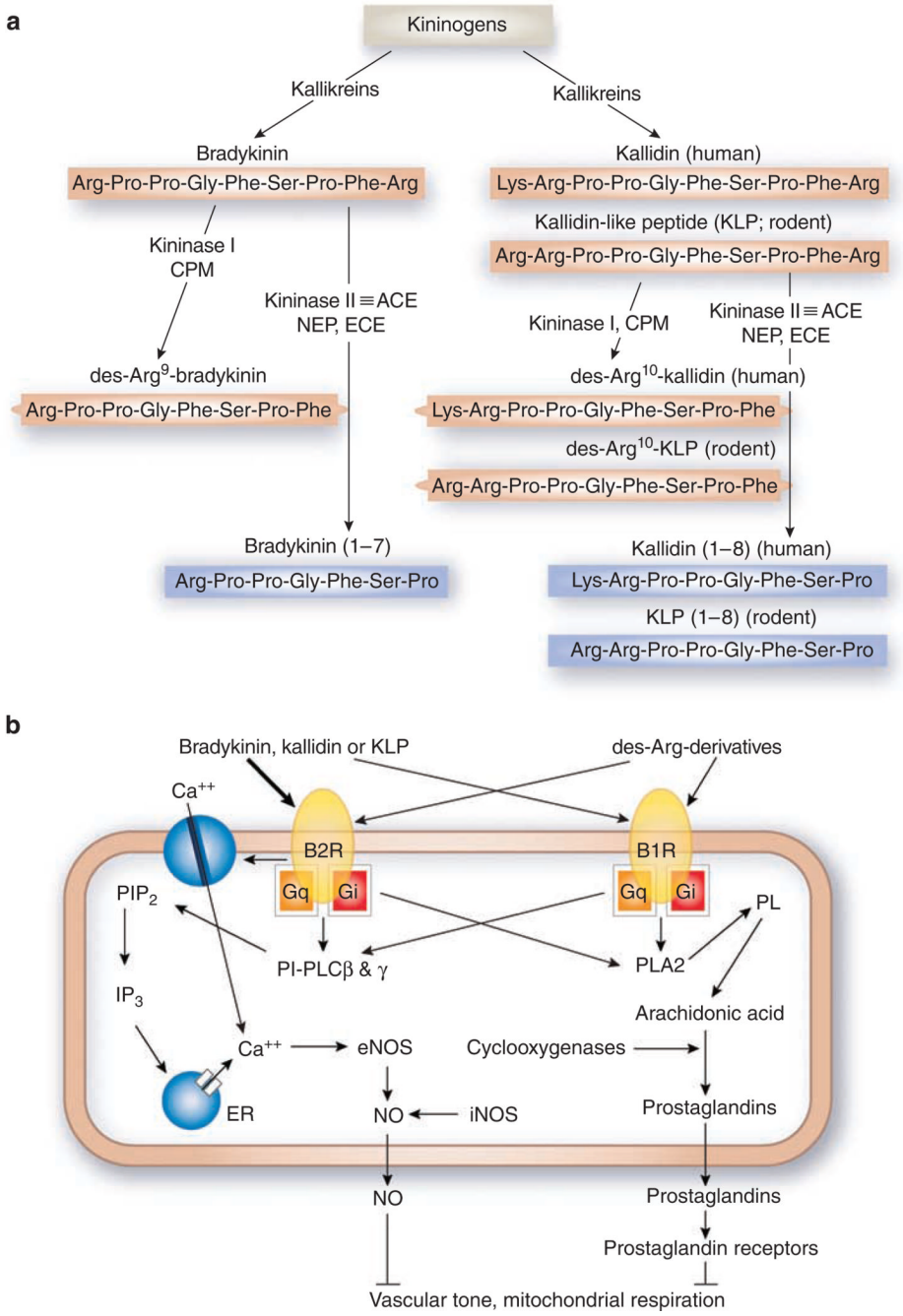

Figure 1. Components and signaling of the KKS

(**a**) Biosynthesis and metabolism of kinins. CPM, carboxypeptidase-M; ACE, angiotensin I-converting enzyme; NEP, neprilysin (endopeptidase 24.11); ECE, endothelin-converting enzyme; red, active peptides; blue, inactive peptides. (**b**) Binding of kinins to bradykinin receptors and two intracellular mechanisms for suppression of oxidative metabolism. The thickness of arrows arising from the kinins indicates the relative potency of each peptide to elevate intracellular calcium concentrations. PIP_2, phosphatidylinositol-4,5-bisphosphate; PI-PLC, phosphatidylinositol-specific phospholipase C; IP_3, 1,4,5-inositol triphosphate; ER, endoplasmic reticulum; PL, phospholipids; PLA2, phospholipase A2.

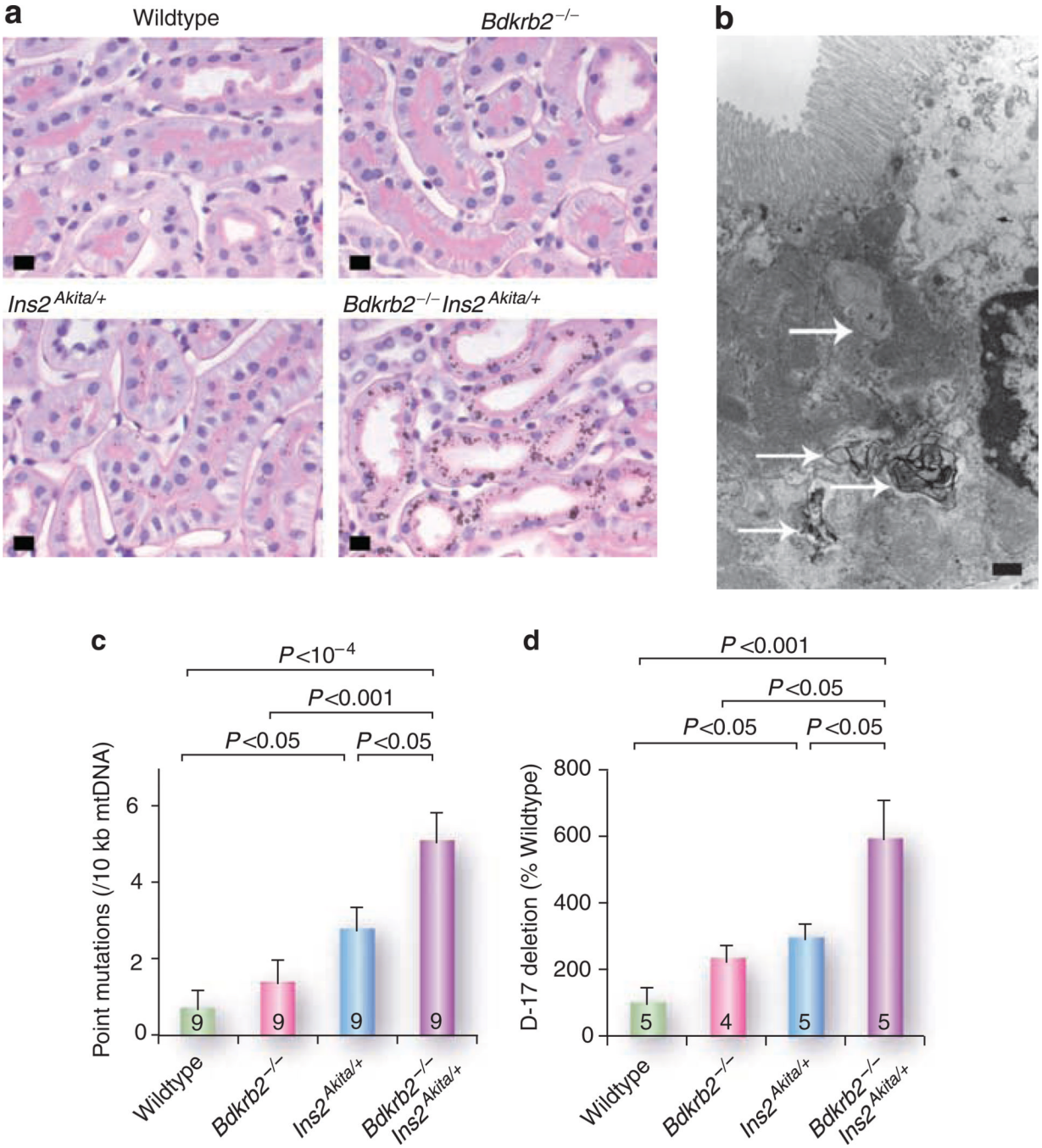

Figure 2. Senescence-associated indices in the kidney of B2R-null and/or Akita diabetic mice

(a) Periodic acid-Schiff-stained kidneys of 12-month-old male mice (bar = 10 μm). Note the intracellular pigmented vacuoles in the cytoplasm of the proximal tubular epithelial cells of the Akita diabetic mice (*Ins2* $^{Akita/+}$), and their greater prominence when the diabetic mice also lack B2R (*bdkrb2*$^{-/-}$). (**b**) Transmission electron micrograph of a renal proximal tubule cell from a 12-month-old doubly mutant mouse (bar= 1 μm). There are numerous phagolysosomes containing lipid debris with focal lamination (arrows). (**c**) Frequencies of point mutations in mitochondrial DNA. (**d**) Relative proportion of D-17 deletions in mitochondrial DNA.