The Renal Kallikrein-Kinin System

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THE KIDNEY contains several hormonal systems—kallikrein-kinin, renin-angiotensin, prostaglandin—which serve as renal self-regulatory mechanisms and may have extrarenal effects as well. Although the functions of the kallikrein-kinin system are incompletely defined, there is evidence that it may regulate renal blood flow, modulate tubular salt and water transport, and participate in the pathogenesis of a number of human diseases, such as hypertension. Unfortunately, many components of the renal kallikrein-kinin system are not adequately characterized and many current assays are of uncertain specificity. Hence, at present we must be cautious in interpreting available observations. Greater awareness of the potential physiological and clinical importance of the renal kallikrein-kinin system has led to increased research in this area. Recent progress in purification of components and in development of more specific assays should lead to important insights in the near future.

Because this is a rapidly evolving field, it is important to note that this review was completed in July, 1978. To limit the reference list within the compass of a Brief Review, only relatively recent references are cited in most cases, even if original or older investigations are more extensive.

Components of the System (Fig. 1)

The basic elements of the kallikrein-kinin system were first described by Werle in 1937 (Werle, 1937). Kallikreins are proteinases which liberate vasoactive peptides, the kinins, from protein precursors or kininogens. The kinins are rapidly inactivated by kininases. There are two types of kallikreins, plasma and glandular. These differ in mechanisms for activation, immunological identity, physicochemical properties such as molecular size and charge, rates of reaction with natural and synthetic substrates, and degree of inhibition by a number of antagonists (Erdos, 1976; Pisano, 1975; Spragg and Austen, 1977).

The Plasma Kallikrein System

The plasma kallikrein system is much better characterized than the renal system. Although not directly pertinent to this discussion of renal kallikrein, the plasma system deserves brief review for comparative purposes. Plasma kallikrein is found only in an inactive form, prekallikrein, a basic protein whose molecular weight is 107,000 and pI is 8.5–8.9 (Spragg and Austen, 1977). Prekallikrein and Hageman factor reciprocally activate one another in a feedback mechanism that also requires high molecular weight kininogen. Initial activation of Hageman factor may occur on exposure to any of a variety of negatively charged surfaces, such as basement membranes. Activated Hageman factor, and especially a fragment split from it during activation, are efficient activators of prekallikrein in plasma. Kallikrein, in turn, is the most potent activator of Hageman factor in plasma. Activated Hageman factor initiates the coagulation and fibrinolytic cascades in blood. Thus, kallikrein is an intrinsic part of the blood coagulation mechanism; a deficiency of plasma prekallikrein (Fletcher factor) leads to a prolonged clotting time. Plasma kallikrein has at least two additional functions, chemotaxis of neutrophilic leukocytes and liberation of the vasoactive peptide, bradykinin, from plasma kininogen. Plasma contains three inhibitors which can modulate kallikrein activity, α2 macroglobulin, C1 INH (the inhibitor of the first component of complement), and the antithrombin III-heparin complex (Spragg and Austen, 1977).

Two forms of kininogen are found in plasma, a high molecular weight type (mol wt, about 120,000) and a low molecular weight form (mol wt approximately 80,000). (Estimates of the molecular weight of the kininogens, especially the low molecular weight form, vary in the literature.) The former appears to be the preferred substrate of plasma kallikrein. In addition to its role as a substrate from which bradykinin is released, intact high molecular weight kininogen forms a complex with either pre-
kallikrein or factor XI and directs their surface deposition and interaction with Hageman factor. A deficiency of this kininogen, variously named Fitzgerald, Williams, or Flaujeac traits, leads to deficiencies of blood coagulation and fibrinolysis, as well as of kinin formation.

Bradykinin, the kinin released from kininogen by plasma kallikrein, is a nonapeptide (Fig. 2), strongly basic (pI > 10) by virtue of the arginine residues at either end of the molecule. It induces vasodilation, increases capillary permeability, and causes contraction of some intestinal and bronchial smooth muscle. Some of these actions appear to be mediated in part by prostaglandins, whose synthesis is stimulated by bradykinin. Bradykinin is rapidly destroyed by peptidases. In plasma, the principal inactivation enzymes are kininase I, which splits arginine, and kininase II, which splits the Phe-Arg dipeptide from the C-terminal end of bradykinin. (Kininase II also is known as angiotensin-converting enzyme, since it splits a His-Leu dipeptide from the C-terminal end of bradykinin. (Kinase II also is known as angiotensin-converting enzyme, since it splits a His-Leu dipeptide from angiotensin I to form the vasoactive peptide, angiotensin II.)

In summary, the plasma kallikrein system is characterized by the presence of the proenzyme prekallikrein, which, when activated by Hageman factor, releases bradykinin from high molecular weight kininogens. The system is modulated by kallikrein inhibitors and kininases. Kallikrein and high molecular weight kininogen are important activators of blood coagulation and fibrinolysis; kallikrein is also chemotactic. (More complete descriptions of the plasma kallikrein mechanism and bibliographies can be found in the following references: Erdos, 1976; Pisano, 1975; Spragg and Austen, 1977; Pisano and Austen, 1976.)

Assays

Some understanding of current assays is essential to appreciate problems and progress in this field. The activity of the renal kallikrein-kinin system usually is inferred from measurements of urinary kallikrein. The implicit assumption that kallikrein is the rate-limiting step in the system, while reasonable, is unproven. Moreover, urinary excretion may not reflect renal synthesis accurately, because some kallikrein may leave the kidney in blood or lymph or be catabolized in the kidney or urine. Most kallikrein methods measure its enzymatic activity, not its concentration. In most renal studies, kallikrein activity has been determined by esterase assays, which measure the ability of kallikrein to split synthetic arginine esters at an alkaline pH. Such methods are simple and, if the substrate is radioabeled, quite sensitive (Margolius et al., 1974a; Beaven et al., 1971). Several groups have reported that urinary alkaline esterase activity correlates well with urokinin-related biological activities, as determined by bioassays (Levy et al., 1977; Margolius et al., 1974a; Seino et al., 1977). However, recent studies have demonstrated that urine contains non-kallikrein esterases which contribute significantly to measured activity (Geiger et al., 1977; Ole-MoirYoi et al., 1977a). Kallikrein activity also can be determined by measuring the rate at which kinin is liberated from substrate added to urine or kidney test samples. Because they are tedious, kinin bioassays or immunoassays have been less widely used in studies. Moreover, all these methods which measure kallikrein activity are subject to interference by kallikrein inhibitors and kininases present in urine and kidney extracts. Immunoassays which measure kallikrein concentration, rather than activity, recently have been reported (Carretero et al., 1978; Mann and Geiger, 1977). Whereas immunoassays for kallikrein are highly specific, they probably measure inactive pro-kallikrein and inhibitor-complexed kallikrein. Therefore, immunoassays should be combined with functional methods in physiological and clinical studies. When such combinations are used, apparent changes in urinary kallikrein in specific diseases or functional states may prove to be due to excretion of an inactive enzyme or to changes in inhibitors or non-kallikrein esterases (Carretero et al., 1978).

The activity of the kallikrein-kinin system also can be assessed by measurement of urinary kinin excretion, using bioassays or immunoassays. Such measurements have the advantage that they measure the biologically active part of the hormonal system directly. In a recent study (Vinci et al., 1978), urinary kallikrein and kinin changed in op-

**Figure 1** The renal kallikrein system is diagrammed by terms in capitals and heavy arrows on the right side of the figure. The plasma kallikrein system also includes these sequences and, in addition, the steps shown by lower case lettering and light arrows. Reactions which limit activity of the system are shown by cross-hatched arrows. (See text for more details.)

**Figure 2** Structure of the principal kinins. Sites of cleavage by kininases are shown by cross-hatched arrows.
The Renal Kallikrein System

Some organs, such as the pancreas and the colon, contain prekallikrein, but to date only the active enzyme has been isolated from the kidney and salivary glands (Pisano, 1975). Possibly a pro-enzyme is activated by the procedures required to free it from renal tissue. In favor of this possibility is a recent preliminary report that 25-75% of urinary kallikrein is excreted in the form of an inactive precursor (Corthorn et al., 1977). Renal (and other glandular) kallikreins are acidic glycoproteins, with a molecular weight in the 27,000-43,000 range and a pI of about 4 (Pisano, 1975). As noted earlier, plasma kallikrein is a larger, more basic glycoprotein, with a molecular weight of 107,000 and a pI of about 8.7.

There is substantial evidence that kallikrein is synthesized by the kidney. The amount of the enzyme in a 24-hour urine collection from a rat is about eight times the content of kallikrein in its kidneys (Nustad, 1970a). The isolated rat kidney releases more kallikrein into the perfusate and the urine than can be accounted for by the minor decrease in renal kallikrein content which occurs during perfusion, implying synthesis (Robiero et al., 1976). Kidney slices incorporate radiolabeled leucine into kallikrein (Nustad et al., 1975). Suspensions of rat renal cortical cells secrete kallikrein into the bathing medium (Margolius et al., 1976). Margolius et al. (1976) found that the rate of secretion in this system is enhanced by aldosterone and decreased by spironolactone. They suggested that kallikrein may be an aldosterone-induced protein.

Three-quarters or more of renal kallikrein is found in the cortex (Nustad, 1970b; Scicli et al., 1976a). Nearly all the rest is in the outer strip of the outer medulla; little or no kallikrein is in the inner strip of outer medulla or the inner medulla (Nustad, 1970b). Isolated glomeruli contain low concentrations of kallikrein (Scicli et al., 1976a; Ward et al., 1977). These findings suggest that kallikrein originates from tubular epithelium of cortical nephron segments. This view recently has been supported by immunofluorescence experiments, which show that antibody to rat kallikrein localized in epithelial cells of the segment of the distal tubule between the macula densa and the collecting ducts (Orstavik et al., 1976). Kallikrein appears to be concentrated in an apical rim of cytoplasm just below the luminal membrane of these cells, suggesting possible positioning for secretion into the urine. This result agrees with stop-flow experiments suggesting distal secretion of kallikrein (Scicli et al., 1976b) and fits with the absence of the enzyme in a rat kidney tumor derived from proximal tubular cells (Ward et al., 1976b). The localization of kallikrein within cells is uncertain. Using differential centrifugation and density gradient separation techniques, some investigators find that the enzyme is associated with plasma membranes and endoplasmic reticulum (Nustad, 1970b; Ward et al., 1975; Ward et al., 1976a; Ward et al., 1976b). Ward and associates (1976b) have suggested that kallikrein is synthesized on the endoplasmic reticulum, then reoriented to a plasma membrane for subsequent release. Other workers (Baggio et al., 1975; Carvalho and Diniz, 1966) find that kallikrein is associated with lysosomes.

Urinary kallikrein appears to be identical with renal and other glandular kallikreins in molecular characteristics such as weight, pI, and response to various natural and synthetic inhibitors (Kaizu and Margolius, 1975; Nustad, 1970a). It is immunologically cross-reactive with renal and pancreatic but not with plasma kallikrein (Kaizu and Margolius, 1975; Nustad et al., 1975; Ole-MoiYo, 1977a; Ole-MoiYo, 1977b). Thus it seems certain that urinary kallikrein is not of plasma origin; it is probable that urinary kallikrein is synthesized by the kidney, although urinary excretion of other glandular kallikreins cannot be ruled out. There is some evidence that kallikrein may enter renal lymph (DeBono and Mills, 1974) and that it may be catabolized by the kidney (Mills et al., 1975).

Glandular kallikrein activity is modulated by inhibitors present in many organs, including lung, pancreas, submandibular glands, and kidney (Hochstrasser et al., 1974; Werle et al., 1973). Most are small proteins, 5,000-20,000 in molecular weight, which inhibit a number of proteinases, including trypsin and plasmin, as well as kallikrein. Aprotinin (trasylol), an example of this type of inhibitor, is prepared from bovine lung. Two such nonglycosylated proteinase inhibitors have been found in urine (Hochstrasser et al., 1974). A specific kallikrein inhibitor has recently been isolated from kidney (Geiger and Mann, 1976).

Although studies with fully purified enzyme are lacking, it appears that both forms of kallikrein are split by glandular kallikreins, but low molecular weight kininogen is the preferred substrate (Jacobson, 1966). Kininogens are probably synthesized by the liver (Bryan et al., 1972).
Glandular kallikreins release the decapeptide kallidin, or lysyl-bradykinin (Fig. 2), from kininogen (Webster and Pierce, 1963), whereas plasma kallikrein releases bradykinin, as noted earlier. An aminopeptidase (Webster and Pierce, 1963) present in kidney converts some kallidin to bradykinin by removing the N-terminal lysine. Both kinins appear in urine (Hial et al., 1976). Elevation of blood bradykinin concentration 100-fold increases urinary bradykinin only 2-fold (Nasjletti et al., 1975). This suggests that urinary bradykinin is formed in the kidney, not derived from blood kinin. Urine contains a third kinin, methionyl-lysyl-bradykinin, which apparently is formed in urine after voiding by action of urinary pepsin on kininogen (Hial et al., 1976). Although unlikely to be of physiological significance, formation of this substance in urine is of interest because it indicates that a substrate smaller than low molecular weight plasma kininogen may be present in urine. Since the latter is negatively charged and 80,000 in molecular weight, it is unlikely to pass the glomerular filtration barrier in significant amounts.

Kininases I and II, described earlier in connection with the plasma kallikrein, are also widely distributed in tissues (Cicilini et al., 1977; Erdos, 1976). Kidney has very high kininase activity, most of which is kininase II (Ward and Mills, 1975). Kininase II can be detected by fluorescent antibody staining throughout the cytoplasm of proximal and distal tubular epithelium; lower concentrations are present in collecting duct cells and glomeruli (Hall et al., 1976). Differential centrifugation of homogenized kidney shows that the brush border of the proximal convoluted tubule contains by far the highest kininase activity (Ward et al., 1975; Ward et al., 1976a, 1976b). Most of the bradykinin microinfused into proximal tubules is promptly destroyed, while bradykinin injected into distal tubules is excreted intact (Carone, 1976). Angiotensin is hydrolyzed by the same proximal tubular mechanism (Oparil et al., 1976). Presumably, as Ward et al. have suggested (Ward et al., 1975, 1976a, 1976b), the luminal position of kininases on the proximal brush border provides a mechanism for destruction of filtered kinins. This accounts for the fact, noted earlier, that changes in blood kinin levels have little effect on urinary levels (Nasjletti et al., 1975). Kinins formed within the kidney probably escape destruction by this mechanism because they are released by kallikrein, which is secreted by the distal tubule. Except when production is very high (McGiff, 1977), kinins released in the kidney are probably excreted in the urine or inactivated by renal or plasma kininases and act, therefore, as local rather than systemic hormones.

In summary, renal kallikrein releases the kinin, kallidin, from plasma kininogens. Kallikrein apparently is present in distal tubules in an activated form. Activity of the enzyme is modulated by renal inhibitors. Kallidin is rapidly metabolized by renal kininases, which limit intrarenal action of the kinins.

Relations among Kallikrein, Renin, and Prostaglandin Systems

Although evidence for some of the individual interactions shown in Figure 3 is limited, it is quite clear that there are multiple and complex relations among these three hormonal systems in the kidney. Since the juxtaglomerular apparatus, in which renin is synthesized, and the macula densa area of the distal nephron, the probable site of kallikrein synthesis, are anatomically and functionally linked, it is not surprising that these two hormonal systems interact extensively. As already noted, aldosterone may stimulate kallikrein synthesis or secretion (Margolius et al., 1974a 1976). It follows that the renin-angiotensin system, which stimulates aldosterone synthesis, should increase kallikrein release indirectly via aldosterone. There is some evidence (MacFarlane et al., 1974a) that angiotensin II also stimulates kallikrein release directly. Moreover, angiotensin II increases prostaglandin synthesis (McGiff, 1977; Terragno et al., 1976), and prostaglandin E can increase urinary kallikrein excretion (Obika and Mills, 1976). Thus, there are three possible mechanisms by which the angiotensin-aldosterone system can enhance the activity of the kallikrein-kinin system directly via aldosterone or angiotensin and indirectly via prostaglandins. Reciprocally, the kallikrein system can stimulate the renin-angiotensin and prostaglandin mechanisms. Recently it has been reported that human urinary kallikrein converts inactive pro-renin to active renin, and it is possible that kallikrein is a physiological activator of the renin-angiotensin system (Sealey et al., 1978). Kinin increase the release of prostaglandins from the perfused kidney (Colina-Chourio et al., 1976; McGiff et al., 1972), from renal medullary interstitial cells (Zusman and Keiser, 1977), and from blood vessels (Blumberg et al., 1976).
Biochemically, they activate phospholipases, which supply increased arachidonic acid, the rate-limiting precursor, to the prostaglandin synthetic pathway (Hong and Levine, 1976; McGiff, 1977; Terragno et al., 1976). Since prostaglandins can stimulate renin release (Larsson et al., 1974; Weber et al., 1976), it follows that the kallikrein-kinin system can stimulate the renin-aldosterone system indirectly via enhancement of prostaglandin synthesis. The prostaglandins appear to occupy a central role in this complicated scheme of relations among the three hormonal systems, in that they reciprocally stimulate and are stimulated by both the kallikrein and the renin mechanisms. The latter systems are also linked by the role of kininase II or converting enzyme, which limits the activity of the kallikrein system while activating the renin mechanism.

Not all of the interactions shown in Figure 3 are necessarily important physiological control mechanisms. For example, although both the kallikrein and renin systems stimulate renal prostaglandin synthesis, Terragno et al. (1976) cite evidence that, under basal conditions, the kallikrein system is the principal determinant of prostaglandin synthetase activity. Moreover, it is by no means clear that the interactions shown in Figure 3 are direct effects of the hormonal components. A number may be mediated indirectly, through effects of the hormones on renal function. For example, prostaglandins increase renal blood flow and alter tubular handling of salt and water; enhanced kallikrein and renin release may be mediated by these changes rather than by a direct biochemical action of prostaglandins.

In summary, there are complex interactions among the kallikrein, renin, and prostaglandin hormonal systems of the kidney. Whereas the details remain to be established, it is clear that the physiological and clinical role of the renal kallikrein-kinin system cannot be determined without reference to this hormonal interplay.

**Physiological Role**

Renal kallikrein has no known action on the kidney analogous to the direct involvement of plasma kallikrein in blood coagulation. Only kinins are known to influence kidney function directly. Some possible limitations of most studies of the renal actions of kinins should be kept in mind. They are usually performed with bradykinin, not kallidin. Recognized differences (Reis et al., 1971) between these kinins in potency and duration of action may be due to more rapid hydrolysis of bradykinin (Dorer et al., 1974; Johnson and Erdos, 1977). Elevation of renal kinins by infusion may not have the same effect as increases produced by the action of kallikrein, since the latter is localized in the distal tubule.

Infusion of kallidin (Webster and Gilmore, 1964) or bradykinin (Barraclough and Mills, 1965; Stein et al., 1972; Willis et al., 1969) increases renal blood flow but has no systematic effect on glomerular filtration rate (GFR). Direct measurements show that most of the decrease in renal vascular resistance is in the afferent arteriole, but a smaller decrease in efferent resistance also occurs (Baylis et al., 1976). Kinins, like other vasodilators, increase deep-cortical blood flow disproportionately (Istkowitz and Campbell, 1976; Stein et al., 1971). Medullary blood flow, derived from the deep cortical circulation, probably increases as well. Kinins and other vasodilators decrease the hydraulic conductivity of the glomerular filter, a phenomenon of unknown mechanism which accounts for the failure of GFR to rise (Baylis et al., 1976). Disproportionate hemodynamic changes in deep nephrons (Stein et al., 1972), whose GFR may not be as blood flow dependent, may also play a role.

Sodium (Barraclough and Mills, 1965; Stein et al., 1972; Webster and Gilmore, 1964; Willis et al., 1969), calcium, and magnesium (Schneider et al., 1973) excretions increase during kinin infusion. Unlike some other vasodilators, bradykinin does not decrease proximal sodium and water reabsorption in superficial nephrons available for micropuncture (Schneider et al., 1973; Stein et al., 1972). Hence, the increase in sodium excretion must be due either to inhibition of distal reabsorption or to changes in deep nephron function. The disproportionate increase in deep cortical flow (Istkowitz and Campbell, 1976; Stein et al., 1971) would fit either with a heterogeneous deep nephron response (Stein et al., 1972) or with an effect of increased medullary blood flow on reabsorption in Henle's loop. The latter factor may also play a role in the vasopressin-resistant diuresis and decreased urinary osmolality caused by kinins (Barraclough and Mills, 1965; Stein et al., 1972; Webster and Gilmore, 1964; Willis et al., 1969).

Several of the physiological effects of kinins may be related to an increase in intrarenal prostaglandin levels. Blockade of prostaglandin synthesis by indomethacin partly prevents the vasodilatory action of kinins in isolated blood vessels (Messina et al., 1975) and the isolated perfused dog kidney (McGiff et al., 1975), but not in the isolated cat (Chapnick et al., 1977) or rabbit (Fink et al., 1977) kidneys. Indomethacin completely blocks the vasopressin-resistant reduction in urinary osmolality caused by kinin infusion (McGiff et al., 1975). This may be due in part to decreased medullary blood flow, as noted above. Probably more significant is interference with the role of prostaglandins as physiological antagonists of vasopressin (Anderson et al., 1975) at the tubular level. Prostaglandin E2 is natriuretic (Johnston et al., 1967; Tannenbaum et al., 1975) and inhibits sodium reabsorption by the collecting duct (Stokes and Kikko, 1977). It seems likely, therefore, despite contrary data for the isolated perfused dog kidney (McGiff et al., 1975), that increased prostaglandin synthesis contributes to the...
natriuretic effect of kinins. Adding to the complexity is the observation that prostaglandins can stimulate renin release (Larsson et al., 1974; Weber et al., 1976). Since angiotensin is vasoconstrictor and antinatriuretic, the effects of kinins and prostaglandins on the kidney may depend on the prior state of the renin-angiotensin system.

Although it is clear that administered kinins can alter renal blood flow and sodium and water excretion, the role of kinins as physiological regulators of the renal circulation or electrolyte and water excretion is uncertain. Kinins in vena caval blood just above the entrance of the renal veins increased during saline infusion in intact but not in nephrectomized dogs, suggesting increased renal release of kinins in response to volume expansion (Marin-Grez et al., 1972). Anti-bradykinin antiserum (which should also bind kallidin) decreased sodium excretion after saline infusion by 50% in rats (Marin-Grez, 1974), suggesting that kinin release within the kidney is a cause of the natriuresis of volume expansion. Consistent with this view are reports that kallikrein excretion varies directly with dietary salt in man (Adetuyibi and Mills, 1972), and increases during saline infusion in dogs (DeBono and Mills, 1974) and rats (Croxatto et al., 1975; Croxatto et al., 1976; Godon and Damas, 1974). However, other investigators have found an inverse relation between dietary salt and kallikrein excretion in man (Levy et al., 1978, Margolius et al., 1974a) and rats (Johnston et al., 1976a; Johnston et al., 1976b) and reported that saline infusion has no effect on kallikrein excretion in man (Levy et al., 1978, Margolius et al., 1974a). These conflicting views are highlighted by contrasting interpretations of the response to mineralocorticoids. All agree that kallikrein excretion increases in subjects (Adetuyibi and Mills, 1972; Margolius et al., 1974a) and dogs (Marin-Grez et al., 1973) on high salt intakes during chronic mineralocorticoid administration. Some investigators interpret the increase as a response to progressive expansion of extracellular volume, consistent with their view that the kallikrein-kinin system is part of the effector mechanism for natriuresis (Marin-Grez et al., 1973). They point out that kallikrein excretion does not increase during the first few days of mineralocorticoid administration, suggesting that the response is not to the hormone but to progressive volume expansion due to initial sodium retention. Margolius et al. (1974a) cite the following observations to support their view that a direct hormonal effect is involved. They find: (1) kallikrein excretion increases on a low salt diet, (2) this increase is inhibited by spironolactone, and (3) aldosterone stimulates kallikrein secretion by kidney cell suspensions (Kaizu and Margolius, 1975; Margolius et al., 1976). It is impossible to come to a firm conclusion from the conflicting data. Nonspecificity of the assays may be the problem. For example, extracellular expansion might increase true kallikrein excretion, whereas aldosterone might increase another esterase in urine. Both would be interpreted as an increase in "kallikrein" with the esterase assay used by most investigators.

On one observation there is unanimity: kallikrein excretion increases during the action of diuretics (Croxatto et al., 1975; Croxatto et al., 1976; Croxatto et al., 1976; Johnston et al., 1976a, 1976b; Nielsen and Arrigoni-Martelli, 1977; Nielsen et al., 1976; Olsen, 1977; Olsen and Ahnfelt-Ronne, 1976) in both dogs and rats. Furosemide and bumetanide, which act on the loop of Henle, thiazides, which act on the distal tubule, and acetazolamide, which acts proximally, are all effective, so the increase in kallikrein excretion probably is not a direct pharmacological action. Vasodilators, including bradykinin, acetylcholine, isoproterenol and substance P, also increase urinary kallikrein (MacFarlane et al., 1974b; Mills et al., 1976), so varied a group as to suggest a class rather than a specific drug effect. The cause of increased kallikrein excretion with either group of agents is uncertain. All increase sodium excretion; it is possible that a change in urine flow, salt concentration, or pressure in the distal tubule, the apparent site of synthesis, stimulates kallikrein secretion. Renal blood flow is increased by furosemide and the vasodilators, but not by thiazides or acetazolamide, so increased blood flow itself is probably not the sole mechanism. Plasma renin concentration is increased by diuretics, especially if volume losses are not replaced. Concurrent administration of a /3-adrenergic blocker enhances the effect of thiazides on kallikrein excretion while decreasing hyperreninemia (Nielsen et al., 1976), which suggests that increased renin release is not the key factor. Since kallikrein excretion can increase within a few minutes after a diuretic is administered (Olsen and Ahnfelt-Ronne, 1976), volume depletion and/or increased aldosterone activity seem unlikely mechanisms. Whatever the stimulus to kallikrein release, the increase in kallikrein activity may contribute to the vasodilator and natriuretic effects of some diuretics and vasoactive agents.

With one exception (Margolius et al., 1974a), investigators have found that kallikrein excretion is increased by water loading in man (Levy et al., 1978) and animals (Croxatto et al., 1975; Croxatto et al., 1976; Mills and Ward, 1975; Mills et al., 1976). The increase is less than that induced by saline or diuretics, although the effect of a maximum water diuresis has not been reported. The mechanism is unknown, but one may speculate that changes in urine flow or composition at a distal tubular kallikrein secretory site may be a factor. Kinins released by kallikrein presumably potentiate the water diuresis by stimulating the synthesis of prostaglandin E₂, a natural vasopressin inhibitor, as noted above.

In summary, kinins are potent renal vasodilators, which increase salt and water excretion. The physiological role of the kallikrein-kinin system is uncertain. It probably modulates vasopressin action and may regulate the renal circulation. Whereas it
is probable that the system helps control sodium excretion, it is unclear at present whether it functions as a natriuretic hormone during extracellular volume expansion or as part of an aldosterone-stimulated antinatriuretic mechanism. Activation of the kallikrein-kinin system by diuretics and vasodilators may modulate the effects of these agents on the kidney.

Hypertension

Over 40 years ago, hypertensive patients were found to excrete subnormal amounts of urinary kallikrein. Surprisingly, the two early reports stimulated no further publications until Margolius and associates (1974b) confirmed the observation a few years ago. They found that urinary kallikrein was about half of normal in patients with essential hypertension, a finding since supported by others (Levy et al., 1977; Seino et al., 1975). Kallikrein excretion did not vary with dietary sodium over a wide range of normal intakes (Margolius et al., 1974b; Seino et al., 1975) but increased somewhat during severe salt restriction, although not as much as in normals (Margolius et al., 1974b). Margolius and his associates (1974b) did not distinguish between black and white subjects; however, recent reports suggest that race may be a factor. In epidemiological surveys, black children have lower urinary kallikrein concentrations than white children (Zinner et al., 1976). Levy et al. (1977) found that kallikrein excretion is greatly reduced in black men, whether normotensive or hypertensive; both groups excreted even less kallikrein than white hypertensives. With salt restriction, kallikrein excretion increased to low normal in black normotensives and white hypertensives but was virtually unchanged in black hypertensives. The subjects studied by Seino et al. (1975) presumably were all Japanese; as in the study by Margolius and associates (1974b), there was a clear difference between normotensive and hypertensive subjects. On the other hand, Lawton and Fitz (1977) were unable to find any reduction in kallikrein excretion in a small group of white subjects with "normal renin" essential hypertension. They suggest that kallikrein excretion in hypertension may be correlated with plasma renin and that admixture of patients with low renin hypertension may account for reduced excretion in unclassified hypertensives in previous studies. However, patients with primary aldosteronism, whose plasma renin is suppressed, have greatly elevated urinary kallikrein (Lechi et al., 1976; Margolius et al., 1974b; Seino et al., 1975, 1977), an observation not consistent with any simple relation between plasma renin and kallikrein excretion in hypertension. Spironolactone decreases kallikrein excretion in primary aldosteronism (Margolius et al., 1974b, Seino et al., 1977) but not in essential hypertension (Seino et al., 1977).

Kallikrein excretion has been studied in several forms of experimental hypertension in rats. Carreno et al. (1978) measured kallikrein excretion in rats bred to be susceptible or resistant to the hypertensive effect of salt. Using a specific immunosay, they found a moderate reduction in kallikrein excretion in susceptible rats. Enzymatic kallikrein activity (kinin liberation by urine in vitro) was much more severely reduced than excretion of kallikrein protein measured by immunosay. These investigators suggest that susceptible rats excrete subnormal amounts of kallikrein and that some of the enzyme is either bound to an inhibitor or is in an inactive form. They speculate that deficient activity of the kallikrein-kinin system may alter sodium and water excretion in susceptible rats and thereby promote hypertension during high salt intake. Kallikrein excretion is also decreased in genetic hypertension and "one-kidney" Goldblatt hypertension (renal arterial stenosis and contralateral nephrectomy) (Croxatto et al., 1976; Geller et al., 1975; Lechi et al., 1976b; Pisano et al., 1974). Kallikrein activity in renal tissue is also decreased (Favaro et al., 1975; Lechi et al., 1976b). Only in experimental hypertension due to mineralocorticoids and high salt intake is kallikrein excretion increased (Keiser et al., 1976a). In "two-kidney" renovascular hypertension (arterial stenosis with contralateral intact kidney), two studies conflict (Johnston et al., 1976b; Keiser et al., 1976a); one reporting increased, the other decreased, excretion.

As in normals, diuretics increase kallikrein excretion in hypertensive patients (O'Connor et al., 1977) and animals (Johnston et al., 1976b; Nielsen and Arrigoni-Martelli, 1977; Nielsen et al., 1976); β-adrenergic blockers potentiate this effect in dogs (Nielsen et al., 1976). Hydralazine also increases kallikrein excretion in rats (Nielsen and Arrigoni-Martelli, 1977). As noted earlier, changes in kallikrein excretion may be secondary to alterations in urine flow or sodium excretion rather than direct effects of the drugs themselves. It is possible that these agents exert their antihypertensive effects in part by stimulating the kallikrein-kinin system.

In summary, there is evidence that activity of the renal kallikrein system is deficient in some patients with essential hypertension and in many animal models. However, such defects may be limited to particular subclasses of essential hypertension in man. It is even possible that defects in the kallikrein system play a role in the pathogenesis of some forms of hypertension. There appear to be racial differences in kallikrein excretion, which conceivably could account in part for the higher frequency and severity of hypertension in blacks. Stimulation of the kallikrein system may be one mechanism of action of some important antihypertensive agents.

Kidney Disease and Related Conditions

Data are limited, but some of the scattered observations are provocative. Adetuyibi and Mills (1972) reported that kallikrein excretion was increased in four patients with advanced chronic renal
failure. Cannella et al. (1973) found kallikrein excretion to be normal in a group of 21 patients with varying degrees of renal failure due to chronic glomerulonephritis. Kallikrein excretion per milliliter of GFR is greatly increased in both studies; this phenomenon may help maintain normal sodium excretion and high blood flow to surviving nephrons in chronic renal failure. Mitas et al. (1978) reported that kallikrein excretion was markedly decreased in hypertensive patients with chronic renal failure. The severity of hypertension in these individuals correlated well with the degree to which kallikrein excretion was reduced, suggesting a possible cause and effect relation. Kallikrein excretion was even lower than in patients with essential hypertension of comparable severity. [The presence of hypertension was not specified in the study by Cannella et al. (1973); two of the four patients of Adetuyibi and Mills (1972) had moderate hypertension.]

In experimental chronic glomerulonephritis in rats (Godon and Damas, 1974), kallikrein excretion was normal; blood pressure was not reported. During the first several weeks of experimental nephritis and nephrosis in rats, kallikrein excretion was reduced (Glasser and Michael, 1976); observations during the chronic phase were not described. Kallikrein excretion is reported to be low in rats with nephrotoxic acute renal failure (Werle and Vogel, 1960), in dogs with acute (Bevan et al., 1974) or chronic (Keiser et al., 1976) renal arterial clamping, and in rats with chronic ureteral obstruction (Barton and Schacter, 1974). It is conceivable that decreased activity of the kallikrein-kinin system is one factor that leads to the intrarenal vasoconstriction characteristic of acute renal failure.

Abnormalities of the plasma kallikrein system have been reported in cirrhosis (Bagdasarian et al., 1974) and the hepatorenal syndrome (Wong et al., 1977). In mild cirrhosis, plasma prekallikrein activity (by specific immunoassay) and plasma esterase activity were reduced proportionately by about one-half. In severe cirrhosis, immunological kallikrein was not further depressed, but functional kallikrein by the esterase method decreased to 8% of normal. Although several other interpretations are possible, the investigators suggest that the liver in advanced cirrhosis secretes an inactive molecule. It is possible that renal salt retention in cirrhosis is due partly to decreased activity of the kallikrein-kinin system. In patients with the hepatorenal syndrome, "functional plasma pre-kallikrein" (esterase) was reduced still further to about 30% of that in patients with comparably severe cirrhosis, and plasma bradykinin was also decreased. As would be expected, plasma renin activity was greatly increased in both groups. The authors suggest that combined vasodilator (kinin) deficiency and vasoconstrictor (angiotensin) excess may cause the increase in renal vascular resistance which is a key pathogenetic mechanism in hepatorenal syndrome. Although these observations are provocative, evaluation of the renal kallikrein system may prove more informative.

In Bartter's syndrome of hypokalemia, hyperreninemia, hyperaldosteronism, and normal blood pressure, kallikrein excretion is extraordinarily high (Halushka et al., 1977; Lechi et al., 1976a; Vinci et al., 1978). Kallikrein excretion falls during treatment with spironolactone (Lechi et al., 1976a) or prostaglandin synthetase inhibitors (Halushka et al., 1977; Vinci et al., 1978), although not always to normal (Halushka et al., 1977; Lechi et al., 1976a). Both types of drugs decrease aldosterone activity, spironolactone directly and prostaglandin synthetase inhibitors indirectly by decreasing prostaglandin-mediated renin release and thereby angiotensin-stimulated aldosterone secretion. Thus, the effects of both types of drugs fit the hypothesis that elevated kallikrein in Bartter's syndrome is, at least in part, caused by hyperaldosteronism. Vinci et al. (1978) report that urinary kinins are subnormal despite the elevated kallikrein levels. Moreover, kinins increased during treatment with prostaglandin synthetase inhibitors, which decreased kallikrein excretion. The authors suggest that urinary kallikrein does not control kinin excretion in Bartter's syndrome. However, as noted earlier, the relation between urinary excretion and renal production of kinins is uncertain. The interrelations of the kallikrein-kinin, renin-angiotensin-aldosterone, and prostaglandin systems in Bartter's syndrome have been reviewed recently (McGiff, 1977).

In pregnancy, kallikrein excretion increases to twice normal during the first trimester, then gradually falls to normal, nonpregnant levels in the last trimester (Elebute and Mills, 1976). Increased aldosterone secretion, also a characteristic hormonal change during pregnancy, is a possible cause of the increased kallikrein excretion. Kallikrein excretion in hypertensive women in the third trimester was significantly reduced. Whether this reduction is pathogenetically significant or a secondary change is not known.

Conclusions

Characterization of the components of the renal kallikrein-kinin system is incomplete, and methods for assessing the activity of the system at its sites of biological action are not yet available. Nevertheless, enough information already has accumulated to indicate that the renal kallikrein-kinin system is an important part of an intrarenal hormonal regulatory complex which also includes renin-angiotensin, prostaglandins, and aldosterone. It seems quite likely that the kallikrein system is a significant factor in the control of the renal circulation and of salt and water excretion. It is possible that abnormalities of this system play a role in the pathogenesis or pathophysiology of some forms of hypertension, kidney disease, and fluid and electrolyte dis-
orders. The results of previous investigations appear to justify the current rapid expansion of research in this area.

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