Generation of Fibrinolytic Activity by Infusion of Activated Protein C into Dogs

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ABSTRACT Bovine-activated protein C, administered intravenously to dogs, increases the rate of lysis of whole blood clots. Protein C, bovine prothrombin, and diisopropylfluorophosphate-inactivated protein Cₐ do not increase the rate of lysis. Repeated infusions of protein Cₐ sustain rapid blood clot lysis, but neither elevate circulating fibrin-split products nor decrease circulating plasminogen levels. The administration of protein Cₐ results in the elevation of the levels of lysine-adsorbable plasminogen activator activity in the plasma. When partially purified concentrates of this activator are added to normal dog blood at the levels seen following protein Cₐ injection, the rate of clot lysis is similar to that seen after protein Cₐ injection. The addition of protein Cₐ to citrated whole blood in vitro, with the subsequent neutralization of protein Cₐ with antibodies, results in increased rates of lysis when plasma made from the treated blood is reinfused into the animal. The generation of fibrinolytic activity is dependent on both cellular and plasma components of blood. A model of protein Cₐ fibrinolytic activity has a minimum of two components: a secondary messenger formed by protein Cₐ action on blood cells and plasma, and the subsequent appearance of plasminogen activator in the animal in response to that messenger.

Evidence for the activation of protein C in vivo has recently been presented by Marlar and Griffin (4) who showed that a group of patients lacking an inhibitor to activated protein C exhibited simultaneous Factor VIII and Factor V deficiency. We have now shown the presence of an endothelial cell surface cofactor for thrombin-catalyzed protein C activation that accelerates the thrombin-catalyzed activation of protein C at least 20,000-fold (5, 6). These observations provide evidence for protein C activation in humans and provide a plausible mechanism by which this activation can occur.

In addition to its function as an anticoagulant, activated protein C has been shown by Zolton and Seegers (7, 8) to enhance fibrinolysis, at least in part, by lowering the levels of fibrinolytic inhibitors. Our studies (9) have confirmed that fibrinolytic activity can be generated both in vivo and in vitro in response to activated protein C. We have recently described a quantitative method for using lysine-agarose to assay plasminogen activator levels (10). We have now used this assay to further characterize the in vivo fibrinolytic state induced by activated protein C.

METHODS

Reagents. Bovine blood was the generous gift of the Cornell Packing Company, Oklahoma City, Okla. QAE-Sephadex Q-50, SP-Sephadex C-50, porcine heparin grade II, and soybean trypsin inhibitor were purchased from Sigma Chemical Co., St. Louis, Mo. d-valine-leucine-lysine-p-nitroanilide (S2251) was purchased from Ortho Pharmaceuticals, Raritan, N. J. Benzamidine HCl was purchased from Aldrich Chemical Co., Milwaukee, Wis. Acrylamide of electrophoresis grade was purchased from Eastman Laboratory and Specialty Chemicals, Rochester, N. Y. All other reagents were of the highest grade commercially available.

Preparation of affinity columns. Lysine (11) and heparin (12) were coupled to Bio-gel agarose A 15 (Bio-Rad Laboratories, Richmond, Calif.) following cyanogen bromide (CNBr) activation (13).

Preparation of proteins. All proteins were of bovine origin. Protein C (14, 15) and prothrombin (16) were isolated by published methods. Thrombin was prepared by activation
of prothrombin with Factor Xa, Factor Va, phospholipid, and Ca²⁺ as described (16). Bovine and canine plasminogen were prepared by the method of Deutsch and Mertz (11).

Protein C (20 mg, 3 mg/ml) was activated with bovine thrombin (1 mg, 0.15 mg/ml) in 2 mM Tris buffer, pH 7.4, containing 50 mM NaCl at 4°C for 16 h. The activation mixture was then brought to pH 6.0 by the addition of 2(N-morpholine) ethane sulfonic acid to a final concentration of 20 mM. The mixture was then chromatographed on a 0.9 × 60 cm sulfoethyl Sephadex C-50 column equilibrated in 50 mM NaCl, 1 mM benzamidine, 20 mM 2(N-morpholine) ethane sulfonic acid, pH 6.0. Activated protein C was not retained. Fractions containing protein C₅ were then chromatographed on a column of QAE Sephadex Q-50 (0.9 cm × 2 cm), and the column washed with 0.1 M NaCl, 20 mM Tris HCl, 1 mM benzamidine HCl, pH 7.4. The protein C₅ was then eluted from the column with a linear NaCl gradient (0.1–0.7 M) in the Tris-benzamidine buffer. Activated protein C anticoagulant activity was monitored by determining the prolongation of a Factor Xa clotting time (17).

Animal studies. Mongrel dogs were anesthetized with sodium nembutal administered intravenously. Protein solutions were administered by injection into a foreleg vein. Blood samples were withdrawn via a 2-in 18-gauge intravenous catheter (Becton-Dickinson & Co., Rutherford, N. J.) inserted into the external jugular vein. The catheter was occluded with a 2-in Safedwell catheter (Becton-Dickinson & Co.) when not in use. The catheter was cleared by aspirating and discarding 2–3 ml of blood before samples were drawn.

Fibrinolysis studies in whole blood clots. Whole blood (2.5 ml) was collected, added to a 17 × 100-mm polystyrene tube containing 280 µl of 3.8% citrate, pH 5.5, and mixed immediately on a Vortex mixer. To this was added 10 µg 1251-human fibrinogen with a specific activity of ~167 µCi/mg and the blood again mixed. The blood was clotted with 5 U bovine thrombin in 100 µl of 0.15 M NaCl, 20 mM Tris HCl, pH 7.4. The thrombin was added while vortexing the blood. The blood was allowed to clot at room temperature for 5 min and then the clot was freed from the wall of the tube by rinsing the clot with a round wooden applicator stick. The clots were then incubated in a 37°C water bath and at 30 min, 1, 2, and hourly intervals thereafter, 100-µl samples of serum were withdrawn from around the clot and placed in 400-µl plastic microcentrifuge tubes (Bio-Rad Laboratories). The tubes were centrifuged for 5 min in a Beckman microfuge B. 30 µl serum was then removed from above the pellet of shed erythrocytes, placed in a Biovial, and 125I-fibrin-split product content was determined by counting the sample in a Beckman gamma counter. Visible clot lysis was monitored by gently tipping the tubes at hourly intervals. Lysis was judged to be complete when the organized clot no longer visible.

Plasminogen activator assay. Plasminogen activator activity in canine plasma was measured using lysine-agarose and canine fibrin agar plates. Human plasma (10). Using this method, canine plasma was passed through lysine-agarose columns and the adsorbed plasminogen activator activity was eluted from the columns with ammonium thiocyanate-containing buffer. Plasminogen activator activity was then quantitated by measuring the conversion of plasminogen to plasmin on bovine fibrin agar plates.

Preparation of concentrated plasminogen activator. Concentrated preparations of lysine-adsorbable plasminogen activator were made from blood obtained 5 min after the intravenous injection of 5 µg protein C₅ per milliter of normal canine plasma. The addition of protein C₅ was then chromatographed on a 0.9 × 10-cm lysine-agarose column. After washing, the plasminogen activator was eluted from the column as described at a rate of 3 ml/h. The activator activity was measured on fibrin agar plates and fractions containing the activator were pooled. The pool was placed in dialysis tubing and the tubing covered with dry Sephadex G-50 at 4°C. After the activator activity was concentrated 6–10-fold, the sample was removed from the dialysis bag and stored at –80°C until used. Aliquots were dialyzed for 4 h at 4°C immediately before addition to normal canine blood.

Preparation and assay of anti-bovine protein C antibodies. Rabbits were inoculated with protein C in Freund's complete adjuvant at weekly intervals for 3 wks and then with protein C in Freund's incomplete adjuvant 1 wk later. Serum was obtained after the final inoculation and the IgG fraction purified. The IgG fraction was then concentrated by ammonium sulfate precipitation and dialyzed against 0.15 M NaCl, 0.02 M Tris-HCl, pH 7.4. The IgG was then chromatographed over Ultrogel A234 in the same buffer that removed all contaminating plasminogen activator activity, as assayed by incubation of the IgG with plasminogen on fibrin agar plates.

The antiprotein C-IgG was assayed using the Xa-one stage assay. Under the conditions of this assay, the control clotting time of 29 s was prolonged to >80 s by the addition of 5 µg protein C₅ per milliter of normal canine plasma containing 5 µg protein C₅ completely corrected the prolongation of the Xa clotting time.

Preparation of canine plasma and washed blood cells. Citrated plasma was prepared from citrated blood by centrifugation at 1000 g for 10 min at 25°C. Washed blood cells were prepared by resuspension of the cellular sediment in 5 vol of Hanks' HEPES buffer containing 1 part 3.8% sodium citrate, pH 5.5, per 9 parts buffer. The resuspended cells were centrifuged 5 min at 1,000 g and the washing was repeated. The sedimented cells were resuspended with Hanks' HEPES buffer-containing citrate to the original blood volume before use.

Estimation of plasma plasminogen activator molecular weight. 200-µl samples of lysine-adsorbable plasminogen activator containing 0.5 NH₄SCN were made 0.1% in sodium dodecyl sulfate (SDS) at room temperature and electrophoresed at 50 mV at 25°C on 10% acrylamide gels containing 0.1% SDS (18). Gels were cut into 2-mm slices, crushed with a glass rod, and eluted with 200 µl of 20-mM Tris buffer, pH 7.4, containing 10 mg/ml bovine albumin, 1 mg/ml bovine plasminogen, and 0.15 M NaCl for 2 h at 4°C. The plasmin substrate, S-2251 (30 µl of 3 mM S-2251) was added and the mixture incubated 2 h at 37°C. The reaction was stopped by the addition of 400 µl of 50-mM acetic acid. The hydrolysis of the S-2251 was then quantified by monitoring absorption at 405 nM after centrifugation to remove gel fragments.

RESULTS

Effect of activated protein C on whole blood clot lysis. The intravenous administration of activated protein C resulted in an increase in the rate at which whole blood clots lyses (Fig. 1). Whole blood clots made from blood drawn 5 min after the administration of 3 µg protein C₅ per milliter of normal canine plasma decreased more rapidly than clots formed from blood before protein C₅ injection. The rate of clot lysis gradually decreased after the injection of protein C₅ and by 35–40 min, had reached preinjection levels. The addition of the same levels of protein C₅ in

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Figure 1. Effect of protein C\(_a\) in vitro and in vivo on whole blood clot lysis. The release of \(^{125}\text{I}-\)fibrin-split products is shown from clots made from blood drawn before (A), at 5 (B), 10 (C), and 20 min (D) after the intravenous injection of 3 \(\mu\)g protein C\(_a\)/ml dog plasma. Release of fibrin-split products from a clot formed from blood that had the same level of protein C\(_a\) added in vitro is shown (E). The time at which complete visible clot lysis occurred is indicated (L) on each curve. Visible lysis did not occur in the preinjection sample or the in vitro addition sample in 36 h. Similar results were obtained with three other dogs.

Fibrinolytic activity in vitro caused a small, but reproducible increase in the rate of clot lysis. Visible clot lysis correlated with the release of fibrin-split products. Complete lysis of the clots made from blood drawn 5 min after protein C\(_a\) injection occurred 60–80 min after thrombin addition. The clots made before protein C\(_a\) injection, or by the addition of protein C\(_a\) in vitro took longer than 36 h to lyse. Addition of equivalent levels of protein C\(_a\) to unanticoagulated blood in vitro gave an increase in the rate of lysis similar to that seen by addition of protein C\(_a\) to citrated blood (data not shown).

The fibrinolytic response of dogs to protein C\(_a\) administration was found to follow a positive dose-response curve (Fig. 2). All dogs had a measurable response to 0.5 \(\mu\)g protein C\(_a\)/ml. There was an increase in the rate of lysis of the postprotein C\(_a\) clots as the in vivo dose of protein C\(_a\) was increased. One dog was given 10 \(\mu\)g protein C\(_a\) per milliliter plasma volume. The clots made following this dose completely dissolved in 35–40 min and demonstrated an 18-fold increase in \(^{125}\text{I}-\)fibrin-degradation product release.

We wished to determine if the fibrinolytic response seen after protein C\(_a\) injection was specific for activated protein C or whether class-similar proteins could produce the same response. The proteins selected were bovine prothrombin, diisopropylfluorophosphate, inhibited protein C\(_a\), and the zymogen, protein C. A control injection of saline was also run. Only the protein C\(_a\) increased the rate of clot lysis significantly above preinjection rates.

The effect of repeated infusions of protein C\(_a\) on fibrinolysis in the dog was also examined. Repeated doses of protein C\(_a\) were administered at 30-min intervals for 5 h. (Fig. 3). An increased rate of clot lysis was observed and sustained over the 5-h period. When the infusions were stopped, the rate of clot lysis returned to control levels. Plasminogen levels did not fall during the 5-h period, suggesting that plasminogen was not being converted to plasmin in the circulation. There was no change in the platelet count, suggesting that a condition like disseminated intravascular coagulation was not initiated by the protein C\(_a\). Fibrin-split products in the plasma, as detected by staphylococcal agglutination were not elevated and remained \(<10 \mu\text{g/ml}\). This suggested that neither fibrinogen nor fibrin was being degraded.

No change occurred in the animal’s systolic, diastolic, or mean arterial blood pressures during the experiment, nor did body temperature, pulse, or respiratory rate change. These findings suggested that the mechanism by which protein C\(_a\) increased fibrinolysis...
nolytic activity did not involve the release of pyrogens in the blood or production of systemic shock or anaphylaxis. The dog used in this experiment showed no evidence of bleeding during or after the experiment at venipuncture sites. The animal’s hematocrit remained stable during and after the experiment, suggesting that internal hemorrhage did not occur.

Neutralization of protein Ca before clot formation. As noted above, optimal lytic response required intravenous infusion of protein Ca. Addition of equivalent levels of protein Ca to citrated blood in vitro gave a comparatively small increase in the rate of clot lysis. This suggested that protein Ca, itself, might not be necessary in the clotted blood for the enhancement of lysis to occur. To examine this possibility, we administered protein Ca intravenously to a dog, drew blood from the animal, and then added sufficient rabbit IgG directed against protein C to neutralize the protein Ca (Fig. 4). The dog blood treated with the IgG had a rate of clot lysis very similar to the untreated blood. The IgG did not affect the rate of lysis of pre-protein Ca control blood clots. This suggested that protein Ca was not having a direct effect in the formed clot but in the circulation.

Effect of protein Ca on circulating lysine-adsorbable plasminogen activator levels. Because protein Ca did not cause clot lysis directly, we examined the canine plasma to determine if plasminogen activator activity was generated as a result of protein Ca injection. Specifically, we looked for the appearance of lysine-adsorbable plasminogen activator activity in the dog’s plasma. Radcliffe and Heinz (19) have found that human lysine-adsorbable plasminogen activator activity increases after exercise and we (10) have found

![Figure 3](image-url) **Figure 3** Effect of repeated infusions of protein Ca. A dog was infused with 3 \( \mu \text{g} \) protein Ca per milliliter plasma volume at 30-min intervals. The relative rate of \(^{125}\text{I}-\text{fibrin-splitted product release was monitored (■). Plasminogen levels (Δ) and platelet counts (●) are shown. Each dose of protein Ca was given over a 20-s period. Arrows indicate protein Ca infusions. A second animal showed similar results over a 180-min experimental period.**

![Figure 4](image-url) **Figure 4** Effect of neutralizing protein Ca on the rate of clot lysis. A dog was injected with 5 \( \mu \text{g} \) protein Ca per milliliter plasma volume. Blood was drawn 5 min after protein Ca administration and was divided into 2 aliquots. One aliquot had sufficient rabbit IgG directed against protein C added to neutralize protein Ca activity (●), while the second had the same volume of Tris-saline added (■). Both samples were clotted, as was a sample of canine blood taken before protein Ca injection (Δ). The addition of IgG alone to canine blood did not change the rate of clot lysis (data not shown). Two other animals showed similar results under the same conditions.
elevated levels in humans with cirrhosis and following venous occlusion and exercise. The levels of lysine-adsorbable plasminogen activator were measured before and after protein Cₐ administration to a dog (Fig. 5). A fivefold increase in lysine-adsorbable plasminogen activator was observed 5 min after protein Cₐ injection. The lysine-adsorbable plasminogen activator level decreased over the following 30 min. When the equivalent level of protein Cₐ was added to citrated whole blood, no elevation of lysine-adsorbable plasminogen activator was observed.

We wished to determine if a dose-response relationship existed between the level of protein Cₐ administered to the dog and the level of lysine-adsorbable plasminogen activation generated, (Fig. 6). A positive dose-response relationship did exist. Within the concentration range tested, the response was not saturated. The highest dose used, 10 μg/ml, resulted in the complete lysis of the clots in 25–30 min and a 4.8-fold rise in the plasminogen-activator level.

Whereas the increase in lysine-adsorbable plasminogen activator following protein Cₐ administration suggested that this plasminogen activator might be involved in the increased rate of clot lysis observed, any number of other plasminogen activators or profibrinolytic activities might also be generated by the protein Cₐ. To determine if the levels of lysine-adsorbable plasminogen activator observed were sufficient to give these increases in the rate of clot lysis, concentrated lysine-adsorbable plasminogen activator (made from the plasma of a dog infused with protein Cₐ) was added to normal canine blood in vitro before clot formation (Fig. 7). The canine blood had 0.06 U intrinsic activator activity per milliliter and had a relatively slow rate of fibrin-split product release. Lysine-adsorbable plasminogen activator was added to the blood at levels approximating those seen after protein Cₐ infusion. The exogenous plasminogen activator produced whole blood clot lysis rates in vitro comparable to those observed following protein Cₐ administration in vivo.

Estimation of molecular weight of plasminogen activator by SDS gel electrophoresis. Lysine-adsorbable plasminogen activator was obtained from plasma samples prepared before and after injection of 3 μg protein Cₐ per milliliter canine plasma volume. The molecular weight of the plasminogen activator was determined on SDS gels as described (Methods). The molecular weight was estimated at 70,000–72,000 (Fig. 8). This value was in agreement with estimates made using Ultrogel AcA34 of 62,000–66,000.

Neutralization of protein Cₐ added to blood in vitro. The generation of plasminogen activator could be due to either a direct or indirect effect of protein Cₐ on plasminogen activator production. The indirect mechanism could involve formation of a product in blood, which then leads to the release/generation of plasminogen activator. To examine the latter possibility, we added protein Cₐ to blood in vitro and then neutralized the protein Cₐ with antibodies before re-injection into the dog (Fig. 9). Although the protein Cₐ activity was neutralized by the IgG, an increase in the rate of lysis occurred, and a 290% increase in

**Figure 5** Change in lysine-adsorbable plasminogen activator activity after protein Cₐ addition in vivo and in vitro. At time 0, 5 μg protein Cₐ per milliliter was injected into a dog (●) and the levels of lysine-adsorbable plasminogen activator measured at times up to 30 min. The same level of protein Cₐ was added to citrated canine blood (■) and lysine-adsorbable plasminogen activator assayed at the times indicated. Similar results were obtained with three other dogs.
plasminogen activator level was observed. When the protein \( C_a \) was neutralized with IgG before addition to the blood, no increase in the rate of lysis and no increase in plasminogen activator occurred. These results suggest that the protein \( C_a \) generates a secondary messenger in blood, which in turn is involved in the appearance of plasminogen activator and the increased rate of clot lysis.

To investigate which components of blood were necessary to generate the secondary messenger, protein \( C_a \) was added in vitro to washed blood cells alone, plasma alone, and to recombined plasma and blood cells. The protein \( C_a \) added in vitro was then neutralized with antiprotein C antibodies and the supernate from the cells, the plasma and supernate from the recombined cells plus plasma were sequentially injected into the dog (Fig. 10). The washed cell supernate did not increase fibrinolytic activity. The administration of protein \( C_a \)-treated plasma resulted in a

FIGURE 7  Effect on clot lysis of supplementation of normal canine blood with concentrated lysine-adsorbable plasminogen activator. Citrated whole blood from a donor dog was supplemented with concentrated plasminogen activator. The citrated donor canine blood (△) that did not have additional plasminogen activator added had 0.06 U lysine-adsorbable plasminogen activator per milliliter. The blood was supplemented by adding plasminogen activator to final concentrations of 0.13 U/ml (□), 0.20 U/ml (○), 0.27 U/ml (◊), and 0.34 U/ml (■). The citrated blood samples were clotted and the release of \(^{125}\text{I}-\text{fibrin-split products determined. Two duplicate experiments using blood from two other dogs gave comparable results.}

FIGURE 8  Estimation of the molecular weight of plasminogen activator. Lysine-adsorbable plasminogen activator was prepared from plasma samples taken before and after infusion of 3 µg/ml protein \( C_a \) into the dog. Samples (200 µl) were electrophoresed on 10% polyacrylamide gels in SDS as described (Methods). (Top panel-before infusion; bottom panel-postinfusion). Molecular weight markers (indicated by arrows from left to right) were phosphorylase B, bovine serum albumin, ovalbumin, and chymotrypsinogen. Plasminogen activator activity was measured as described in Methods. Urokinase was used to construct a standard curve.

FIGURE 9  Treatment of blood in vitro with protein \( C_a \) followed by addition of antiprotein C IgG. Protein \( C_a \) was added to 50-ml citrated whole blood and incubated for 5 min at 37°C. The blood cells were precipitated by centrifugation for 10 min at 1,000 g and sufficient antiprotein C IgG (1.9 ml) was added to the 28 ml of resulting plasma to inhibit the protein \( C_a \). The plasma was incubated 5 more min at 25°C and then injected into the dog and the relative rate of clot lysis recorded before and after plasma administration (○). A control was also run which consisted of mixing the same amounts of protein \( C_a \) and antiprotein C IgG together for 5 min before adding the mixture to the same volume of blood (■) and injecting the plasma into the animal. Similar results were obtained using two other animals.
6.5-fold increase in the rate of fibrin degradation product release. The infusion of the supernate from the protein Cₐ-treated recombined cells and plasma yielded a 10.3-fold increase in fibrinolytic activity. Although addition of protein Cₐ to the cells did not result in increased fibrinolytic activity, the cells did augment the effect seen by adding protein Cₐ to plasma alone.

**DISCUSSION**

The in vivo administration of physiologic levels of activated protein C causes a dramatic increase in the rate of whole blood clot lysis. The fibrinolytic response to activated protein C is specific; class-similar proteins such as prothrombin do not enhance the rate of clot lysis. The active site of protein Cₐ is necessary for the enhancement of clot lysis because neither diisopropylfluorophosphate-inhibited protein Cₐ nor the zymogen protein C enhance clot lysis. This enhanced ability to lyse clots formed in vitro appears to result primarily from an elevation of circulating plasminogen activator levels. When lysine-adsorbable plasminogen activator is added to normal blood in vitro to elevate the plasminogen activator levels to those seen in vivo following protein Cₐ infusion, the rate of clot lysis of the supplemented blood is similar to that of the blood taken from animals receiving protein Cₐ. Whereas other plasma proteins may contribute to the increased rate of clot lysis, the rise in the levels of this plasminogen activator are sufficient to cause the increase in lytic activity. The plasminogen activator does not cause intravascular fibrinogenolysis, as evidenced by normal levels of plasminogen and no elevation of fibrinogen degradation products during prolonged infusions of protein Cₐ. This suggests that the plasminogen activator may function more efficiently in the presence of fibrin in the formed blood clot. This suggests a role of protein Cₐ in clot lysis different than either urokinase or streptokinase, both of which activate circulating plasminogen, which subsequently degrades fibrinogen. The thrombolytic effect of protein Cₐ may protect the animals from bleeding episodes during and after protein Cₐ administration. In over 150 protein Cₐ administrations to dogs, none have shown evidence of bleeding and none have died. Following the administration of protein Cₐ, the effect on clot lysis is of limited duration and the plasminogen activation generated is cleared from the plasma.

The origin of the lysine-adsorbable plasminogen activator is unknown. However, the molecular weight

**FIGURE 10** Treatment of washed cells, plasma, and whole blood in vitro with protein Cₐ. Protein Cₐ (3 µg/ml fluid volume) was added to 50 ml washed blood cells, 50 ml citrated plasma, and to recombined washed blood cells and plasma. The three fractions were incubated for 5 min at 37°C and then centrifuged at 1,000 g for 10 min. The supernates were collected and sufficient anti-protein C antibodies were added to neutralize protein Cₐ activity. The three supernatants were then injected intravenously into the dog at 30-min intervals. Addition of protein Cₐ in vitro was timed to precede injection by 20 min for each sample. Order of addition was (a) supernate from washed cells (▲), (b) plasma (★), and (c) supernate from recombined cells and plasma (■). Blood drawn before the injections was also assayed (●). A duplicate experiment in another animal gave similar results.
of 70,000 suggests it may be related to vascular plasminogen activator described by others (20, 21). Vascular plasminogen activator is released into the blood by various physiologic events, including ischemia (22), pyrogenemia (23), and physical exercise (24). The administration of protein Ca causes the elevation of plasminogen activator activity without changing the animals’ pulse, respiratory rate, blood pressure, or temperature. Therefore, it is doubtful hypotension or shock play a role in the action of protein Ca.

Protein Ca does not appear to directly release plasminogen activator from the vasculature. Our preliminary results indicate that when oxygenated buffer containing protein Ca is used to perfuse isolated dog tissues, little or no plasminogen activator activity is released from the blood vessel walls. However, when protein Ca is added to blood in vitro with subsequent neutralization of the protein Ca with antibodies, the rate of clot lysis is increased when the blood is reinjected into the animal. The addition of protein Ca to plasma alone is sufficient to generate the fibrinolytic activity, but the effect is more marked when protein Ca is added to a mixture of plasma and blood cells. These findings suggest that protein Ca generates a secondary messenger, or family of messengers, which in turn cause the elevation of circulating plasminogen activator activity.

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