SOME EFFECTS OF PROTEOLYTIC INHIBITORS ON TISSUE INJURY AND SYSTEMIC ANAPHYLAXIS*

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Most forms of bodily injury are associated with a marked increase in proteolytic activity. It has been suggested that during conditions of tissue damage (1) and systemic shock (2) activation of proteolytic mechanisms results in the formation of vasoactive products which are responsible for many of the manifestations of injury. Attempts have been made (3, 4) to establish the importance of particular proteolytic factors by treating animals with chemical inhibitors which under *in vitro* conditions act on particular proteolytic systems. Most of these experiments have provided suggestive but inconclusive information, primarily because of the unknown biological activity of the inhibitors employed. An important recent advance has been the demonstration that competitive substrates can be used to inhibit particular proteolytic enzymes or the endogenous activators of these systems (5).

The present study is concerned with the application of such inhibitors as a means of elucidating the chemical mechanisms concerned with a number of different injury phenomena. Included in the group of inhibitors tested for a protective action, are soybean trypsin inhibitors (6), tosylarginine methyl ester (5), and epsilon aminocaproic acid (lysine with the alpha amino group deleted) (7)—all of which are general inhibitors of the tryptic type of proteolytic enzymes. Studies with enzymes of the trypsin class have shown that they are activated from their inactive precursors by splitting off one of the basic amino acids, arginine or lysine (5). The activators of plasmin appear to be more closely associated with lysine substrates (8). These inhibitors were studied, not because of our conviction that these particular enzymes are involved, but rather because the chemical basis of their action has been worked out.

We were primarily concerned with two facets of the problem—the possible involvement of proteolytic enzymes in the disruptive hemorrhage developing during severe injury to the skin, and the relation of the increased proteolytic activity of the blood to the systemic toxemia following lethal trauma or stress. In addition, an attempt was made by such treatment to circumvent the lethal manifestations of acutely lethal endotoxemia and anaphylaxis.

The effect of agents on the inflammatory process has been difficult to evaluate because of the subjective nature of the end-point. The local accumulation of a blue dye complexed to plasma albumin (9) does not represent an adequate expression of the overlapping series of changes which develop during tissue injury. We therefore employed several forms of tissue damage which lend themselves to more definite evaluation by virtue of the fact that they terminate in local hemorrhage and necrosis. Included in this category are the lesions produced by bacterial endotoxins (10, 11) and antigen-antibody interactions (12).

The present paper demonstrates that animals treated with competitive inhibitors of proteolysis were protected against the development of several different types of injury; rabbits were resistant to several forms of local hemorrhagic necrosis; mice survived normally lethal anaphylaxis; and rats and mice withstood better acutely lethal doses of bacterial endotoxin.

Materials and Methods

Tissue injury was studied in the rabbit (1.7 to 2.0 kg. body weight), using a number of different experimental models. A dermal Shwartzman reaction was produced by injecting 100 μg. of an *Escherichia coli* endotoxin intracutaneously (13) and subsequently at 18 hours a similar amount of endotoxin intravenously. The second model was a modification of the Shwartzman lesion, as described by Thomas (11). Skin sites were prepared by injecting 0.2 ml. containing 100 μg. epinephrine intracutaneously and giving 100 μg. of endotoxin concurrently, either by an intravenous route, or by combining the bacterial extract with the epinephrine. Such areas develop a typical erythema, edema, and hemorrhagic necrosis within 4 to 6 hours.

In view of reports in the literature linking the local manifestations of antigen-antibody interaction to activation of proteolytic enzymes (14), the Arthus reaction was studied in rabbits. A reverse Arthus reaction was induced by injecting rabbit antibody to ovalbumin into the skin and shortly thereafter injecting the antigen intravenously. Such reactions develop rapidly and within 3 to 5 hours are hemorrhagic and edematous.

Anaphylaxis was studied in the mouse and guinea pig. Male and female mice of the Swiss-Webster strain, weighing between 20 and 30 gm. were used. They were injected intravenously with 0.5 ml. of a solution of solubilized antigen-antibody complexes (rabbit antiovalbumin) which contained a total of 1.5 mg. of antibody protein. The complexes were prepared[1] by adding antigen to known amounts of antibody, centrifuging down the precipitate, washing the precipitate and then redissolving it in 20 × antigen excess. The resulting complex, when injected into control mice, was 60 per cent lethal within 6 hours.

In the guinea pig, rabbit antibody to crystalline ovalbumin was injected intravenously and within a few minutes the antigen (ovalbumin) was given intravenously. Anaphylaxis developed within a few minutes and the doses were so adjusted as to provide LD_{50} to LD_{60} value.

Passive cutaneous anaphylaxis was studied in the guinea pig according to the method of Ovary (15). In brief, six separate skin sites were sensitized by the injection of 0.1 ml. of a solution containing 0.02 μg. of rabbit anti-ovalbumin antibody N per ml. After a 3 hour latent period, the animal received intravenously 70 μg. of ovalbumin protein (2 times crystallized, Worthington Chemical Co.) together with 0.25 ml. of a 1 per cent solution of Evans blue dye to demarcate the resulting injury.

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[1] Courtesy of Frederick Miller, Department of Pathology, New York Medical Center.

The systemic effects of bacterial endotoxin were studied in two ways. Acutely lethal amounts of extracts of *E. coli* or *Salmonella enteritidis* were given intraperitoneally to mice, rats, and rabbits.

Two suitably spaced injections of bacterial endotoxins produce a systemic reaction in rabbits, the generalized Shwartzman reaction, which has been reported to be accompanied by changes in the proteolytic activity of the blood (16). The Shwartzman reaction was induced by an intravenous injection of 100 μg. *E. coli* lipopolysaccharide and 18 to 24 hours later by a second injection of a similar amount of bacterial extract. The animals were sacrificed 24 hours later and the kidneys then examined for evidence of bilateral cortical necrosis and fibrinoid deposits.

Traumatic shock was induced by rotation of rats in the Noble-Collip drum (17). The *E. coli* and *Salmonella typhosa* endotoxins were prepared by Difco Company according to the method of Landy and Johnson described in reference 18. The soybean trypsin inhibitor was prepared by Worthington Chemical Co., and epsilon aminocaproic acid (EACA)[2] by Aldrich Chemical Company. Tosylarginine methyl ester (TAME)[3] was synthesized in the laboratory.

RESULTS

I. Dermal Reaction Produced by Mixtures of Epinephrine and Bacterial Endotoxin

For purposes of orientation, our initial experiments were confined to the dermal reactions produced by mixtures of 100 μg. epinephrine and 100 μg. bacterial endotoxin. This combination produces hemorrhagic necrosis in the skin of rabbits with a high degree of reproducibility (85 to 90 per cent).

1. Soybean Trypsin Inhibitor (SBTI)[4].—

Soybean trypsin inhibitor has been reported to suppress inflammatory reactions (18). SBTI was injected intravenously into rabbits in doses ranging from 5 to 30 mg./kg. and at time intervals ranging from 2 to 3 hours before to 3 to 5 hours after the dermal challenge.

A positive inhibitory effect was obtained only when the SBTI was given just prior to dermal injection, and in amounts of at least 25 to 30 mg./kg. As shown in Table I, animals which received 30 mg. of SBTI 15 minutes before the dermal injection, showed a significant reduction in both the severity and incidence of hemorrhagic necrosis (38 per cent, only 10/24 positive). The majority of skin sites became edematous but within 3 hours had reverted to an almost normal appearance.

In another group of six rabbits, the inhibitor was added to the epinephrine-endotoxin vehicle and injected locally. Here only a modest decrease in the number of hemorrhagic and necrotic sites was encountered (10/18 experimental, as compared with 10/12 controls).

It was found that maximal protection was afforded in instances when the

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[2] EACA, epsilon aminocaproic acid.
[3] TAME, tosylarginine methyl ester.
[4] SBTI, soybean trypsin inhibitor.

inhibitor was given intravenously from 5 to 10 minutes before injecting the skin sites. No effect on the reaction was observed when the inhibitor was given as late as 30 minutes after the epinephrine mixture had been injected into the skin.

2. *Tosylarginine Methyl Ester (TAME)*.—

In order to rule out non-specific properties of the soybean extracts and to delineate further the system involved, two synthetic inhibitors were studied. The fact that it had been necessary to inject the SBTI in advance of the damaging agent suggested that a rapidly activated process was involved. TAME has been found (5, 6) to have a more prompt inhibitory effect than SBTI on proteolytic systems under *in vitro* conditions. It was therefore anticipated, if

TABLE I

Effect of SBTI on Dermal Reaction in Rabbit Induced by Mixtures of Endotoxin and Epinephrine

Time of administration relative to challenge*	SBTI dose‡	Positive lesions
	mg.	
—	—	8/8 (100%)
—	—	14/16 (88%)
—	—	10/12 (83%)
15 min. before	30 (i.v.)	10/24 (38%)
60 min. before	30 (i.v.)	5/8 (82%)
Mixed with endotoxin	30 (i.d.)	10/18 (55%)
30 min. after	30 (i.v.)	14/18 (77%)

* 100 μg. epinephrine + 100 μg E. coli endotoxin i.d. in 0.2 ml.

‡ per 1.7 to 2.0 kg. rabbit.

an autocatalytic reaction was involved, that TAME should be more effective than SBTI in counteracting the reaction to injury.

TAME in doses of 15 to 20 mg./kg. clearly suppressed the development of hemorrhagic lesions following mixtures of epinephrine and endotoxin (100 μg. of each). As indicated in Table II, optimal protection was achieved in animals which received TAME for 2 successive days together with a booster dose of 20 mg. just before the intradermal test injection.

A major premise in these experiments is the assumption that these agents are in fact acting as inhibitors of proteolysis. This thesis was put to test by using tosylarginine (TA),[5] the product of proteolytic action upon TAME, which, although chemically similar, does not act as a proteolytic inhibitor. Tosylarginine in comparable amounts (10 to 25 mg.) had no effect on the dermal reaction to injury (9/10 positive lesions with epinephrine-endotoxin mixtures).

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[5] TA, tosylarginine.

3. *Epsilon Aminocaproic Acid (EACA).*—

Inspection of the various combinations in our biological test system suggested that a two-step phenomenon might be involved in the development of the injury reaction following epinephrine and endotoxin. Since TAME was more effective when given during the initial period of the skin preparation, it seemed plausible to assume that the inhibitor might be acting by interfering

TABLE II

Effect of TAME on Dermal Reaction in the Rabbit Induced by Mixtures of Endotoxin and Epinephrine

Dose* (mg.)	No. of injections	Positive lesions‡
15 (i.v.)	3§	17/20 (85%)
20 (i.v.)	1	8/20 (40%)
20 (i.d.)	1, locally	16/20 (80%)

* per 1.7–2.0 kg. rabbit.

‡ Mixture of 100 μg. *E. coli* endotoxin and 100 μg. epinephrine, 0.2 ml. i.d.

§ 12 hours apart, last just prior to skin challenge.

TABLE III

Effect of EACA on Dermal Reaction in the Rabbit Induced by Mixtures of Endotoxin and Epinephrine

Dose*	No. of injections‡	Positive lesions§
mg.		
10 (i.v.)	1	4/14 (28%)
10 (i.v.)	3	3/6 (50%)
50 (i.v.)	3	1/8 (12%)
100 (i.v.)	2	4/14 (28%)
0.1 (i.d.)	1	11/12 (91%)
—	—	20/24 (63%)

* per 1.7 to 2.0 kg. rabbit.

‡ at 24-hour intervals, last just prior to skin challenge.

§ Mixture of 100 μg. *E. coli* endotoxin and 100 μg. epinephrine, 0.2 ml., i.d.

with an activator mechanism. Inasmuch as activators of this type are usually present only in small amounts, once the process starts and a substantial concentration of the proteolytic enzyme is produced, a substance such as TAME would be rapidly hydrolyzed to TA and no longer effective. Comparatively high doses of TAME would therefore be required to interfere with proteolysis *per se.*

Recently, it has been reported (19) that it is possible to interfere with the activator step in the plasminogen or trypsinogen reaction almost preferentially

with EACA. This substance is also more effective than TAME in inhibiting the initial phase of the reaction as opposed to the proteolytic step *per se.*

EACA, in intravenous doses ranging from 500 µg. to 10 mg./kg. was found to be effective in the rabbit in counteracting the hemorrhagic lesions produced by mixtures of epinephrine and endotoxin (Table III). There was no apparent correlation between the dose used and the degree of protection afforded. On the other hand, addition of EACA (0.1 to 1.0 mg.) directly onto the skin site not only was ineffective, but increased the resulting area of hemorrhage and necrosis.

TABLE IV

Effect of Proteolytic Inhibitors on Various Forms of Injury

	Animal	Challenging agent	Positive reactions		
			Controls	EACA*	TAME*
Dermal Shwartz-man	Rabbit	100 µg. *E. coli* endotoxin (i.v.) + 100 µg. *E. coli* (i.v.)	12/14	6/10	8/12
Arthus reaction	Rabbit	Ovalbumin, rabbit anti-ovalbu-min	8/8	13/16	3/4
PCA	Guinea pig	Ovalbumin, rabbit anti-ovalbu-min	13/16	21/27	7/7
Generalized Shwartzman	Rabbit	2 doses of 100 µg. *E. coli* endo-toxin (i.v.) 18 hours apart	8/10	9/12	9/12
Acutely lethal endotoxemia‡	Rat	2000 µg. *E. coli* endotoxin (i.v.)	9/12	6/10	9/10
	Rabbit	250 µg. *E. coli* endotoxin (i.v.)	8/12	8/10	9/10
	Mouse	200 µg. *E. coli* endotoxin (i.v.)	10/12	6/10	6/10

* EACA, 30 mg. (i.v.), TAME, 25 mg. (i.v.), for 48 hours preceding challenge and just prior to test dose, except when noted otherwise in the text.

‡ Rats weighed between 120 and 175 gm.; rabbits between 1.7 and 2.0 kg.; mice between 20 and 30 gm.

4. Other Forms of Injury.—

We then turned our attention to several other forms of tissue damage, including the classic dermal Shwartzman phenomenon, and the passive Arthus reaction in the rabbit. As indicated in Table IV, neither of these two phenomena was modified by the administration of either TAME or EACA.

The generalized Shwartzman phenomenon was produced in two groups of rabbits (12 in each), one of which received TAME (25 mg./kg.) and the other EACA (30 mg./kg.) as indicated in Table IV. In these instances the inhibitors were given intravenously for 2 days prior to the priming dose of *E. coli endotoxin,* at hourly intervals for 6 hours thereafter, and in conjunction with the provoking dose of endotoxin.

There was no evidence, either gross or microscopic, of a diminished deposition of fibrinoid material in the blood vessels, or of a lessened incidence of bilateral cortical necrosis of the kidneys.

Single large doses of bacterial endotoxin are rapidly lethal in most laboratory animals, causing prostration and death within 4 to 12 hours. In view of known species differences in the reaction to bacterial extracts, endotoxic shock was studied in the rat, rabbit, and mouse.

In each case (see Table IV), the animals received an amount of endotoxin designed to produce an LD_{60} to LD_{75} in controls. Both TAME and EACA were used. The two inhibitors were given both before and for several hours after the endotoxin. The rats and mice looked manifestly better, showing no diarrhea, ruffling of the hair, and did not have the usual tendency to huddle in the corner of the cage. All of the animals survived for at least 9 to 10 hours and the only deaths occurred overnight. Mortality figures were more favorable in the rat and mouse than in the rabbit (Table IV).

TABLE V

Effects of Proteolytic Inhibitors on Lethal Drum Shock in the Rat*

	Dose‡	Lethality
	mg.	
Controls	—	24/32 (75%)
TAME	25 (i.v.)	12/24 (50%)
EACA	30 (i.v.)	10/24 (41%)
EACA	30 (i.v.) twice	8/24 (33%)

* 700 turns in Noble-Collip drum.

‡ per 1.7 to 2.0 kg. rabbit.

Passive cutaneous anaphylaxis was produced in the skin of 18 guinea pigs. Eight animals were pretreated with 30 mg. EACA intravenously at 20 hours before and again 1 hour before the challenge. Four animals received 30 mg. of TAME (intravenously) just before the PCA[6] tests. In no case (Table IV) was the size or intensity of the tissue damage diminished appreciably.[7]

The influence of EACA and TAME on traumatic shock was studied in the rat following rotations in the Noble-Collip drum. Here again, as in endotoxic shock, both of the inhibitors gave significant protection against the lethality of drum trauma (Table V). The animals appeared less cyanotic and much more alert when removed from the drum. Deaths were associated with extensive hemorrhage into the intestinal tract and congestion and swelling of the liver.

5. Systemic Anaphylaxis.—

Mice, which received 0.5 ml. of the solubilized antibody-antigen complex (see Material and Methods), were divided into two groups: one was given 30 mg. EACA in saline (intraperitoneally) and the other an equivalent volume of saline alone (intraperitoneally).

[6] PCA, passive cutaneous anaphylaxis.

[7] We are indebted to Dr. Zoltan Ovary for this experiment.

The data in Table VI indicate that animals receiving EACA showed a high degree of protection, particularly when the material was administered some 8 to 14 hours previous to the anaphylaxis. Untreated mice died within 2 to 4 hours. The majority of the treated mice showed only minor symptoms,— diarrhea, apprehension, but no cyanosis, respiratory embarrassment, or shock.

Comparable studies were carried out in the guinea pig, using an ovalbumin-rabbit anti-ovalbumin anaphylaxis. However, in a series of 36 guinea pigs (250 to 300 gm.) treated with EACA (see Table VI)—either in the form of a single injection just prior to the challenge, or several injections for 24 hours prior to the shock—only slight protection was demonstrated against the lethal course of the reaction.

TABLE VI

Effect of EACA on Acutely Lethal Anaphylaxis*

	Controls	Treated
Mouse	15/20 20/22	6/21‡ 14/22
Total	35/42 (83%)	20/43 (44%)
Guinea pig	12/12 10/12 11/12	9/12§ 9/12 10/12
Total..............	33/36 (89%)	28/36 (77%)

* i.v. injection solubilized antigen-antibody complex (as indicated in text).

‡ 30 mg. EACA i.p., 8 hours prior to anaphylactic challenge.

§ 25 mg./100 gm. body weight.

6. General Effects of TAME and EACA.—

It was necessary to establish whether EACA and TAME acted on other biological mechanisms, some of which are known to alter the susceptibility to tissue damage and to shock.

A. Pharmacological properties: The two inhibitors were found to have no effect on the mean blood pressure as recorded by mercury manometer in rats, rabbits, or guinea pigs (6 each) anesthetized with pentobarbital (30 mg./kg.). Tests were also conducted on the pressor or depressor action of intravenously administered vasoactive agents,—epinephrine, norepinephrine, acetylcholine (mecholyl), histamine, and 5-hydroxytryptamine. In no case did treatment with these inhibitors interfere with the intensity or duration of the blood pressure changes with the above drugs.

Measurements of coagulation time were carried out in a series of 12 rats and 10 rabbits on whole blood collected directly from the exposed carotid artery

into No. 60 polyethylene catheter tubing (method of Spaet, 20). Only a minor decrease in coagulation time was observed transiently in animals which had received high doses of TAME (30 mg./kg.) and EACA (40 mg./kg.). On the other hand, a definite effect was noted on the disturbed coagulability of the blood which is induced by systemic endotoxin.

In untreated animals, endotoxin (50 to 100 μg., intravenously administered) caused a biphasic change—an initial short lived period (15 to 20 minutes) of hypercoagulability followed by a more protracted (45 to 120 minutes) hypo-coagulable phase. Rats and rabbits pretreated with 30 mg. EACA/kg. showed an attenuation of both the hyper- and hypocoagulable phase following lethal amounts of endotoxin.

Blood counts (WBC, RBC, and platelets) revealed no significant change in either the number or distribution of cells.

The status of the reticulo-endothelial system (RES) has been shown to be an important factor in the systemic response to various forms of stress and endotoxemia (21–25). In the current studies, measurements were therefore made of the efficiency of the RES by the rate of clearance of colloidal material from the blood stream, using the method of Biozzi, Benacerraf, and Halpern (26). Neither TAME nor EACA had any significant effect on the phagocytic index measured from 1 to 4 hours after the administration of these agents in a group of 6 rats and 6 rabbits.

B. Local inflammatory reaction: The fact that the severity of tissue injury is related to the availability and release of vasoactive amines within the affected site led us to determine whether or not the inhibitors under investigation acted to deplete tissue stores of these amines. For this purpose, xylene was used to produce a local inflammatory reaction and the magnitude of the effect measured by the rate of appearance and intensity of the local blueing reaction in the region (9).

The skin of three rats and three rabbits was shaved and several sites in each were painted with xylene and Evans blue (0.5 ml. of a 1 per cent solution) injected intravenously. The development of the exudative blueing reaction was essentially similar in controls and in animals treated, as previously, with 30 mg. of EACA or 35 mg. of TAME.

DISCUSSION

Several basic questions must be answered in any discussion of the data. First, are the materials used acting under *in vivo* conditions to inhibit proteolysis? Secondly, is the protective action in a particular form of injury related to this inhibitory action? Thirdly, are the observed effects due to an indirect action unrelated to proteolytic phenomena?

The fact that each of three different inhibitors, EACA, TAME, and SBTI, in common, suppress the development of the same forms of local injury and

systemic stress is highly suggestive evidence that proteolytic mechanisms are in fact important determinants of such reactions. Although these substances are known under *in vitro* conditions to inhibit enzymes of the trypsinogen-trypsin and plasminogen-plasmin class (7, 8), the animal experiments cannot be used by themselves to implicate a particular enzyme system in the response to tissue injury (27).

Reports in the literature make it obvious that the manifestations of tissue injury are mediated by several separate pathways. The data provided by the present study delineate one of these pathways. Inasmuch as several basically different forms of tissue damage are affected by EACA and TAME, it is probable that a common mechanism exists through which most forms of injury pass before diverging into a series of end-reactions.

Several ancillary observations further strengthen our contention that the protective action of EACA or TAME in our experiments is related to their effect on activation of proteolysis. Neither of these drugs has any demonstrable musculotropic or vasotropic action on the blood vessels. In addition, tosylarginine, a chemically similar substance without proteolytic inhibitory properties, has no effect on the course of tissue injury.

In view of species differences and conditions peculiar to particular forms of injury or systemic stress, it is obvious that generalizations in this regard are not warranted. This is clearly indicated by our finding that EACA prevents the lethal consequences of systemic anaphylaxis in the mouse but not appreciably in the guinea pig. Inasmuch as antihistaminics are effective against anaphylaxis in both species (28–30), it is probable that different pathways are involved in the release of the mediators responsible for the toxic reaction.

The majority of experiments in the present series were carried out with epsilon aminocaproic acid (EACA) because of its superior properties as an inhibitor of proteolytic activation under *in vitro* conditions, and because it was found to be well tolerated in contrast to the toxicity of soybean trypsin inhibitor (SBTI) and tosylarginine methyl ester (TAME) in optimal effective concentrations. EACA, thus, was able to provide a more favorable test of the relative importance of proteolytic factors in the genesis of tissue injury.

With respect to the mechanisms of action, it should be noted that both TAME and EACA were especially effective against tissue lesions which involved epinephrine as an initiating factor. A similar relationship would seem to be indicated by the protective action of these two inhibitors against the lethal and toxic manifestations of systemic shock and endotoxemia,—conditions in which the release of vasoactive amines is prominently involved (31).

The data offer some insight into the biochemical processes which may be involved in the reaction to injury. Many of the published reports (32–35) emphasize the formation through proteolytic activity of vasoactive polypeptides—agents such as bradykinin, kallicrein, and substance P are believed

responsible for the vascular dilation and capillary damage encountered in all forms of injury. In this regard, EACA or TAME may act, as they do under *in vitro* conditions (34), to inhibit the formation of these polypeptides. Other workers have emphasized the formation and release of histamine or H substance during injury (36). Neither TAME nor EACA has a direct antihistaminic action. It has been reported (37) that epinephrine causes the release of plasminogen activator. It should be emphasized that the role of plasminogen activator may be much wider than that implied by its known function in regard to the specific enzyme plasminogen. Furthermore, epinephrine has been shown to activate other enzymatic systems, including thromboplastin and trypsin (37).

The observation that epinephrine-activated lesions were inhibited by EACA, whereas neither the classical local Shwartzman nor the Arthus reactions were affected, suggests a basic difference between the two phenomena. Both the Arthus and the dermal Shwartzman reactions involve a period of preparation characterized by heavy infiltration of the tissue with leucocytes which subsequently degenerate and fragment coincident with the onset of local hemorrhage (38). The destruction of these cells undoubtedly liberates proteolytic enzymes in an already active form. EACA is not an effective inhibitor of the proteolytic enzymes themselves, although it does prevent the activation of such enzymes from their precursors.

Miles and Wilhelm (39) have reported that soybean trypsin inhibitor diminishes the local increase in permeability following several forms of tissue damage. SBTI is known to interfere with the activation of thrombin and plasminogen. In the current studies, the inflammatory response of the skin to xylene or to bacterial extracts was not affected by EACA or TAME. On the other hand, those lesions, which subsequently progressed to the point at which vascular disruption, hemorrhage, and necrosis appeared, were clearly interrupted before this phase of the reaction developed. The two aspects of the phenomenon may therefore involve different enzyme systems.

SUMMARY AND CONCLUSIONS

A study was made of the development of various forms of local and systemic injury in animals treated with inhibitors of proteolytic activity. The agents used were tosylarginine methyl ester (TAME), epsilon aminocaproic acid (EACA), and soybean trypsin inhibitor (SBTI).

1. Hemorrhagic necrosis in the skin of the rabbit following intradermal epinephrine in combination with bacterial endotoxin (either intravenous or local) was clearly suppressed by EACA, TAME, and SBTI, given systemically. Tosylarginine (TA) was ineffective.

2. No effect was observed on the classical Shwartzman reaction, the local Arthus phenomenon, or inflammation induced by xylene.

3. The lethal effects of systemic anaphylaxis in the mouse, acute endotoxemia in the rat and mouse, and drum shock in the rat are suppressed by EACA and TAME.

4. There was no effect on the generalized Shwartzman phenomenon in the rabbit and on anaphylaxis in the guinea pig.

5. The effects of EACA or TAME on the injury reactions under investigation were not due to a pharmacological or chemical action on vascular behavior *per se*.

6. The data provide corroborative evidence for a proteolytic step in injury phenomena which may be mediated through some common activation system.

7. The working hypothesis is advanced that local or systemic stress through the release of epinephrine may result in an increase of a circulating activator of proteolysis and that this in turn may give rise to the release of vasoactive substances,—possibly histamine, serotonin, or a polypeptide.

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