The Resistance of Fibrinogen and Soluble Fibrin Monomer in Blood to Degradation by a Potent Plasminogen Activator Derived From Cadaver Limbs

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The effect of a cadaver-derived vascular plasminogen activator (VA) on the degradation of fibrinogen, soluble fibrin monomer, and fibrin was studied and compared with the effect of equivalent fibrinolytic potencies of streptokinase (SK), urokinase (UK), and plasmin. The proteolytic activity of the three activators and plasmin was determined by a standard fibrin plate assay and was expressed in CIA units from a UK reference curve. Fibrinogen degradation was measured by clottable protein determinations and by an electrophoretic technique sensitive to small changes in the molecular weight of fibrinogen. When VA was incubated in plasma, no degradation of fibrinogen occurred, whereas rapid fibrinolysis took place after the plasma was clotted. By contrast, equivalent potencies of SK, UK, and plasmin caused extensive fibrinogenolysis. Since the plasmin added and that formed by the three activators had equivalent fibrinolytic activity, the failure of VA to induce fibrinogen degradation was attributed to antiactivators rather than antiplasmins. VA activity in plasma was consumed by clotting, whereas the antiactivator activity remained in the serum, suggesting dissociation of the VA-antiactivator complex on the fibrin clot. Fibrinogen and its soluble derivatives resisted degradation by VA in plasma because a solid phase appeared necessary for the complex to dissociate. The findings indicated that the degradation of fibrinogen or soluble fibrin in blood as a result of plasminogen activation by VA was unlikely to occur due to a large excess of antiactivator activity. Alternative pathways for their catabolism are discussed.

The degradation of fibrinogen utilizing plasmin or plasminogen activators has been studied by numerous investigators. Streptokinase (SK) and urokinase (UK) are the only activators commercially available in purified form and, therefore, have been the most commonly used. Despite certain differences in the mechanism of plasminogen activation, the final proteolytic effect of all activators is generally considered equivalent since it is dependent on plasmin elaboration. Nevertheless, significant physiologic differences in the specificity of plasminogen activators may be caused by certain inhibitors found in blood which are not present when fibrinogen degradation is studied in a purified system. Fibrinogenolysis is readily induced by SK and UK both in vitro and in vivo. Little is known, however, about the effect of naturally occurring plasminogen activator in blood on circulating fibrinogen. The purpose of this study was to evaluate the effect of a cadaver-derived vascular plasminogen activator (VA) on the degradation of fibrin, fibrinogen, and soluble fibrin monomer complexes. The results were compared with those obtained with...
equivalent fibrinolytic potencies of SK, UK, and plasmin. The VA was obtained from cadaver limbs by a perfusion method described by Aoki and von Kaulla. The VA was obtained from cadaver limbs by a perfusion method described by Aoki and von Kaulla. Plasma electrophoresis, using a technique whereby small changes in the molecular weight of fibrinogen could be measured directly, was used to determine fibrinogen degradation.

MATERIALS AND METHODS

Cadaver-derived vascular plasminogen activator (VA) was obtained from three adult cadavers by the limb perfusion method of Aoki and von Kaulla. The extractions were performed 18-24 hr after death. Cannulae were introduced and secured in the femoral artery and vein. The limb was perfused with 500 ml of 0.85% saline. By the end of the perfusion, the venous outflow was virtually clear of blood. A second perfusion with 500 ml of barbital-buffered saline (pH 7.42, 54°C) was started. After 100 ml had been introduced, the venous outflow was clamped, and the remainder of the solution was allowed to run into the limb. Fifteen minutes after the completion of this step, the venous cannula was opened and the contents drained into a collecting flask. The limb was perfused with an additional 1000 ml 0.85% saline. Immediately after collection, the cellular elements were removed by centrifugation (8000 g for 30 min) in the cold. The protein content of the supernatant was 1.1-3.2 mg/ml. The extract containing the plasminogen activator was obtained by fractionation of the supernatant in the cold with ammonium sulfate to 45% saturation. The mixture was centrifuged for 30 min (8000 g, 0°C) and the precipitate suspended in 10 ml 0.85% saline. The preparation was dialyzed overnight in the cold against 0.06 M Tris, 0.09 M NaCl buffer, pH 7.4. The final product did not induce lysis of heated, plasminogen-free fibrin plates (Hyland Laboratories, Costa Mesa, Calif.)

Streptokinase (SK) (Streptase, Hoechst Pharmaceuticals, Somerville, N.J.) was made up in 0.85% saline.

Urokinase (UK) prepared from human urine (ICN Nutritional Biochemicals, Cleveland, Ohio, containing 5000-10,000 Ploug U/mg) was made up in 0.85% saline.

Plasmin (Novo Industri A/S Copenhagen, containing 3.4 Novo U/mg) was a trypsin-activated pig plasmin which has been shown to be free of trypsin activity.

A plasminogen-free purified bovine preparation of thrombin (Sigma Chemical Co., St. Louis, Mo.) was used and made up in 0.85% saline. Unless otherwise stated, the concentration used was 100 NIH U/ml.

Hirudin (Veb Arzneimittelwerke, Dresden, E. Germany) was made up in 0.85% saline to contain 10 U/ml.

Protamine sulfate, 1% (Eli Lilly Co., Indianapolis, Ind.), was used in the concentrations described below.

Euglobulin clot lysis (ECL) was performed according to the method of Kowalski et al.

Platelet-poor plasma (PPP) was obtained from blood collected from five normal donors into 3.8% sodium citrate (9:1) and centrifuged for 5 min (3000 g). Following centrifugation, the plasma was pooled and stored at -20°C in plastic test tubes.

Plasma clot lysis time was performed on mixtures of 1.0 ml PPP, 0.2 ml activator, and 0.1 ml thrombin. The time of complete clot lysis after incubation at 37°C was recorded.

Fibrin plate assay for the determination of fibrinolytic activity was modified from Astrup and Müllertz. Human fibrinogen (grade L, AB Kabi, Stockholm, containing plasminogen and factor XIII), 150 mg per 100 ml of barbital-acetate-buffered saline (0.005 M sodium acetate, 0.005 M sodium barbital, 0.15 M sodium chloride, pH 7.42), was used in the preparation of the plates. The same batch of fibrinogen was used for all experiments. This fibrinogen contained plasminogen in sufficient excess to enable activator activity to be measured. 0.03 ml of VA, SK, UK, or plasmin was placed on the plate in triplicate, and perpendicular lysis zone diameters were measured after 18 hr incubation at 37°C. The area of lysis of each zone was determined by multiplying the perpendicular diameters.

Determination of Fibrinolytic Potency in CTA Units

A reference curve of fibrinolytic activity was constructed utilizing UK in concentrations from 1 to 500 Ploug U plotted against the corresponding fibrin plate lysis areas. Ploug units were con-
PLASMINOGEN ACTIVATOR

converted to CTA (Committee on Thrombolytic Agents) units using a conversion factor of 1.25. The fibrinolytic potency of VA, SK, UK, and plasmin used in the experiments described below was determined by measuring the fibrin plate lysis area obtained and then finding the corresponding CTA units from the UK reference curve.

Fibrinogen

Fibrinogen was determined by a modification of the thrombin-clottable protein method of Swaim and Feders. Aprotinin, 0.1 ml (Trasyol, FBA Pharmaceuticals, N.Y.), was added to each sample before clotting with 1 ml thrombin-calcium solution (thrombin, 50 NIH U/ml in 0.15 M CaCl₂).

Electrophoresis

Sodium dodecyl sulfate polyacrylamide electrophoresis utilizing 3.5% gels was performed according to the method previously described. Densitometric scans of the stained gels were performed (Densicord Model 542A, equipped with an integrator, Photovolt, N.Y.).

Preparation of Soluble Fibrin Monomer

Thrombin (0.03 ml, 10 NIH U/ml) was added to 10 ml PPP. After 5 min incubation (37°C), 0.2 ml hirudin was added. The presence of fibrin monomer was determined by the addition of protamine sulfate according to the semiquantitative, serial dilution method previously described using a 30-min period of incubation.

Effect of Incubation on VA-induced Fibrinolytic Activity in Plasma

Plasma clot lysis times were determined before and after 18 hr of incubation (37°C) in order to evaluate the loss of VA-induced fibrinolytic activity during this period of time. A series of three experiments was performed in which 1.0-ml aliquots of PPP were mixed with equal volumes of VA (200 CTA U/ml). Thrombin, 0.1 ml, was added to three aliquots immediately and to an additional three aliquots after 18 hr of incubation. Clot lysis times were determined.

Determination of VA, SK, UK, and Plasmin Activities in Plasma on the Substrates Fibrinogen, Soluble Fibrin Monomer, and Fibrin

The potencies of the activators and plasmin expressed as CTA units per milliliter which were used in these experiments ranged as follows: VA, 1–1000; SK, 5–1000; UK, 5–250; and plasmin 5–50. One milliliter of the activator or plasmin solution was added to equal volumes of PPP or PPP containing soluble fibrin monomer. The fibrinogen content of each mixture was determined initially and after 18 hr incubation (37°C). To additional 1-ml aliquots of PPP, 0.1 ml thrombin was added simultaneously with the activators or plasmin. The protein content of the fibrin clot formed was determined immediately, and in other aliquots it was determined after 18 hr incubation. The solutions containing fibrin monomer were additionally tested with the semiquantitative serial dilution protamine sulfate method. Each of the experiments was done in triplicate.

The electrophoretic pattern of fibrinogen in the PPP before and after incubation was examined. VA, SK, UK, or plasmin (200 CTA U/ml, final concentration) was added to the PPP before incubation. For this experiment, blood was collected into heparin (10 U/ml) as well as citrate. A single potency (200 CTA U/ml) of VA, SK, UK, and plasmin was used in these experiments. The clot lysis time and euglobulin lysis time of the mixtures were determined before incubation.

The Effect of VA, SK, UK, and Plasmin on the Degradation of Fibrin and Fibrinogen in Saline

Fibrin clots were made up from 1 ml human fibrinogen (Kabi, Stockholm, Sweden; 2 mg/ml in barbital-buffered saline) and 0.1 ml thrombin to which 0.2 ml of activator or plasmin solution ranging in potency from 5 to 200 CTA U/ml was added simultaneously. The clots formed were incubated (37°C) and the time of complete lysis recorded. Each experiment was done in triplicate.

Ten milliliters of human fibrinogen solution (2 mg/ml in barbital-buffered saline), 1.0 ml normal saline, and 2.0 ml of activator or plasmin solution of sufficient potency to give a clot lysis time of 15–20 min were mixed and incubated (37°C). One-milliliter aliquots were removed at intervals
and the fibrinogen content determined. The incubation time required for complete fibrinogenolysis (no clottable protein remaining) was recorded.

**Activator or Plasmin-induced Fibrinolytic Activity in Plasma, Serum, and Lysate**

To 1-ml aliquots of PPP, 0.5 ml solution of VA, SK, UK, or plasmin (200 CTA U/ml) plus 0.1 ml saline was added (final concentration, 62 CTA U/ml) and the fibrinolytic activity of the mixture determined on fibrin plates. To an identical series of samples, 0.1 ml thrombin was added instead of saline. The fibrin clot was wound out and fibrinolytic activity of the serum determined by the fibrin plate assay. In a third series, the clots formed after addition of thrombin were incubated (37°C) until complete clot lysis had occurred. The fibrinolytic activity of the lysate was then determined. Each set of experiments was performed in triplicate.

In addition, the fibrinolytic activity remaining in the supernatant after precipitation of plasma fibrinogen with protamine sulfate (0.2 ml) and after paracoagulation of fibrin monomer by the addition of protamine sulfate (0.2 ml of a 1:10 saline dilution) was also determined. This experiment was performed with VA only.

**Estimation of Fibrinogenolysis Inhibitor Content of Plasma and Serum**

For these experiments, PPP and serum from the same donor were used. Serum was obtained from whole blood which was collected into glass test tubes and incubated (37°C) for 2 hr. Dilutions of PPP and serum (1:10-1:100) were made up with barbital-buffered saline. One-tenth milliliter fibrinogen (3.4%) was added to 0.9 ml of the PPP and serum dilutions as well as to 0.9 ml barbital-buffered saline. Two-tenths milliliter VA, SK, UK, and plasmin (100 CTA U/ml) was added to each of the mixtures and incubated (37°C) for 2 hr. At the end of this time, the fibrinogen content of each sample was determined. Each experiment was performed in triplicate.

**RESULTS**

**Determination of Fibrinolytic Potency**

The UK-induced fibrin plate lysis zones ranged from 120 sq mm (1 CTA U/ml) to 2000 sq mm (500 CTA U/ml). When the lysis zones were plotted on log-log paper against the corresponding UK activity in CTA units, a straight line was obtained. This UK curve was used as the standard reference to determine the fibrinolytic potency in CTA units of the various activator or plasmin solutions used in the experiments. In each case, the fibrin plate lysis zone was measured, and the corresponding CTA units were taken from the reference curve.

When dilutions of the VA preparations ranging from undiluted to 1:1,000 were plotted on log-log paper against the corresponding fibrin plate lysis zone obtained at each dilution, a straight line was obtained. However, the slope of these lines was not parallel to the UK curve. The three undiluted VA preparations ranged in activity from 100 to 1000 CTA U/ml.

**Effect of Incubation on VA-induced Fibrinolytic Activity in Plasma**

Incubation (37°C) of plasma containing VA (200 CTA U/ml) resulted in a slight loss of fibrinolytic activity, the plasma clot lysis time increasing from a mean initial value of 10 min (range, 8-14) to 45 min (range, 40-48) after 18 hr incubation.
Effect of VA, SK, UK, and Plasmin on Fibrinogen, Soluble Fibrin Monomer, and Fibrin Degradation in Plasma

Incubation with VA failed to result in any change in the clottable protein content of plasma or plasma containing soluble fibrin monomer. The latter gave a gel with protamine sulfate at a 1:40 dilution initially and again after 18 hr incubation. In contrast to this lack of effect on fibrinogen and soluble fibrin monomer in all the experiments conducted, VA induced complete lysis of fibrin even at the lower range of potencies used (5 CTA U/ml) (Fig. 1).

Incubation with SK resulted in loss of almost half the clottable protein in plasma and in plasma containing soluble fibrin monomer at a mean potency of 300 CTA U/ml and all of it at 1000 CTA U/ml. There was associated reduction (1:10 gel) and finally loss of the protamine sulfate paracoagulation reaction with fibrin monomer. Fibrin clot lysis occurred at a mean SK potency of 150 CTA U/ml (Fig. 1).

Incubation with UK caused complete loss of clottable protein in plasma containing soluble fibrin monomer at a mean potency of 188 CTA U/ml and in plasma itself at 250 CTA U/ml. Fibrin clot lysis occurred at a mean potency of 62 CTA U/ml of UK (Fig. 1).

**Fig. 1.** The degradation of fibrinogen, fibrin monomer, and fibrin in plasma by increasing amounts of activators and plasmin. The fibrinogen concentration was determined after 18 hr of incubation at 37°C. Fibrin was most sensitive to degradation by VA, whereas fibrinogen and fibrin monomer were not digested by this activator.
Incubation with plasmin caused degradation of fibrinogen and fibrin monomer into unclottable and unparacoagulable fragments at a potency of 44 CTA U/ml. Complete fibrin clot lysis occurred at the same potency (Fig. 1).

Changes in the Electrophoretic Pattern of Plasma Fibrinogen
After Incubation With VA, SK, UK, and Plasmin

The electrophoretic pattern of clottable protein in the plasma before incubation consisted of a high-molecular-weight (HMW) and lower-molecular-weight (LMW) band as previously described. A nonclottable protein was seen migrating in the region of the HMW fibrinogen which was subtracted before calculating the content of this fibrinogen fraction. After 18 hr incubation with VA (200 CTA U/ml), the electrophoretic pattern remained the same. Densitometric scans of the gels showed no quantitative change in the areas corresponding to the HMW and LMW fibrinogen. Measurement of fibrinolytic activity of this plasma gave a euglobulin clot lysis time of 3 min and a plasma clot lysis time of 10 min (Figs. 2 and 3A).

Incubation of plasma with SK and UK (200 CTA U/ml) resulted in degradation of fibrinogen into two major lower-molecular-weight fragments. Eoglobulin clot lysis times were 11 min and 9 min, respectively. Plasma clot lysis times were 14 min and 12 min for the SK- and UK-containing samples (Figs. 2 and 3B).

Incubation of plasma with plasmin (200 CTA U/ml) resulted in extensive proteolysis, including apparent degradation of the unclottable protein migrating in the region of the HMW fibrinogen (Figs. 1 and 2B).
**Fig. 3.** (A) Densitometric scans of gels of plasma before (0 hr) and after (18 hr) incubation with VA. The corresponding serum is shown, and the fibrinogen bands are identified (hatched area). A major protein (α2-macroglobulin) migrates in the region of the higher-molecular-weight fibrinogen band. (B) Densitometric scans of plasma after 18 hr incubation with SK, UK, and plasmin. The point of origin is aligned with (A). SK and UK caused degradation into two major lower-molecular-weight fractions. Plasmin caused extensive proteolysis of fibrinogen. There was also loss of the α2-macroglobulin band.

The Effect of VA, SK, UK, and Plasmin on the Degradation of Fibrinogen and Fibrin in Saline

The mean CTA units of activator required to give a fibrin clot lysis time of 15–20 min were as follows: VA, 20; SK, 5; UK, 50; and plasmin, 1. The mean times required for lysis of all fibrinogen in saline using these amounts of the activators were 120, 60, 45, and 20 min, respectively (Table I).

Activator or Plasmin-induced Fibrinolytic Activity in Plasma, Serum, and Lysate

The fibrin plate lysis zones indicated the following mean fibrinolytic activity (CTA U/ml) in the plasma samples containing the activators: VA, 58; SK, 54; UK, 60; and plasmin, 45. After clotting with thrombin, the serum fibrinolytic activity (CTA U/ml) was as follows: VA, 5; SK, 2; UK, 2; and plasmin, 15. Following lysis of the clots, the lysate fibrinolytic activity was VA, 60; SK, 65; UK, 80; and plasmin, 45 CTA U/ml.

The supernatants from the PS-precipitated plasma and the PS-paracoagulated sample with VA added contained 5 and 2 CTA U/ml, respectively.

**Table 1. Activator-induced Fibrinolysis Versus Fibrinogenolysis in Saline**

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<th>Fibrin Clot Lysis (min)</th>
<th>Fibrinogen Lysis (min)</th>
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<td>VA</td>
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<td>Plasmin</td>
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In the saline-fibrinogen mixtures, no clottable protein remained after 2 hr incubation with VA, SK, UK, or plasmin.

When the saline was replaced by the diluted plasma or serum, fibrinogen degradation was inhibited, and no loss of clottable protein occurred after 2 hr incubation with VA, even at a dilution of 1:100 of either plasma or serum. By contrast, complete fibrinogenolysis occurred with SK at plasma or serum dilutions greater than 1:10. Incubation with UK resulted in a 50% loss of fibrinogen at a plasma dilution of 1:60 and a serum dilution of 1:50. At a 1:100 dilution, 75% loss of fibrinogen occurred in plasma and 95% loss in serum. Incubation with plasmin resulted in lysis of all fibrinogen even in the lowest dilution of plasma or serum.

**DISCUSSION**

The activators of plasminogen are specific proteinases responsible for the conversion of plasminogen to the enzyme plasmin. They are present in many tissues as well as blood, urine, and certain microorganisms. Plasminogen activators from various human sources have been shown to be immunologically similar, the largest concentration of human activator being located in the endothelium of small blood vessels. This vascular plasminogen activator is believed to be the principal source of the plasminogen activator found in blood. Although vascular plasminogen activator is considered an important regulator of intravascular fibrinolytic activity, little is known about its biologic properties. After death, this activator is responsible for the fibrinolytic activity of postmortem blood.

The plasminogen activator obtained from the cadaver limb perfusate is believed to be derived primarily from the vascular endothelium, although contamination with extravascular tissue activator cannot be excluded. No attempt was made to purify the activator, since Aoki and von Kaulla found that this results in a substantial loss of activity. It is, therefore, quite possible that the VA used in this study was not derived from a single source. The principal aim of the study was to examine certain properties of the most potent human plasminogen activator activity that could be extracted from cadavers by this method.

A serum inhibitor has been described which was shown by Aoki and Kawano to preferentially inhibit vascular activator over UK. A similar specificity of the plasma and serum inhibitor for VA was found in the present study. It is believed that the specificity of this antiactivator is largely responsible for preventing fibrinogen degradation by VA.

The enzymatic potencies of the three activators and plasmin were calculated on the basis of their activities on a standard plasminogen-rich fibrin plate. Their potencies expressed as CTA units were also found to be roughly equivalent where fibrinogen was the substrate in a purified system. It was only in plasma that major differences between VA and the other activators and plasmin were found.

The electrophoretic technique used has been shown to be highly reproducible.
and permits direct visualization and quantitation of the heterogeneous composition of fibrinogen in blood. Utilization of this method avoids the possibility that degradation of fibrinogen occurs in vitro during its isolation and purification. Analysis of several hundred plasma samples has shown that plasma fibrinogen invariably consists of two major fractions differing slightly in molecular weight but not clottability. A nonclottable protein which has been identified as \( \alpha_2 \)-macroglobulin migrates in the region of the HMW fibrinogen. Subunit chain analysis showed that the LMW component had the characteristics of a derivative of HMW fibrinogen caused by limited plasmin degradation. We were unable, however, to demonstrate conversion of HMW to LMW fibrinogen by naturally induced fibrinolytic activity occurring after venous occlusion, physical exercise, or death. Similarly, in the present study, VA failed to induce any degradation of HMW or generation of LMW fibrinogen. By contrast, extensive degradation of fibrinogen was found and has been demonstrated in vivo in patients treated with SK or UK.

The enzymatic activity generated by VA in plasma was found to induce rapid lysis of fibrin or precipitated fibrinogen even at relatively low potencies, whereas it failed to degrade fibrinogen or soluble fibrin monomer at the highest potency used. No change in the clottable protein concentration in plasma occurred after 18 hr incubation with 1000 CTA U/ml of VA. By contrast, complete lysis of fibrin clots made from the same plasma occurred with as little as 5 CTA U/ml. More precisely, there was no change in the electrophoretic pattern of plasma fibrinogen after incubation with a concentration of VA sufficient to give a plasma clot lysis time of 10 min. These findings could not be explained by a rapid loss of proteolytic activity during incubation, since the activity was only slightly diminished at the end of the period of incubation. Neither could the absence of fibrinogenolysis be attributed to an artifact caused by citrate, since the same results were obtained in blood collected into heparin.

The presence of substantial fibrinogenolysis by equivalent potencies of SK, UK, and plasmin suggests that absence of a similar activity by VA in plasma is due to specific antiactivator activity rather than to antiplasmins. Antiactivator activity sufficient to prevent fibrinogen but not fibrin degradation by VA has been found in both serum and plasma even when they are diluted 100-fold with saline. Lower dilutions inhibit UK- and still lower dilutions inhibit SK-induced fibrinogenolysis, indicating a high degree of specificity of the blood anti-activator for VA.

Aoki and Kawano have shown that activator-inhibitor interactions are non-stoichiometric, suggesting a reversible inhibition. Our findings are consistent with their observations and indicate that the activator-inhibitor complex is dissociated on the surface of fibrin or precipitated fibrinogen. The inhibitor, but not the VA, is found in serum, indicating that the VA remains bound to the fibrin where it converts plasminogen to plasmin causing fibrinolysis as originally suggested by Mülertz. A solid phase such as fibrin or precipitated fibrinogen appears to be required for dissociation of the activator-inhibitor complex to occur. This mechanism permits intravascular fibrinolysis while at the same time protecting fibrinogen from degradation. Soluble fibrin monomer is found similarly to resist degradation in VA-enriched plasma. This observation is in keep-
ing with our previous findings that in experimental animals soluble fibrin monomer is not lysed in the circulation but rather is cleared from the blood by a process involving intravascular precipitation and subsequent degradation.\textsuperscript{22}

In saline, all three activators were found to have a higher affinity for fibrin over fibrinogen as previously demonstrated for SK and UK.\textsuperscript{23} However, the relative affinity of VA for fibrin was significantly greater than that of SK or UK. This property together with the specificity of antiactivator for VA helps to explain the differences in the activity induced by VA and SK or UK.

In conclusion, the degradation of fibrinogen in man may differ importantly from that which occurs in purified systems or that induced by the infusion of SK or UK. The cadaver-derived plasminogen activator has been shown to have properties distinct from SK or UK which are largely attributable to the specificity and abundance of antiactivator in blood. These results are consistent with our previous observations that only fibrinolysis but not fibrinogenolysis occurs after venous occlusion,\textsuperscript{17} exercise,\textsuperscript{18} or death.\textsuperscript{19} The apparent specificity of the antiactivator for VA suggests that VA consists predominantly of a single plasminogen activator which has properties similar to the activator released during venous occlusion, physical exercise, or after death. The antiactivator in plasma is not consumed in the process of clotting and is probably identical to the serum inhibitor described by Aoki and von Kaulla.\textsuperscript{14}

It has been postulated that direct intravascular fibrinogenolysis by plasmin represents a major catabolic pathway of fibrinogen\textsuperscript{24} and accounts for its heterogeneity in blood.\textsuperscript{25} The present findings do not completely exclude this possibility. An alternative pathway of plasminogen activation, such as via the activation of factor XII,\textsuperscript{26} may result in activity differing from that induced by VA. Alternatively, it may be postulated on the basis of the present study that physiologic fibrinogen degradation by plasmin requires an intermediate solid phase. This permits dissociation of the activator from the inhibitor, allowing plasmin formation to occur. Although plasmin thus formed is likely to be bound to antiplasmin, these latter complexes have been shown by Harpel and Mosesson to retain limited fibrinogenolytic activity.\textsuperscript{27} Our finding that fibrinolytic activity reappears in the lysate following fibrin digestion is consistent with this hypothesis. However, to prove the existence of this pathway of fibrinogen catabolism, further investigation is needed.

REFERENCES


The resistance of fibrinogen and soluble fibrin monomer in blood to degradation by a potent plasminogen activator derived from cadaver limbs

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