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# TISSUE KALLIKREINS AND KININS: REGULATION AND ROLES IN HYPERTENSIVE AND DIABETIC DISEASES

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## INTRODUCTION

Important progress toward understanding the regulation and roles of tissue kallikreins[1] and their peptide products was made over the past few years. Kallikrein and kininogen genes were cloned, sequenced, and their regulation explored (1–4), new structural characteristics of kallikreins and kininogens described (5, 6), new sites of kallikrein and kallikrein-like enzyme localization or gene expression detected (7–9), new functions suggested for the substrates, enzymes, and peptide products (10–12), a new kininogen, two new kinins, and kinin receptor blockers discovered (13–16), and additional kallikrein-kinin system abnormalities described in association with major diseases such as hypertension and diabetes mellitus (17, 18). These and other findings (e.g. roles in inflammatory disorders) (19) show that research on tissue kallikrein-kinin systems is bursting into its most important period. This progress signals the development of new drugs, designed to focus upon the activities of tissue kallikrein system components. Only some of the notable papers concerning the molecular genetics, structural and functional characteristics of the substrate and enzyme proteins, kinin interactions with cellular receptors and messengers, and new findings in the aforementioned diseases can be reviewed in support of this contention.

[1]"kallikrein" always refers to the tissue serine proteinases of which renal kallikrein is representative, and not to the plasma enzyme of the same name, unless stated otherwise. Other members of the (tissue) kallikrein gene family are called "kallikrein-like" enzymes.



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## MOLECULAR GENETICS AND STRUCTURE

Molecular genetic and protein structural studies of kallikreins and kininogens have provided some surprises. Using a cDNA library to male mouse submaxillary gland, Richards et al (20) compared cloned inserts of abundant sequences to the sequence of porcine pancreatic kallikrein. There was extensive homology (~57%) in one cDNA insert *(pMK-1)* and important similarities between it and other submaxillary serine proteinases, e.g. γ-subunit of NGF, EGF-binding protein, and γ-renin. *pMK-1* was used to probe mouse submaxillary DNA and many EcoR1 fragments that hybridized with *pMK-1* were found (21). All were on murine chromosome 7. The first fully sequenced kallikrein family gene *(mGK-1)* consisted of five exons and four introns and although closely similar to *pMK-1* (~84% homology), neither it nor the next gene, *mGK-2*, exactly represented the genomic *pMK-1* sequence. *mGK-1* was transcriptionally active. Surprisingly, blot analyses and examination of all clones indicated about 25 different mouse kallikrein genes (21). Many of these are tightly clustered in a head-to-tail arrangement with strong conservation of restriction sites. These initial findings raised the possibility that large and highly homologous kallikrein gene families in mammals might code the synthesis of a family of closely related enzymes involved in processing many peptide hormone precursors that contain kallikrein-susceptible basic or dibasic amino acid cleavage sites (22). The recent data bearing upon this hypothesis, especially in relation to human kallikrein, is noted below.

The surprise elicited by a potentially huge kallikrein gene family was accentuated by studies of the kininogen gene. Working with a bovine liver cDNA library, Nakanishi et al found that mRNAs for *HMW* or *LMW* forms of prekininogens were transcribed from a single gene (23). In human prekininogens, the mRNA and protein sequences of these two kininogen forms were found to be identical throughout the 5'-untranslated region, the amino terminal signal peptide, past the kinin sequence, and to begin to diverge at residue 402, with the *LMW* form terminating at 427, and the HMW form continuing to 626 amino acids (24). The entire human kininogen gene was sequenced (2). Analyses of total DNA indicated also only one kininogen gene in the human genome. Its structural organization was elegantly detailed, and permits the conclusion that the functional diversity of kininogens as kallikrein substrates, thiol proteinase inhibitors, binding proteins, or cofactors in contact activation processes is ultimately the result of alternative RNA processing pathways for this gene (6). The results of the alternative processing of this, or the rat T-kininogen gene in pathological circumstances are mentioned below.

Studies of kallikrein or kininogen gene expression as affected by disease, drugs, or hormones have been confined thus far to liver (25), major salivary glands (26), kidney (27, 28), and pituitary (4, 29, 30). Nevertheless, it has

become easier to explore hypotheses about differences in levels of tissue kallikrein system components (e.g. in disease, or as sex dimorphism) and relations between these differences and some functional property of the cells wherein the genes are being expressed. These experiments are being carried out with androgens (31, 32), estrogens (29, 30), glucocorticoids (33), insulin (18), dopamine (4), and bacterial lipopolysaccharide (25), and are measuring mRNA, precursor and active protein, and synthesis rates—in some cases in association with tissue and cell functional behavior. Some specific and recent examples are mentioned below. These studies are leading toward the identification of some of the genomic regulatory features associated with the expressed genes of those cells (1, 3).

Some newly appreciated structural features of the protein and peptide components have been reported. The amino terminal, heavy chain portions of each of the mammalian kininogen molecules are essentially identical to the major acute phase protein ($\alpha_1$-MAP) in the rat (6, 34) and the cysteine proteinase inhibitor in humans (10, 35). Thus, inhibition of lysosomal cathepsins or platelet calpain (36), for example, may precede or accompany kinin liberation at sites of inflammation, or the contact activation promoted by the carboxy terminal light chain of *HMW* kininogen. These properties extend the functional diversity of the single gene kininogen products already characterized as substrates or binding proteins for kallikreins.

The T-kininogens and the T-kinin, ile-ser-bradykinin (so-called because of their discovery subsequent to trypsin treatment of plasma) (13) and their two genes are unique to the rat. The proteins are not susceptible to kallikrein attack (37, 38) but respond dramatically to systemic inflammatory stimuli (25). Their previously undetected existence (in large concentrations) in rat plasma may have contributed to searches for other related proteins and peptides. Most recently, reports of $^3$Hyp-lys-bradykinin have appeared (15, 39). This peptide represents about 25% of the trypsin-, or kallikrein-releasable kinin from human plasma, but its origin and pharmacology remain to be explored.

## *Kallikrein Genes*

The number of kallikrein gene family members is now known to vary widely: in the mouse there are 24 genes (40); the rat from 8–17 (27, 41); the hamster about three (42); and the human four, of which two are expressed, the renal or "true" kallikrein gene (1, 43, 44) and a prostate-specific antigen (45). The two other human genomic sequences (43, 44, 46) have significant (~70% or greater) homology to renal pancreatic kallikrein and to the prostate antigen genomic sequences, but their expression has not yet been reported. At least in mouse or rat, the genes of family members are very closely related, creating some problems in detecting individuals with large probes complementary to

highly conserved regions, and thus require the use of gene-specific, short oligonucleotide probes complementary to regions variable amongst family members (26, 27, 47, 48). With these, and with monoclonal antibodies of characterized specificity (49–51), the study of the expression of individual genes and their regulation has begun.

Each of the kallikrein genes studied thus far has five exons and four introns. In the largest gene family discovered, that of the mouse, identity between the predicted amino acid sequences of two of the analyzed exons of all 24 genes reaches at least 75% (40), but 12 are inactive pseudogenes and 12 are functional (1). Identification of the gene for bona fide tissue kallikrein is still somewhat uncertain because there is no protein sequence information on mouse-tissue kallikrein. However, the mouse gene called *mGK-6* by van Leeuwen et al (47) is likely to be the only gene encoding true kallikrein and is expressed in kidney, as well as in salivary glands, pancreas, and spleen. Importantly, *mGK-6* expression is not detected in mouse brain (47), in contrast to developing information about true kallikrein gene expression in rat brain (4, 7). Other genes code for γ-NGF *(mGK-3)* and α-NGF *(mGK-4)* (52), three EGF-binding proteins (*mGK-13, -22,* and *-9*) (48), and γ-renin *(mGK-16)* (40). All of the genes are located on chromosome 7, possibly forming a single genetic locus inseparable from each other and the genetically defined, linked *Tam-1, Prt-4,* and *Prt-5* loci (40).

In the rat, identification of the mRNA and gene for true kallikrein is based upon comparisons of derived amino acid sequences from nucleotide sequence (53), with purified enzyme amino acid sequence. From at least eight genes in the rat family, four distinct kallikrein mRNAs have been sequenced (54) and can be detected with synthetic oligonucleotide probes complementary to variable mRNA subregions (27). The mRNAs encode enzymes that are between 74–86% identical and include what is probably true tissue kallikrein and other kallikrein-like proteases, including tonin, and a prostate antigen with some homology to an enzyme called esterase A (51). The kallikrein mRNA (called PS) is present at high levels in the pancreas and kidney as well as the submaxillary gland (54).

The human gene family is the smallest in size of any evaluated, with the possible exception of the hamster (42). Human DNA or cDNA libraries from kidney (43), pancreas (44), fetal liver (46), prostate (45), and parotid (1) have served as starting points in searches for human kallikrein family genes. Cloning and sequence analysis of the cDNAs for human kidney or pancreatic kallikreins disclosed a coincidence of sequence (43, 44). Subsequent genomic blot analyses with the human renal kallikrein cDNA probe detected only about three distinct bands. With the recent full characterization of the human renal kallikrein gene (1), it is now clear that human kallikrein is encoded by a single gene with the usual five exons of sequence identical to that reported from

pancreatic and renal cDNA, except for a possible polymorphic site at position 162, changing a glu to lys. The gene is located on the long arm of chromosome 19 at, or close to, the region called 19q13.2–q13.4; this region is known to carry several genetic markers (cytochrome P-450s, transforming growth factor-$\beta$, etc.) which, like the kallikrein gene, also appear on chromosome 7 in the mouse. These markers map to a region adjacent to the kallikrein gene or the previously mentioned *Tam-1* locus. Thus, at least the renal kallikrein gene (and perhaps the entire family because there is only one chromosomal region of in situ hybridization using a cDNA probe able to detect all kallikrein genes) in humans is in a chromosomal location analogous to that of the mouse. The reported existence of restriction fragment length polymorphisms (RFLPs) in the kallikrein gene of the first few individual human DNAs evaluated (43), suggests that further questions about RFLPs at this site may be helpful in clarifying the association of abnormal kallikrein with certain human diseases. On the other hand, earlier notions about a large family of closely related genes and enzymes serving multiple proteolytic processing functions now seem less likely to apply in man than in mouse (22).



## *Kininogen Genes*

This subject has recently been reviewed comprehensively (6). Most fascinating is the elucidation of the different modes of mRNA generation for either *HMW* or *LMW* kininogen from the *K* kininogen gene, or the *LMW* forms from the two additional T-kininogen genes also present in the rat (3). In this study, chimeric kininogen genes were constructed by exchanging equivalent restriction fragments amongst the genes and then introducing them into heterologous COS cells for expression, a method that allowed the gene sequences controlling the form of the mRNA produced to be identified and located. This precise characterization of the genetic mechanisms for alternative mRNA production of a kallikrein system component also demonstrates the first application of a strategy to understand the molecular basis of important functional differences in closely related proteins (55). It seems likely that similar strategies will be applied to other system components (e.g. kinin receptors, kininases) as they are more clearly characterized.

## *Regulation of Gene Expression*

Recent work has begun to dissect the relative expression of individual kallikrein gene family members, notably in mouse salivary glands (26) and in the rat pituitary (4, 29, 30, 56). The mouse kallikrein gene designated *mGK-6* and considered to encode the renal, or bona fide kallikrein (47) is expressed constituitively in the gland. It does not show sexual dimorphism, and is unresponsive to, or even repressed by testosterone (26), a finding reminiscent of the testosterone effect upon renal kallikrein synthesis rate (31). In contrast,

*mGK-3* (γ-NGF), -4 (α-NGF), -5, and -13 (EGF binding protein) do show sexual dimorphism (47) and testosterone treatment can induce expression in females to levels approaching those in male animals. Testosterone induction of these genes appears to accompany a differentiation of striated duct cells to granular convoluted tubule cells. The time course of induction of these genes confirms the impression gathered in previous studies (32) that large doses of hormone, administered for up to a week, are required to observe concomitant changes in gene expression and tissue morphology. This suggests that hormonal regulation of these kallikrein-like proteins is probably not a result of a direct action on genes or their regulatory elements. Nevertheless, the tissue specificity of the responses suggests associations between the induced enzymes and cell proliferation and differentiation.

The presence of kallikrein-like activity in the rat anterior pituitary (57) is known to reflect gene expression of authentic tissue kallikrein as assessed by radioimmunoassay of the enzyme, identification of kallikrein mRNA using different cDNA probes (4, 29, 30, 56), and nucleotide sequence analysis of pituitary-derived cDNA clones (4). There is clear sex dimorphism with mRNA and enzyme levels manyfold higher in the female anterior lobe. Estrogen treatment of intact or gonadectomized males or ovariectomized females markedly increases both RNA (~50-fold) and enzyme protein (20–90-fold) (29, 30). As with mouse salivary gland kallikrein-like enzymes and androgen, this induction of bona fide kallikrein gene expression by estrogen requires several days of hormone administration.

Dopamine is another neuroendocrine influence on rat pituitary kallikrein gene expression (4, 58). Three days of treatment with bromocryptine decreases anterior pituitary mRNA levels in both males and females, and neurointermediate lobe levels in males. Haloperidol, the dopamine antagonist, causes an increase in neurointermediate lobe kallikrein mRNA in both sexes and in the anterior lobe of females. The noted changes in kallikrein gene transcription are accompanied by parallel changes in pro-opiomelanocortin (POMC) mRNA in the neurointermediate lobe, of interest because of the proposals about roles for this kallikrein in some aspect of POMC-processing or peptide secretion (4). Although these studies of pituitary kallikrein demonstrate clear effects of hormones upon rat-tissue kallikrein gene expression, there is no evidence for a direct effect of these, or other hormones upon kallikrein genes, per se.

Additional candidate hormones for such a role in regulating the renal kallikrein gene include: mineralocorticoids, now probably unlikely to directly affect expression (59); insulin (18); glucocorticoids (33); and perhaps vasopressin, based upon the very rapid changes in urinary kinin excretion that can be produced in humans, dogs, and in Brattleboro rats with hereditary diabetes insipidus (60).

Clues to likely candidates for activation (or perhaps, repression) of true kallikrein gene transcription may come from the identification of four distinct regions of promotor sequence homology upstream of both the mouse and human kallikrein gene. These elements might represent sites for tissue or cell-specific regulation of expression by binding some specific factors (1).

The regulation of kininogen gene expression, especially in relation to acute inflammatory stimuli (25, 61–64), is also under intensive study, and has been recently reviewed (6, 19).

### *Protein Structure and Function*

Present interest in structure-function relationships in the kallikrein-kinin system stems from two sets of findings. The first is that kallikrein gene families encode a series of highly homologous proteins that may bind to, and/or process some known (e.g. *EGF*, *NGF*, kininogens) (11, 47, 48, 52) or candidate proteins (POMC, atriopeptin precursor, apolipoprotein B-100) (4, 65–67). The second is that K kininogen gene products (and in addition, T-kininogen gene products of the rat) are multifunctional proteins generated by diverse, alternative RNA-processing mechanisms (6). These observations provide a tremendous opportunity to explore the regulation and roles of kallikrein family enzymes or specific domains of kininogens in the spectrum of physiological or pathophysiological events with which tissue kallikrein-kinin system components have been associated over the years. In addition to the inflammatory processes, other events deserving of mention include: exocrine and endocrine secretion; muscular contraction and relaxation; neural hyper-, and depolarization; and epithelial ion transport. There is now a feasibility to (and of course, precedent for) proposals that hold specific sequence, and tertiary structural features of tissue kallikrein system components to be crucial to their functional capability or aberrency in disease. Nevertheless, most studies of kallikrein-kinin involvement in the processes just mentioned are pharmacologic, in that they explore responses "downstream" of exogenous kinin administration.

## CELLULAR AND TISSUE RESPONSES TO KININS

The action of human plasma kallikrein upon human *HMW* kininogen results in bradykinin (BK) liberation, while human tissue kallikreins liberate lysyl-BK or (hyp$^3$)-lysyl-BK from either *HMW* or *LMW* human kininogens; in the rat, BK is thought to be the only product. A number of other kinins have been described in various nonmammalian tissues with biological activities generally similar to mammalian kinins, but, with few exceptions, they contain the nonapeptide BK sequence.

Biological activities demonstrated for kinins include effects upon the car-

diovascular system, smooth muscle, ion transport, eicosanoid synthesis, cell proliferation, capillary permeability, and pain production. The biological significance of many of these findings have been questioned repeatedly, and the true physiological or pathophysiological functions of kinins, as opposed to their activities in vitro, have been difficult to ascertain. Now, some of this ambiguity is being resolved as kinin receptors and their blockers, cell messenger systems, cell functional responses, and kininase activities are the subjects of increased scrutiny.



## *Receptors and Second Messengers*

Characterization of kinin receptors has not proceeded as rapidly as studies with other peptide hormone receptors. Earlier suggestions of two distinct classes of kinin receptors now appear to be in doubt. The so-called "$B_1$" receptor is difficult to observe ordinarily and seems to appear only after tissue trauma, such as exposure of rat urinary bladder to Triton X-100, or intravenous injection of E. coli lipopolysaccharide to rabbits (68).The analog des-arg$^9$-BK is an order of magnitude more potent for $B_1$-mediated rabbit aorta contractile response, yet is proving to have little, if any, activity separable from BK on a broad range of kinin-sensitive systems presently being categorized as exhibiting "$B_2$" receptors. However, any implication that $B_2$ receptors constitute a homogeneous class should be dispelled quickly, as recent efforts are suggesting both the existence of multiple kinin receptors, and the appearance of some new, more reasonable classification scheme in the near future.

These efforts have been stimulated by the painstaking, and finally fruitful efforts of Vavrek and Stewart (16). Their sequence-related, competitive inhibitors of the classic kinin responses (hypotension, smooth muscle contraction) are being widely used. These substituted D-phe$^7$-BK analogs are capable of blocking a broad range of kinin effects in whole animals and isolated tissue including: venoconstriction and $PGE_2$ release by BK in the rabbit ear (69); nociception (69–73); kinin-induced vascular permeability (69, 74–77); smooth muscle contractions (69, 77–79); and attenuating hypotension, presumably kinin-associated (80); as well as increasing normal blood pressure (81, 82), which is discussed more extensively below. Several of the compounds have some degree of agonist activity or show different antagonist potencies for the same response, and this is being more clearly categorized in various isolated cell systems. For example, in N1E-115 murine neuroblastoma cells, a commonly used kinin antagonist in smooth muscle, [thi$^{5,8}$,D-phe$^7$]-BK, is an agonist for phosphoinositide turnover (83). [D-arg$^o$-hyp$^3$-D-phe$^7$]-BK is 14-fold more potent in inhibiting BK-stimulated prostaglandin synthesis in Swiss 3T3 cells than in bovine pulmonary artery endothelial cells (84).

Kinin receptor properties are also being evaluated in rat renomedullary interstitial cells (85), *ras*-transformed NIH 3T3 cells (86), rat peritoneal mast cells (87), and bovine uterine myometrial membranes (88). Manning et al have continued their earlier studies to demonstrate high affinity kinin binding sites in many guinea pig tissues (89). In ileum, kidney, and heart two sites with $K_d$s in the 7–18 pM and 800–900 pM range were found and pharmacologically characterized with the usual in vitro binding techniques. Autoradiographic studies with [$^3$H]-BK have localized binding sites to various sensory neurons in guinea pig and dog (73), including dorsal but not ventral roots, subsets of cells in the dorsal root ganglion and trigeminal and stellate ganglia. Using the N1E-115 cells, a series of kinin antagonists in certain smooth muscle assays were also evaluated in binding assays and shown to compete to various extents with [$^3$H]-BK (83). Again, heterogeneity of kinin receptors appears to be the rule as, for example, [D-$\beta$-(2-naphthyl) alanine, thi$^{5,8}$, D-phe$^7$]-BK was noted to be a potent antagonist in rat uterus and inactive in guinea pig ileum (73).

The structural characterization of a kinin receptor is beginning to be made, with the use of either photoaffinity probes (90) or a new cloning strategy (91), but no success has been reported, thus far.

Kinin receptors are now known to be coupled directly or indirectly to the array of messenger systems that translate ligand binding into cell functional responses. BK causes an increased low $K_m$ GTPase activity and adenylate cyclase inhibition in NG108–15 cells, both of which are blocked by pertussis toxin and may involve some G-protein (92). Evidence further suggests that different G-proteins may be involved in different cells since pertussis toxin does not decrease kinin-induced increase in GTP hydrolysis, inositol phosphate, or $PGE_2$ formation in some cells (93–95) but does so in others (96, 97). In these and other reports, G-proteins were implicated in kinin effects on phosphoinositide metabolism, prostaglandin synthesis, and calcium mobilization. For example, in neural cell lines, activation of a phosphodiesterase results in breakdown of phosphatidylinositol 4,5-bisphosphate ($PIP_2$) and large increases in inositol 1,4,5-trisphosphate ($IP_3$) within seconds after kinin addition (98–101), paralleled by an increase in intracellular calcium (100): both of these are inhibited by phorbol ester (102). Stimulation of phosphoinositide hydrolysis by BK or kallidin also occurs rapidly in vascular endothelial cells (103) and renal papillary collecting tubule cells (104). In fibroblasts, BK-stimulated $PGE_2$ synthesis was potentiated by phorbol esters and an analog of diacylglycerol, even under circumstances where protein kinase C activity was abolished or inhibited (105).

Kinin-induced cellular responses studied thus far always involve calcium movements (106). Effects seem to involve both influx of extracellular calcium and mobilization of intracellular stores (107). Recent studies of bovine aortic

or pulmonary arterial endothelial cells disclosed a rapid initial spike and an increased steady state level of cytosolic free $Ca^{++}$ that do not occur via voltage-sensitive calcium channels. That is, calcium channel-blocking drugs do not abolish either phase of the kinin response and no electrophysiological evidence for the presence of such channels was obtained (108, 109). Interestingly, $^{86}Rb$ efflux parallels the changes in cytosolic $Ca^{++}$ (110). The initial change in free cytosolic $Ca^{++}$ induced by kinin was not abolished by $Ca^{++}$-free extracellular media suggesting liberation from intracellular stores in these or other (111) cells. However, the changes evoked by kinin are almost completely blocked by a kinin $B_2$-receptor antagonist (109).

Cyclic nucleotides have also been implicated as mediators of kinin responses; changes in cAMP or cGMP have been demonstrated in various smooth muscle or ion transporting systems (112), epithelial cell populations (113), and neuronal cell lines (114). Finally, there continue to be many reports of kinin-induced synthesis and release of various eicosanoids. It seems that while many cellular responses to kinins involve eicosanoids, not all do, and it is possible to discern effects of kinins upon, for example, epithelial chloride secretion independent of eicosanoid synthesis (115, 116). Mediation of cellular structural and functional responses to kinins by eicosanoids, or indeed any of the messengers mentioned above is presently in a state similar to that for many other neurotransmitters and hormones. That is, it is not yet possible to assign an accurate sequence to these events or to define fully how they interrelate.

## *Cell Structural and Functional Responses*

Cell systems in vitro are being used to study cell functional responses to kinins, and some recent papers of interest are noted. After the first definition of the direct effects of kinins upon ion transport in epithelial tissue (12), many studies have explored kinin-induced chloride movements and cotransport events in epithelia (117, 118). Fewer studies have used cultured cells, but these are now allowing clearer assessments of kinin effects upon epithelial cells, per se. Kinin receptors seem to be present on either apical or basolateral surfaces of pig renal papillary-collecting tubule cells or human colonic adenocarcinoma cells (119, 120). Their stimulation leads to electrogenic ion transport, probably net chloride secretion. In MDCK cells, BK leads to a rapid change in potassium conductance and hyperpolarization that is apparently mediated by $Ca^{++}$-activated, $K^+$ channels in basolateral membranes (121, 122). These rapid changes in epithelial ion transport are accompanied by striking acute changes in epithelial tissue and cellular structure (123). These effects in epithelial cells raise important new questions about relations between kinin-induced messenger generation and cell structural-functional responses.

The issue of a kallikrein-kinin, renin-angiotensin connection was highlighted again by studies that histochemically localize renal tubular cell kallikrein in intimate connection to the juxtaglomerular apparatus (124, 125), and by another study showing kinin-induced renin secretion from isolated rat glomeruli (126). The implications for the regulation of renal hemodynamics and blood pressure homeostasis are obvious.

Kinin-induced cell proliferation, DNA, and protein synthesis were noted years ago. These effects in fibroblasts are probably not prostaglandin-mediated, and can also be produced by des-Arg$^9$-BK as well as BK (127). In contrast, BK and des-Arg$^9$-BK stimulate an osteoclast-mediated bone resorption that is prostaglandin-dependent, and may have important implications for rheumatoid or periodontal inflammatory diseases (128, 129). In addition, the long-appreciated association of bradykinin receptors with afferent neural pathways (130, 131) has now been reinforced by studies documenting the analgesic activities of the new kinin antagonists (71–73).

### *Regulation by Kininases*

Some additional insight into kinin catabolism by enzymes other than kininase I (carboxypeptidase N) or kininase II (angiotensin I-converting enzyme) (132, 133) has been gained. Neutral endopeptidase 24.11, like kininase II, cleaves the C-terminal phe-arg from kinins. The enzyme is strongly inhibited by phosphoramidon, and is also found in renal tissue (134) and intestinal smooth muscle (135). It is further reported to constitute one half to two thirds of the total kininase activity in rat urine (136). Infusion of phosphoramidon to rats results in an increase in urinary volume, sodium, and kinin excretion (136). Other candidate in vitro kininases include a porcine renal postproline cleaving enzyme (EC 3.4.21.26) (137), and a rat brain endopeptidase that is able to cleave the phe$^5$-ser$^6$ bond of kinins (138).

Most studies of kininases measure the effects of the various inhibitors, or in vitro enzymatic activities upon kinins or synthetic substrates. There is little information thus far on how these activities are regulated in situ, or on their roles in modulating tissue kallikrein-kinin system activities.

## ROLES IN DISEASE

Advances in the study of relations between tissue kallikreins, kinins, and disease are being made at a modest rate, compared with the molecular genetic and cell biologic findings noted above. However, there are clear abnormalities in levels of tissue kallikrein-kinin system components in some of mankind's commonest diseases (e.g. hypertension, diabetes, allergic and inflammatory disorders), even if their meanings are unclear. Some of the recent studies not reviewed elsewhere (19) are noted.

## *Rat Hypertension*

Studies of kallikrein-kinin system components in hypertensive and prehypertensive conditions continue, albeit slowly, to elaborate upon abnormalities discovered almost twenty years ago (139, 140). Arbeit & Serra (141) used several techniques to prove that active and total urinary kallikrein excretion was lower in Dahl S versus R rats, even on low salt diets and as early as 7 weeks of age. This marked difference in urinary kallikrein between Dahl rat strains has been extended by studies showing lower renal tissue levels and rates of kallikrein synthesis in the Dahl S versus R as early as 2 days of age (142, 143). A similar abnormality was also noted in careful studies of young SHR and Wistar-Kyoto by Ader et al (17). In animals as young as 4 wk the reduced active, and total urinary kallikrein excretion is accompanied by a lower excretion of sodium and water and leads to a cumulative retention of each in the SHR as pressure rises. These continuing studies of tissue kallikrein levels in hypertensive rats are being supplemented by measurements of other system components. Altered sodium intake produced changes in urinary and renal tissue immunoreactive kinins analogous to those of kallikrein, with low salt intake significantly increasing and high salt reducing the kinin levels (144). In DOCA-salt hypertensive rats, the arterial plasma level of kinins was 50% lower than in water-drinking controls, but the nonhypertensive, 1% saline-drinking group also had an arterial plasma kinin concentration similarly lowered (145). When kinin was infused into the saline-drinking groups, the attained arterial plasma kinin levels were significantly lower than in water-drinking rats. This difference was diminished after captopril. The data suggest kinin degradation may be altered by a change in salt intake, regardless of blood pressure or DOCA.

Studies of the effects of kinin antagonists on blood pressure have begun. Gavras and colleagues (81, 82, 146) found that the competitive kinin antagonist [Hyp$^3$, thi$^{5,8}$, D-phe$^7$]-BK could increase the blood pressure of some, but not all, anesthetized male Wistar rats (82). In some two-kidney, one-clip renovascular hypertensive rats made normotensive with either saralasin, sodium nitroprusside, or the kininase II inhibitor enalapril, a significant increase in blood pressure occurred only in the last group upon infusion of the same kinin antagonist (81). A more recent study using arterial infusions of [Hyp$^3$, D-phe$^7$]-BK and unanesthetized normotensive rats (146) disclosed immediate 20–30 mmHg rises in arterial pressure still evident in nephrectomized or adrenalectomized animals, suggesting that agonistic activities of the compound at renal or adrenal kinin receptors to affect catecholamine release, etc, were not responsible for the blood pressure elevations. These and other data (147) are establishing the value of kinin receptor blocking agents for studies of kallikrein-kinin involvement in blood pressure homeostasis.

## *Human Hypertension*

Urinary kallikrein excretion in patients with essential hypertension continues to be controversial. Data from some studies still show reduced excretion (148–152), whereas no difference from that in normotensives is found in others (153–155). The heterogeneity of evaluated populations or methods, and the presence of renal disease are some of the factors contributing to a lack of uniformity in demonstrating an abnormality widely detected in hypertensive animal models. Several of the studies unable to show altered kallikrein excretion in hypertensive patients have found other system abnormalities. Lieberthal et al (153) found urinary kallikrein specific activity (kininogenase activity per $\mu$g enzyme) is lower in essential hypertensives than normal subjects. Weinberg et al (154) showed reduced urinary kininogen and kinins in a similar hypertensive population. The latter finding was extended in comprehensive studies of a Japanese population by Nakahashi et al (151), who found that low-renin hypertensive patients had not only the lowest urinary kinin, but also the highest urinary levels of "total" kininase and kininase I. In epidemiological evaluations, evidence for shared environmental effects upon urinary kallikrein in Utah families was detected in one study (155), and in another, urinary kallikrein levels measured in newborn infants and one month later were negatively related to their blood pressures at each time (156).

Few recent studies of the effects of drugs on kallikrein-kinin measurements in human hypertension have been carried out (157). Of interest is a report (158) of the effects of the serine protease inhibitor aprotinin on urinary kallikrein and sodium excretion in 24 hypertensive men. On a high salt intake, a significant reduction in both urinary kallikrein activity and sodium excretion occurred, but an equivalent reduction in active kallikrein was produced during chronic low salt diet without change in sodium excretion. This finding highlights ambiguities about the human renal kallikrein-kinin system that may be resolved if kinin receptor antagonists can reach clinical investigation.

## *Diabetes Mellitus*

Urinary kallikrein excretion was found to be significantly greater in poorly controlled (hemoglobin $A_{1c} > 11\%$), insulin-dependent diabetics than in either well-controlled diabetics or normal subjects (159). Glycemic control was associated with a fall in kallikrein excretion. One subsequent study in insulin-dependent, poorly controlled diabetics did not confirm the finding (160), and another found significantly decreased urinary kallikrein in type 2 (non-insulin dependent) diabetics with nephropathy, compared with control subjects, patients with diabetes without detectable nephropathy, or patients

without diabetes and with nephropathy (161). A small, but significant increase in plasma kinin level was observed in diabetics with orthostatic hypotension and not seen appropriate in control populations (162). Collectively, these findings may not rouse much interest in kallikrein or kinins in diabetes, a state not too dissimilar from that which might be reached by considering kallikreins in only human hypertension. However, a series of recent studies in diabetic animal models establish clearly that kallikrein synthesis and regulation is abnormal in the diabetic rat kidney (18, 163–165). Further, insulin is a potent, and rapidly acting stimulus to renal kallikrein synthesis (18). The localization of renal kallikrein to the connecting tubule directly adjacent to the afferent arteriole of the same nephron (124, 125) raises the possibility of participation of this enzymic abnormality in the aberrent tubuloglomerular feedback
hyperfiltration states are also associated with dramatic changes in renal kallikreins and kinins, and the evaluation of kallikrein inhibitors and kinin receptor blockers in these states will add a great deal to the understanding of system roles in renal hemodynamics and glomerular filtration.

There is more to be learned about the interesting relation between renal kallikreins, kinins, and vasopressin (166, 167) as dramatically illustrated in rats with diabetes insipidus (60), but the physiological significance of the findings is unclear, and again points to the likely value of specific kallikrein inhibitors or kinin receptor blockers.

## SUMMARY

The spectrum of cellular, or whole animal responses to kinins has expanded enormously in the last five years. The molecular basis for these consequences of kinin-kinin receptor interactions is being glimpsed as a series of cascading and parallel biochemical events occurring either in the same or adjacent, but communicating, cells of the same tissue (e.g. epithelial, endothelial, muscle, neural). Although it is likely that even more kinin-induced events remain to be discovered, like the interesting effects of the peptides on osteoclastic activity (128), a greater challenge in this field is the gathering of insights into precisely how the regulation of system component gene expression is coordinately carried out to allow component protein synthesis, transport and processing when necessary, and limitation of activities, when required. With these insights, we will be more able to understand the meaning of many observations of tissue kallikrein-kinin system abnormalities in common human diseases. This understanding will then make more obvious the drug design strategies to be used to stimulate or replace, and modulate or inhibit kallikrein-kinin system components in those diseases.

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