Biochemical Pharmacology, 1963, Vol. 12, pp. 511–519. Pergamon Press Ltd., Printed in Great Britain.

IMMUNOLOGICAL PROPERTIES OF THE KALLIKREINS

MARION E. WEBSTER, EMILY W. EMMART, WILLIAM A. TURNER, HIROSHI MORIYA* and JACK V. PIERCE

National Heart Institute and National Institute of Arthritis and Metabolic Diseases, Bethesda, Md., U.S.A.

(*Received* 2 *November* 1962; *accepted* 21 *December* 1962)

Abstract—Rabbits immunized with crude or partially purified human urinary and pancreatic kallikreins developed antibodies which inhibited the vasodilator activity of the kallikreins in dogs. The antigenic heterogeneity of the kallikreins was shown by the presence of multiple precipitin bands in agar gel. The kallikrein–antikallikrein band was tentatively identified with the use of purified kallikreins. Antibody to human urinary kallikrein showed cross reaction both in agar gel and by inhibition of the vasodilator activity with human pancreatic kallikrein but not with dog urinary or hog pancreatic kallikrein. The normal inhibitor in rabbit serum could be differentiated from antibody by its stability to acetone and to storage at pH 3·0. Unlike the normal inhibitor, antibody was not competitively inhibited by *p*-toluenesulfonyl-L-arginine methyl ester (TAMe) and was a more potent inhibitor of the vasodilator activity than the TAMe activity of kallikrein.

THE kallikreins are hypotensive enzymes of endogenous origin which exert their pharmacological effect by enzymatic action on kallidinogen, an α_2-globulin in plasma, to release the polypeptide kallidin.[1] The kallikreins derived from various sources, such as saliva, urine, pancreas, and plasma, can be distinguished by the use of proteolytic inhibitors.[2, 3] The immunological properties of the kallikreins have not yet been explored. This communication describes the antigenicity of the urinary and pancreatic kallikreins of man, and the capacity of antisera to precipitate and inhibit kallikreins prepared from various sources.

METHODS

Preparation of kallikreins. Crude human urinary kallikrein (6 Frey units (FU)/mg) was prepared from normal human urine by adsorption to and elution from XE-64 (IRC-50) and diethylaminoethyl cellulose (DEAE-C) as previously described.[4] Further purification was achieved by ethanolic fractionation at pH 7·5.[5] The 0·35-mole fraction of ethanol contained 44% of the partially purified kallikrein at 29 FU/mg. Alternatively, the crude human urinary kallikrein was purified by acetone fractionation at pH 7·5, as described by Moriya *et al.*,[6] followed by ammonium sulfate fractionation. After removal of the ammonium sulfate by gel filtration with Sephadex G-75, this more highly purified preparation contained 50 FU/mg. Electrophoretically and ultracentrifugally homogeneous human urinary kallikrein was prepared by chromatography of this preparation on DEAE-Sephadex A-50. The final product contained 430 FU/mg. Details of this latter method will be described elsewhere.[7]

* Present address: Tokyo College of Science, Tokyo, Japan.

Crude human pancreatic kallikrein, 4 FU/mg, was prepared by adsorption on DEAE-C as described by Webster and Pierce.[8] Acetone fractionation gave a partially purified product containing 20 FU/mg. Purification by ammonium sulfate fractionation, gel filtration with Sephadex G-75, and chromatography on DEAE-Sephadex A-50 resulted in a "pure" preparation containing 250 FU/mg, which gave a single peak in the ultracentrifuge. Nonetheless, electrophoresis on cyanogum gel disclosed the presence of two protein components. In further studies on the purification of this enzyme the conditions for chromatography on DEAE-Sephadex A-50 were improved, and a pure preparation was obtained containing 550 FU/mg.[7]

Crude dog urinary kallikrein (3·3 FU/mg) was obtained by dialyzing dog urine overnight against running tap water and drying the dialyzed solution from the frozen state.

Highly purified hog pancreatic kallikrein (240 FU/mg) was prepared as previously described by Moriya.[9]

Immunization of the rabbits

(*a*) *Urinary kallikrein* (*crude and partially purified*). In the initial experiments Dutch rabbits were injected intravenously or intraperitoneally with doses of 5 to 15 FU two to three times weekly, and the animals bled 1 week after the last injection. The crude human urinary kallikrein (6 FU/mg) was highly toxic for rabbits; seven of nine rabbits, injected with 10 to 700 FU, died within 24 hr after the last injection. The preparation produced a markedly elevated β-globulin and high serum cholesterol levels (150 to 286 mg/100 ml). One of the rabbits (K-11), which received 300 FU of the crude urinary kallikrein intravenously, survived 3·5 months, whereas another rabbit (K-44), which received 700 FU of the same preparation intraperitoneally, survived 7·5 months. As measured by their ability to inhibit the vasodilation by crude urinary kallikrein in dogs, the serum of rabbit K-11 contained no antibody, and the serum of rabbit K-44 possessed a low level.

Previous studies in dogs[10] had suggested that it was possible to separate the toxin from the human urinary kallikrein by ethanolic fractionation. It was thought that the low level of antibody to human urinary kallikrein might be due to its suppression by the toxic effects of the crude preparation. To date two rabbits (K-100 and K-200) have been immunized with the partially purified kallikrein prepared by ethanolic fractionation (29 FU/mg). Intraperitoneal injections of approximately 15 FU were at first given weekly and then increased to two or three times a week. This product evoked no discernible toxicity.

(*b*) *Pancreatic kallikrein.* Crude human pancreatic kallikrein (4 FU/mg) was used to immunize rabbit K-66; a partially purified human pancreatic kallikrein (20 FU/mg) was used for rabbit K-300. Both preparations were nontoxic to the rabbits.

Demonstration of antibody development. The changes in per cent γ-globulin of the serum of rabbits during immunization were determined by electrophoretic analyses by the Spinco paper strip technique. The per cent γ-globulin was calculated from Analytrol recordings of serum samples before immunization and of samples obtained at intervals during the course of inoculations. Because the enzyme preparations were not homogeneous, the increase in per cent γ-globulin gave no measurement of specific antibody to the kallikreins but merely showed that antibody to some antigenic material had been formed.

Precipitin reactions of the various sera were studied in agar gel by the Ouchterlony[11] plate technique. The position of the precipitin bands is recorded by the distance in millimeters from the antigen cup to the precipitin line as measured by a glass-etched millimeter scale placed below the petri dish (Fig. 1). As has been demonstrated by Darcy[12] and others, the ratio of antigen to antibody as well as time influences the rate of migration of the bands. The continuity of the precipitin bands, therefore, establishes their identity.

The development of antibody specific for the kallikrein was determined by measuring the capacity of the antiserum to inhibit the vasodilator activity of kallikrein. To 1·0 ml of a suitable dilution of rabbit antiserum in 0·1 M Tris buffer, pH 7·5, was added 0·5 ml of kallikrein containing 0·3 FU/ml, and the mixture was incubated for 2 hr at 37°. The residual active kallikrein was determined by its ability to cause increased blood flow after injection into the femoral artery of dogs.[13] Serum from normal Dutch rabbits also was capable of inhibiting human urinary and pancreatic kallikreins: a 1 : 10 dilution caused greater than 50% inhibition of the kallikrein activity. An increase of the inhibitor level over that shown by the normal preimmunization serum was therefore taken as evidence for the presence of antibody.

RESULTS

Development of antibodies. Serum samples of rabbits, which had been injected with crude and partially purified human urinary and pancreatic kallikreins, were examined

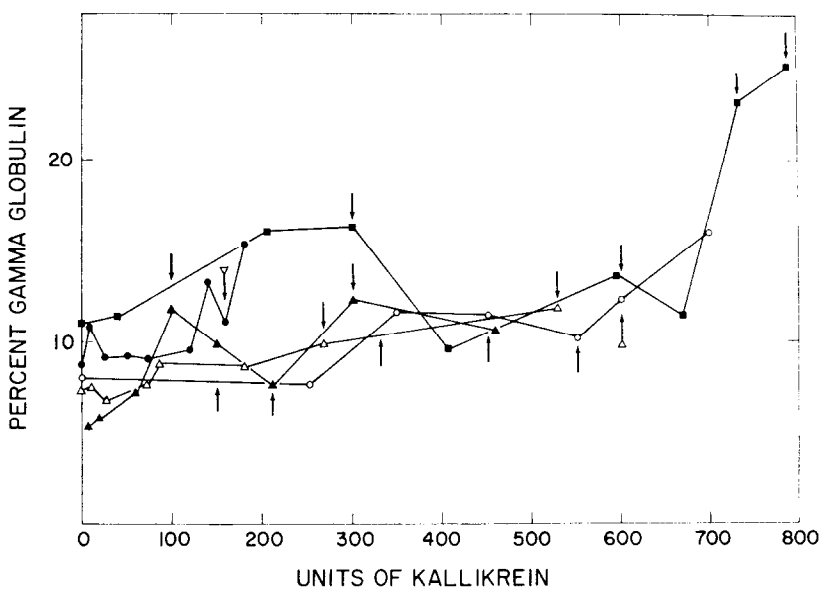

FIG. 2. Units of kallikrein injected and time of withdrawal of successive serum samples in rabbits immunized with crude and partially purified human kallikreins. The data are plotted against the per cent γ-globulin determined for each serum on the basis of the total protein content of the serum. ○ = Rabbit K-44, antibody to crude urinary kallikrein (6 FU/mg); ●, ▲ = rabbits -K100 and K-200, antibody to partially purified urinary kallikrein (29 FU/mg); ■ = rabbit K-66, antibody to crude pancreatic kallikrein (4 FU/mg); △ = rabbit K-300, antibody to partially purified pancreatic kallikrein (20 FU/mg); ↓ = antibody-antigen precipitins in agar. ⥮ = antibody-antigen cross reaction in agar, antibody to human urinary kallikrein and human pancreatic kallikrein as antigen.

at various intervals for increase in per cent γ-globulin, for the formation of precipitin lines in agar with homologous antigen, and for capacity to inhibit the vasodilator activity of kallikrein. Rabbit K-44, which by intraperitoneal injection received a crude, toxic preparation of urinary kallikrein (6 FU/mg) over a 7-month period, showed a gradual elevation of the per cent γ-globulin protein after the administration of a total of 250 FU (Fig. 2). The serum of this animal drawn 5 months after the onset of immunization or after the injection of 450 FU showed a single band in agar 6 mm from the antigen cup (Fig. 3). After a total of 600 FU had been administered, the serum of this rabbit with homologous antigen produced several wide diffuse bands in agar (Fig. 4, cup 1), showing the presence of multiple antigens in the crude kallikrein preparation. The identity of the band responsible for the union of kallikrein with its antibody could not be determined from this preliminary experiment. However, it seemed likely that antibody to the kallikrein had been formed, inasmuch as serum specimens from this rabbit throughout the period of immunization showed an increased ability to inhibit the vasodilator activity of kallikrein as compared to normal serum (Table 1).

TABLE 1. ABILITY OF RABBIT ANTISERUM TO INHIBIT THE VASODILATOR EFFECT OF HUMAN KALLIKREINS

Rabbit no.	Immunized with kallikrein derived from:	Index of purity (FU/mg)	Inhibition titer*
K-44	Urine	6	20
K-100		29	640
K-200		29	30
K-66	Pancreas	4	10
K-300		20	30
Control†			10

* Reciprocal of dilution of the most potent antisera giving greater than 50% inhibition of the kallikrein.

† Serum from normal Dutch rabbits or preimmunization serum.

Nontoxic, partially purified human urinary kallikrein (29 FU/mg) was administered intraperitoneally to rabbits K-100 and K-200. The serum of rabbit K-100 showed an abrupt rise in per cent γ-globulin after the administration of only 140 FU of kallikrein (Fig. 2). Nitrogen determinations on washed specific precipitates followed the standard precipitin curve in the presence of antibody excess, and the serum of this rabbit gave strong precipitin reactions in agar gel with homologous antigen (Fig. 6, cup 4). Rabbit K-200 showed a more gradual rise in per cent γ-globulin with successive inoculations. Precipitin reactions in agar gel obtained with serum from bleedings of this rabbit and homologous antigen, produced a maximum of three precipitin bands which appeared more slowly and were weaker than those formed by rabbit K-100. Appearance of precipitable antibody in agar was paralleled by increases in the ability of the serum to inhibit the vasodilator effect of kallikrein. Rabbit K-100 produced high levels of antibody so that its serum could be diluted 1 : 640 as compared to 1 : 10 for controls. On the other hand, serum from rabbit K-200 showed only a moderate rise in specific antibody (Table 1).

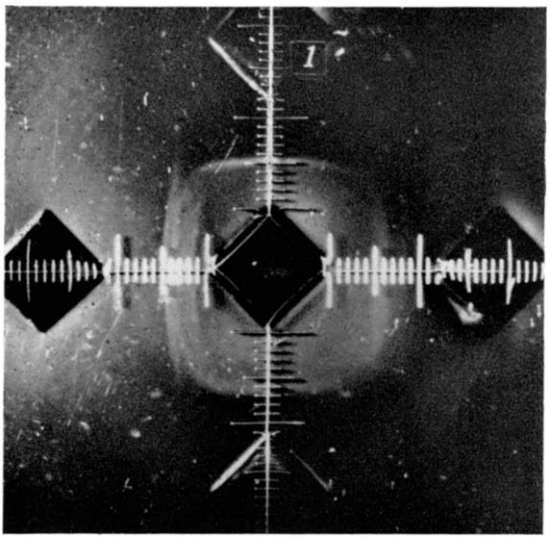

FIG. 1. Method of measuring distance from antigen to precipitin band, using glass plate etched with mm scale through which precipitin lines in Ouchterlony plate are observed. Position of precipitin band has a constant relationship to the distance from the antigen at any given dilution of the antisera.

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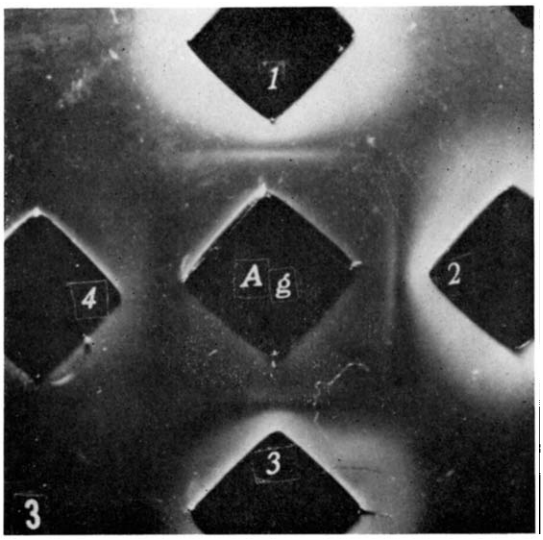

FIG. 3. Crude human urinary kallikrein (Ag) 7·5 FU in center cup with homologous antisera from rabbit K-44 undiluted, cup 1; 50% dilution, cup 2; 25% dilution, cup 3; control serum, cup 4. Photographed after 3 weeks in agar.

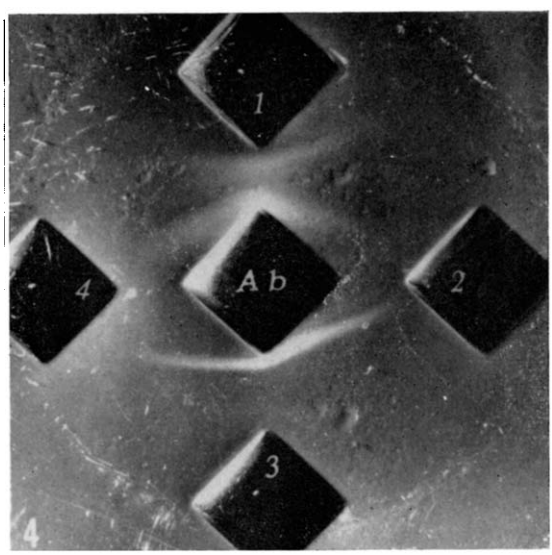

FIG. 4. Antisera (Ab) to crude human urinary kallikrein (6 FU/mg) in center cup, and homologous antigen 7·5 FU, cup 1; cross reaction with antibody to crude pancreatic kallikrein 7·5 FU, cup 3; cups 2 and 4 saline. Of the precipitin bands which formed between center cup and homologous antisera, two were at first visible, later three. Precipitin reactions with human pancreatic kallikrein (cup 3) at first gave wide diffuse band at 8 mm, this later appeared as two bands, one at 8 mm and one at 9 mm. Photographed after 10 days in agar; precipitin bands faintly visible at 5 days.

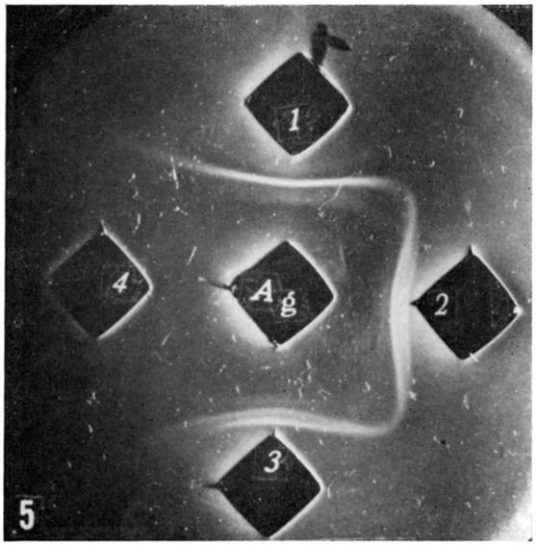

FIG. 5. Crude human pancreatic kallikrein, 7·5 FU, in center cup; cups 1, 2 and 3 contained homologous antisera in two-fold serial dilution; cup 4, control, normal serum. Cup 1, sharp band at 8 mm, faint one at 10 mm; cup 2, bands at 8, 9, and 10 mm; cup 3, bands at 8 and 10 mm; and cup 4, negative. Photographed after 19 days in agar.

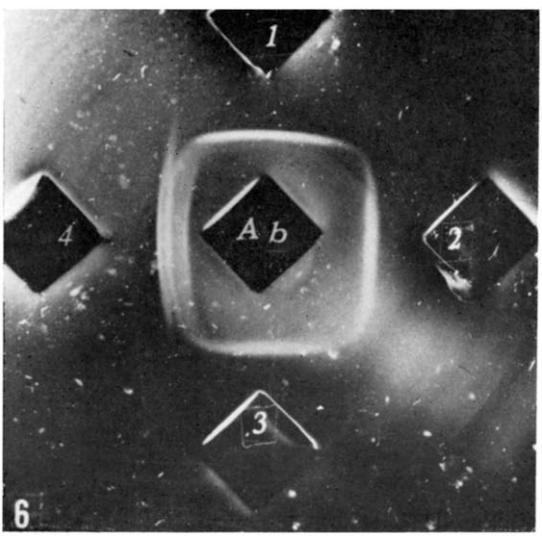

FIG. 6. Antibody to partially purified human urinary kallikrein, 29 FU/mg, in center cup; cup 1, 3·6 FU; cup 2, 1·8 FU; and cup 3, 0·9 FU of highly purified human urinary kallikrein (50 FU/mg). Cup 4, partially purified human urinary kallikrein; 5·2 FU, which precipitated with homologous antibodies in center cup resulting in three distinct bands at 5, 6, and 8 mm and a faint band at 7 mm. The band at 6 mm is continuous with the single 6-mm band obtained with highly purified kallikrein in cups 1, 2, and 3.

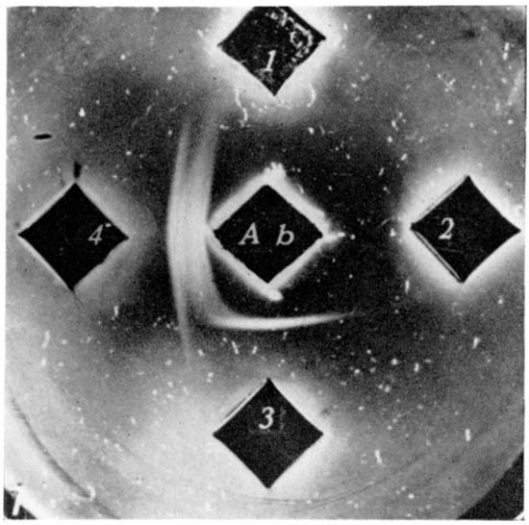

FIG. 7. Antibody to partially purified human urinary kallikrein, center cup; cup 1, dog urinary kallikrein, 3·6 FU; cup 2, highly purified hog pancreatic kallikrein, 3·6 FU; cup 3, highly purified human urinary kallikrein, 3·6 FU; cup 4, partially purified human pancreatic kallikrein, 3·6 FU. Neither crude dog urinary kallikrein nor highly purified hog pancreatic kallikrein crossed with antibody to human urinary kallikrein.

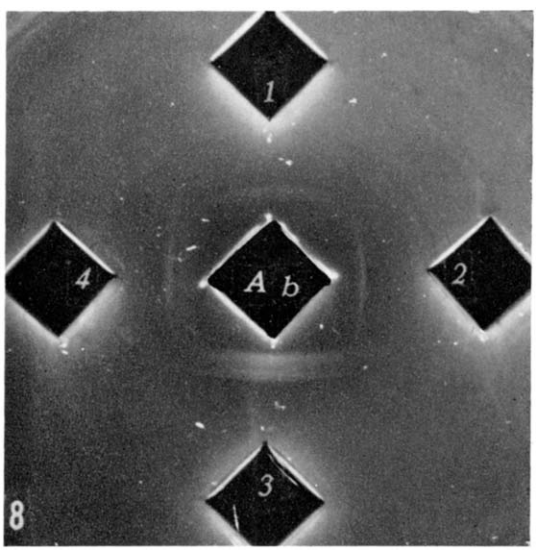

FIG. 8. Antibody to partially purified human urinary kallikrein, center cup; cup 1, "pure" human pancreatic kallikrein, 3·6 FU; cup 2, pure human urinary kallikrein, 3·6 FU; cup 3, partially purified human urinary kallikrein, 3·6 FU; cup 4, partially purified pancreatic kallikrein, 3·6 FU.

After the administration of 300 FU of crude, nontoxic pancreatic kallikrein (4 FU/mg), the serum of rabbit K-66 was found to contain antibodies which precipitated with homologous antigen. A partially purified pancreatic kallikrein having 20 FU/mg, administered to rabbit K-300, required 250 FU to obtain similar results. The sera of rabbits receiving either crude or partially purified pancreatic kallikrein produced a single precipitin band at 8 mm from the antigen cup during early development of the antibody; but as immunization continued, multiple bands appeared (Fig. 5). Despite its production of strong precipitin bands in agar (Fig. 5), serum from rabbit K-66 failed to show an increase in inhibitor level over that given by normal serum (Table 1). On the other hand, antiserum from rabbit K-300 showed a moderate formation of antibody as measured by this technique.

Discrimination between antibody and normal inhibitor. The foregoing data indicate that antibody to the kallikreins had been produced. However, since normal serum at a 1 : 10 dilution contains an inhibitor that will effectively block the vasodilator activity of 0·1 FU of kallikrein, antibody to the kallikrein could be measured only when inhibition was greater than that produced by the preimmunization serum. The possibility was recognized that, instead of antibody, the rabbits had been stimulated to produce greater amounts of the normal inhibitor to the kallikrein. Therefore, methods were sought to distinguish between antibody and the normal inhibitor. Heating the antiserum for one-half hour at 56° did not alter its ability to inhibit human urinary kallikrein. Also this procedure failed to destroy completely the normal inhibitor in rabbit serum even when the time was extended to 3 hr. Treatment of the serum with 20% acetone for 4 hr at room temperature, as used for activation of human plasma kallikrein,[8] destroyed the normal inhibitor in rabbit serum but retained to a significant extent the inhibition by antibody (Table 2). Similarly, adjusting the serum to pH 3·0

TABLE 2. DESTRUCTION OF NORMAL INHIBITOR TO HUMAN URINARY KALLIKREIN AND RETENTION OF ANTIBODY

Sera	Dilution	% inhibition after treatment			
		None	Acetone*	pH 3·0†	TAMe‡
Preimmunization (normal)	1:5	>50	0	0	0
	1:10	>50			
	1:20	34			
After immunization (rabbit K-100)	1:80	>50	>50	>50	>50
	1:160	>50	34	45	>50
	1:320	30	7	18	12

* To 1·0 ml of serum was added 0·25 ml acetone and the mixture allowed to stand at 24° for 4 hr.

† The serum was adjusted to pH 3·0 with dilute HCl and stored for 10 min at 37°, then readjusted to pH 7·5.

‡ Measurements were made in presence of 0·02 M TAMe (*p*-toluene sulfonyl-L-arginine methyl ester).

and storing for 10 min at 37° (cf., Gaddum and Horton[14]) also was effective in eliminating the normal inhibitor with retention of antibody. Previous studies[4] have shown that the inhibitor in human plasma could be blocked by the action of various arginine esters. In the present work, 0·02 M TAMe also was found capable of preventing the action of the normal inhibitor in rabbit sera, but had no effect on the union of

antibody and antigen (Table 2). These three methods of interfering with the normal inhibitor permitted the discrimination between its action and the specific inhibition by antibody; the findings leave little doubt that antibody to the kallikreins had been produced.

Treatment of the serum with acetone or by adjustment of the pH to 3·0 was not appropriate for the detection of small concentrations of antibody. Acetone itself, at low dilutions, can cause vasodilation in the dog and, in those experimental animals which are least sensitive to the action of kallikrein, the presence of acetone tends to obscure the experimental results. Treatment of normal rabbit sera at pH 3·0 to destroy the inhibitors simultaneously causes the activation of small quantities of a substance capable of increasing blood flow in the dog. This latter substance, presumably rabbit serum kallikrein, can cause interference with the dog bioassay, particularly at a 1 : 2 dilution of rabbit serum. For these reasons measurement of antibody in the presence of TAMe provided the most convenient method for the detection of small concentrations of specific antibody. TAMe exerts some effect on the vasodilator activity of the added kallikrein, and the concentration of rabbit serum proteins can modify these results. However, in the presence of TAMe, the vasodilator activity furnished by a dilution of normal rabbit serum compared to that given by the same dilution of immunized rabbit sera can provide an estimate of trace amounts of antibody. For example, a 1 : 2 dilution of antiserum from rabbit K-66, which by the previous method had failed to show detectable antibody, now gave greater inhibition of the kallikrein than normal serum.

Cross reaction between antibody to human urinary kallikrein and the other kallikreins. Human urinary kallikrein and hog pancreatic kallikrein have been differentiated by Werle *et al.*[3] as regards their inhibition by egg white. We have confirmed that human urinary kallikrein is readily inactivated by egg white, and found that human pancreatic kallikrein is only slightly affected. These observations suggest that human urinary and pancreatic kallikreins differ in structure. It was therefore possible that antibodies to the kallikreins were specific inhibitors to their homologous antigens. However, as shown in Table 3, human pancreatic kallikrein gave the same pattern of inhibition with

TABLE 3. ABILITY OF NORMAL AND IMMUNE RABBIT SERA TO INHIBIT THE KALLIKREINS

Kallikreins derived from:	Normal serum		Immune serum*	
	Dilution	Inhibition (%)	Dilution	Inhibition (%)
Human urine	1:10	>50	1:640	>50
	1:20	31	1:1,280	35
Human pancreas	1:10	>50	1:640	>50
	1:20	34	1:1,280	40
Dog urine	1:80	>50	1:80	>50
	1:160	48	1:160	41
Hog pancreas	1:20	>50	1:20	>50
	1:40	44	1:40	47
	1:80	29	1:80	34

* Rabbit K-100 immunized with partially purified human urinary kallikrein.

normal and immune sera as was obtained with human urinary kallikrein. In contrast, the kallikreins derived from other species—i.e. dog urinary kallikrein and hog pancreatic kallikrein—were not inhibited by antibody to human urinary kallikrein, as similar dilutions of normal and immunized rabbit serum inhibited the kallikreins to the same extent. Dog urinary kallikrein was much more readily inhibited by normal rabbit serum than were the other kallikreins. The failure of antibody to inhibit the biological activity of kallikrein from species other than man was paralleled in agar gel tests by the absence of precipitin bands when dog urinary and hog pancreatic kallikreins were tested with antiserum from human urinary kallikrein (Fig. 7, cups 1 and 2). On the other hand, either crude (Fig. 4, cup 3) or partially purified (Fig. 7, cup 4) human pancreatic kallikrein gave multiple precipitin bands with this antiserum. These data show clearly that multiple antigens common to both kallikreins are present in the partially purified kallikrein preparation. One or more of these common antigens may be present in normal human serum. However, reaction of this antiserum with human serum as the antigen showed the presence of only one faint band at approximately 3 mm from the antigen cup and this only in the cup containing undiluted serum. There appeared to be no continuity between this band and the bands formed by reaction of homologous antigen.

Identification of reaction between kallikrein and its antibody in agar gel. The multiple bands which appeared in cross reactions between human pancreatic kallikrein and antibody to partially purified urinary kallikrein prevented identification of the specific kallikrein-antikallikrein band. Therefore, more highly purified kallikrein preparations were examined for their ability to precipitate with antisera. As shown in Fig. 6 (cups 1, 2, 3), a preparation of human urinary kallikrein at 50 FU/mg gave only one precipitin band at 6 mm from the antigen cup. This band merged with a band at 6 mm which

TABLE 4. ABILITY OF ANTIBODY TO INHIBIT TAME ACTIVITY OF HUMAN URINARY KALLIKREIN*

Serum	% Inhibition of kallikrein activity as measured by TAMe	Vasodilation
Normal	0	0
Immune	36	98

* Sera were treated at pH 3·0 as described in Table 2. Then 0·4 ml of rabbit serum was mixed with 6 FU human urinary kallikrein in 0·033 M Tris buffer, pH 7·5, containing 0·85% NaCl (final volume 1·5 ml), and incubated for 2 hr at 37°. Values are corrected where necessary for esterase activity of serum.

formed with homologous antigen (Fig. 6, cup 4). In another experiment, this same single precipitin band (Fig. 7, cup 3) also joined with one or more of the bands that formed with partially purified pancreatic kallikrein (Fig. 7, cup 4). However, positive association of this band with the enzyme could not be made until pure kallikrein preparations were available. As shown in Fig. 8, "pure" human pancreatic kallikrein (250 FU/mg; cup 1) and pure human urinary kallikrein (430 FU/mg; cup 2) formed single precipitin bands with antibody to partially purified human urinary kallikrein which were continuous and the same distance from the antigen cup. These single bands

joined by the kallikreins probably represent the kallikrein-antikallikrein reaction and are in contrast to the multiple bands formed with the partially purified kallikreins (cups 3, 4).

Ability of antibody to cause inhibition of esterolytic activity of kallikrein. Evidence has been presented to suggest that the kallikreins are capable of digesting N-substituted arginine esters.[4] Measurements were therefore made of the ability of antibody to inhibit the hydrolysis of the synthetic substrate TAMe by human urinary kallikrein. As is shown in Table 4, normal rabbit serum, which had been treated at pH 3·0 to destroy normal inhibitor, failed to cause inhibition of kallikrein activity as measured by TAMe assay or by vasodilation in the dog. Serum from immunized rabbits, on the other hand, produced inhibition as judged by both methods of assay; antibody inhibited 36% of the TAMe activity and 98% of the vasodilator activity.

DISCUSSION

The kallikreins are present in most mammalian tissues and are especially abundant and readily demonstrated in urine, pancreas, plasma, and saliva, and throughout the intestinal tract. The kallikreins, either directly or by enzymatic release of kallidin, are known to exert at least five pharmacological effects—smooth muscle stimulation, vasodilation, increase in capillary permeability, migration of leukocytes, and stimulation of pain fibers.[15] They were thought originally to be one proteinase, but evidence has recently been obtained showing that the kallikreins vary in their susceptibility to proteolytic inhibitors and, therefore, in structure. All the kallikreins, however, exert their pharmacological activity by enzymatic action on kallidinogen, an α_2-globulin in plasma, to release the polypeptides, kallidin I and II. Recent investigations[16] have indicated that kallidin I is identical with the nonapeptide bradykinin, which has the sequence H.Arg.Pro.Pro.Gly.Phe.Ser.Pro.Phe.Arg.OH,[17] and that kallidin II is a decapeptide differing from kallidin I only by having an additional N-terminal lysine residue. Whether all kallikreins release the same mixture of peptides is not known. However, both peptides have similar biological activities.

The present studies have shown that human urinary and pancreatic kallikreins are antigenic for rabbits. Precipitin bands obtained with the antisera in agar gel attest to the antigenic heterogeneity of the partially purified kallikreins used for immunization of the rabbits. These multiple bands presumably due to the presence of other proteins in the kallikrein preparations, do not interfere with the formation of the precipitin band between kallikrein and its antibody or with the inhibitory effect of antisera on the kallikrein as measured by bioassay. When a preparation of pure human urinary kallikrein was tested in agar gel against antibody to partially purified urinary kallikrein, a single band was formed, thus tentatively establishing the identity of the line of precipitation responsible for the union of kallikrein with its antibody. The antibody produced to human urinary kallikrein readily inhibited the vasodilator activity of human pancreatic kallikrein. Furthermore, "pure" human pancreatic kallikrein formed a single precipitin band which merged with the band formed by pure human urinary kallikrein.

Although variations to inhibitors between the kallikreins derived from urine and pancreas were reported as early as 1954, the possibilities of species variation of the kallikreins has not been recorded. Human urinary and hog pancreatic kallikrein have been differentiated by the failure of the pancreatic kallikrein to be inhibited by egg

white.[3] We have confirmed these observations and have noted that human pancreatic kallikrein is only weakly inhibited by egg white. Recently Werle has found (personal communication) that the bovine parotid lymph gland inhibitor (Trasylol) inhibits human urinary and hog pancreatic kallikrein but not dog urinary kallikrein. We have noted that human pancreatic kallikrein also is readily inhibited by Trasylol. Antibody to human urinary kallikrein, on the other hand, failed to inhibit the vasodilator activity of either dog urinary or hog pancreatic kallikrein and also failed to give cross reaction in agar gel diffusion tests. It appears likely, therefore, that whether derived from from different sources or from different species, the structure of each kallikrein differs significantly.

The normal inhibitor in rabbit serum can readily be differentiated from antibody, since antibody is stable to acetone and to acid treatment of the sera and is not inhibited competitively by TAMe. Since the kallikreins are capable of digesting this and other arginine esters,[4] the failure of substrate to prevent the union of antibody and antigen suggests that this antibody inhibits in a noncompetitive manner. Although antibody readily inhibited the vasodilator activity of human urinary kallikrein, only moderate inhibition of its esterolytic activity was found with TAMe. Antibody, therefore, is a more potent antagonist for the large molecular weight substrate (kallidinogen) found in the dog than for the smaller ester (TAMe). These data are in agreement with the observations of Cinader[18] who noted that enzymes with substrates of large molecular weight are more strongly inhibited by antibody than are enzymes with substrates of small molecular weight. These observations, together with the fact that antibody inhibits both urinary and pancreatic kallikreins, constitute evidence for one of the mechanisms postulated by Cinader[18]; namely, that antibody is not oriented solely to the enzymatically active center of the molecule but is near to it and consequently produces steric hindrance.

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