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Golgi Complex Function in the Excretion of Renal Kallikrein (41502)

ENRIQUE BRANDAN,[1] MIREYA ROJAS, NORA LOYARTE, AND FERNANDO ZAMBRANO[2]

Departamento de Biología, Facultad de Ciencias Basicas y Farmacéuticas, Universidad de Chile, Casilla 653, Santiago, Chile

Abstract. A Golgi complex rich-fraction containing both *N*-acetylglucosamine galactosyltransferase and kallikrein activity has been isolated from kidney of rats previously treated with colchicine, a secretion inhibitor, followed by the administration of high-sodium solutions, to stimulate biosynthesis or activation of renal kallikrein. After the treatment, *N*-acetylglucosamine galactosyltransferase and kallikrein activities were increased in the Golgi complex, about 18- and 24-fold, respectively, as compared to the homogenate. Low kallikrein activity was found in the crude light mitochondrial fraction from treated animals, whereas a high level of activity was observed in the microsomal fraction. The inverse situation was found in rats treated only with high-sodium solution. Results suggest that kallikrein is probably transported by microsomal elements, particularly by the Golgi complex. Furthermore, the evidence seems to indicate that the kallikrein activity reported in the plasma membrane and/or in the lysosomal fraction is due to kallikrein secretion, in the form of intact granules, which have sedimented with these two fractions.

Glandular kallikreins are a group of related kinin-forming enzymes present at least in the major exocrine glands and in the kidney. Previous studies on the role of the kallikrein–kinin system in the kidney identify kinins as potent vasodilators which cause natriuresis and diuresis when injected into the renal artery (1).

It is known that urinary kallikrein is a glycoproteic enzyme and that the renal kallikrein is secreted into the urine at the level of the distal tubule (2, 3). Moreover, both the renal and urinary enzymes are immunologically and electrophoretically similar (4). Renal kallikrein is also a glycoprotein and it is found in cells in the form of dense granules, which are transported to the extracellular space by some undefined mechanism by microsomal elements (5).

The localization of renal kallikrein is subject of dispute. Some authors refer it to the lysosomal fraction (6, 7), others to the microsomal fraction (8, 9), and still others to the plasma membrane fraction of kidney homogenates (10). In an attempt to clarify the apparently conflicting findings, we have isolated and characterized different subcellular fractions from rat kidney, using both differential and density gradient centrifugation techniques. Our approach was to increase the intracellular amount of kallikrein by a combination of two treatments: salt loading, that we showed increases the activation or the biosynthesis of this enzyme (11), and colchicine treatment, that inhibits the liver secretion by a reduction of the microtubule content of the cells (12, 13).

Our results indicate that Golgi complex participates in the intracellular transport and probably in the biosynthesis and/or activation of renal kallikrein.

Material and Methods. Female Sprague–Dawley rats, 200 to 300 g, fed *ad libitum* were used. Animals were treated with colchicine and then loaded with a 0.342 *M* saline solution at 5% body weight by gavage (14). The unanaesthetized animals were decapitated 2, 4, 8, and 10 min after treatment, then exsanguinated, and their kidneys quickly removed, decapsulated,

[1] Present address: Departamento de Biología Celular, Universidad Católica de Chile, Casilla 114-D, Santiago, Chile.

[2] To whom all correspondence should be addressed.

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and collected in ice-cold 0.25 *M* sucrose. Colchicine was given ip by way of two consecutive injections, 60 and 15 min before killing the animals. Two doses of colchicine were used in two groups of rats: 0.5 and 1.0 mg per 100 g body weight, respectively. UDP-Gal (Calbiochem, Los Angeles, Calif.) and UDP-Gal uniformly labeled with ^{14}C in the sugar moiety (New England Nuclear Corp., Boston, Mass.) were used. The latter was diluted with carrier to a specific activity of about 1 mCi/nmole.

Cell fractions were prepared from a pool of kidneys blotted, weighed, and minced with scissors. Centrifugations were carried out in Beckmann ultracentrifuge, and all operations were carried out at 0–4°. The Golgi complex fraction was prepared by the method previously described (15), with some modifications. About 10 g of minced tissue was suspended in 2 vol of 52% sucrose containing 0.1 *M* sodium phosphate, pH 7.1, and homogenized with three full strokes at 1000 rpm using a 50-ml glass Potter–Elvehjem type homogenizer with an i.d. of exactly 1 in and a Teflon pestle machined to a diameter of 0.974 in. The homogenization was repeated using a pestle with a diameter of 0.982 in. The homogenate was filtered through four layers of cheesecloth and adjusted to 43.7% sucrose with homogenizing medium. Seven to nine milliliters of homogenate were placed in a tube and overlaid with sufficient 37.8% sucrose to bring the total volume to 15.4 ml. This was then successively overlaid with 6.3 ml of 36% sucrose then with 6.3 ml of 33% sucrose and finally with 7 ml 29% sucrose. The step gradient was then centrifuged for 60 min at 25,000 rpm in a SW 25.1 rotor. The Golgi complex rich-fractions 1 and 2 were obtained from the material appearing at the 29/33% and 33/36% sucrose interfaces, respectively. The fractions were diluted with 1/2 vol of cold distilled water and the membranes recovered by centrifugation at 30,000 rpm for 1 hr in a 30 rotor.

The light crude mitochondrial, the light mitochondrial, and the microsome fractions were prepared by the methods of Stein *et al.* (16) as modified by Fleischer and Kervina (17). About 10 g of minced tissue was suspended in 4 vol of 0.25 *M* sucrose solution containing 0.01 *M* Hepes, pH 7.4, and homogenized with three full strokes at 1000 rpm using a 50-ml Potter–Elvehjem type homogenizer (i.d. of glass vessel, 1 in) and a Teflon pestle machine to a diameter of 0.974 in and followed with three full strokes with a pestle machined to 0.988 in. The homogenate was filtered through a 110-mesh nylon monofilament bolting cloth. The homogenate was differentially centrifuged, and a low spin pellet (1000*g* for 10 min), a light crude mitochondrial, a microsomal, and a soluble fraction were successively isolated. The light mitochondrial fraction was obtained by further purification of the crude fraction resuspended in 0.25 *M* sucrose–0.01 *M* Hepes–0.001 *M* EDTA, pH 7.4, and recentrifuged for 10 min at 18,000 rpm in a 40 rotor. The upper light portion (mostly heavy microsomes) of the pellet was separated and discarded. The lower brown portion, enriched in mitochondria, was resuspended in the same solution and centrifuged again for 10 min at 14,000 rpm with the same rotor. The residual upper layer was again removed. A lysosomal fraction was obtained from the light crude mitochondrial fraction by step sucrose gradient centrifugation according to the method of Maunsbach (18). The fraction, resuspended in 0.3 *M* sucrose–0.001 *M* EDTA, pH 7.0, was layered on a linear sucrose gradient (0.3–2.1 *M*) containing 0.001 *M* EDTA and was centrifuged for 180 min at 24,500 rpm in a SW 25.1 rotor. The most dense band is enriched in lysosomes.

Microsomes were further fractionated into a smooth and a rough fraction by a modification (17) of the method of Dallner (19) as described for liver. Microsomes, resuspended with 0.25 *M* sucrose–0.015 *M* CsCl solution, were layered into tubes containing 1.3 *M* sucrose–0.015 *M* CsCl and centrifuged for 180 min at 49,000 rpm in a 50 rotor. The rough microsomes sediment as a pellet and the smooth microsomes remain at the interface.

Glucose-6-phosphatase was determined according to the method of Swanson (20), except that incubations were carried out for

5 and 10 min. Succinate–cytochrome *c* reductase activity was determined as previously described (21). Acid phosphatase activity, using β-glycerophosphate as substrate, was measured by the method of Besseys *et al.* (22), except that inorganic phosphate was measured using the method of Chen *et al.* (23). Galactosyltransferase was determined as previously described by Fleischer (24).

Kallikrein activity was measured using two different methods:

(a) By its stimulating effect on uterine contractility (25), using bradykinin as a standard. Activity is expressed as kallikrein equivalent to nanograms of bradykinin per milligram of protein. Trasylol (Aprotinin), was used as inhibitor of the stimulating effect of the enzyme in the bioassay (25).

(b) By the esterase activity shown by kallikrein, using benzoyl-L-arginine ethyl ester (BAEE) as substrate (6). The colorimetric reaction was measured by the method of Brown (26) and expressed as micromoles BAEE hydrolyzed per minute per milligram of protein.

Proteins were determined by Lowry's procedure (27), using crystalline bovine serum albumin as a standard. Phosphorus was determined by the method of Chen *et al.* (23).

Undiluted aliquots of the sucrose interphase containing the Golgi complex were fixed with 1/10th of 25% glutaraldehyde made up in 0.2 *M* sodium cacodylate at pH 7.4, immediately after isolation from the step gradient. The other cell fractions were fixed by treating an aliquot with an equal volume of 5% glutaraldehyde in 0.25 *M* sucrose and 0.2 *M* sodium cacodylate, pH 7.4. After standing overnight in the refrigerator, the samples were centrifuged at 20,000 rpm for 15 min in a 40 rotor and the supernatant discarded. Pellets were then washed twice by suspension in 0.25 *M* sucrose and recentrifuged. Finally the pellets were fixed with 1% osmium tetroxide, dehydrated, embedded, and sectioned as previously described (28).

Results. The activity of kallikrein in kidney homogenate, in light crude mitochondrial, and in microsomal fractions isolated by differential centrifugation, is shown in Table I. The kidney homogenate of loaded rats shows the highest kallikrein activity 8 min after salt loading. This activity is five times higher than that obtained in nonloaded rats. Ten minutes after salt loading, the kallikrein activity shows only a twofold increase with respect to that of nonloaded rats. The activity measured 4 min after salt loading in light crude mitochondrial and in microsomal fractions, isolated from the same homogenate, increased 17- and 26-fold, respectively, as compared to the values obtained in the same fractions from nonloaded rats. After 8 min of salt loading the corresponding values were increased 56- and 42-fold, respectively. It is necessary to indicate that kallikrein activity in homogenate as well as in light crude mitochondrial and in microsomal fractions, from kidneys of rats sacrificed 2, 4, 8, and 10 min after sham gavage submission, were similar to that of nonloaded rats.

Table II describes the effect of a pre-

TABLE I. RENAL KALLIKREIN ACTIVITY OF LIGHT CRUDE MITOCHONDRIAL AND MICROSOMAL FRACTIONS OBTAINED FROM SODIUM-LOADED RATS

		Fractions	
	Homogenate	Mitochondrial	Microsomal
Nonloaded rat	1.07 ± 0.10	0.75 ± 0.08	0.23 ± 0.03
2 min after treatment	2.87 ± 0.29	1.97 ± 0.16	0.72 ± 0.08
4 min after treatment	4.52 ± 0.41	12.63 ± 1.04	6.11 ± 0.07
8 min after treatment	5.39 ± 0.52	42.30 ± 2.97	9.80 ± 1.06
10 min after treatment	1.95 ± 0.20	21.32 ± 1.94	7.41 ± 0.79

Note. Results are mean values ± SD of three experiments. Kallikrein activity is in each case expressed as kallikrein equivalent to ng of bradykinin/mg of protein, determined by bioassay in cell fractions obtained from half of pooled kidneys of 10 loaded rats.

TABLE II. DISTRIBUTION OF RENAL KALLIKREIN ACTIVITY IN KIDNEY SUBCELLULAR FRACTIONS OBTAINED FROM SODIUM-LOADED RATS

	Salt loaded		Salt loaded and colchicine			
			0.5 mg/100 g body wt		1.0 mg/100 g body wt	
	Total protein (mg)	Enzyme activity	Total protein (mg)	Enzyme activity	Total protein (mg)	Enzyme activity
Homogenate	763.8	4.52	614.6	4.31	694.1	4.47
1000*g* pellet	339.4	5.69	286.2	5.88	293.4	5.68
Light crude mitochondrial fraction	78.9	12.63	60.2	3.61	79.9	1.92
Lysosomes	10.1	0.14	5.8	0.15	7.6	0.15
Microsomes	61.1	6.11	60.5	10.11	59.2	18.72
Supernatant	248.3	0.28	211.9	0.01	231.6	0.05

Note. Values are in each case the average of three experiments obtained in cell fractions from half the kidney pool of 10 salt-loaded rats, after 4 min of treatment. Kallikrein activity, measured by bioassay, is expressed as in Table I. Supernatant is the fraction of the homogenate which does not sediment at 100,000*g* after 1 hr.

treatment with two doses of colchicine and salt loading for 4 min, in the distribution of kallikrein activity in different fractions. The highest activities were found in the microsomal fractions which increased nearly two- (0.5 mg colchicine) and threefold (1.0 mg colchicine). The inverse situation is observed in rats not treated with colchicine, which show a higher activity in the light crude mitochondrial fraction than in the microsomal fraction. Since the composition of a subcellular fraction depends on the method of isolation, it should be indicated that the light crude mitochondrial fraction as well as the microsomal fraction, isolated by differential centrifugation, are mixed fractions. Thus, in addition to mitochondria, the light crude mitochondrial fraction includes lysosomes, granules, and heavy microsomes. On the other hand, the microsomal fraction is mostly composed of fragmented rough and smooth endoplasmic reticulum, including membranes of the Golgi complex. As shown in Table II, in sodium-loaded rats, the pretreatment with colchicine induces an increase of activity in the microsomal fraction and a decrease in the light crude mitochondrial fraction. In spite of the different kallikrein content, the protein distribution in the different fractions is only slightly affected, even in rats treated with a high dose of colchicine. Furthermore, the low kallikrein activity in lysosomes, isolated from the light crude mitochondrial fraction, remains unaffected by the colchicine treatment (see Fig. 3).

Figures 1 and 2 illustrate the morphology of the microsomal and the light crude mitochondrial fractions obtained from sodium-loaded rats treated and not treated with 1.0 mg of colchicine, sacrificed 4 min after treatment with salt. A similar and typical morphology in both microsomal fractions is observed, consisting essentially of closed and empty vesicles, ruptured vesicles, membrane fragments, and vesicles marked by ribosomes attached to their membrane. On the other hand, the difference in morphology between both mitochondrial fractions is quite clear. Although both are rich in mitochondria, the fraction obtained from kidneys of rats not treated with colchicine shows a larger number of dense bodies than the one obtained from colchicine-treated rats. Since similar activity of acid phosphatase was found in both fractions, we suggest that the difference in morphology observed is due to the presence of granules of secretion or secretory vesicles. Thus, the light crude mitochondrial fraction of colchicine-nontreated rats seems to be richer in granules or vesicles

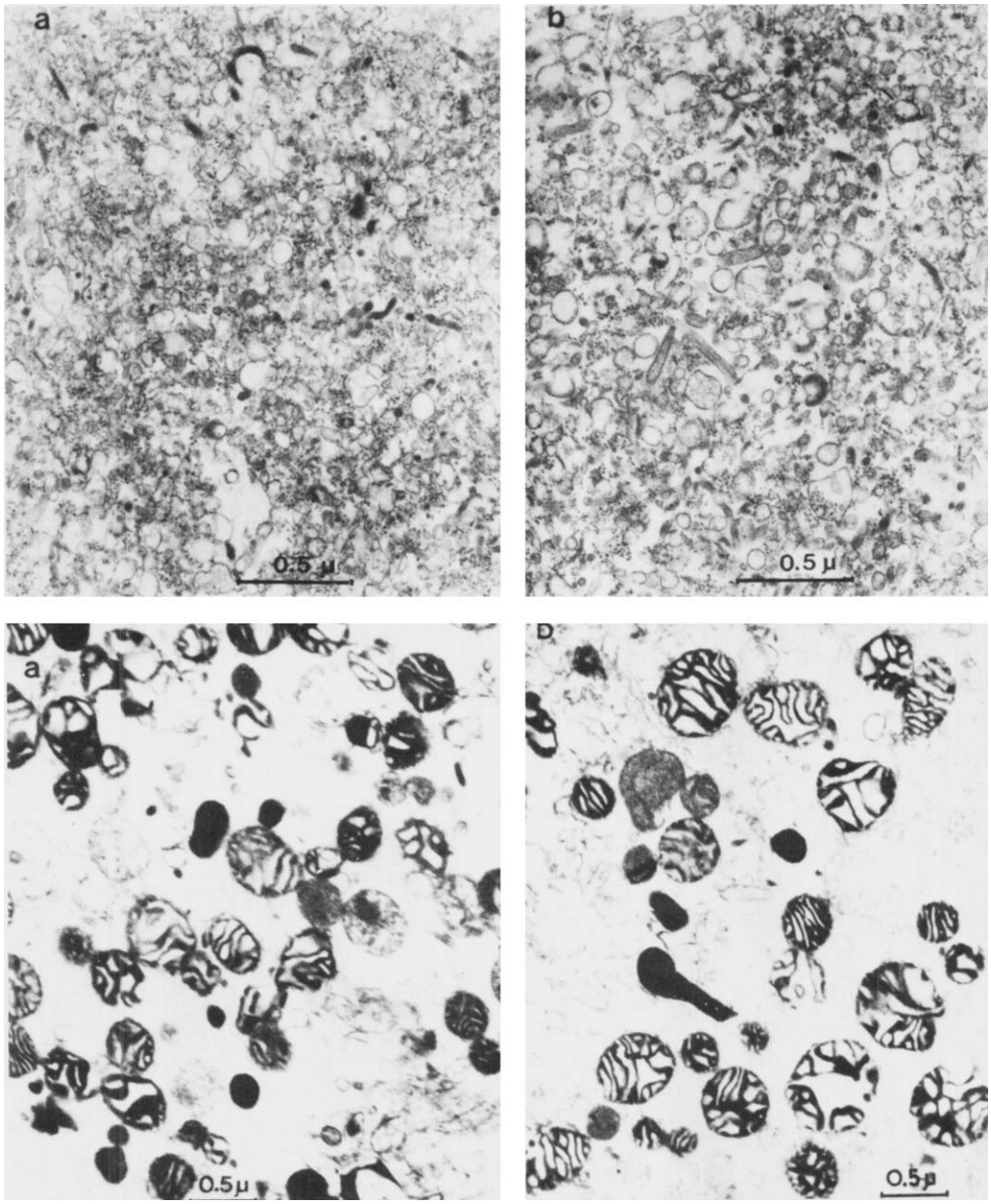

FIG. 1. Electron micrograph of a microsomal fraction obtained from sodium-loaded rat kidney homogenate. (a) Untreated with colchicine ×16,480; (b) treated with colchicine, ×16,480.

FIG. 2. Electron micrograph of a light crude mitochondrial fraction obtained from kidney homogenate of sodium-loaded rat. (a) Untreated with colchicine, ×17,600; (b) treated with colchicine ×17,600.

than the mitochondrial fraction of colchicine-treated rats.

Table III illustrates the kallikrein activity of the microsomal fraction and its components, obtained from pooled kidneys of 10 rats, treated with 1.0 mg of colchicine, and killed 4 min after sodium loading. Since most kallikrein activity resides in micro-

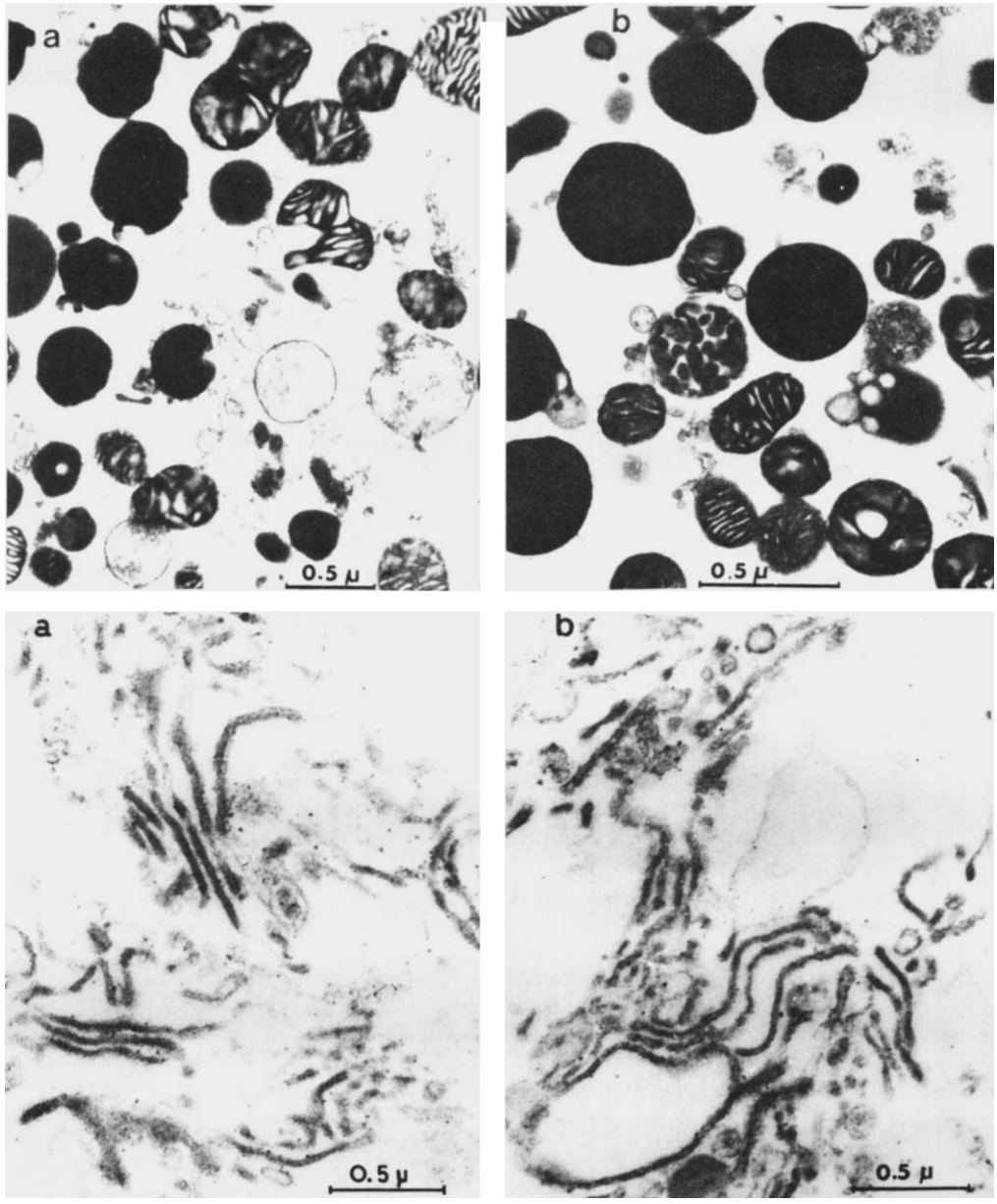

FIG. 3. Electron micrograph of a lysosomal fraction obtained from the light crude mitochondrial fraction of Fig. 2. (a) Untreated with colchicine, ×18,400; (b) treated with colchicine, ×18,400.

FIG. 4. Electron micrograph of a Golgi complex-rich fraction (equivalent to fraction 1 + 2) obtained by step sucrose gradient of sodium-loaded rat kidney homogenate. (a) Untreated with colchicine, ×16,800; (b) treated with colchicine, ×16,800.

somes (see Table II), the enzyme was measured in total microsomes as well as in microsomes divided into smooth and rough fractions, obtained from half the pooled kidneys. Kallikrein activity was also measured in the Golgi complex-rich fractions 1 and 2, obtained from the remaining half of the pooled kidneys. The kallikrein activity in

TABLE III. RENAL KALLIKREIN ACTIVITY OF DIFFERENT COMPONENTS OF THE MICROSOMAL FRACTION OF SALT-LOADED COLCHICINE-TREATED RATS

	Colchicine (1.0 mg/100 g wt)	
	Total protein (mg)	Enzyme activity
Homogenate	672.0	4.36
Microsomes	54.45	18.83
Rough microsomes	39.94	2.90
Smooth microsomes	10.74	70.24
Golgi complex-rich fraction 1	1.74	134.26
Golgi complex-rich fraction 2	2.79	175.35
Supernatant	220.42	0.04

Note. Fractions were isolated from the same pool of kidneys from 10 treated rats. Values are the mean of three experiments and are expressed as in Table I. Total microsomes, smooth and rough microsomes, and the supernatant were obtained from half the pooled kidneys. The remaining tissue was used to isolate Golgi complex-rich fractions. The protein content of each fraction is referred to as the whole homogenate.

the smooth microsome fraction was almost 24-fold higher than that in the rough microsome fraction. The activity in the smooth microsome fraction was almost four times higher than that in the total microsomes. The smooth microsome fraction (Table V) shows a galactosyltransferase activity which indicates a 12% of contamination with Golgi complex, from the transferase activity found in Golgi complex-rich fraction 2 (Table V). Golgi complex-rich fractions 1 and 2 have the highest kallikrein activity. The combined Golgi complex fractions contain 71 and 96% of the kallikrein found in total microsomes and in smooth microsome fractions, respectively.

TABLE IV. DISTRIBUTION OF RENAL ESTERASE ACTIVITY IN DIFFERENT COMPONENTS OF THE MICROSOMAL FRACTION OF SALT-LOADED COLCHICINE-TREATED RATS

Fraction	Esterase activity
Homogenate	0.145 ± 0.016
Microsomes	0.280 ± 0.019
Rough microsomes	0.160 ± 0.014
Smooth microsomes	0.970 ± 0.010
Golgi complex-rich fraction 1	1.800 ± 0.150
Golgi complex-rich fraction 2	1.905 ± 0.186
Supernatant	0.025 ± 0.003

Note. Values represent the mean ± SD of three preparations obtained from 10 pooled, colchicine-pretreated, and sodium-loaded rat kidneys. Enzyme activity is expressed as μmole BAEE hydrolyzed/min/mg of protein, at 37°.

In addition, the stimulating effect of the different fractions on uterine contractility were 100% sensitive to trasylol.

A similar distribution of activities in all the mentioned fractions was obtained when the activity of esterase was determined, as is shown in Table IV. Again, in animals previously treated with colchicine, the two Golgi complex fractions show the highest level of kallikrein activity.

Since the Golgi complex-rich fractions have low yields of proteins, both assays used to measure activity of kallikrein were not sufficiently sensitive to measure it in Golgi complex-rich fractions of nonloaded rats, in the absence of an enzymatic induction. For this reason we could not measure it in these fractions.

Since the amount of protein in the Golgi complex fractions 1 and 2 was rather low, both fractions were combined. The morphologies are shown in Fig. 4, and indicate that they derive predominantly from the Golgi complex. The Golgi complex fraction from colchicine-treated rats seems to contain more loaded Golgi complex elements.

As shown in Table V, the Golgi complex-rich fractions 1 and 2, isolated from sodium-loaded rat kidneys, were enzymatically unique compared to other purified cell fractions (see Fig. 5). Thus, these fractions exhibit the highest level of activity for galactosyltransferase, and appear

TABLE V. DISTRIBUTION OF "MARKER ENZYMES" IN PURIFIED SUBCELLULAR FRACTIONS OF SODIUM-LOADED RAT KIDNEYS

Fractions	Total protein (mg)	Amount of phosphorus per mg protein (μg)	Succinate cytochrome *c*	Glucose-6-phosphatase	Acid phosphatase	Galactosyl transferase[a]
Homogenate	763.8	13.40	0.145	0.029	0.564	11.92
Light mitochondria	49.8	10.42	0.753	0.012	0.604	N.D.
Lysosomes	10.1	13.02	0.290	0.051	2.143	N.D.
Smooth microsomes	12.2	28.17	0.018	0.275	0.343	38.16
Rough microsomes	43.4	34.29	0.024	0.253	0.101	8.70
Golgi-rich fraction 1	2.3	32.64	0.027	0.059	0.061	235.29
Golgi-rich fraction 2	4.4	30.64	0.010	0.041	0.166	312.19
Supernatant	250.2	13.69	0.000	0.022	0.281	N.D.[b]

Note. Values are the mean of three preparations from five pooled rat kidneys, obtained after 4 min of salt loading.

[a] Nanomole/hr/mg of protein at 37°; all other activities expressed as μmole/min/mg of protein at 32°, except glucose-6-phosphatase which was carried out at 37°.

[b] Not detected.

to be about 70 and 75% pure, respectively.

The glucose-6-phosphatase activity present in smooth microsomes shows that the Golgi complex fractions contain about 15 to 20% endoplasmic reticulum contamination. The acid phosphatase activity indicates that they are contaminated 3 to 8% with lysosomes. Succinate cytochrome *c* reductase activity suggests that they are also contaminated (1 to 4%) with mitochondria. From the galactosyltransferase activity found in Golgi complex-rich fraction 2, it appears that smooth and the total microsomal fractions are contaminated with the Golgi complex, about 9 and 12%, respectively. On the other hand, the level of acid phosphatase activity in the light mitochondrial fraction appears to indicate a 28% contamination with lysosomes.

The same marker enzymes, in purified subcellular fractions of sodium-loaded rats and sodium-loaded rats pretreated with low and high doses of colchicine were measured, and the activities did not differ significantly from the values presented in Table V. In general, the values illustrated in this table are quite similar to the specific activity of the same marker enzymes of purified organelles isolated from untreated or from unloaded rat kidneys (29).

Discussion. Due to previous evidence suggesting different sites of subcellular localization of kallikrein biosynthesis and secretion we focused our efforts on localizing the organelles possibly involved in the biosynthesis and/or activation of renal kallikrein. In order to analyze the conflicting evidence available on the subject, we have isolated different subcellular fractions from kidneys of rats submitted to two different treatments. Our purpose was to inhibit the secretion process and to obtain an increase in renal kallikrein content.

As we reported previously (11) acute NaCl loading induces in the rat a rapid and considerable increase of renal kallikrein that could be due to the existence of a sodium receptor in the gastrointestinal tract or to a rapid change in the release mechanism of renal kallikrein following sodium absorption.

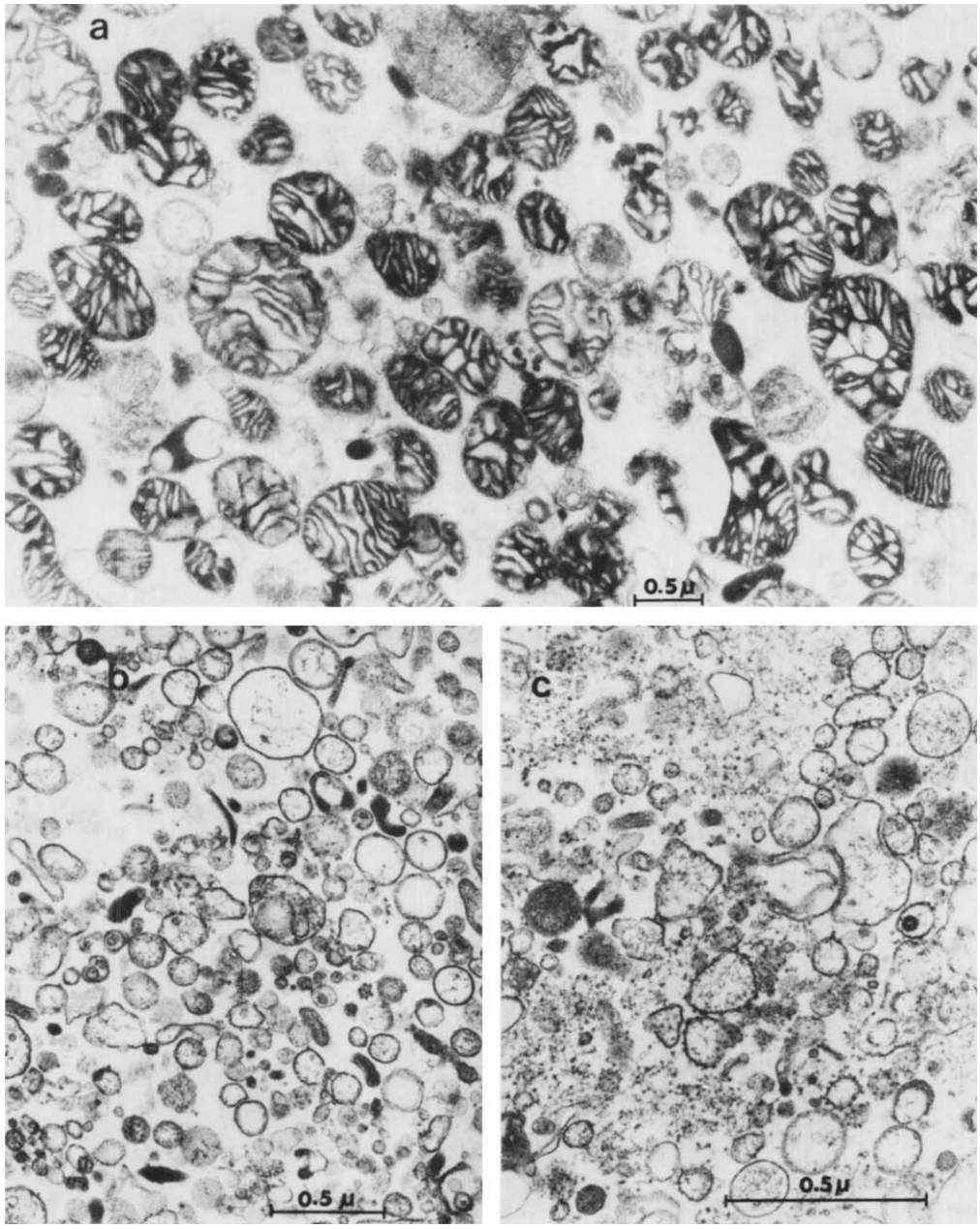

FIG. 5. Electron micrograph of a purified fraction obtained by fractionation of a kidney homogenate from a sodium-loaded rat. (a) Mitochondria-rich fraction, ×19,200; (b) smooth microsome-rich fraction, ×25,600; (c) rough microsome-rich fraction, ×29,600.

Our present results demonstrate that subcellular fractions, isolated by differential centrifugation from sodium-loaded animals not treated with colchicine (Table I), have a high activity of kallikrein in the crude light mitochondrial fraction. This fraction has a significant lysosomal contamination as is indicated by its acid phos-

phatase activity (Table IV). Based on the phosphatase activity, our results agree with those obtained by Carvalho and Diniz (6) and Baggio *et al.* (7). These authors postulated that lysosomes are the site where most of the kallikrein activity resides. However, results obtained by Chiang *et al.* (30) and Geipert and Erdös (5) suggest that kallikrein is secreted into the extracellular space in the form of dense granules and probably through fusion with the plasma membrane. Our studies seem to indicate that this mechanism may also occur in kidney cells. When using colchicine, a secretion inhibitor that does not interfere with the protein transport from the rough endoplasmic reticulum to the Golgi complex (13), the enzyme activity was recovered in the microsomal fraction, specifically in the Golgi complex-rich fraction. This indicates that the action of colchicine at the Golgi complex level may affect the secretion of granules containing kallikrein. Moreover, the acid phosphatase activity remains unaffected by colchicine treatment, indicating that lysosomal contamination in the light crude mitochondrial fraction of nontreated and colchicine-treated rats was similar. The fact that high kallikrein activity appears in the microsomal fraction after colchicine treatment is in agreement with results obtained by Nustad and Rubin (9). These authors have reported that kallikrein is mainly localized in this membranous fraction. Microsomes, isolated according to our procedure (29), mostly include fragmented rough and smooth endoplasmic reticulum as well as Golgi complex membrane. For this reason, we associate the site of biosynthesis and/or activation of kallikrein with the Golgi complex.

Studies by Redman (13) have shown that colchicine, administered to whole rat, blocks the secretion of serum albumin in rat liver cells blocking the release of this component from the Golgi complex. Our data suggest that colchicine administered to whole animal also blocks the release of kallikrein from this organelle in kidney cells. Carvalho and Diniz (6) and Baggio *et al.* (7) reported a high level of kallikrein activity in the lysosomal fraction. We suggest that their results can be explained by the fact that kallikrein is secreted as secretory granules, which are physicochemically similar to the kidney lysosomes, and that after homogenation both sediment together.

Renal kallikrein is a glycoprotein with a yet not determined carbohydrate sequence. Nevertheless, it is clear that glycoproteins are synthesized in the rough endoplasmic reticulum (peptidic backbone), and then transported to the Golgi complex (31). In this last organelle, sugars are added stepwise to the nonreducing end of the carbohydrate chains of the glycoprotein, by the specific action of galactosyl or syalyl transferase, using nucleotide sugar glycosyl as donors (32). It is most likely that kallikrein is not only concentrated but also modified in the Golgi complex by the addition of a terminal sugar.

The evidence offered in this paper supports the idea that kallikrein is transported within renal cells by a system of secretory vesicles derived from the Golgi complex. Our data also suggest that lysosomes may not play an important role in this process. On the other hand, since our data indicate that kallikrein activity is present in microsomes, and particularly in the Golgi complex, the biosynthesis and/or activation of renal kallikrein seems to occur in these organelles.

Finally, our results provide a feasible explanation for the apparently conflicting results on the sites of renal kallikrein biosynthesis and/or activation.

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