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KALLIKREIN - LIKE ENZYME IN PURIFIED RAT RENAL EXTRACTS CONTAINING RENIN

H. R. CROXATTO and G. E. NOE

SVMMARIVM — Renina, quae per consuetas vias ex muris renibus apparatur, habet enzymam, quae ex callicreinarum genere est; haec enzyma efficit ut in sero fiant peptides ad kininarum familiam pertinentes.

The finding of a significant decrease in the excretory rate of urinary kallikrein in renal hypertensive rats [1] prompted us to investigate whether a similar enzyme could occur in the animal's kidneys, under different experimental conditions. Results here reported not only show that a kallikrein-like activity occurs in the kidney, but also that this enzyme is identifiable in a purified fraction that contains almost all the extractable renin activity. Then, the question arises whether renin itself can be a kinin releaser substance. Evidence is given that, besides renin, a kallikrein similar to the urinary enzyme occurs in the renal extracts.

Paper presented by H. E. GIOVANNI BATTISTA MARINI BETTOLO, Pontifical Academician, on April 13th, 1972 during the Plenary Session of the Pontifical Academy of Sciences.

MATERIAL AND METHODS

Renal extracts. The technique used by BRAUN MENÉNDEZ et al [2] to purify renin from kidneys was employed with a single modification: the last step of this procedure — acidification (pH 2.5) with HCl, filtration, and neutralization — was substituted by gel filtration, which does not affect the renin yield. Three different batches of 1.400, 700 and 450 g of kidneys from normal adult rats of both sexes were employed. Before processing, the organs were kept frozen at — 30°C for several months. A fourth batch of 150 g was obtained from anesthetized animals. The kidneys were perfused with a Tyrode solution through a cannula inserted in the abdominal aorta, until no blood was seen in the fluid flowing from the renal vein. After one month storage in a freezer, these kidneys were submitted to the same purification procedure as the non perfused ones. After thawing, but still cold (2°C), the kidneys were ground in a Waring blender and suspended in 3 volumes of 6% NaCl could solution. Toluene was added as a preservative.

The kidney pulp was stirred for several hours and kept overnight in the cold chamber. After skimming the fats off, the extract was filtered and the residue stirred once again, during four hours, with an additional volume of NaCl solution. After filtering, the residue was discarded. The two filtrates were combined, acidified to pH 4 with acetic acid, and centrifuged. The supernatant was filtered by suction through a Büchner funnel. To precipitate the proteins, 39 g ammonium sulphate per 100 ml of the filtrate was added. This precipitate was collected by slow filtration in a Büchner funnel and dissolved in a small volume of distilled water (1/20th of the previous volume). It was dialyzed against tap water for 48 h to get rid of the ammonium sulphate and, finally, dialyzed against 0.9% NaCl plus. 003 M ethylene diamine tetracetate

(EDTA) for 48 h, in the cold room. The maroon solution obtained contains only traces of angiotensinase.

Gel filtration

Further purification was achieved by gel filtration in a column (50 × 25 cm) of Sephadex G — 100 embedded in ammonium acetate, .05M, pH 7.8. An aliquot of the extract, corresponding to 100 g of kidneys, was introduced in the column and the effluent solution was collected in separate volumes of 8 ml. Kallikrein and renin activities were determined in each fraction by means of the biological assays described below. The residue was dissolved in .5 M ammonium acetate and filtered again in the same Sephadex G—100 column. The effluent solutions corresponding to the peak of the activities ere dialyzed against distilled water and concentrated to small volume at low pressure.

Renin and kallikrein determinations

Besides testing their direct effect on rat blood pressure, the presence of the enzymes was detected indirectly through the formation of either angiotensin or kinins, or both, when interacting with the adequate substrates. In the case of renin, an incubation assay with angiotensinogen obtained from rat serum, according to 6 first steps of SKEGGS method [3], was adopted. Dog serum, previously incubated at 37°C for 96 h in sterilized glass flasks, was used as the substrate to detect kininogenase activity. Dog serum was found to be a suitable substrate to explore the kininogenase activity of rat urinary kallikrein [4]. Since rat urinary kallikrein in very small doses produces contractions in the rat uterus, even in the absence of the substrate, most of our study was devoted to investigate kallikrein activity through the direct oxytocic ef-

fect of renal extracts. A correlation was found between uterine contraction and kallikrein contents of the extracts.

To get further evidence on the occurrence of kallikrein-like activity in the renal extracts, the following experiments were undertaken:

1) Inhibitory effect of Di-isopropyl fluor phosphate (DFP) on the pharamacological features of the extracts. DFP blocks the kininogenase activity of kallikreins obtained from different sources [5, 6] including purified rat urinary kallikrein [4].

2) Inhibitory effect of apronitin (Trasylol) upon kinin-forming ability.

3) Identification of the peptides formed in the enzyme-substrates interaction, by using either pepsin or carboxypeptidase B for a differential peptide destruction: the first enzyme inactivates angiotensin type peptides without damaging kinins; in contrast, the latter destroys bradykinin-like peptides without inactivating angiotensins.

4) Effect of renal extract upon isolated cat jejunum in the presence of either dog serum or angiotensinogen as the substrate. This preparation responds specifically to bradykinin or kallidin but not to angiotensin [7]. Furthermore, the effect of apronitin (500-5000 U) and DFP inhibitors of kallikrein was also investigated in this preparation.

Biological assays

Arterial blood pressure.

Male Sprague-Dowley rats (180-200 g), normal or nephrectomized 14-16 hours before, were anesthetized with diallyl barbiturate urethane solutions (.2 g per 100 g b.w.) [1]. Intravenous infusions were given through a polyethylene catheter introduced in a femoral vein. Blood pressure changes

were registered with a Grass Polygraph (model 500) and a Statham transducer connected with a carotid artery. The direct effects of renal extracts, both treated and non-treated with DFP, were studied: *a*) before and after ganglionic blockade, by administering pentolinium tartrate (.5 mg per 100 g g.w.) through peritoneal or intramuscular route; and *b*) before and after i.v. injection of apronitin.

Blood pressure changes induced by the extracts were compared to those produced by standard solutions of angiotensin II and bradykinin. The effects of pepsin upon peptides formed by the action of renal extracts upon angiotensinogen and dog serum were also studied in this preparation. At the end of the incubation period (2-15 min), the mixture of enzymes and substrates was acidified to pH 5.2, heated for 10 min in a boiling water bath and centrifuged. The clear supernatant solution was then neutralized and injected.

Effect upon isolated rat uterus

The isolated rat uterus was found a suitable preparation to investigate the occurrence of renin and kallikrein activities in the extracts. These activities were identified through the formation of either angiotensins or kinins. Both peptides, that have an oxytocic effect on the rat uterus, can be released in the bath by introducing sub-threshold doses of the rat renal extract and one of the substrates, i.e., angiotensinogen or dog serum. The contractions of an isolated rat uterus horn, immersed in 25 ml oxygenated Tyrode solution [4] or suspended in air according to the technique of superfusion [8], were recorded by means of a strain gage connected to a Grass Polygraph. The direct effect of the renal extracts and of the active substances released by the enzymes from the substrates were tested and evaluated by using standard solutions of angiotensin and bradykinin as controls. In this bioassay, the

effect on uterus contraction of different kallikrein inhibitors, namely: apronitin, carboxypeptidase B and DFP, was also studied.

Isolated cat jejunum

Strips of cat jejunum immersed in a Krebs solution, according to FERREIRA adn VANE [7], were used. Experiments to test the effect of renal extracts, substrates and inhibitors, were carried out following the same pettern as described for the experiments on rat uterus.

Effect of pepsin

The incubation of small amounts of renal extract with the substrates gives rise to peptides that display an oxytocic effect. The effect of pepsin was investigated in order to clarify whether angiotensin or kinins, or both, were involved in this pharmacological action. At the end of the incubation period (15 min) when the action upon the uterus is maximal the extract and substrate mixtures were acidified to pH 5.2 with HCl, and then boiled for 5 min. The proteins were precipitated and centrifuged, the supernatant acidified to pH 2.5, and 2 mg of pepsin was added. After four hours incubation at room temperature, followed by neutralization, the biological assays were performed. A similar mixture of enzyme and substrate, submitted to the same procedure but without pepsin, was used as control. Both final solutions were assayed on rat blood pressure, isolated rat uterus, and cat isolated jejunum.

Effect of carboxypeptidase B

This enzyme, which inactivates kinin very rapidly [9], also proved to be an inhibitor of the oxytocic effect of pure

rat urinary kallikrein [4]. It was used in two main experimental situations: A) Prior to the introduction of renal extract, doses of 50-500 mcg were added to the fluid bathing the uterus, to test its inhibitory effect upon the direct oxytocic activity of the extract. The magnitude of this effect was evaluated by introducing different doses of bradykinin 2-4 min after the addition of carboxypeptidase B; B) Furthermore, the effect of this enzyme was assayed on incubated mixtures of renal extract, dog serum and sodium phosphate, prepared in small tubes. After incubating for 2-10 min at room temperature, the mixture was tested on isolated organ preparations.

Effect of apronitin

This substance was used in all the bioassays, to investigate its action upon the extracts: *a*) their direct oxytocic effect; *b*) the kinin-releasing ability; *c*) the blood pressure changes. Apronitin (500-1000U) was added to the solution bathing the isolated organs. Similar amounts were added to the mixtures of extracts and substrates. In blood pressure assays, 5000-20000 U were injected i.v.

Effect of DFP

A solution of DFP .02M in isopropyl alcohol, stored at — 30°C, was used. It was added to the extract in order to obtain a final concentration of .001 M — .0005 M. After standing at room temperature for 12-16 hours, the extract was assayed to study the changes in: *a*) Kininogenase activity, and *b*) angiotensin-generating activity. In the latter case angiotensinogen was used as the substrate. Results were compared to those of control renal extracts which were treated only with the solvent (isopropyl alcohol). Moreover, these extracts were directly introduced into the rat circulation to explore their effects on blood pressure.

Protein determination

The total protein contents of renal extracts was determined by LOWRY et al's method [10].

MATERIALS

Pepsin 3 x crystallized and carboxypeptidase B were purchased from Nutritional Biochemicals Corp., Cleveland, Ohio.

Apronitin (Trasylol) ampoules 20,000 U ml, was provided by Bayer.

Pure DFP was generously supplied by Dr. GORDON VAN ARMAN.

Angiotensin-II-valyl-5 was provided by CIBA.

Bradykinin was a gift of Prof. J. STEWART.

RESULTS

The purification procedure here described allows to obtain an extract that contains not only most of the extractable renin of kidney but also shows that an important amount of kallikrein-like enzyme is present in this organ.

Assays on rat blood pressure

In pentolinium-treated rats, the intravenous injection of the purified extracts elicits the typical pressor-response curve of renin (Fig. 1A). Only rarely a transient hypotensive reaction, before the prolonged rise of blood pressure, can be observed. The hypotensive reaction can be abolished if a high dose of apronitin (10.000 U) is administered immediately before the extract injection. In rats with normal blood pres-

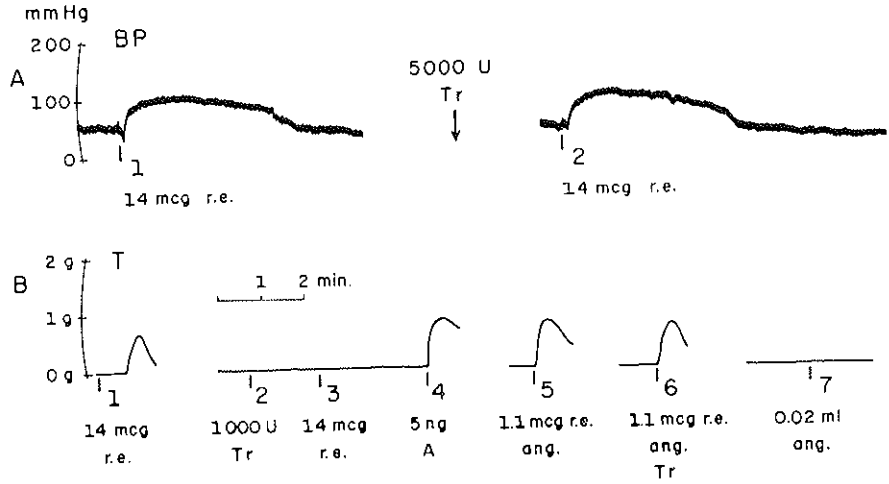

FIG. 1 — A. Blood pressure changes produced by the intravenous injections of a renal extract in ananesthetized rat under pentolinium tartrate In 1 and 2, .05 ml of the renal extract (r.e.) containing 14 mcg of total protein. Inmediately before 2, 5000 U of apronitin (Tr) were given i.v. B. Oxytocic effect upon isolated rat uterus. T indicates muscular tension in g. In 1, 14 mcg of the same extract used in A experiment, is introduced in the bath. After washing, in 2,3 and 4 are successively introduced: 1000 U apronitin (Tr) 14 mcg r.e. and 5 ng angiotensin (A). In 5, a mixture, incubated for 15 min. (pH 7.8) containing subthreshold dose (1.1 mcg) of r.e. plus .04 ml of angiotensinogen is introduced; in 6, the same amount of a similar mixture but containing 1.000 U of apronitin; in 7, .04 ml of angiotensinogen are introduced. In this and in the following figures the amounts of r.e. used are indicated by their respective total protein contents.

sure non treated with pentolinium, the extract usualy produces a biphasic reaction. A moderate and brief drop of the pressor curve is followed by a more intense and sustained rise of blood pressure. When the extract is previously incubated with DFP, the hypotensive reaction is suppressed or consistently diminished (Fig. 2). The incubation of the extract with angiotensinogen gives rise to a strong vasopressor substance (Fig. 3), similar to angiotensin, which is completely destroyed by pepsin. In contrast, the injection of the deproteinized incubated

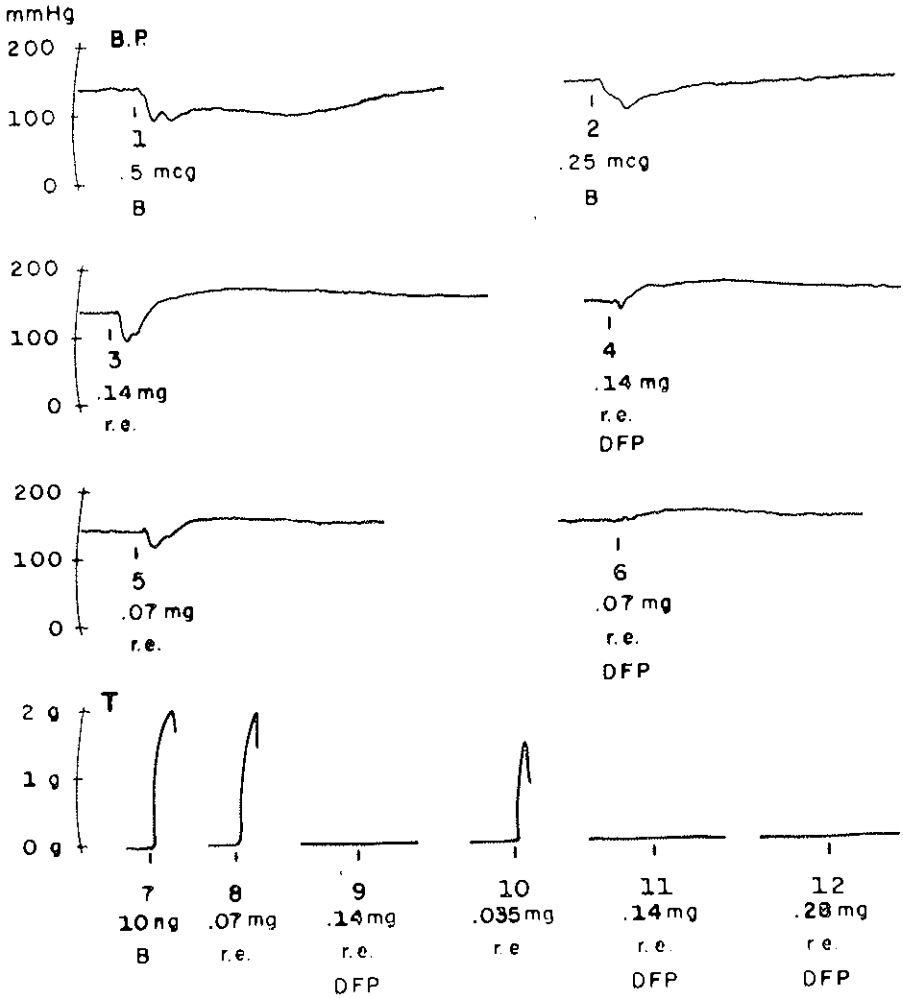

FIG. 2 — In the upper part: Blood pressure changes in a rat with no pentolinium and below, isolated rat uterus contractions. Injection in: 1 and 2, .5 mcg and .25 mcg of bradykinin (B) respectively. In 3 and 5, .14 and 0.07 mg of r.e.; in 4 and 6 the same amounts of r.e. previously in contact with DFP for 16 hours. In the bath are introduced in: 7, 10 ng of bradykinin; in 8, .07 mg of r.e.; in 9, .14 mg of r.e.; in 11 and 12, .14 mg and .28 mg of r.e. treated with DFP, respectively.

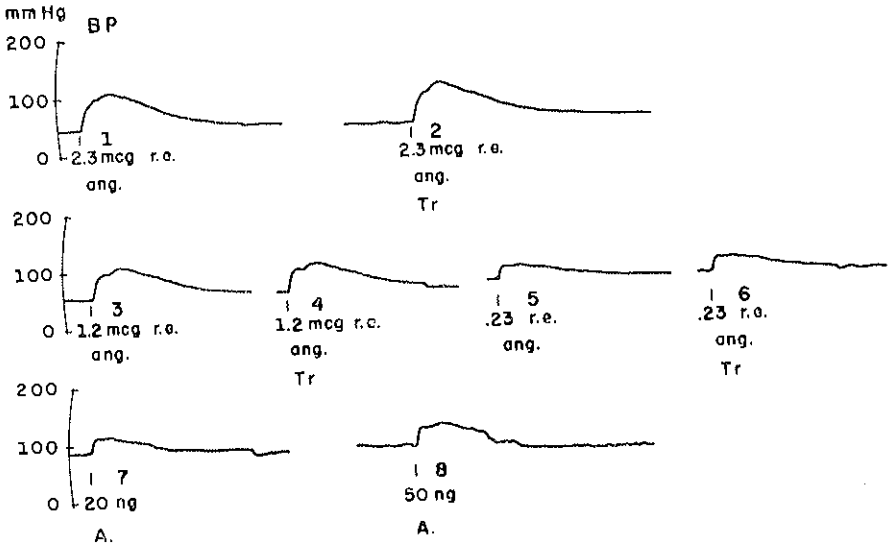

FIG. 3 — Blood pressure changes in rat under pentolinium blockade. The following injections were made: in 1,3 and 5, incubated mixtures of r.e. and angiotensinogen (4 hr) after deproteinization (boiling 10 m, pH 5,2) and neutralization. For 2.3 mcg of r.e., .08 ml of angiotensinogen was used. In 2,4 and 6 the same mixtures incubated in the presence of 500 U of apronitin (Tr). In 7 and 8, 20 ng and 50 ng of angiotensin (A).

mixtures of renal extracts and dog serum produces a moderate rise or a biphasic change in blood pressure.

When the mixtures are submitted to pepsin hydrolysis only a depressor effect is obtained. After ganglionic blockade, the same doses of the samples non treated with pepsin induce a greater increase in blood pressure; but the sample hydrolized with pepsin brings about a less conspicuous drop of the pressure level (Fig. 4). Similar responses to pepsin treated extracts under ganglionic blockade are observed when moderate doses of bradykinin are injected [11].

Apronitin inhibits the formation of depressor substances when the renal extracts are incubated with dog serum. However, when the renal extract is incubated with angiotensinogen,

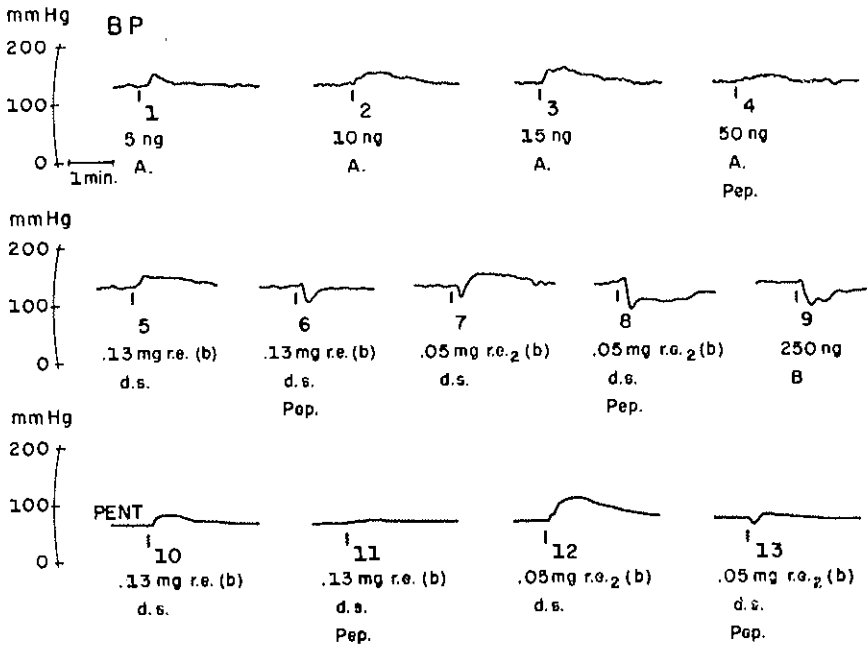

FIG. 4 — Blood pressure changes in rat, before and after pentolinium (PENT) blockade. The following injections were made: in 1, 2 and 3, 5 ng, 10 ng and 15 ng of angiotensin; in 4, 50 ng of angiotensin incubated with pepsin; in 5 and 7, incubated mixtures of r.e. ad dog serum (d.s.) for 15 min., which were deproteinized at the end of the incubation period. In 6 and 8, similar mixtures treated with pepsin after deproteinization; in 9, 250 ng of bradykinin. After PENT, in 10 and 12, the same mixtures as in 5 and 7; and in 11 and 13, the mixture as in 6 and 8.

apronitin does not hinder the formation of angiotensin-like peptides. As it can be seen in figure 3, the injections of deproteinized mixture previously incubated, either with or without apronitin, produce the same rise in blood pressure.

Renin activity of the renal extracts

An amount of renal extract containing 12-15 mcg of protein elicits an increase of 50 mm Hg in blood pressure in a 180-200, rat under ganglionic blockade.

Assays on the rat uterus

All the purified renal extracts, before and after gel filtration, including the one obtained from kidneys in which blood had been excluded, contain a non dialyzable principle that produces contractions on the rat uterus. The oxytocic effect is dose-dependent, but a contraction corresponding to that of 10 ng bradkinin can be obtained with a dose of the most pure extract containing .07 mcg of protein (Fig. 2).

The oxytocic effect can be attributed to the kallikrein present in the extract, since similar effects have been obtained with low doses of the purest rat urinary kallikrein [4]. The following results give good evidence that a kallikrein-like enzyme is responsible for this effect. Apronitin inhibits the direct oxytocic effect (Fig. 5) and it interferes as well with the formation of oxytocic substances when introduced in the

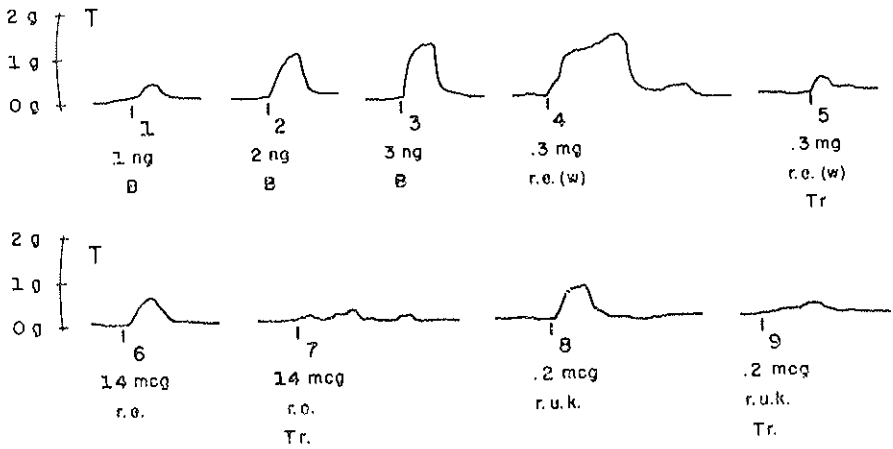

FIG. 5 — Effect on isolated rat uterus superfused with Tyrode solution. Are introduced in: 1,2 and 3, 1 ng, 2 ng and 3 ng of bradykinin. In 4, .3 mg of r.e.; in 5, .3 mg of r.e. mixed with 500 U of apronitin (Tr); in 6, 14 mcg of r.e.; in 7, 14 mcg of r.e., mixed with 500 U apronitin; in 8, .2 mcg of rat urinary kallikrein (r.u.k.) and in 9, .2 mcg r.u.k. mixed with 500 U apronitin.

mixtures that contain the extract plus dog serum (Fig. 6). The action of apronitin is as effective as that observed when the inhibitor is introduced in the mixture of urinary kallikrein plus dog serum. However, apronitin does not inhibit angiotensin-like peptides when introduced into renal extract- angiotensinogen mixtures (Fig. 1-B). DFP completely abolishes the oxytocic activity. The extract pre-treated with DFP, entirely loses its ability to originate kinins from dog serum substrate (Fig. 7). Under the same experimental conditions, the extract keeps all its capacity to form angiotensin, when the DFP-treated extract is incubated with angiotensinogen. This is in keeping with the results obtained on blood pressure with renal extracts submitted to DFP action (Fig. 2). The

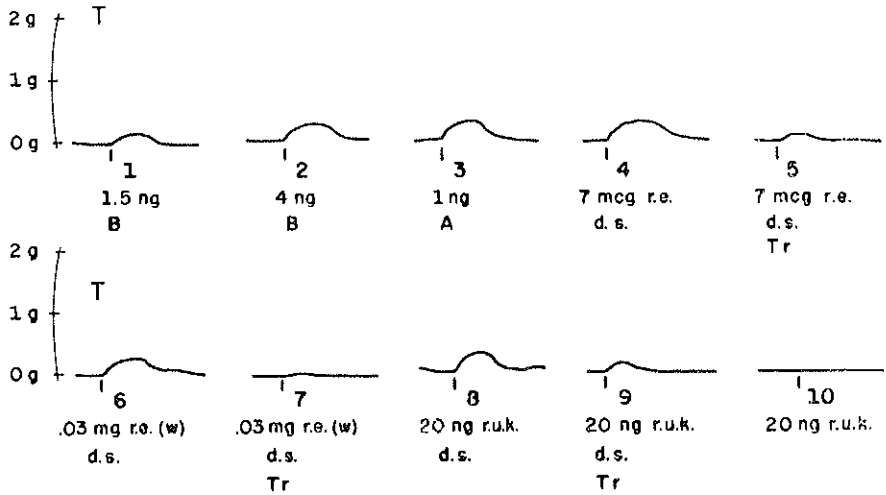

FIG. 6 — Effect on isolated rat uterus superfused with Tyrode solution. Introduced in: 1 and 2, 1.5 ng, and 4 ng of bradykinin (B); in 3, 1 ng angiotensin, in 4, 7 mcg of r.e. incubated with .01 ml of dog serum; in 5, the same mixture as in 4 but incubated with 500 U of apromitin; in 6, .03 mg of r.e. incubated with dog serum; in 7, the same as in 6, incubated with 500 U of apronitin; in 8, 20 ng of rat urinary kallikrein incubated with dog serum; in 9, the same misture as in 8, incubated with 500 U of apronitin; and in 10, 20 ng of rat urinary kallikrein.

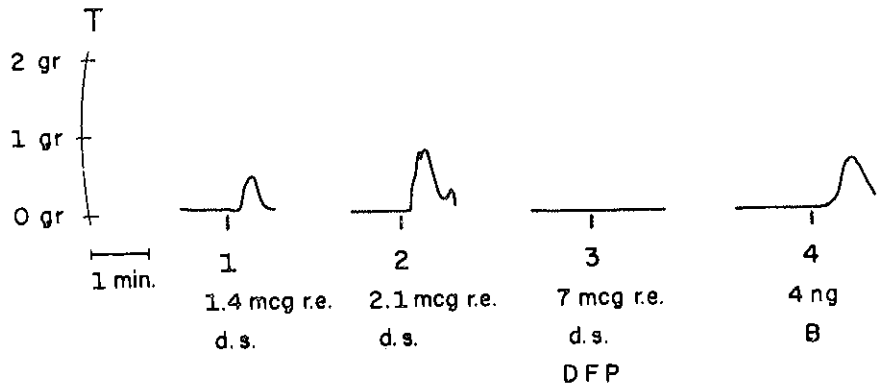

FIG. 7 — Effect of r.e. on isolated rat uterus superfused with Tyrode. Are introduced in: 1 and 2, 1.4. mcg and 2.1. mcg of r.e. incubated for 2 minutes with .005 ml dog serum respectively; in 3, 7 mcg of r.e. treated with DFP for 16 hours incubated 2 minutes with .025 ml dog serum; and in 4, 4 ng bradykinin.

oxytocic effect is, in this case, obviously explained by the release of an angiotensin-like peptide during this reaction. The peptide is completely inactivated by pepsin and resists the action of carzoxypeptidase B.

The latter enzyme, in doses of .05 mg, abolishes the direct oxytocic effect of a threshold amount of the extract upon the uterine muscle. In addition, it may completely abolish the contractile effect of a mixture of a sub-threshold dose of the extract and dog serum interacting in the bath (Fig. 8). Contrariwise, the oxytocic peptides liberated during the reaction are resistant to pepsin hydrolysis (Fig. 9).

Cat jejunum

No direct effect was observed on this preparation when the extract was introduced in the bath. The result is different if a small amount of dog serum (.05 ml) containing

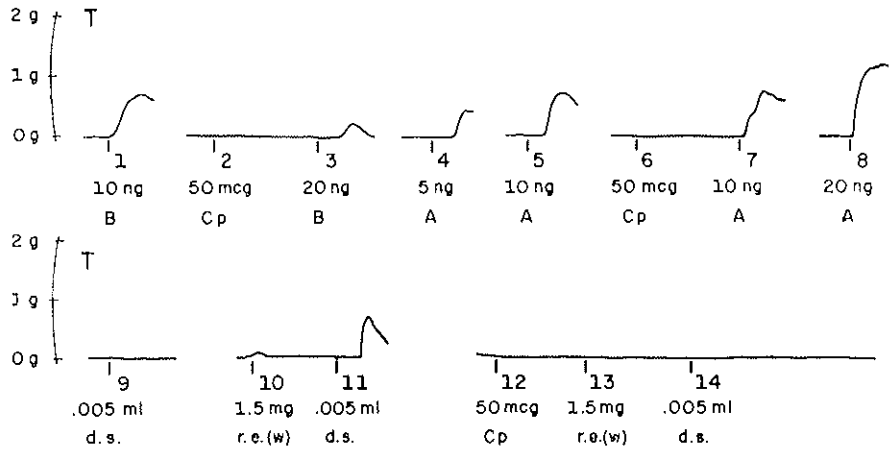

FIG. 8 — Effect on isolated rat uterus. Studies on carboxypeptidase B action. Are introduced in: 1, 10 ng of bradykinin; in 2, 50 mcg carboxypeptidase B (Cp); in 3, 20 ng of bradykinin; in 4 and 5, 5 ng and 10 ng angiotensin (A); in 6, 50 mcg Cp. and in 7, 10 ng angiotensin; in 8, 20 ng angiotensin; in 9, .005 ml dog serum (d.s.); in 10, 1.5. mg r.e.; in 11, .005 ml. d.s.; in 12, 5 mcg Cp; and in 13, 1.5 mg r.e. and in 14, .005 ml d.s.

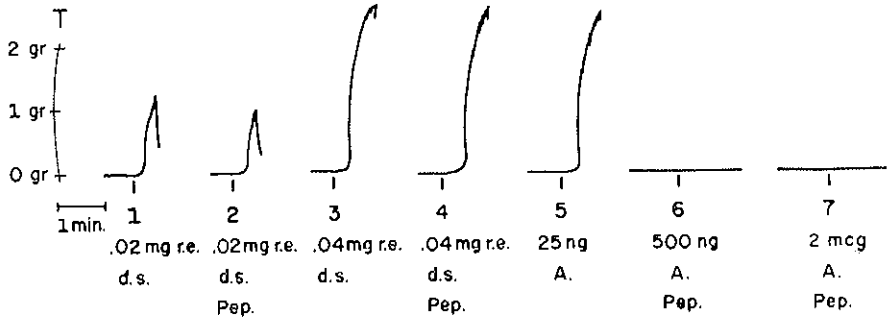

FIG. 9 — Effect on isolated rat uterus. Studies on pepsin (pep) action. Are introduced in: 1, .02 mg of r.e. incubated with .05 ml dog serum, 2 minutes; before introducing in the bath, the mixture was acidified, deproteinized, centrifuged and neutralized; in 2, the same amount of the similar mixture hydrolyzed with pepsin (4 hr.); in 3, the same as in 1, using twice as much as in 1; in 4,the same as in 3, incubating with pepsin; in 5, 25 ng of angiotensin; in 6, 500 ng of angiotensin incubated with pepsin; and in 7, 2 mcg of angiotensin incubated with pepsin.

kininogen is added after the extract, or viceversa, or both at the same time. Then, an intensive and progressive contraction occurs. This effect, which cannot be due to angiotensin, is easily blocked by carboxypeptidase B, or by apronitin or by DFP (Fig. 10).

Discussion

The experiments give evidence that the rat kidney extract contains a kininogenase. The occurrence of this enzyme has been described by different investigators, [12, 13]. More recently, Nustad [14], has demonstrated that the kallikrein

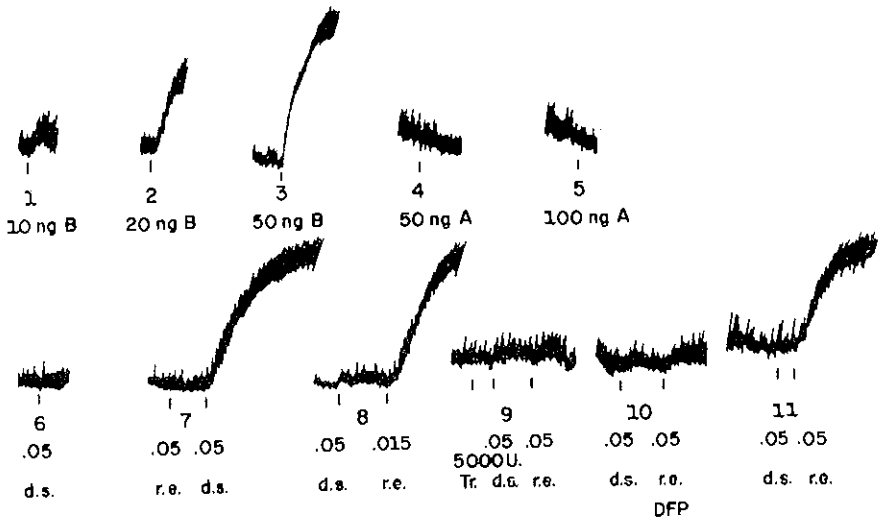

Fig. 10 — Effect upon isolated cat jejunum. Studies on apronitin (Tr) and DFP action. Are introduced in: 1,2 and 3, 10 ng, 20 ng and 50 ng of bradykinin; in 4 and 5, 50 ng and 100 ng of angiotensin respectively; in 6, .05 ml of dog serum (d.s.); in 7, .05 ml of renal extract (r.e.) and .05 ml of dog serum; in 8, .05 ml of d.s. and .015 ml r.e.; in 9,5000 U of apronitin, .05 d.s. and .05 ml r.e.; in 10, .05 ml d.s. and .05 ml r.e. previously treated with DFP; and in 11, .05 d.s. and .05 r.e. .05 ml of the r.e. contained 55 mcg of protein.

activity in the rat kidney is similar to urinary kallikrein in regard to the pH optimum, to the effect of some inhibitors and to the ability to form kinins, which is in agreement with our finding. Although NUSTAD [14] gives no data on renin activity, his results show that the kallikrein activity is mainly located in the kidney cortex. It is well known that renin is also localized in that zone. It must be pointed out that the dominant pharmacological feature of the extracts is their potent renin-type pressor effect on the rat. This property is to be expected since the purification procedure here employed is similar to that proposed by BRAÚN MENÉNDEZ et al [2] to obtain purified renin from kidney. The kallikrein reaction of the blood pressure is practically anulled, particularly in hypotensive pentolinium-treated rats. This result is not strange, since in hypotensive rats, bradykinin induces a biphasic or a predominant hypertensive reaction, instead of the typical drop on the pressure level [11]. Kinetics studies on peptide formation through the interaction of enzymes and rat plasma substrates can provide a quantitative correlation with blood pressure changes. It is likely that a competitive effect upon the vessels, between angiotensin and the bradykinin formed by the simultaneous action of renin and kallikrein, should resolve in favour of the angiotensin effect. Among other reasons, the vasoconstrictor peptide has a longer life than bradykinin in the blood stream.

The complete inhibitory effect of DFP on kininogenase activity with no reduction of renin activity precludes Ng's assumption [15] that renin itself would be responsible for the kinin formation. Although they are of a similar molecular size, since both activities appear in the same peak after gel filtration, it is likely that each of these enzymatic activities should belong to two separate molecular entities. Further progress in the purification procedure should solve the question of whether renin shares the kinin forming ability of kininogenases.

It must be mentioned that purified renin extracts from kidneys of other animal species, prepared according to other methods, [16, 17] also exhibit the ability to release kinins in the presence of blood substrates [18]. Since these extracts have been widely used to explore the role of renin under a great variety of conditions, it is important to consider that kallikrein would have been participating, or interfering, in some of the typical reactions induced by renin, if no specific kallikrein inhibitors had been used. After gel filtration, the kallikrein activity was not significantly different in the extracts from perfused and non perfused kidneys. It can be assumed that the kinin forming activity is not due to one of the plasma kallikreins that would have been incorporated by blood into the extracts.

Our results that point to the kidneys as the possible origin of urinary kallikrein, are in keeping with NUSTAD'S findings [14]. Whatever conclusion may be drawn, either that kidneys produce kallikrein or that their kinin forming activity represents a transient stage between blood and urine enzyme, the question of its physiological meaning still stands. There is ample room for speculation, but we can assume that kidneys could participate in homeostasis, not only with renin, but also with kallikrein release.

* * *

Abstract

Ral renal extracts, prepared according to BRAUN MENENDEZ'S technique to purify renin [2], extensively used to explore the role of renin, contain a kallikrein-like enzyme. After filtration in Sephadex G-100, both renin and kininogenase activities appear in the same peak. The purified fraction induces a renin-type reaction on blood pressure; when acting upon angiotensinogen it gives rise to angiotensins

whereas when acting on kininogen (dog serum) it releases kinin-like peptides. The formation of kinins is inhibited by kininogenase inhibitors, such as DFP and apronitin, but the angiotensin liberation is not affected. These results preclude that renin is responsible for the generation of kinins. The presence of kallikrein accounts for both the direct oxytocic effect upon rat uterus and the biphasic effect on the blood pressure of normotensive rats. The kinin forming activity of the renal extract was demonstrated by using bioassays (isolated rat uterus, cat jejunum, rat blood pressure), enzyme inhibitors, and the differential effect of pepsin and carboxypeptidase B on formed peptides.

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Key words

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