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The relationship between kidney and urinary kininogenase

K. NUSTAD

Institute of Surgical Research, Rikshospitalet, Oslo 1, Norway

Summary

1. Rat kidneys which were perfused with saline contained both kininogenase (KGA) and kininase activity. These activities were separated by gel filtration on a Sephadex G-100 column. The kininase activity was excluded from the column whereas the KGA activity was retained. Kidney KGA activity was primarily found in the sedimentable fraction of the homogenate.

2. The kidney KGA activity was compared with the urinary KGA activity, and the following properties were found to be the same: molecular dimension, pH optimum, effect of inhibitors, and ability to liberate kinins from kininogens.

3. A urinary sample collected over 24 h contained about 8 times the KGA activity found in the corresponding kidneys at the end of the collection period. The urine : kidney ratio for alkaline phosphatase was about 0·01.

4. The ability of kidney and urinary samples to hydrolyse *N*-α-benzoyl-L-arginine ethyl ester (BAEE) at pH 8·5 paralleled the KGA activity.

Introduction

It was originally assumed that the nondialysable hypotensive substance in urine, urinary kinin-forming enzyme or kininogenase (KGA), originated from the pancreas (Frey, 1929). This substance was therefore referred to as kallikrein (Kraut, Frey & Werle, 1930). Subsequent studies made this origin of urinary KGA unlikely (Beraldo, Feldberg & Hilton, 1956 ; Moriya, Pierce & Webster, 1963).

A small amount of KGA activity was demonstrated in kidney extracts by Kraut *et al.* (1930). Later Werle & Vogel (1961) showed that the KGA activity increased when kidney extracts were treated with trypsin. Carvalho & Diniz (1963, 1964, 1966) demonstrated KGA activity in kidney homogenates after incubation at pH 5. These findings pointed to the kidney as a possible origin of urinary KGA. However, the relationship between urinary and kidney KGA activity is still not clear. Similar inhibition patterns were observed for these activities by Carvalho & Diniz (1966). Werle & Vogel (1961), however, found that the kidney KGA was inhibited by soybean trypsin inhibitor (SBTI) whereas the urinary KGA was not. The aim of the present study has been to compare the kidney and urinary KGA activities in more detail.

Preliminary communications covering part of this work were given at the F.E.B.S., 4th Meeting, Oslo, 1967, and at the Bayer Symposium on Plasma Kinins, Helsinki, 1969.

Methods

Kidney homogenates

Adult male and female rats of a local strain weighing 200 to 300 g were used. Food was withheld 20 h before the experiment. Water was supplied *ad lib.* The rats were kept in a cage that allowed collection of urine. A midline abdominal incision was made under ether anaesthesia and the aorta isolated. The needle (23 gauge) of a scalp vein set (Portland Plastic Ltd., Hythe, Kent) was introduced into the lower aorta. The aorta above the renal artery was clamped and the caval vein opened. At the same time perfusion of the kidneys was started with ice cold saline containing 0·3% sodium citrate. Each pair of kidneys was perfused with 60 ml of the solution during a 10 to 15 min period. The kidneys were removed and weighed. Homogenates were prepared in ice cold 0·25 M sucrose containing 0·05 M Tris HCl, pH 7·5, with a Dounce homogenizer (five strokes with a loose fitting pestle and ten strokes with a tight fitting pestle). After homogenization the volume of the homogenate was adjusted to a final concentration of 1 g wet weight of tissue per 5 ml solution (1 : 5 homogenate). Suitable dilutions were made when necessary.

Preparation of materials for gel filtration

Kidney homogenates. The homogenates were solubilized before gel filtration by adding deoxycholic acid to a final concentration of 0·5%. After centrifugation at 4° C (9×10^6 $g \times$ min), in a Spinco ultracentrifuge, Model L2, rotor 50, the supernatant was gel filtered. When KGA activity was determined by bioassay, usually 1 ml of a solubilized 1 : 10 homogenate was applied to the column. Four times more material was applied to the column when hydrolysis of *N*-α-benzoyl-L-arginine ethyl ester (BAEE) was determined. In these latter experiments, one volume of the solubilized kidney extract was dialysed against 500 volumes of the buffer used to equilibrate the Sephadex column. Dialysis was carried out at 4° C with Visking dialysis tubing (Visking Dept., Union Carbide International Comp., New York, U.S.A.) of 24 Å pore size. The buffer was replaced once during the 6 h dialysis period.

KGA activity was also determined in the soluble and sedimentable fraction of the homogenate. In these experiments a 1 : 10 homogenate was centrifuged at 4° C (9×10^6 $g \times$ min) and 1 ml of the supernatant was gel filtered without further treatment. The sediment was suspended in 0·25 M sucrose, solubilized by deoxycholic acid, and gel filtered as described above.

Rat urine and dog plasma kininogen preparation. One ml of either urine or kininogen preparation (see below) was gel filtered after removal of insoluble materials by centrifugation at 4° C (9×10^6 $g \times$ min).

Rat plasma. Plasma was taken by heart puncture under ether anaesthesia into a plastic syringe containing sodium citrate to give a final concentration of 0·3%. After centrifugation at 22° C (4×10^4 $g \times$ min in an International Centrifuge, Model V, rotor 253), 1 ml of plasma was gel filtered. Contact with glass was avoided throughout the procedure.

Blue dextran. The marker (2 mg) was added to all samples to be gel filtered.

Gel filtration

Gel filtration was carried out on a Sephadex G-100 column (AB Pharmacia, Uppsala, Sweden) at room temperature (20°–24° C). The column (1·9 × 50 cm) was equilibrated and eluted with 0·05 M Tris HCl, pH 8·5, containing 0·1 M KCl. Sodium azide, 3×10^{-3}M, was added to prevent bacterial growth. This buffer is later referred to as the " gel filtration buffer ". A flow rate of 10 ml/h was obtained by a LKB peristaltic pump (type Perpex 10 200). Fractions of 3 ml were collected.

Preparation of kininogen

Jacobsen (1966a, b) showed that both rat and dog plasma contain two kininogens, substrates 1 and 2. Preliminary experiments showed that substrate 2 prepared from dog plasma reacted well with urinary KGA from rats, whereas it was found impractical to prepare kininogen from rat plasma (see below). Therefore, our standard kininogen preparation was made from citrated dog plasma, according to a slight modification of the method used by Amundsen, Nustad & Waaler (1963). Instead of filtering the plasma through a column of silica powder, massive contact with silica powder was obtained as described by Jacobsen (1966a). This substrate preparation showed no kininase activity and did not spontaneously form kinins when diluted. Gel filtration of this substrate preparation on the Sephadex G-100 column was performed. Eluted fractions were incubated with gel filtered rat urinary or kidney KGA. Only one peak of kininogen was recovered, and the elution pattern corresponded to that of substrate 2 present in untreated dog plasma as described by Jacobsen (1966a). No peak corresponding to substrate 1 was observed. The kininogen preparation was diluted 1 : 4 with 0·33 M Tris HCl, pH 8·5 or the appropriate buffer where stated, before use. The kininogen content of 1 ml of this diluted kininogen preparation was equivalent to about 1 μg of bradykinin.

In some experiments kininogen was prepared from rat plasma. Gel filtration on the Sephadex G-100 column of untreated rat plasma showed that a kininogen which formed kinin-like material when incubated with gel filtered rat urinary or kidney KGA, was eluted in fractions corresponding to those where the dog plasma substrate 2 was eluted. Rat plasma contained far less of this kininogen than dog plasma. Similar findings were made by Jacobsen (1966b). Therefore rat plasma was found to be an unsuitable source for preparation of kininogen.

Plasma kinin activity

Bioassay of plasma kinin activity was performed on an isolated rat uterus preparation suspended in an organ bath (8 ml) containing de Jalon solution at 29° C (de Jalon, Bayo & de Jalon, 1945). The uterus was taken from rats which had been given an intraperitoneal injection of stilboestrol (250 μg/kg) 20 h before death. The isolated uterus was stored at 4° C in de Jalon solution 24 h before use as recommended by Jacobsen (1966a). Samples of 0·1 to 0·3 ml were added to the organ bath every 5 min and were washed out by overflow after 1 min contact time. The dose range was usually 1 to 20 ng bradykinin added to the organ bath. Kinin activity in test samples was measured in bradykinin equivalents. When carboxypeptidase B was added to the test samples, the uterus contracting activity disappeared.

Enzyme assays

KGA activity. KGA activity was determined by incubation of aliquots of test samples with a kininogen preparation and subsequent measurement of the kinins formed by bioassay on the isolated rat uterus. With kidney homogenates it was necessary to control the kininase activity (Erdös & Yang, 1966) before the KGA activity could be assayed. No such precautions had to be taken with urinary samples because no kininase activity was found in the dilution of rat urine used for the KGA assay.

In most experiments on kidney homogenates, the influence of the kininase activity on the KGA assay was avoided by a gel filtration procedure which separated the KGA from the kininase activity (see **Results**). An inhibition of the kininase activity was obtained by preincubation of 0·1 ml of diluted homogenate (1 : 500 homogenate in the " gel filtration buffer ") with 0·1 ml mecaptoethanol (3×10^{-2}M) and 0·1 ml dipyridyl (2×10^{-3}M) in the same buffer. KGA activity was then measured in appropriate dilutions (using the " gel filtration buffer ") of either rat urine, gel filtered kidney homogenates or kininase-inhibited homogenate. Samples from the sources mentioned, 0·1 to 0·3 ml, were incubated with 0·1 ml of the diluted kininogen preparation in 0·15 M Tris HCl (final concentration), pH 8·5, for 4 min at 37° C and then bioassayed.

The KGA activity was expressed as ng of bradykinin equivalents formed per min. One unit of KGA activity was defined as the amount of enzyme forming kinins equivalent to 1 μg of bradykinin in 1 min under the described conditions.

When the effect of pH on the KGA activity was tested, the assay was modified as follows: The enzyme solution (gel filtered rat urinary or kidney KGA) was diluted (1 : 5 to 10) with a 0·05 M solution of the buffer to be used. A 0·33 M solution of the same buffer was used to dilute the dog plasma kininogen preparation. The KGA assay was then performed as described above. Tris maleate buffers, 0·15 M, were used at pH 6·5, 7·5, and 8·5, whereas 0·15 M glycine NaOH buffers were used at pH 8·5, 9·0, and 9·5.

Effect of inhibitors on the KGA activity was measured by preincubation of gel filtered KGA (0·3 ml) with inhibitor (0·1 ml) in the " gel filtration buffer " at 37° C. After 1, 6 and 11 min preincubation 0·1 ml was removed and tested for KGA activity as described.

Kininase activity

Kininase activity. Kininase activity was measured by incubation of 0·1 ml of enzyme dilution with 0·1 ml of synthetic bradykinin (200 ng/ml) in the " gel filtration buffer ". After 4 min at 37° C the kinin activity remaining in the incubate was assayed on the isolated rat uterus. The kininase activity was expressed as ng bradykinin destroyed per min in the incubate. One unit of kininase activity was defined as the amount of enzyme which inactivated 1 μg of bradykinin per min under the assay conditions.

BAEE esterase activity. Esterase activity was determined by the modified Hestrin (1949) method (Roberts, 1958, 1960). The incubation mixture (1·0 ml) consisted of 3×10^{-2}M BAEE, 0·50 ml enzyme solution, and 0·20 M buffer solution, which was either Tris HCl, Tris maleate, or glycine NaOH. Tris HCl pH 8·5 was used in all experiments except when the pH dependency of the reaction was being

tested. BAEE esterase activity of kidney homogenates was determined in presence of 0·1% Triton X-100. Appropriate dilutions of rat urine were made with distilled water, whereas kidney homogenates were diluted with 0·25 M sucrose. The dilutions were such that no more than 50% of the BAEE present was hydrolysed during the incubation period of 60 min at 37° C. The dilutions were found to be necessary in order to obtain a straight line relationship between ester hydrolysis and the amount of enzyme present. One unit of BAEE esterase activity was defined as the amount of enzyme which would hydrolyse 1 μmol of ester per min under the conditions of the assay.

The influence of pH on the BAEE esterase activity of kidney homogenates and urine was determined using a final buffer concentration of 0·20 M Tris maleate at pH 5·5, 6·5, 7·5, and 8·5, or 0·20 M glycine NaOH at pH 8·5, 9·0 and 9·5.

The effect of inhibitors on the BAEE esterase activity at pH 8·5 of kidney homogenates or untreated urine were determined after preincubation of dilutions with inhibitor for 10 min at 37° C. BAEE was then added and the assay carried out as described above.

Alkaline phosphatase. Alkaline phosphatase was assayed in a final incubation mixture of 1·0 ml by the method of Bonting, Tsoodle, de Bruin & Mayron (1960). One unit of alkaline phosphatase activity was defined as the amount of enzyme which caused the decomposition of 1 μmol of substrate per min under the conditions of the assay.

Proteins. Proteins were determined by the method of Lowry, Rosebrough, Farr & Randall (1951) using bovine serum albumin as standard.

Reagents

Blue Dextran 2000 (Pharmacia, Uppsala, Sweden); albumin, bovine, fraction V (Sigma Chemical Company, St. Louis, Missouri, U.S.A.); albumin, egg, grade III (Sigma); *N*-α-benzoyl-L-arginine ethyl ester HCl (BAEE) (Sigma); carboxypeptidase B from hog pancreas (Sigma); deoxycholic acid, sodium salt (Sigma); Triton X-100, octyl phenoxypolyethoxyethanol (Sigma); trypsin inhibitor from soybean, type I-S (Sigma); trypsin inhibitor from ovomucoid, type II-0 (Sigma); myoglobin, horse (Nutritional Biochemicals Corporation, Cleveland, Ohio, U.S.A.); bradykinin, BRS 640 (Sandoz, Basel, Switzerland); Padutin®, hog pancreatic kallikrein, 10 Frey units per ampoule (Bayer, Leverkusen, W. Germany); aprotinin, proteinase inhibitor (Trasylol, Bayer).

Results

KGA activity of rat urine

When rat urine was incubated with the kininogen preparation, the formation of kinin-like material was proportional to the time of incubation within the first 10 to 12 min of the incubation period. In all later experiments a standard incubation period of 4 min was used. The pH optimum of the KGA activity was on the alkaline side (Fig. 1). Since no increase in activity was observed above pH 8·5, this pH was used in all later experiments.

The concentration of KGA activity in urine varied considerably. Generally it was found that the concentration of KGA activity was inversely related to the volume of urine excreted (Table 1). After gel filtration of rat urine on a Sephadex G-100 column, all the KGA activity was recovered in one peak (Fig. 2). The recovery of KGA activity was 93%. The molecular weight, calculated according to Andrews (1965), was 38,500. The effect of inhibitors on the KGA activity is shown in Fig. 3. The values given in Fig. 3 are calculated from the inhibition observed after 11 min preincubation of KGA and inhibitor. Higher concentrations of aprotinin than shown gave an immediate, complete inhibition of the KGA activity in samples removed after 1 min preincubation of enzyme with inhibitor. Lower concentrations of aprotinin caused a gradually increasing inhibition with time. Soybean trypsin inhibitor (SBTI) inhibited the KGA activity in high concentrations only, whereas ovomucoid trypsin inhibitor (OTI) did not inhibit the KGA activity of rat urine.

BAEE esterase activity of rat urine

Rat urine contained considerable BAEE esterase activity. The pH dependency of urinary esterase activity is shown in Fig. 1. The esterase activity of untreated urine as well as of fractions after gel filration showed similar pH dependencies. The ester was increasingly unstable above pH 8·5 under the test conditions used, as shown by reduced amounts of BAEE in blanks above this pH. This possibly

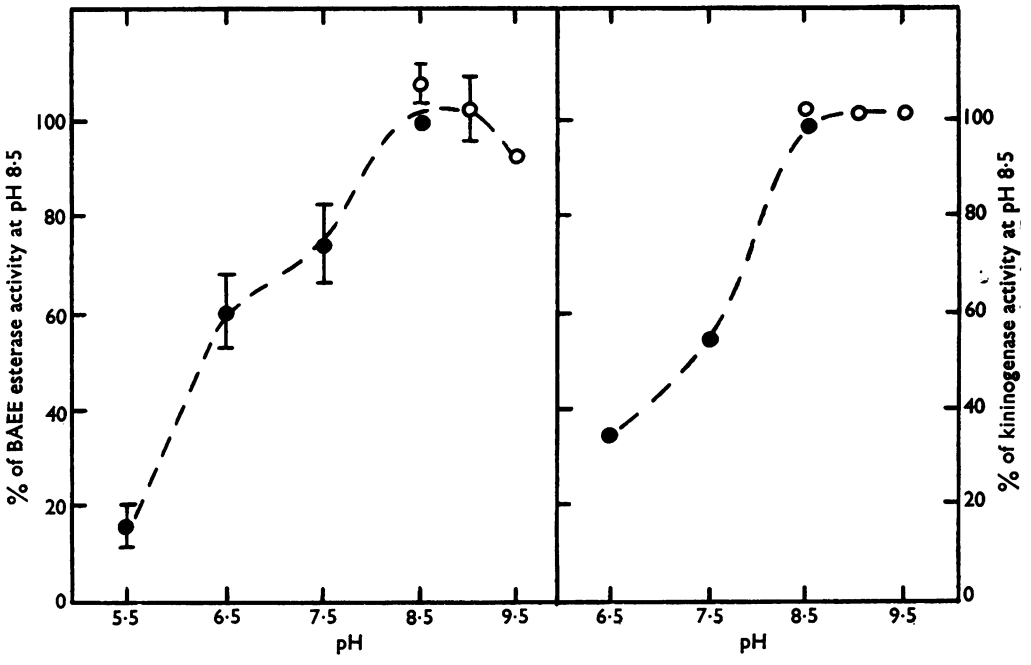

FIG. 1. Effect of pH on kininogenase and BAEE esterase activities of rat urine. The figure to the left shows the effect of pH on the BAEE esterase activity of untreated rat urine. Values are given as means + S.D. of three to five experiments. The figure to the right shows the effect of the pH on the KGA activity of gel filtered rat urine. The KGA activity is illustrated with a single typical experiment. ●, Activities assayed in 0·15 M Tris maleate buffer; ○, activities assayed in 0·15 M glycine NaOH buffer.

accounts for the lower pH optimum observed when compared with that found by Mares-Guia & Diniz (1967). In all later experiments pH 8·5 was used. The concentration of BAEE esterase activity varied directly with the KGA activity (Table 1). Gel filtration on a Sephadex G-100 column revealed that all of the BAEE esterase activity was recovered in the same fractions as the KGA activity (Fig. 2). The recovery of BAEE esterase was 95%. The inhibitor pattern of the BAEE esterase was similar to that observed for the KGA activity (Fig. 3).

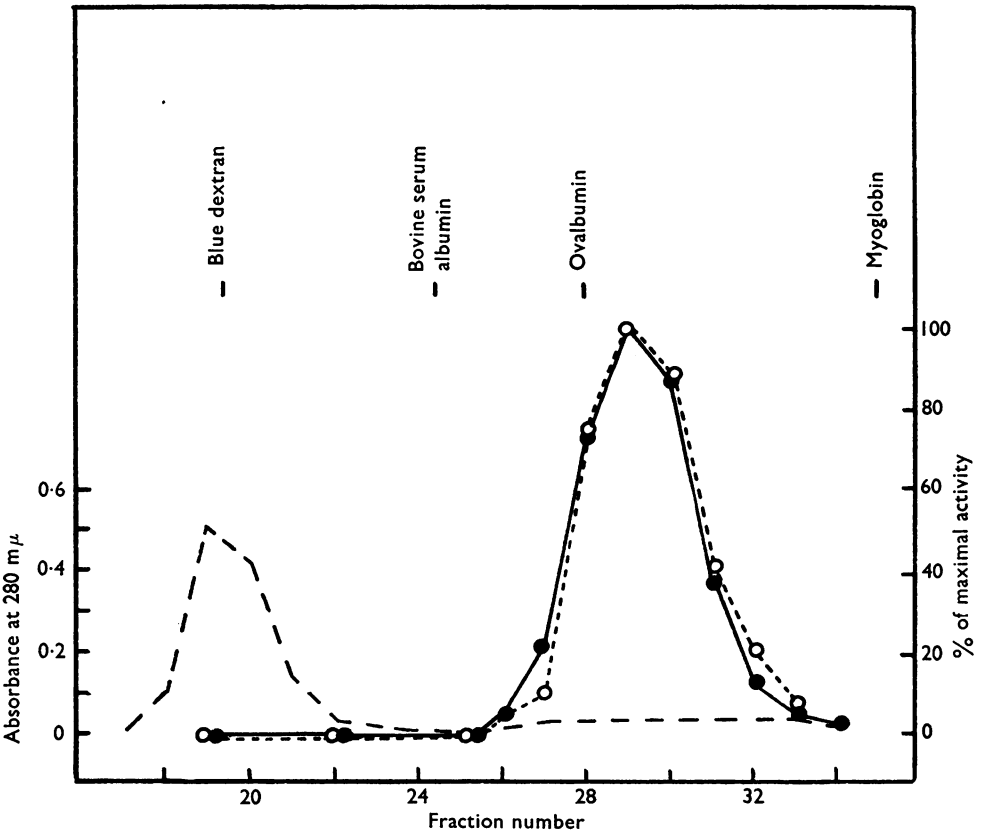

FIG. 2. Gel filtration of rat urine on a Sephadex G-100 column. Rat urine, 1 ml, was applied to the column. Fractions of 3 ml were collected. The elution maxima of reference proteins and blue dextran are also shown. ●——●, Kininogenase (KGA) activity (100% = 2·4 units/fraction); ○···○, BAEE esterase activity (100% = 0·30 units/fraction); ----, absorbance at 280 nm.

TABLE 1. *Urinary excretion of kininogenase (KGA) and BAEE esterase activities in rats with high and low diuresis*

No. of animals	Urine excretion ml/24 h	Kininogenase activity		BAEE esterase activity	
		Units/ml	Units/24 h	Units/ml	Units/24 h
2	1·2	30·0	36	2·45	2·94
3	3·0	10·0	30	0·90	2·70
3	14·0	5·4	76	0·32	4·48
3	15·0	3·8	57	0·30	4·50

KGA activity in rat kidney homogenates

When untreated kidney homogenates were incubated with the kininogen preparation, no kinin activity could be measured. When kininase inhibitors were present, KGA activity could be demonstrated. The reproducibility of the assay of KGA activity in whole homogenates in the presence of kininase inhibitors was not satisfactory, although the method was found to be fairly accurate when used on subcellular fractions of the homogenate (Nustad, 1970). It was therefore necessary to separate the KGA activity from the kininase activity of the homogenate. This separation was obtained by gel filtration of solubilized kidney homogenates on a Sephadex G-100 column. The kininase activity was eluted in the void volume of the column ; the KGA activity was retained and separated from the kininase activity (Fig. 4). A similar pattern was observed using a Sephadex G-75 column. The elution pattern of the kidney KGA activity was similar to that of the urinary KGA activity (Fig. 2). Repeated extractions of a 1 : 10 kidney homogenate with deoxycholic acid, followed by gel filtration of the extracts, showed that more than 90% of the KGA activity recovered was present in the first extract. In six experiments the KGA activity assayed after gel filtration varied between 3·0 and 7·7 units per g wet weight (average 4·6). Before gel filtration and with kininase inhibitors, the average KGA activity was about one-half this value.

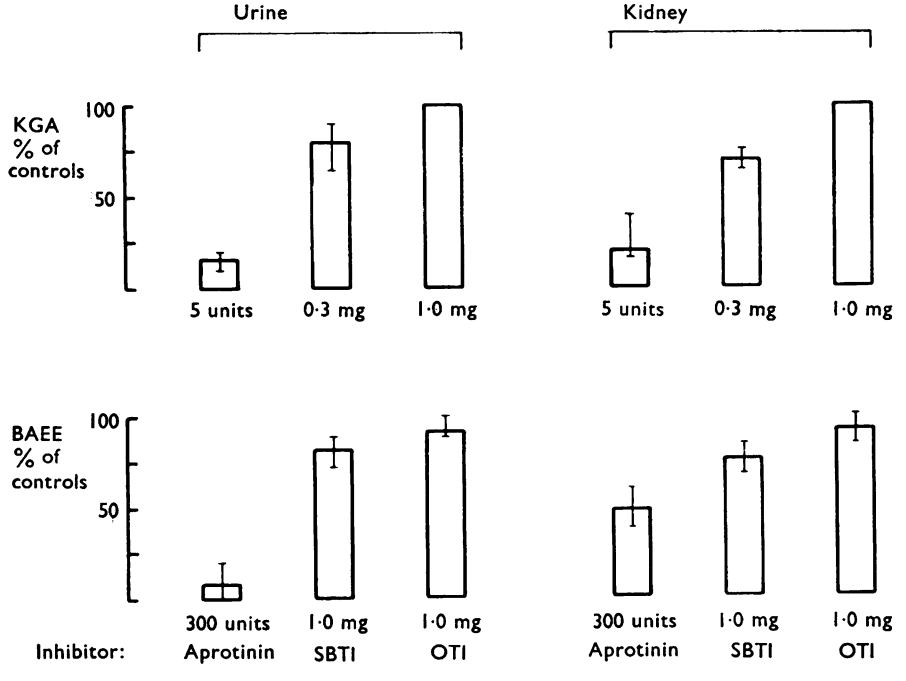

FIG. 3. Effect of inhibitors on the kininogenase (KGA) and BAEE esterase activities of rat urine and kidney homogenates. KGA and BAEE esterase activities are presented as per cent of controls after incubation with aprotinin, soybean trypsin inhibitor (SBTI) or ovomucoid trypsin inhibitor (OTI). Values are given as means of three to five experiments with absolute range indicated. Control activities: KGA 0·01 units and BAEE esterase 0·12–0·20 units. The amount of aprotinin in the incubation medium is given in biological inhibitor units (Frey *et al.*, 1968).

When the kidney homogenate was centrifuged and the sediment and supernatant assayed for KGA activity, 84% (range 63 to 94%, $n=4$) of the KGA activity was recovered in the sedimentable fraction. Repeated washings of the sediment with 0·25 M sucrose containing Tris HCl (0·05 M) at pH 6·5, 7·5 or 8·5 did not solubilize further significant amounts of KGA activity as tested by gel filtration of the combined supernatants after three washings at each pH.

The kidney and urinary KGA activities were similar with regard to pH optimum, effect of inhibitors (Fig. 3), and ability to form kinins from dog and rat plasma kininogen.

BAEE esterase activity of rat kidney homogenates

Freshly prepared kidney homogenates contain large quantities of BAEE esterase activity. The pH dependency of freshly prepared kidney homogenate was different from urinary BAEE esterase activity as shown in Fig. 5. The pH dependency of the kidney BAEE esterase activity, however, was changed after 6 days' storage of

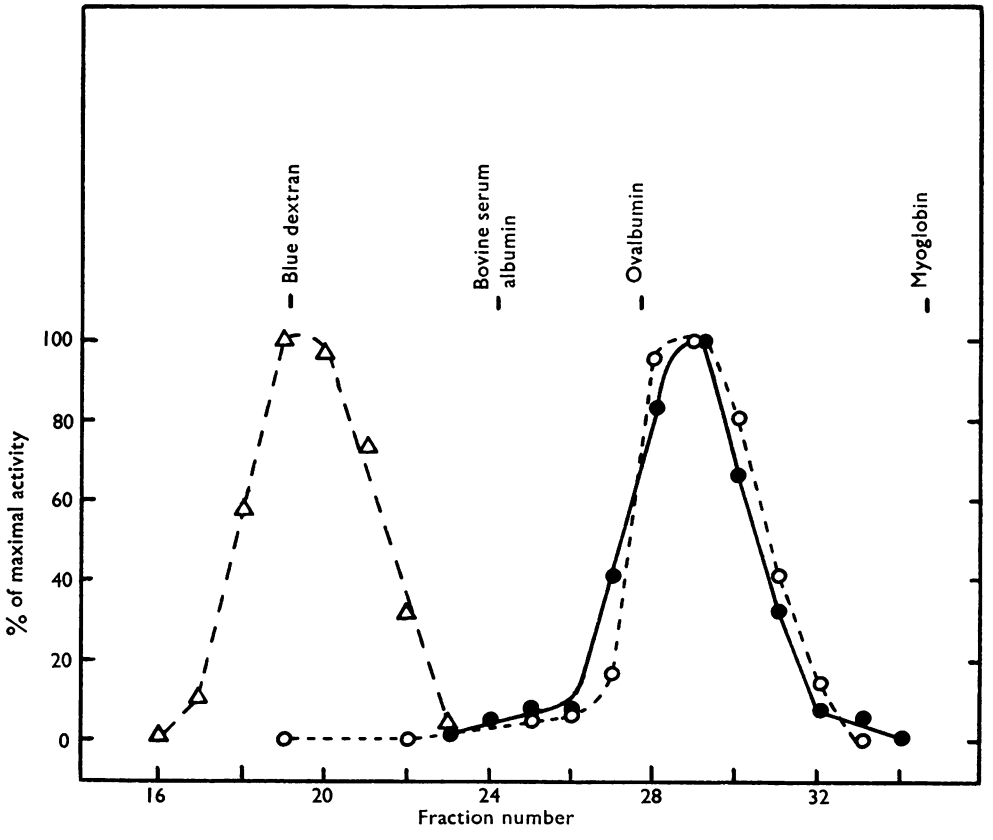

FIG. 4. Gel filtration of kidney cortex homogenate on a Sephadex G-100 column. Kidney homogenate, 2 ml, corresponding to 0·4 g wet weight, pretreated as described under **Methods** was applied to the column. Fractions of 3 ml were collected. The elution maxima of reference proteins and blue dextran are also shown. ●——●, Kininogenase (KGA) activity (100% = 0·45 units/fraction); ○·····○, BAEE esterase activity (100% = 0·05 units/fraction); △- - -△, kininase activity (100% = 1·9 units/fraction).

the homogenate at −20° C. The total activity of the homogenate at pH 6·5 was reduced markedly by this storage while the activity at pH 8·5 was unaffected.

When deoxycholic acid was added to the kidney homogenate and the homogenate centrifuged, more than 90% of the BAEE esterase activity (pH 8·5) was recovered in the supernatant fraction. When the supernatant fraction was gel filtered on a Sephadex G-100 column, the BAEE esterase activity (pH 8·5) was found in the same fractions as the KGA activity (Fig. 4). Recovery of BAEE esterase activity (pH 8·5) was about 70%. When the fractions were tested for esterase activity at pH 6·5

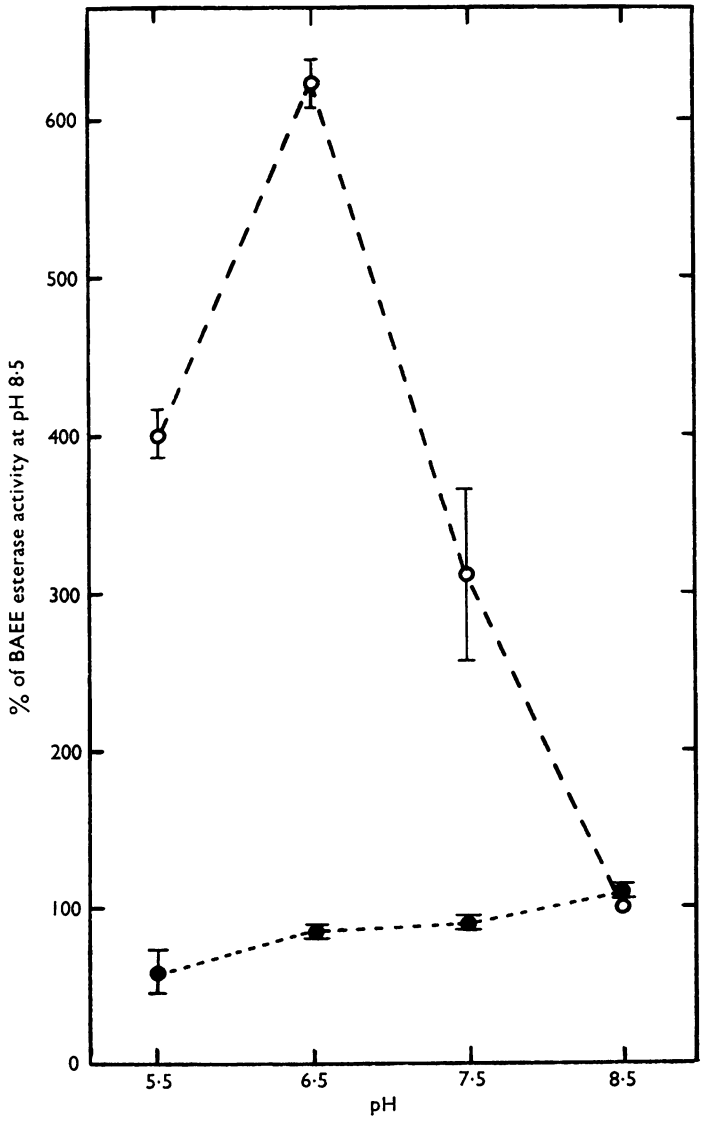

FIG. 5. Effect of pH on BAEE esterase activity of rat kidney homogenates. Values are given as means ± S.D. of three to five experiments. Ordinate: % of activity at pH 8·5 of freshly prepared homogenates. This activity was 0·50 units/g wet weight (S.D. = ±0·15, n=5). ○- - -○, Freshly prepared homogenate; ●- - -●, after storage of the homogenate at −20° C for 6 days.

all of the recovered esterase activity was found in the same fractions containing the pH 8·5 esterase activity ; however, recovery of the pH 6·5 esterase activity was less than 13%.

When the kidney homogenate was centrifuged, 81% (range 56 to 100%, $n=4$) of the BAEE esterase activity was recovered in the sediment as found above for the KGA activity.

The pattern of inhibition of the kidney BAEE esterase activity measured at pH 8·5 was similar to that observed for the urinary BAEE esterase (Fig. 3).

Relationships between amounts of enzyme activities in urine and kidney

Similarities between the KGA activity in urine and that found in the kidney homogenate suggest that the former is derived from the latter. Therefore, the quantitative relation between these activities was determined. The total amount of KGA excreted in urine during a 24 h period was about 8 times the quantity found in the two kidneys at the end of the period (Table 2). A similar relationship was found for the BAEE esterase activity. However, the urine : kidney ratio of alkaline phosphatase was <0·01.

Discussion

The results obtained in the present study show that the KGA found in the kidney homogenate has similar properties to the urinary KGA. The following observations were made: Kidney and urinary KGAs have similar molecular dimensions, pH optima, and are inhibited by the same inhibitions. The different effect of SBTI on urinary and kidney KGA activities observed by Werle & Vogel (1961) could not be reproduced. It was found that very high concentrations of SBTI inhibited both kidney and urinary KGA activities.

The KGA activity present in kidney homogenates was mainly particle bound and was present in kidneys which had been perfused. Thus, it seems reasonable to assume that the KGA activity demonstrated in the kidney homogenates was not derived from plasma or urine present in the kidneys at death. However, the complicated function of the kidney calls for special precaution in interpreting the finding of KGA activity in this organ. It has been shown that several enzymes found in urine have their origin in part from plasma, although most urinary enzymes are thought to be of kidney origin (Raab, 1968).

The possibility that the urinary KGA activity is derived from plasma is questionable because no likely precursor has been demonstrated in plasma. The known plasma KGA (plasma kallikrein) is different from the urinary KGA activity in molecular dimension, electrophoretic mobility, and effect of inhibitors (Frey, Kraut,

TABLE 2. *Quantitative relationship between total activities in kidney and urine*

	Units in urine collected over 24 h	Units in corresponding kidneys	Ratio: Total urinary activity/ Total kidney activity
Kininogenase (KGA)	58±18 (4)	6·8±2·5 (4)	8·5
BAEE esterase	4·5±1·3 (4)	0·60±0·10 (4)	7·5
Alkaline phosphatase	0·9±0·2 (3)	139±22 (3)	<0·01

Values are expressed as means±S.D. Number of experiments is given in parentheses.

Werle, Vogel, Zickgraf-Rüdel & Trautschold, 1968). Also, it has been demonstrated that urinary KGA releases kallidin from kininogen while plasma KGA releases bradykinin (Webster & Pierce, 1963). It is possible, however, that the known plasma KGA could be altered in the kidney or that an unknown plasma KGA is the precursor of the urinary KGA activity. If one of these possibilities were true, the KGA activity present in the kidney could represent a transient stage between plasma KGA and urinary KGA.

The possibility that urinary KGA is formed in and secreted by the kidney has not yet been proved. In a recent review of the KGA-kinin system, however, it was suggested that the urinary KGA originated in the kidney, possibly from the proximal tubular cells (Frey *et al.*, 1968). This suggestion was based on the missing plasma precursor mentioned above, the finding of KGA activity in the kidney, and the fact that experimental tubular damage caused a marked reduction of KGA activity in urine (Werle & Vogel, 1960).

KGA has also been demonstrated in the urine formed by an isolated perfused dog kidney. The kidney, however, was perfused with blood and the concentration of KGA in the urine was far below the normal value (Beraldo *et al.*, 1956). The urine formed by an isolated rat kidney perfused with an artificial medium (modified Eagle basal medium) contained KGA activity only in the initial 0·5 ml sample collected. Later no KGA but markedly elevated kininase activity was found. The consumption of oxygen and the production of acid and carbon dioxide by the kidney preparation was satisfactory throughout the experiments (Semb & Nustad, unpublished observations). Low to zero concentration of KGA and raised concentration of kininase activity in urine was also found after open heart surgery on patients with healthy kidneys. This was presumably due to a transient kidney damage caused by the operation (Ofstad & Nustad, 1969). These observations indicate that the normal appearance of KGA in urine is the result of a process which is easily impaired. This makes it more difficult to prove the origin of urinary KGA, but likely that determination of KGA in urine will be of clinical importance.

The high urine : kidney ratio observed for the KGA activity suggests an active secretion of KGA into urine by the kidney or the excretion from plasma, whereas the smaller amount of alkaline phosphatase in urine is thought to be derived from normal tubular cell turnover in the kidney (Raab, 1968). The possibility that

TABLE 3. *Comparison of rat kidney kininogenase (KGA) activity as determined by various methods*

Treatment of homogenate	Frey units per g wet weight	μg bradykinin eqv. per g protein	μg bradykinin eqv. per g wet weight (absolute range)	Reference
Trypsin added to a water extract	2–8			Werle & Vogel (1961)
Homogenate incubated at pH 5 for 2 h at 37° C		7·2		Carvalho & Diniz (1966)
Homogenate extracted with deoxycholic acid and gel filtered	1·8–4·6*	16·1–41·2†	3·0–7·7	Present study

* The values given have been calculated after determination of the KGA activity of a pancreatic KGA preparation (Padutin®) with known activity in Frey units. It was found that the unit used in the present study corresponded to 0·6 Frey unit (S.D. = ±0·2, $n = 3$).
† The values given were calculated after determination of protein content of the homogenate. It was found that 1 g wet weight contained 187 mg protein (S.D. = ±23, $n = 9$).

urinary KGA is secreted by the kidney would be analogous to the situation demonstrated in the pancreas and in salivary glands because these glands and their exocrine secretions contain large quantities of KGA activity. The analogy is strengthened by the fact that the kidney both morphologically and functionally is a gland. Furthermore, the urinary KGA activity is similar to salivary and pancreatic KGA activities in approximate molecular dimension and is equally affected by most proteolytic inhibitors (Frey *et al.*, 1968). Finally, antibodies prepared against urinary KGA cross-react with pancreatic KGA (Webster, Emmart, Turner, Moriya & Pierce, 1963) and both enzymes form kallidin from kininogen (Webster & Pierce, 1963). The urinary KGA activity is not identical with pancreatic KGA (Moriya *et al.*, 1963), but the similarities are so striking that these enzymes are often referred to as glandular KGAs (Webster & Pierce, 1963).

The estimation of KGA activity by the bioassay is a very laborious method and subject to all of the pitfalls inherent in a biological determination. However, the quantity of KGA activity found in kidney homogenates in the present study after the gel filtration procedure seems to be about the same as that found by others (Table 3). More important is the fact that most of the BAEE esterase at pH 8·5 in rat urine and kidney homogenates seems to be due to the KGA. This is suggested by the fact that KGA activity and BAEE esterase activity were always found in the same fractions and were inhibited by the same inhibitors. The pH optimum of urinary BAEE esterase activity was on the alkaline side, as was that of the KGA activity. Furthermore, it was found that the ratios KGA activity (in Frey units): BAEE esterase activity (in μmol/min) were: 7·2 (range 5·0–9·5, $n=8$) in urine and 6·1 (range 5·4–8·9, $n=6$) in kidney homogenates. These values are in the range reported for human urinary KGA (8·8) and hog urinary KGA (2·3) (Frey *et al.*, 1968). It is also known that the ratio between KGA activity and esterase activity is about the same in crude dialysed urine as in a 1,000-fold purified human urinary KGA preparation (Moriya *et al.*, 1963).

The pH optimum of the kidney BAEE esterase activity suggests that this activity consists of at least two enzymes: one acid unstable esterase with pH optimum about 6·5 and one stable BAEE esterase activity with pH optimum on the alkaline side. The instability of the acid BAEE esterase might explain the loss of this activity after gel filtration of the kidney extract. Although the BAEE esterase activity at pH 8·5 was found to parallel the KGA activity, purification of both kidney and urinary KGA is necessary to evaluate if all the alkaline BAEE esterase activity can be attributed to the KGA.

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