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COMMENTARY

MULTIHORMONAL REGULATION OF RENAL KALLIKREIN

THE INVOLVEMENT OF THE RENIN–ANGIOTENSIN–ALDOSTERONE SYSTEM, THE CORTICOTROPIN–GLUCOCORTICOID SYSTEM, ANTIDIURETIC HORMONE, CATECHOLAMINES AND PROSTAGLANDINS

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Although the presence of kallikrein in urine was detected for the first time more than half a century ago [1], the persistent effort to clarify its physiological role started in the early 1970s. The impulse for this development is probably attributable to the introduction of simple and reproducible methods for the measurement of the enzyme [2–4]. Several excellent reviews on the biochemical, morphological and functional aspects of this progress have been published in recent years [5–15]. Only the information necessary to introduce the subject of the present discussion to those not familiar with the kallikrein–kinin system will be mentioned here. This review deals with a complex but exciting new topic: the influence of hormonal interactions on renal kallikrein.

The renal kallikrein–kinin system

Kallikrein is synthetized in the kidney and in other organs as well [16]. The precise renal site of synthesis has not yet been identified. There is some evidence suggesting that the distal tubular cells produce kallikrein. With the aid of immunohistochemical methods as well as by measurements of the kallikrein activity in homogenates of distal tubules obtained by tubular microdisection the enzyme has been localized in the distal nephron segments [17–19]. In addition, it has been reported that when the urine flow of dogs undergoing an osmotic diuresis is stopped (to amplify the effects of the different nephron segments on urine composition) kallikrein is added to the tubular fluid at the level of the distal tubules [20].

In kidney homogenates both a soluble and a membrane-bound form of the enzyme have been found [21]. Reticuloendoplasmic as well as luminal and basolateral membranes from renal cells appear to contain kallikrein [22, 23]. It is still unclear whether the enzyme is synthesized as such or in the form of a precursor. Human urine, perfusate and urine from isolated rat kidneys and solution bathing rat kidney slices contain a form of kallikrein that can be activated [24, 25]. This, however, could be also explained by the presence of an enzyme–inhibitor complex.

Renal kallikrein is a glycoprotein whose mol. wt has been estimated, by various techniques and in different species, to be between 27,000 and 40,000. Multiple forms of the enzyme with isoelectric points close to pH 4.0 have been found on isoelectric focusing. Kallikrein is a single-chain molecule which has several disulfide bridges. Serine is present in the active center [26].

The enzyme has the ability to release the decapeptide kallidin from both a low (*ca* 50,000) and a high (*ca* 100,000) mol. wt substrate called kininogen. Several urinary and renal enzymes can convert kallidin to the nonapeptide bradykinin or to inactive fragments (Fig. 1). Apart from its kininogenase (i.e. kinin-generating) activity, kallikrein has the ability to hydrolyse synthetic esters of arginine [27, 28]. This property has been exploited for measuring kallikrein activity [2, 3]. Although the esterase and kininogenase activities of urine are known to correlate with each other [29], it has been shown that an important amount of the urinary esterase may be unrelated to kallikrein [30, 31]. Thus, caution must be exercised in evaluating the studies in which the esterase, rather than the kininogenase, activity was used to estimate kallikrein. In the following discussion, the term 'kallikrein' will be used to refer to results obtained with (or corroborated by) the kininogenase assay [4].

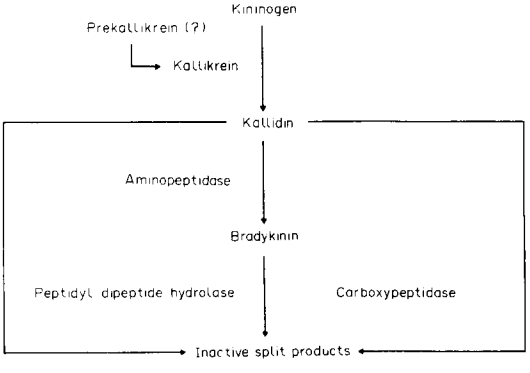

Fig. 1. Scheme of the renal kallikrein–kinin system.

When referring to studies in which the enzyme's activity was estimated by using a synthetic ester of arginine [2, 3] or the synthetic tripeptide D-Val-Leu-Arg-paranitroanilide [32, 33], the terms "esterase" or "amidase" will be used.

Most of the studies concerning the physiological, pharmacological or clinical role of the renal kallikrein–kinin system have relied on the estimation of urinary kallikrein excretion. The daily excretion of the enzyme (urinary kallikrein activity times 24-hr urine volume) was assumed to be an index of its activity in the kidney. Only recently have we been able to verify this assumption [34]. Acute changes of diuresis or renal blood flow may, however, alter this relationship [35, 36].

The dependance of the renal kallikrein activity on several hormones has been discovered in recent years.

The role of the renin–angiotensin–aldosterone system

The stimulatory effect of exogenous mineralocorticoids on kallikrein excretion was reported several years ago [37–39] and it has been confirmed many times thereafter [40–43]. Homogenates of kidneys obtained from mineralocorticoid-treated rats also have increased kallikrein activity [43]. In addition, it has been reported that aldosterone increases the release of an alkaline arginine esterase to the medium by suspended renal cortical cells [44]. This has been contested by Vio *et al.* [45] who did not find a stimulatory effect of aldosterone on kallikrein release by isolated perfused rat kidneys. These authors, however, did not publish the data on electrolyte excretion to demonstrate that the infused mineralocorticoid was biologically effective.

A marked rise in *endogenous* aldosterone is also associated with an increased excretion of alkaline arginine esterase. This has been observed in patients with primary aldosteronism [46–49] and in patients with the secondary aldosteronism of Bartter's syndrome [50, 51]. The question whether variations in the plasma aldosterone concentration within the physiological range also affects the activity of renal kallikrein has been more difficult to answer. It has been repeatedly shown that stimulation of the renin–angiotensin–aldosterone system by sodium depletion leads to increased kallikrein excretion [38, 52–55]. Studies on the suppression of aldosterone by saline loading gave conflicting results. Although prolonged high sodium chloride intake provoked, as expected, a decreased kallikrein excretion [54], a rapid saline loading led to increased rather than to decreased kallikrein activity [56–58]. The first indication that this discrepancy was due to a methodological artifact was given by our finding that diuretics induce only a transient enhancement of kallikrein, which occurs concomitantly with the appearance of the diuresis [35, 59]. This suggested that an acute rise in the urine flow would induce a wash-out of kallikrein from the kidney. The puzzling finding of a "stimulatory" rather than an inhibitory effect of an acute saline load upon kallikrein excretion was finally elucidated when we repeated the load at relatively short intervals. Under these conditions, after an initial "stimulatory" effect which is probably due to a non-specific wash-out, both plasma aldosterone and urinary kallikrein (amidase) excretion are depressed (M. Marin-Grez, G. Schaechtelin, G. Bönner and F. Gross, submitted).

There are, however, several conditions under which the renin–angiotensin–aldosterone system and kallikrein excretion are actually dissociated:

(1) renal hypertensive rats have increased aldosterone and reduced kallikrein excretion [60].

(2) kallikrein (amidase) activity is depressed after an osmotic diuresis, whereas plasma aldosterone tends to increase [35].

(3) water immersion does not affect kallikrein (esterase) excretion in man, in spite of the fact that this manœvre depresses the renin–angiotensin–aldosterone system [61, 62].

(4) rats with hereditary diabetes insipidus (Brattleboro strain) have a stimulated renin–angiotensin system but unchanged kallikrein (amidase) excretion [63].

(5) some patients with primary aldosteronism have normal kallikrein (esterase) excretion [48, 49, 64].

(6) the activity of the renin–angiotensin system is increased in rats with hypertonic dehydration [65], but the opposite happens with the kallikrein (amidase) excretion [63].

(7) patients with essential hypertension may respond with decreased kallikrein (esterase) and increased aldosterone excretion to a low sodium diet [64].

(8) some patients with *low* renin essential hypertension have normal kallikrein (esterase) excretion [48, 49].

(9) Some low renin essential hypertensive patients treated with the aldosterone antagonist spironolactone respond with increased rather than decreased kallikrein excretion [66].

We may conclude that factors other than the plasma aldosterone concentration also play a role in controlling the activity of the renal kallikrein–kinin system.

The role of the corticotropin–glucocorticoid system

We have found that administration of corticosterone to rats (i.e. the principal glucocorticoid in that species) depresses renal kallikrein (amidase) release [41]. More recently, McPartland *et al.* [67] found depression of kallikrein (esterase) excretion in rats treated with a synthetic glucocorticoid. One could speculate that this reduction is actually mediated by a lowering of aldosterone release caused by the negative feed-back between glucocorticoids and corticotropin. This does not seem to be the case. We found that rats injected with adrenocorticotropic hormone (tetracosactid) for 3 days have, along with the enhanced corticosterone, a reduced kallikrein excretion. This occurs in spite of the concomitant marked stimulation of the aldosterone secretion rate. After 10 days of tetracosactid treatment the aldosterone secretion rate was normal and the kallikrein (amidase) excretion was still depressed (G. Bönner, R. Authenrieth, M. Marin-Grez and F. Gross, unpublished).

Glucocorticoids could act by antagonising the effect of endogenous aldosterone at the level of the renal receptors. Their effect could be also indirect as for example by inhibition of the release of anti-

diuretic hormone [68]. It has been reported that this hormone stimulates the release of renal kallikrein (see later).

It is unfortunate that the single report on the influence of adrenocorticotropic hormone on urinary kallikrein excretion in humans refers to an investigation of short duration and thus it only comprised the phase of potent stimulation of aldosterone release but not the ensuing preponderance of glucocorticoid stimulation [52].

Although antidiuretic hormone is chemically and functionally distinct from the corticotropin-releasing factor [69–71], it has the capacity to release corticotropin both *in vitro* and *in vivo* [72, 73]. Thus, a vasopressin-induced stimulation of glucocorticoid release could lead to *depression* of the renal kallikrein activity. Whether this occurs under physiological conditions remains to be proved.

The involvement of the antidiuretic hormone

The first indication that a hypophyseal factor plays a stimulatory role in renal kallikrein release was provided by the report of Croxatto *et al.* [74] that hypophysectomized rats excrete less kallikrein than sham operated controls. These authors reported that administration of the growth hormone partially restored kallikrein excretion. This effect of somatotropin may be related to its capacity to restore aldosterone release in hypophysectomized rats [75].

It has been demonstrated, but not yet confirmed, that an infusion of arginine vasopressin stimulates kallikrein release in both rats and dogs [76]. This effect can neither be due to the non-specific effect of rising urine flow nor be mediated by the renin–angiotensin system, since arginine vasopressin depresses both. The reduced urine flow may be expected to lower rather than to increase the kallikrein excretion. Although one would expect "*a priori*" that antidiuretic hormone induced hyponatremia would promote renin release, the opposite has been found experimentally [77, 78]. The inhibition of renin release by antidiuretic hormone appears to be unrelated to its vasoconstrictor action [78].

Exogenous antidiuretic hormone could stimulate kallikrein excretion either directly or through its natriuretic and/or kaliuretic properties [79]. Mills and Newport [80] could not confirm the kallikrein-stimulating effect of antidiuretic hormone. However, these authors did not report on the duration of the vasopressin infusion. If their experiment lasted only a short period of time they may have missed an effect which perhaps depends on new protein synthesis.

We have studied the role of endogenous antidiuretic hormone by measuring the kallikrein excretion of rats with hereditary diabetes insipidus [63]. These animals have normal kallikrein (amidase) excretion in spite of the absence of circulating antidiuretic hormone. This finding does not necessarily rule out a kallikrein-stimulating role of antidiuretic hormone since other factors could compensate for its absence. However, it is almost certain that vasopressin is not a fundamental factor for the control of renal kallikrein activity since in our study neither control nor diabetes insipidus rats responded with increased kallikrein excretion to vasopressin treatment [63]. The discrepancy between our results and those by Fejes-Tóth *et al.* [76] could be due to differences in the experimental design (anaesthetized vs non-anaesthetized animals, intravenous vs subcutaneous route of administration of the antidiuretic hormone). It is likely that the barbiturate used by the Hungarian scientists inhibited the antidiuretic hormone induced release of corticotropin [81]. This could allow the expression of a direct stimulatory effect which under normal conditions would not take place.

If antidiuretic hormone proves to be a physiological regulator of the renal kallikrein activity, all factors capable of influencing its release could thereby affect the levels of the enzyme. Apart from the usual stimuli responsible for the release of antidiuretic hormone (variations in blood volume, blood pressure or osmolarity), hormonal interactions may be of importance. Antidiuretic hormone release is stimulated by systemic and intracerebral administration of angiotensin and perhaps also by endogenous angiotensin [82]. Therefore an enhanced activity of the renin–angiotensin system, e.g. by sodium depletion, might stimulate kallikrein activity not only through an increased secretion rate of aldosterone but also by an enhancement of the plasma concentration of antidiuretic hormone. Interestingly, reduced vasopressin and aldosterone accompanied the lowering of kallikrein excretion induced by vincristine treatment in a patient with the syndrome of inappropriate secretion of antidiuretic hormone [83].

A complex interaction may exist between the renin–angiotensin–aldosterone system, the corticotropin–glucocorticoid system and the antidiuretic hormone in the control of the renal kallikrein activity (Fig. 2).

The involvement of catecholamines

It has been reported that the kallikrein (esterase) excretion of dogs increases after stopping an infusion

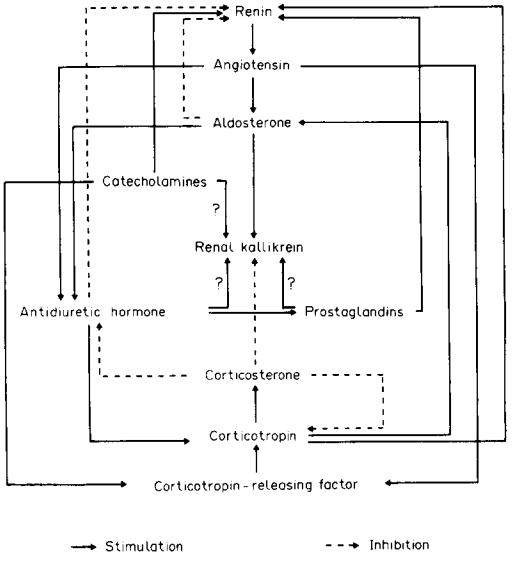

Fig. 2. Known and putative (?) hormonal influences on rat renal kallikrein. Hormonal interactions (direct or mediated) are also depicted. For details see text.

of noradrenaline. This effect was abolished by pretreatment with an α-blocker [84]. Also adrenaline, although only after α-adrenoreceptors have been blocked, was found to stimulate kallikrein excretion [85]. These results are difficult to interpret. It is known that both α- and β-adrenergic stimulation increases the release of salivary gland kallikrein [86, 87]. If the same were true for renal kallikrein one would expect that adrenaline stimulates the enzyme also in the absence of blocked α-adrenoreceptors.

Catecholamines stimulate the release of corticotropin (α- and β-receptors have been implicated [88, 89]) and of renin (β_1-agonists are stimulatory [90]). By altering blood pressure they can also induce changes in the release of antidiuretic hormone [92]. Thus, enhanced adrenergic activity (or exogenous adrenergic agonists) could produce a widely varied picture of renal kallikrein activity depending on the agonist involved and its site of action. Direct or indirect stimulatory and/or inhibitory impulses could counteract each other. Consequently the unaltered kallikrein excretion found during a prolonged systemic infusion of noradrenaline does not necessarily mean that catecholamines are not involved in the regulation of kallikrein activity [92]. It has been reported that both the kininogenase and the esterase excretion of rats with renal denervation are unaffected [92, 93] or only transiently reduced [94]. However, renal nerve stimulation in cats produced a depression of the urinary kallikrein activity. This effect was reversed by adrenergic blockade [94]. It is difficult to reconcile this data with the reported rise of kallikrein excretion brought about by exogenous catecholamines [84, 85] and with the lower renal kallikrein activity found in cats treated with α- and β-adrenergic antagonists [94].

The involvement of prostaglandins

The interaction between the renal kallikrein–kinin system and prostaglandins has been recently reviewed by Nasjletti and Malik [95]. Bradykinin, by increasing the availability of arachidonic acid (apparently through activation of phospholipase A) stimulates renal prostaglandin E_2 synthesis and release [96]. It has been both affirmed and denied that prostaglandins mediate the renal vasodilating, diuretic and natriuretic effects of bradykinin [97–99].

An involvement of prostaglandins in the control of renal kallikrein activity is even less clear. In acute experiments both prostaglandin E_1 and E_2 were able to stimulate kallikrein release in normally hydrated rats and dogs, whereas prostaglandin $F_{2\alpha}$ was able to stimulate kallikrein release in hyperhydrated but not in normally hydrated animals [100, 101].

Additional studies are required to elucidate whether the stimulatory effect of prostaglandins actually reflects increased renal kallikrein activity or merely represents an artifact due to the simultaneous enhancement of urine flow (wash-out?). In this context it is pertinent to note that administration of inhibitors of prostaglandin synthesis to patients with the Bartter syndrome normalizes the (increased) kallikrein excretion [51]. Although it has not been ruled out that the normalization of urinary kallikrein is secondary to the correction of the polyuria and electrolyte disarrangement by the treatment, Vinci *et al.* [51] believe that the lowering of the plasma renin activity (and consequently the aldosterone secretion rate) by the cyclooxygenase inhibitor accounts for the normalization of the urinary kallikrein (esterase) excretion. It is not yet clear whether this explanation is correct, but this reasoning suggests that in a chronic setting prostaglandins could influence renal kallikrein indirectly by controlling renin release [102, 103].

Prostaglandin E_2, through a direct and/or a renin-mediated pathway, could mediate the kallikrein-stimulating effects of other hormones. Antidiuretic hormone, for instance, stimulates the release of prostaglandin E_2 by the medullary interstitial cells of the kidney [104].

Comments

For the sake of conciseness I have mentioned in most instances only one (usually the first) report on the influence of a hormone on the release of another one. The literature confirming these interactions is abundant. Nonetheless, my suggestion that an interaction of several hormones affects the renal kallikrein activity is still highly speculative. Such complex hormonal interactions (Fig. 2) could explain the lack of correlation between plasma aldosterone concentration and kallikrein excretion observed in some clinical and experimental conditions. For instance, the dissociation between these parameters in rats undergoing an osmotic diuresis may be due to a preponderance of glucocorticoids. Mannitol-induced hypovolemia probably stimulates the corticotropin–corticosterone axis in rats. This is suggested by the report of enhanced β-endorphin (which is released concomitantly with corticotropin [105]) and of corticosterone in polyethylene glycol induced hypovolemia [106, 107].

Although in animals with hypertonic dehydration the release of renin and antidiuretic hormone is stimulated [65, 108], we have found that water deprivation depresses the kallikrein (amidase) excreted by Long–Evans rats [63]. This could be also explained by a higher plasma concentration of corticosterone. This steroid rises after water deprivation in potassium-depleted and in sodium- plus potassium-depleted rats [107].

Exogenous administration of mineralocorticoids or a low sodium diet (endogenous aldosterone stimulation) have, to my knowledge, never been reported to fail to stimulate kallikrein excretion *in vivo*. It is, therefore, puzzling that some patients with primary aldosteronism have normal kallikrein excretion [48, 49]. Gláz *et al.* [109] reported that some adrenal hypertensive patients have, along with the aldosteronism, slightly elevated plasma cortisol levels and an enhanced cortisol response to corticotropin. Also, adrenal adenoma cells release not only aldosterone but corticosterone as well when incubated *in vitro* [110]. Although the inhibitory effect of glucocorticoids upon renal kallikrein in humans remains to be confirmed, it is tempting to speculate that patients with "primary aldosteronism" who have normal urinary kallikrein excretion are those who in addition

to the aldosteronism have an increased secretion rate of glucocorticoids. This, if confirmed, would be useful in the clinics where the measurement of urinary kallikrein (which is easier to perform than that of the adrenal steroids) could be applied to detect this subpopulation of patients.

Several groups of investigators have reported that the kallikrein excretion is reduced in rats with established renovascular hypertension [111–113]. This abnormality appears after the development of hypertension and, therefore, seems to be the consequence rather than the cause of the high blood pressure [114, 115]. The preponderance of an inhibitory effect of the increased level of corticosterone found in this condition [116] over the stimulatory effect of the renin–angiotensin–aldosterone system and the putative effect of antidiuretic hormone could play a role in the reduction of renal kallikrein.

Numerous reports on the finding of a reduced kallikrein (esterase) excretion in some patients with essential hypertension have been published to date [46, 48, 117–121]. Perhaps these patients represent a subpopulation with increased glucocorticoid response to corticotropin [109]. This or other mechanism could displace the mineralo-/glucocorticoid ratio in favor of the latter. The importance of the balance between these steroids in the control of the level of kallikrein excretion in patients with essential hypertension has not yet been studied. An alternative possibility is that the lower kallikrein excretion of some hypertensive patients reflects the damage to the kallikrein-producing cells by the chronically elevated blood pressure. Renal damage reduces kallikrein excretion [122]. However, Zinner *et al.* [123] proposed that this abnormality is inherited. Their opinion was based on the observation that (normotensive) children of hypertensive families excreted less kallikrein (esterase) per milligram of creatinin than children of normotensive families. This epidemiological study relied on casual measurements of kallikrein and creatinin. Since the level of the hormones which affect the renal kallikrein activity is subjected to a nyctohemeral rhythm, casual measurements of the enzyme are likely to be inadequate. Thus, before definitive conclusions are drawn, the aforementioned study should be validated by the measurement of the daily excretion of kallikrein in both populations of children.

Some authors have failed to find a significant depression of the kallikrein (esterase) excretion in patients with essential hypertension [49, 120, 124]. This discrepancy is unlikely to be due to methodological problems, since in both negative and positive studies the same alkaline esterase assay was used for the estimation of kallikrein. When it became apparent that black people excrete less kallikrein than whites [49, 125–127], the uneven distribution of races in the earlier studies (more blacks than whites in the hypertensive group) was made responsible for the discrepancy. However, in a more recent study it has been shown that essential hypertensive patients excrete less kallikrein than their race-, sex- and age-matched normotensive controls [19]. Not only the reduced kallikrein (esterase) excretion in some patients with essential hypertension but also the normal kallikrein excretion in others may represent an abnormality. As suggested by numerous physiological and pharmacological studies on the influence of mineralocorticoids on kallikrein excretion, the subpopulation of patients with high plasma renin activity (and consequently an elevated aldosterone secretion rate) should have a stimulated rather than normal renal kallikrein. A kallikrein excretion which is either reduced or inadequate for the coexisting activity of the renin–angiotensin–aldosterone system suggests that in these patients an hormonal abnormality is at play. One is tempted to speculate that these patients produce excessive amounts of either a glucocorticoid, an abnormal mineralocorticoid or a steroid precursor which by its binding to renal mineralocorticoid receptors prevents the stimulatory effect of aldosterone on the synthesis of kallikrein.

Although the plasma aldosterone concentration and the excretion of tetrahydroaldosterone and aldosterone-18-glucoronide have been found to be normal in black people [49, 128], their kallikrein excretion is lower than that of whites. Perhaps the level of renal kallikrein activity (which may reflect the influence of both stimulatory and inhibitory factors on the mineralocorticoid receptors) will prove to be a better index of mineralocorticoid activity than the plasma level of aldosterone or the rate of excretion of the aldosterone metabolites.

It is still obscure why kallikrein excretion remains unaffected after water immersion in man, a condition in which both the renin–angiotensin–aldosterone system and the antidiuretic hormone are depressed and in which the 17-OH-steroids are unaffected [62, 63, 129, 130].

Although in this review I have concentrated on those hormones shown to affect renal kallikrein activity, one cannot avoid mentioning other factors which may also contribute to the level of kallikrein excretion. Perhaps the most important of these factors is the integrity of the kallikrein-synthesizing cells. We and others have shown that the simple manœvre of excising one kidney reduces the kallikrein excretion by 50% [113, 114]. Unilaterally nephrectomized rats excrete less kallikrein (amidase) than sham operated controls up to 30 days after surgery (M. Marin-Grez and G. Schaechtelin, submitted). Other factors which have been proposed to play a role in the regulation of the kallikrein excretion are the arterial blood pressure and the renal concentration of potassium [12, 58]. By using isolated rat kidneys we have found that the influence of perfusion pressure on kallikrein excretion and renal kallikrein activity are secondary to the changes in urine flow and probably of no physiological meaning (G. Bönner, U. Schwertschlag, M. Marin-Grez and F. Gross, submitted). In most of our studies we have found a significant correlation between the excretions of potassium and kallikrein. This suggest that kallikrein (like potassium [131]) excretion depends on the rate of distal tubule fluid flow.

Much has to be learned about the relationships of known (and still unknown) parameters on the regulation of renal kallikrein activity. However, the advance achieved in the last few years suggests an important role for this enzyme in modulating or mediating the action of hormones in the distal segments of the nephron.

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