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THE KIDNEY AND ITS HUMORAL ACTION ON ARTERIAL HYPERTENSION

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Since many years the intuition of some clinicians gave rise the general belief that the kidney is involved in arterial hypertension pathogenesis. The discovery carried out by GOLDBLATT (1937) [15] provided a solid experimental support to this clinical assumption. The simple procedure of placing a clamp in one renal artery which reduces the kidney blood flow, promotes the rise of the arterial blood pressure, which can reach very high level. In the animal, along the weeks, functional and anatomical changes are observed, which are similar to the classical signs described in advanced or malignant hypertension in humans. For the first time arterial hypertension was reproduced in experimental animals, similar in many respects to that frequently observed in patients. From the epidemiological and medical point of view arterial hypertension has

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enormous relevance. Approximately 18% of the world human population suffers of high blood pressure, alteration which is one of the main factors of mortality. It is not surprising that since GOLDBLATT experiment, arterial hypertension has been one of the most active fields on biomedical research. The attention focused in the kidney, was very fruitful, and the evidence was given that this organ acts upon blood pressure through a specific substance, *renin* which is released in the blood. Among other effects, general vasoconstriction and the increase in aldosterone secretion are the two main outcomes of renin release. Contraction of the small arterioles and sodium retention induced by aldosterone could account for the enhancement of blood pressure.

RENIN-ANGIOTENSIN SYSTEM

It is easy to conceive that the discovery of this endocrine function of the kidney, contributed considerably to enlarge and to enrich our physiological concept of humoral interactions among the body organs. But in addition, in the last few years it has been established that the kidney produces not only renin, but also other hormones such as erythropoietin and the 1-25 $(OH)_2$ calciferol. (Fig. 1). The vasoconstrictor effect which results when renin is released by the kidneys is not produced by renin itself but by the peptide set free through the hydrolytic action on the substrate, according to the scheme shown in Fig. 2 A. Renin is elaborated in the juxtaglomerular apparatus, secreted in the blood stream, where it reacts with an a_2-globulin, splitting off a decapeptide angiotensin I. This peptide is practically inactive, but is rapidly converted by the action of a proteolytic enzyme (plasma and tissues) in angiotensin II. The latter, an octapeptide is a potent vasoconstrictor, even stronger than cathecolamines (nor-adrenaline). Renin is the only hormone, up to now described, having proteolytic activity. Its effect on angiotensinogen can be reproduced by pepsin.

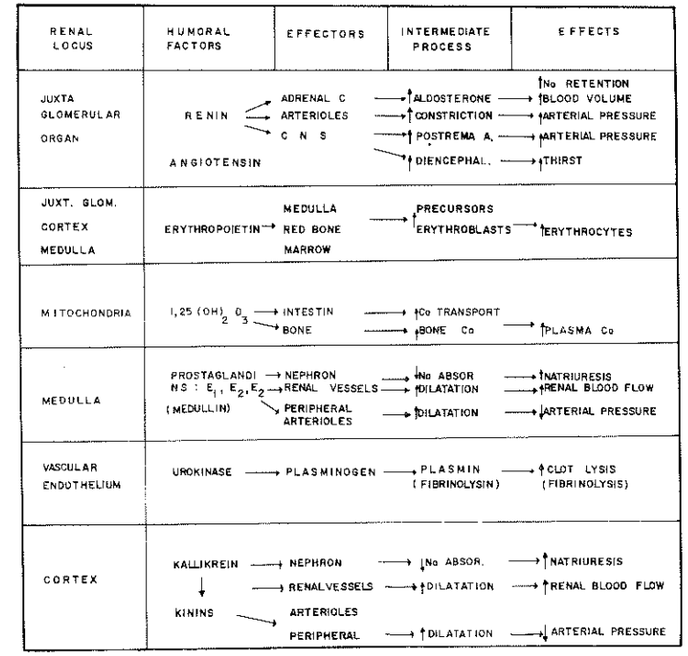

FIG. 1 — Hormonal function of the kidneys.

Experiments carried out, in 1940, allowed us to show that the « digestion » of renin substrate by pepsin, sets free pepsitensin a vasoactive peptide, identical to angiotensin I.

The predominant hemodynamic feature either in experimental or clinical hypertension is an increase in total peripheral resistance due to a vasoconstriction of the arteriolar vessels, effect which is reproduced by angiotensin II infusion in a normal animal. In the hypertensive animal, whose hypertension has been induced by clamping one renal artery, the blood pressure returns rapidly to normal level when the clamp is taken off, indicating that the renal ischemia is causative linked

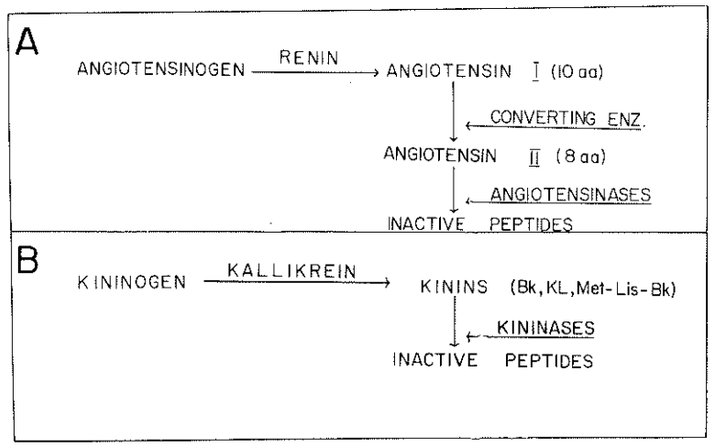

FIG. 2 — In A is shown renin-angiotensin system, and in B., kallikrein-kinin system.

to the elevated blood pressure level. Therefore it is not strange, that the renin theory to account for the mechanism of hypertension, has been extensively explored by many investigators.

HUMORAL VASODEPRESSOR SYSTEM

Although we cannot underscore the role of renin in blood pressure regulation, many observations have showed that either in essential hypertension or in several experimental and genetic hypertension, normal level of renin in the blood is the common occurrence. This fact invalidates the concept that an excess of renin is by itself responsible for the maintenance in these types of hypertension of high blood pressure. However, it has not been ruled out the possibility that the kidney could be "the most important final arbiter" in determining the long basal level of arterial pressure, not only because of renin release,

but also by other complex regulatory mechanism, dealing either with sodium and water excretory action or/and by some other unknown process [17]. In the last decades many efforts have been developed in order to disclose the occurrence in the kidney of some vasodepressor mechanism able to counteract the effects of renin-angiotensin system. Briefly, it has been proposed that kidney can manage blood pressure through two opposite mechanisms: one termed: "prohypertensive", depending on renin-angiotensin system and the other one "antihypertensive", which lowers blood pressure. The blood pressure level might be the result of the interaction between both antagonistic factors. According with this view, it is likely that high blood pressure might occur with normal or sub-normal levels of renin but with an insufficient activity of a vasodepressor system. For some workers the way in which kidney handles electrolytes and water excretion and/or intrarenal circulation can account for the antihypertensive action of this organ [17]. Others have suggested an « incretory » vasodilator function which could antagonize renin. In this latter direction in the last few years an impressive experimental evidence has been growing that the ubiquitous prostaglandins (medullin) and/or a neutral lipid, produced by the kidneys could be effective humoral factors for counteracting vasopressor substances. Furthermore, well documented facts, provide support that the renal kallikrein-kinin system — probably associated with prostaglandins — can be the major mediator for the hypotensive actions of the kidney. This system could be able at least to subserve both sodium-water excretion and intrarenal hymodynamics adjustments. For space reasons, we are not going to discuss prostaglandin [19] and neutral lipid [28] influence in blood pressure regulation. The emphasis of this paper will be directed upon renal kallikrein-kinin system, which has received great concern in our Laboratory since 1968.

RENAL KALLIKREIN-KININ SYSTEM

Kallikreins are defined as endogenous enzymes which specifically liberate a kinin (decapeptide) or bradykinin (nonapeptide). Kallikreins are found in many organs and body fluids but they are different proteins. As a conspicuous exception, renal and urinary kallikrein obtained from the same animal species are very similar or identical. As can be seen in figure 2 B, kallikrein acts upon kininogen, a substrate which is found in the blood plasma (there are at least two substrates, low and high molecular weight kininogens), setting free a peptide kallidin or bradykinin. Both of them have strong vasodilating action and conspicuous diuretic and natriuretic effects when acting upon the kidney, and are finally inactivated by proteases called kininases.

Renin-angiotensin and kallikrein-kinin systems, have striking common features, offering a unique mechanism of vasopeptide generation in circulative blood. Kallikrein, similarly to renin, is a proteolytic enzyme. Both act upon specific plasma substrates, liberating potent vasoactive peptides: the one setting free, vasopressor (angiotensin) and the other vasodepressor (kinins) peptides. In addition to this similar pattern, kininase II is identical to angiotensin I converting enzyme. Angiotensins and kinins have opposite effect upon vessels, whereas kinins acting on the kidney accelerate sodium loss in the urine, contrariwise angiotensin by stimulation of aldosterone secretion, promotes sodium retention. The similarity and peculiarity of both patterns and the antagonistic effects of the end products is keeping with the concept that they are engaged in a common homeostatic regulatory mechanism.

There are experimental data endorsing the idea that kallikrein-kinin system is involved in the mechanism of arterial hypertension.

In our Laboratory conducting studies on vasoactive peptides in the urine of hypertensive rats (one-kidney experimental model) it was disclosed that urinary kallikrein in these animals

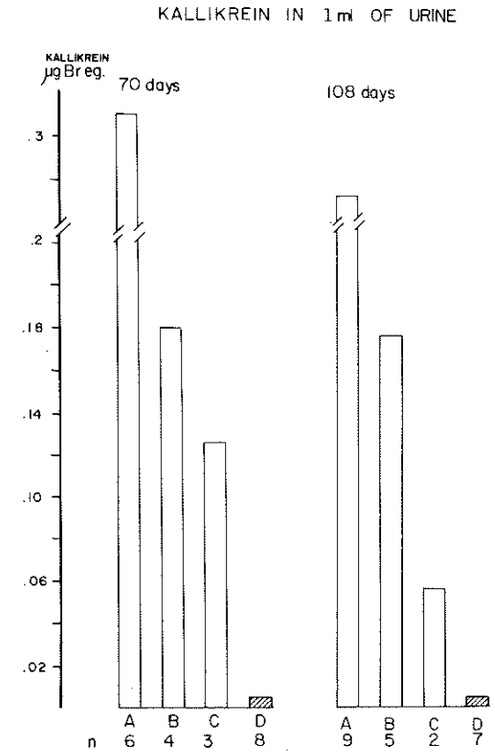

FIG. 3 — Urinary Kallikrein activity in four groups of rats: A, normal rats; B, uninephrectomized; C, fig. in — eight ligature in one kidney and contralateral nephrectomy, with blood pressure below 155 mm Hg; D, rats similarly operated as in C, but with a blood pressure above 155 mm Hg. Kallikrein activity was measured at 70 and 108 days after the operation. (CROXATTO and SAN MARTÍN, 1970).

was significantly reduced as compared to unilateral nephrectomized normotensive rats (Fig. 3). Hypertension was induced in Wistar and Sprague-Dawley strains, either by GROLLMAN procedure or by ligature of both poles in one kidney and contralateral nephrectomy [6, 8]. These results were promptly

confirmed [22] in rats made hypertensive by placing a silver clip in the renal artery of one kidney [21]. Contrariwise in corticoid hypertension (produced by giving high doses of DCA and sodium chloride) higher increase in kallikrein excretion was found. These results showed: a) that a decrease of kallikrein excretion is not a common feature of any type of arterial hypertension and b) that corticoids can influence kallikrein [22]. The diminished kallikrein activity in the urine of the renal hypertensive rats is not due to an abnormal kallikrein or to the occurrence of an inhibitor, but to a lower excretion of the same enzyme produced by normal rats [18]. It is interesting to recall that in experimental hypertension (one-kidney model), where kallikrein excretion is low, renin level in the blood and in the kidney are normal or sub-normal. Different is the situation in the 2-kidneys model where renin is high, but kallikrein in the urine is normal or tends to decrease only after six to seven weeks [2]. Studies carried out in spontaneously hypertensive strain of rats, obtained by selecting inbreeding have shown to PORCELLI and *col.* [32] that kallikrein concentration and excretory rate in the urine were significantly diminished ($p < 0.001$) as compared to those normotensive rats descending from the same ancestors Fig. 4. These results are of particular relevance because by performing kidney-cross transplantation between normotensive and hypertensive rats it was found that the kidney from normotensive rat, normalizes the blood pressure in the hypertensive recipient; and vice-versa, the transplantated hypertensive kidney increases the blood pressure in the normotensive recipient [3]. The latter results demonstrate that the kidney has a primary role in this genetic type of hypertension and advocates for a protagonistic role of renal kallikrein in its genesis.

Several studies in hypertensive patients have shown that in essential hypertension, kallikrein activity in the urine is decreased. ELLIOT and NUZUM [14], publishing a rather ignored paper (1934) were the first to demonstrate — by testing in rabbits — the vasodepressor effect of urine, that kallikrein in

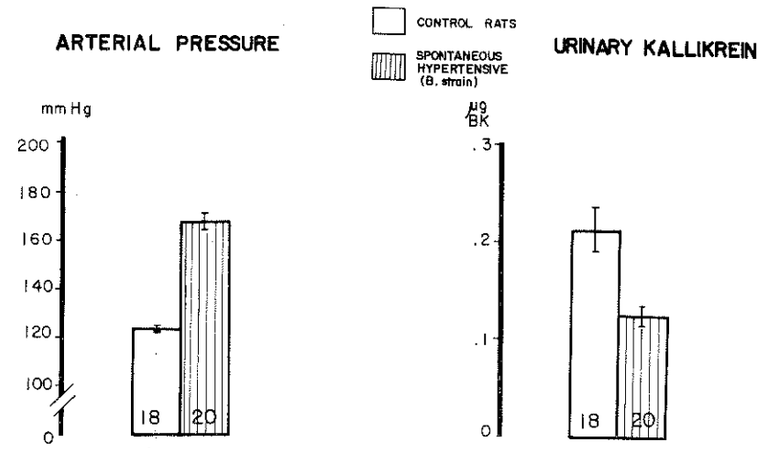

FIG. 4 — Urinary Kallikrein excretion and blood pressure in two groups of rats: normotensive and genetically hypertensive rats. Both groups of rats are descending from the same ancestors. (BIANCHI *et al.*, 1975).

the urine of essential hypertensive patients was significantly less than in normotensive. MARGOLIUS *et al.* [21] using a different method not only confirmed these results but, in addition, they observed very high or normal level of kallikrein excretion in secondary hypertension, pheochromocytome and aldosteronism. GRECO *et al.* [16] also confirmed that in essential hypertension the kallikrein excretory rate is decreased (Fig. 5). More recently, SEINO *et al.* [36] arrived to a similar conclusion. In general, the studies on human beings raise the possibility that the kallikrein system can be of pathogenic significance in human essential hypertension.

For many years the occurrence of kallikrein in the urine represented the renal clearance of a blood kallikrein. However, kallikrein activity has been found in kidneys homogenates and the enzyme appears very similar to urinary kallikrein, at least in the rat [29]. Rat urinary kallikrein has been highly purfied

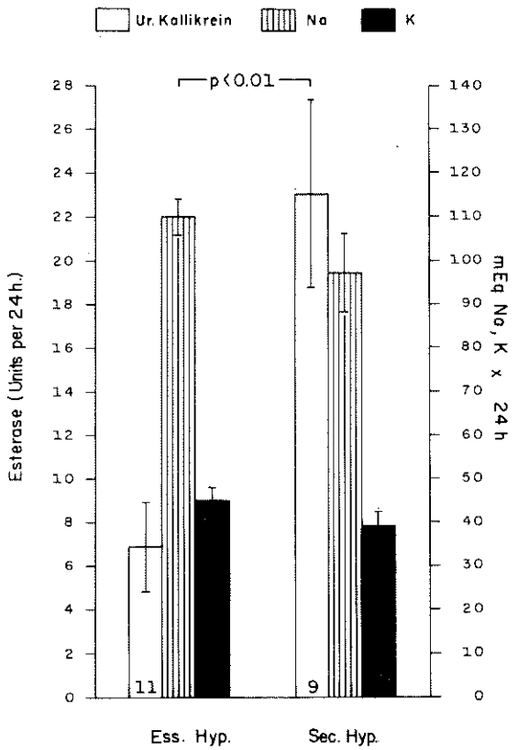

FIG. 5 — Total kallikrein, sodium and potassium excreted in the urine (24 h) in two groups of persons: 11 essential hypertensive and 9 secondary patients. Kallikrein was expressed in esterase activity. Whereas a significant difference between essential and secondary hypertension was demonstrated no difference was found between the latter and normal people. (GRECO *et al.*, 1974).

and its aminoacid composition already given (PORCELLI) confirming its dissimilarity with pancreas kallikrein [31]. The latter has much greater molecular weight and is easily inhibited by sojabean antitrypsin. Accumulated evidence is in favor to the idea that the urinary kallikrein is produced by kidney.

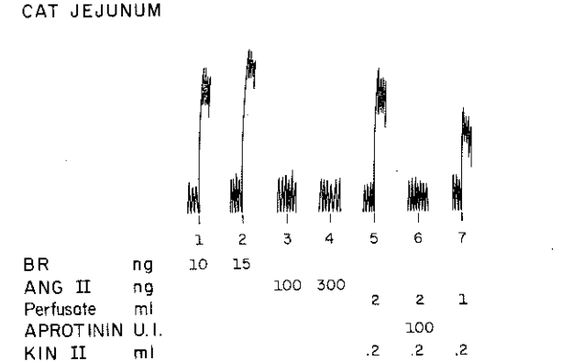

FIG. 6 — Kallikrein activity in the perfusate collected from the vein of an isolated perfused kidney. The activity was tested upon cat jejunum, suspended in Krebs solution in a small bath. In 1 and 2 are introduced 10 and 15 ng bradykinin; in 3 and 4, 100 and 300 ng of angiotensin II, respectively. In 5, is added in the bath a mixture of 2 ml of perfusate with .2 ml of rat kininogen (incubated two min.); 6, the same mixture as in 5, incubated in the presence of the specific inhibitor (aprotinin); and 7, the same mixture as in 5, but using 1 ml of perfusate.

ROBLERO *et al.* [33] in our Laboratory have identified kallikrein in the urine and in the perfusing fluid obtained from a rat isolated kidney, perfused with a solution containing no blood kallikrein precursors. Kallikrein released by the kidney into the perfusate (Fig. 6) is similar to urinary kallikrein in all the parameters investigated. Apparently during perfusion a very active synthesis of kallikrein occurs in the kidney. At the end of the experiment the amount of kallikrein which has disappeared from the organ is much less than the amount found in the perfusate and urine. This difference is even greater when diuresis is stimulated by the addition of furosemide in the perfusing fluid [35]. That the kidney is able to synthetize kallikrein has been demonstrated « in vitro » experiment, incubating kidney slices in the presence of (3_H)-L-leucine. The

newly radioactive synthetized enzyme resembles in many respects the urinary kallikrein [29].

Kallikrein is produced in the renal cortex. It is likely that the most active site where the enzyme is synthetized is the distal tubule close to the macula densa [5]. High kallikrein activity has been found in isolated glomeruli [20]. The location of renal kallikrein in a key zone of the nephron, close to the structure where renin is produced, reinforces the concept that the function of both enzymes are interconnected.

Renal kallikrein

Evidence has been provided that the amount of kallikrein in the kidneys is significantly decreased in hypertensive rats (1 kidney model) as compared to normotensive rats with unilateral nephrectomy [12]. The fall in renal kallikrein is particularly important in rats with high levels of blood pressure (Fig. 7) where the decrease in urinary kallikrein is more striking. In 2 kidney hypertensive rats, no changes in renal kallikrein were observed, in despite of a declining of urinary kallikrein after several weeks of hypertension [2].

Kininogen level in renal hypertension

In our Laboratory evidence has been provided that kininogen on blood undergoes very important changes in different types of renal hypertension in rats [1, 9]. A significant increase in kininogen was observed in hypertensive animals in comparison with sham operated or uninephrectomized control rats (Fig. 8). The increase of kininogen is in keeping with the idea that the substrate level reflects the enzymatic activity, and when the latter is subnormal the substrate rises and vice-versa. Although we have shown [12] that different types of body

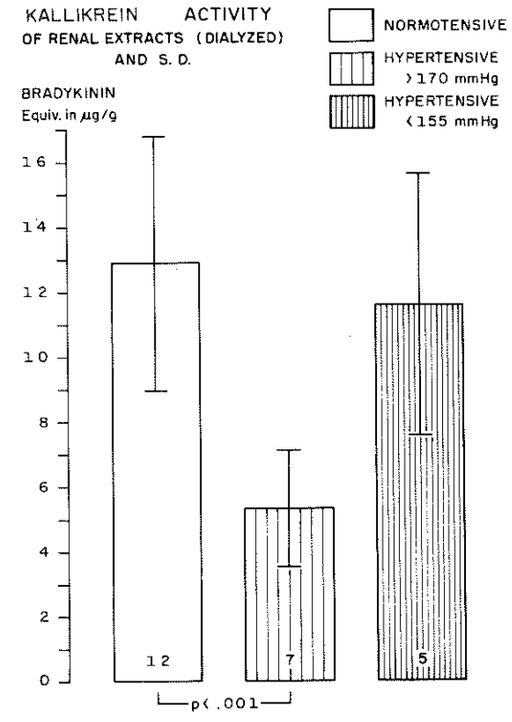

Fig. 7 — Renal Kallikrein, expressed in ng bradykinin equivalent per g of renal tissue, in 12 normotensive uninephrectomized, in 7 severe hypertensive, and 5 moderate hypertensive rats. (One kidney model, poles ligated).

insults such as adrenalectomy, surgical operation, etc., produce a rapid and reversible increase in kininogen of the hypertensive rats, the surgical trauma cannot account for the high kininogen level, since the increase of this substrate instead of declining tends to rise when hypertension reaches its highest levels 6 to 15 weeks after the triggering operation.

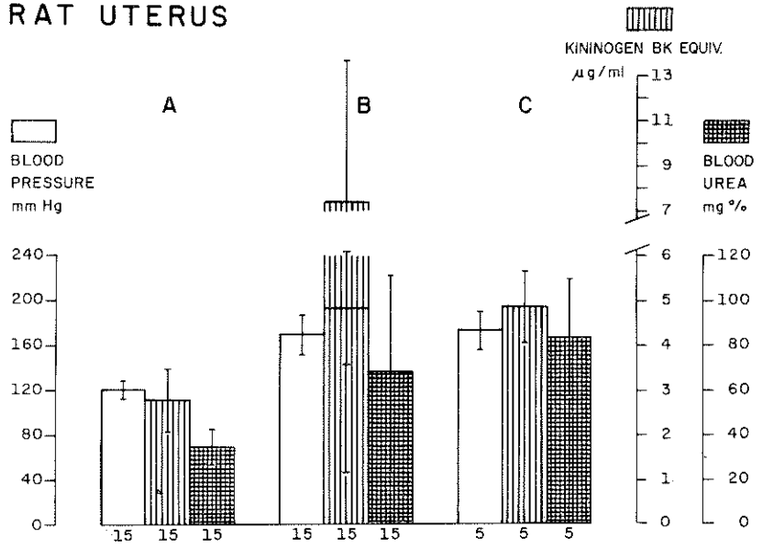

FIG. 8 — Blood Pressure, Plasma Kininogen and Urea, in three groups of rats. A. 15 normal rats. B. 15 hypertensive rats one kidney poles ligature, and C. 5 hypertensive rats (two kidneys Goldblatt model) silver clamps in one renal artery.

PHYSIOLOGICAL AND PHARMACOLOGICAL FACTORS WHICH INDUCE CHANGES IN KALLIKREIN-KININ SYSTEM

Since the discovery of the natriuretic effect of kinins, the prevalent concept has been that kallikrein-kinin system participates in the maintenance of electrolyte and water balance. This assumption is substantiated by the increase of kallikrein excretion which is elicited either by replacing a low sodium diet with a high sodium diet [30] or by acute NaCl overloading [11]. Furthermore kallikrein in the urine and blood kinins in the blood increase when acute loading of NaCl is performed [23].

In the same directions points the inhibitory effect of a

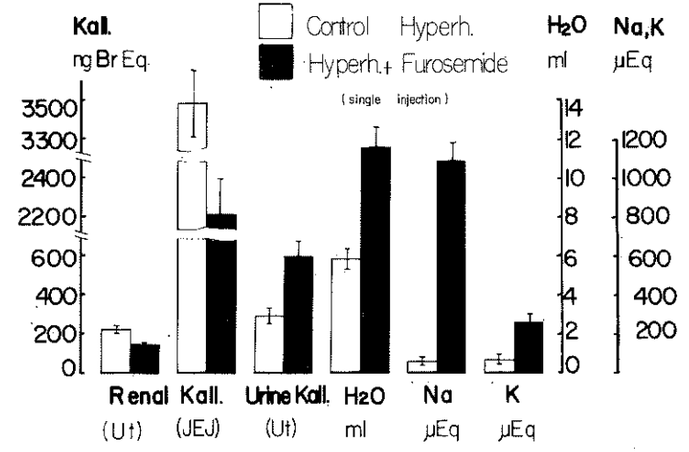

FIG. 9 — Effect of furosemide (10 mg) on urinary kallikrein, sodium, potassium and water, and on renal kallikrein in 6 rats, two hours after the injection. Renal kallikrein was measured using two biological tests: (Ut) direct effect upon rat uterus and (Jej) indirect effect, through kinins formation, using cat jejunum. As control were used rats injected with saline.

specific antibody antagonist on the natriuretic-diuretic actions which occurs when the extracellular space is expanded [24]. According other experiments in rabbits, under low or high sodium diet, kallikrein excretion is positively correlated with urinary volume, but not with sodium excretion [27].

Studies conducted in our Laboratory [11] have shown that either acute water or NaCl overloading, induce very striking changes on kallikrein excretion. Maximal acceleration of kallikrein excretion was observed in normal rats either under hyperhydration or NaCl overloading or administration of diuretics such as furosemide, acetazolamide (Fig. 9). Hypertensive rats, which show in basal conditions low excretory rate of urinary kallikrein, exhibit only moderate increase of this enzyme when diuresis is stimulated giving water. In contrast, in uninephrectomized normotensive rats, hyperhydration is fol-

lowed by a significant increase in kallikrein activity ($p < 0.05$). It is interesting that NaCl loading or furosemide administration which are followed by elevated excretion of kallikrein in the urine reduce considerably kallikrein activity of the kidney (- 50%) (Fig. 9). The amount of kallikrein which appears in the urine in two hours after the diuretic administration is approximately twice as much the amount which disappears from the renal tissue [10, 12]. This result is in keeping with the ROBLERO *et al.* experiments [34] which indicate that kallikrein is rapidly synthetized or activated in the kidneys. In general, these observations are in favor with the concept that the stimuli which increase diuresis and natriuresis promote an activation of the renal kallikrein-kinin system and furthermore that this system is deeply impared in the kidneys of hypertensive rats.

Urinary kallikrein in normal hydrated of hyperhydrated rats, is significantly modified under the effect of natriuretic agents. In additions to the considerable increase of urinary kallikrein produced by furosemide it is necessary to add that other agents such as substance P elevated both kallikrein and Na excretion [26]. In the rat the administration of natriuretic-diuretic hormones, such as oxytocin is followed by a rapid increase in kallikrein excretory rate which can last 120-180 min. after the injection (Fig. 10). A striking exception is the effect of renin. The intraperitoneal injection of renin in a subpressor dose, which elevates Na excretion in the urine, induces a significant decrease in kallikrein excretion, within the 2 hours after the administration. It is not yet clearly stablished the inhibitory mechanisms of renin upon kallikrein excretion; more study will be required in order to clarify whether an impurity in renin preparation can account for this effect.

Hypophysectomy [7] is followed in the rat by a significant fall in urinary kallikrein, particularly several weeks after hypophysis removal. Adrenalectomy in rats, under normal sodium diet is followed by a significant decrease [12]. Although DCA in rats [30] and in dogs [4] elevates kallikrein excretion,

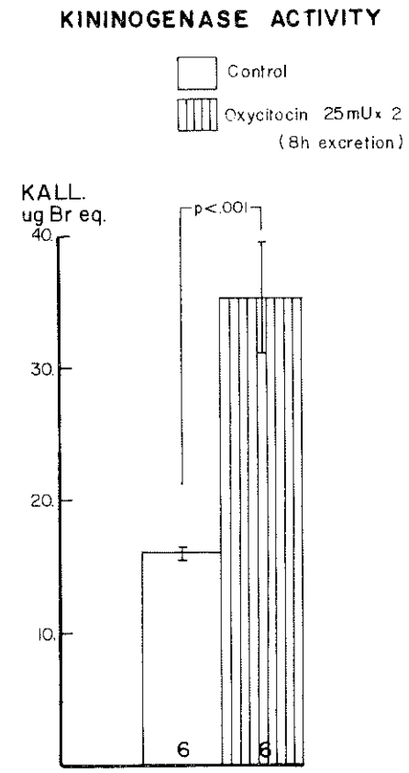

FIG. 10 — Effect of 2 i.p. injections of 25 mU of oxytocin at 2 h. interval on kallikrein activity in the urine excreted by 6 rats during 8 h. after the first injection. As control was used 6 rats injected with saline. The columns correspond to mean values ± s.e. Each sample of urine was incubated for two minutes with rat kininogen II. (CROXATTO *et al.*, 1976).

aldosterone in normal rats has no effects even in large doses (Fig. 11). In adrenalectomized rats, aldosterone (2-150 mcg 100 g. b.w.) enhances kallikrein excretion as compared to control sham operated rats. Hydrocortisone (2 ug per rat)

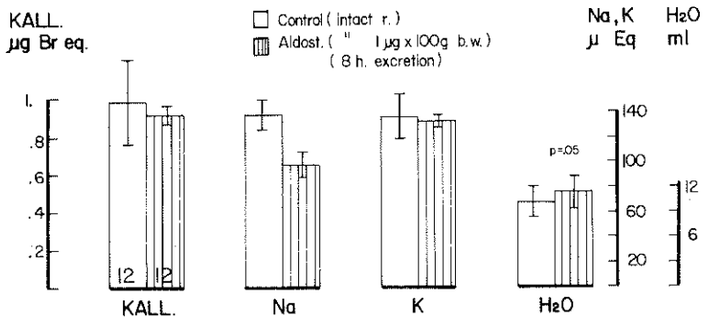

FIG. 11 — Effect of 1 µg of aldosterone per 100 g body weight in normal rats, upon kallikrein, Na and water excretion during 8 h. after i.p. injection.

enhances considerably kallikrein and Na excretion in the rat [12].

The influence of water and electrolytes balance on kallikrein excretion and the changes induced by humoral factors which control fluid and sodium distribution and excretion, indicates that plasma volume can have a decisive role in renal kallikrein regulation.

The decrease in kallikrein excretion in the urine in different types of clinical, spontaneously and experimental hypertension, the significant fall of kidney kallikrein in experimental hypertension characterized by normal or low level of blood renin; the increase in plasma kininogen in experimental hypertensions are indications that renal-kallikrein system is involved in the mechanism of hypertension. Furthermore, the general vasodilator effect, the increase in renal blood flow, the natriuretic-diuretic actions of kinins and the rapid response of renal kallikrein under conditions which require accelerated water and electrolytes excretion, provide further experimental support to the concept that renal kallikrein could be an efficient anti-hypertensive factor. Studies carried out by WONG *et al.* (1975) [37] have demonstrated that in normal subjects angio-

tensin and bradykinin are constantly found in the blood. Both vasoactive peptides exhibit parallel changes when saline is infused or body position is modified. These observations can be logically interpreted on the basis that both antagonistic peptides share blood pressure regulation. But it is still premature to asses whether renal kallikrein can be released to the blood, or stored in kidney, having only the chance to be excreted in the urine. Evidence has been given that renal kallikrein can pass to the interstitial space, since it was found in renal lymph [13]. Furthermore, ROBLERO [33], in aforded mentioned experiments, has proved in isolated perfused kidneys that much more kallikrein passed into the perfusate than in the urine. Against the view that kallikrein has a humoral role controlling blood pressure, are the results obtained by CARRETERO *et al.* [5]. The injection of a kallikrein antibody which prevents the hypotensive effect of an exogenous kallikrein, does not change the blood pressure of a normal rat. He has postulated that renal kallikrein has a regulatory role acting only locally within the kidney.

Extensive research work should be done with kallikrein in connection with renal prostaglandins. Numerous studies have strengthened the view that intrarenal prostaglandins are closely interrelated with kallikrein-kinin system and more recent experiments indicate that prostaglandins modulate the local effects of bradykinin either on renal vessels or in water and electrolytes excretion. Kinins would be able to act on different stages of prostaglandins generation and conversion [25]. These observations contribute, not only to a better understanding of kidney regulation, but also assign more relevance to the renal kallikrein-kinin system as hormone-like factor.

However, it is necessary to recall that kallikrein, in contrast with prostaglandins and bradykinin, can cross safely the lung barrier and appears as one of the most likely candidates to account for the kinins found in the blood even in normal conditions. The occurrence of these vasodilators logically suggest that they can be effective opponents of pressor agents.

The evidence about the occurrence in the blood of a kallikrein of renal origin is still lacking. Complexity derived from the great instability of the pre-kallikrein system in the blood, make the identification of a kallikrein of renal origin a difficult task. But this uncertainty has to be solved in order to answer the question whether renal kallikrein has a regulatory role upon peripheral vessels.

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