Relative Antiplasmin (Antifibrinolysin) Activity of ω-Aminocarboxylic Acids

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ω-Aminocarboxylic acids with carbon chain lengths of three to eight are shown to possess antifibrinolytic activity *in vitro*. ω-Aminohexanoic acid (ε-aminocaproic acid) is the most active compound studied.

IN RECENT YEARS one of the most exciting achievements in therapeutics probably has been the introduction of several enzymes for clinical uses. One of the latest developments is human plasmin (or fibrinolysin) preparation. Plasmin is the enzyme normally present in the blood plasma in its inactive zymogen form, known as plasminogen. In the laboratory, plasminogen may be converted into plasmin by streptokinase or urokinase. Plasmin is used in the treatment of venous thrombosis, such as thrombophlebitis or phlebothrombosis, pulmonary embolism, and topical debridements. However, overdosage of injected plasmin may cause hypofibrinogenemia and uncontrolled hemorrhage. An antiplasmin agent which serves as an antidote in controlling plasma plasmin activity is highly in need.

In 1957 workers in Japan announced the discovery of an antiplasmin (antifibrinolysin) agent known as ε-aminocaproic acid. This compound was patented in Great Britain (1) and distributed by Daiichi Seiyaku Co. of Japan. Remarkable effects were obtained by the Japanese workers on cases of hemorrhagic metropathia, dysmenorrhea, threatened abortion, toxemia of pregnancy, hyperemesis gravidarum, and eczema urticaria and other dermatitis. All of these diseases are thought to be associated with increased plasmin activity. They gave this acid in doses of 10–20 Gm. per day, orally or intravenously, to more than 100 patients and observed no toxic effect (2).

The effect of ε-aminocaproic acid on a large number of clinical coagulation defects was systemically investigated in Sweden in 1960 (3). Enhanced fibrinolysis has been reported in various diseases. Pathological fibrinolysis most probably arises from increased circulating levels of plasminogen activator. Fibrinogen, often given in large quantities under these circumstances, is usually without significant effect because of its rapid destruction by plasmin. Theoretically, control of fibrinolysis by giving an inhibitor of plasminogen activation, followed by the use of fibrinogen, should be satisfactory. In these respects, ε-aminocaproic acid has proved to be promising. Favorable results have been obtained by Nilsson, Sjoerdsma, and Waldenstrom (3) in a limited number of clinical cases.

It has been suspected for some time that activated blood fibrinolysin may also play a prominent role in the pathogenesis of anaphylactic shock. Recently, Burden and co-workers (4) have demonstrated that the spontaneous lysis of clotted blood samples collected during acutely fatal shock in guinea pigs following intravenous injection of peptone, histamine, trypsin, and anaphylatoxin was found to be a regular feature of these reactions just as observed previously in guinea pigs undergoing specific anaphylactic shock (5). With the aid of a potent fibrinolysin inhibitor, it is possible to find out whether increased fibrinolytic activity is the consequence or the cause of anaphylactic shock.

The potential importance of this antiplasmin agent prompted our interest in studying the mode of action of ε-aminocaproic acid. As the first step of approach, we chose to study the relative antiplasmin activity of the homologous series of ω-aminocarboxylic acids. The results of this study are reported in this paper.

METHODS AND MATERIALS

Measurement of Fibrinolytic Activity.—For the measurement of fibrinolytic activity, the method described by Lewis and Ferguson was used with modifications (6). To a small tube add 0.5 ml. of 1% human plasma fraction I (fibrinogen) and 0.5 ml. of borate buffer (0.1*M*, pH 7.5). To a second tube, add 0.5 ml. of enzyme solution (purified human plasmin solution, 150 units) and 0.5 ml. of human thrombin (2 units). Mix the contents of the tubes by pouring back and forth twice, and then incubate at 37°. The amount of fibrinogen in the tube after being acted upon by the excess amount of thrombin present was just enough to form a turbid gelatinous fibrin solution. The amount of plasmin added was sufficient to clarify the turbid solution about 10 minutes after incubation. Buffer alone or buffer with antiplasmin agents was added in a volume of 0.3 ml. to the fibrinogen solution. The final volume in all cases was 2.3 ml. Time for the clarification of the gelatinous fibrin solution was recorded. The effect of concentration of different ω-amino acids was assayed separately at first, since preliminary experimental results indicated that the concentration range varied widely. The effective concentration range for each acid studied has been located with this method. The purpose of this type of study was to supply information for selecting an appropriate concentration at which the activities of all of the acids studied could be compared. Under the conditions described in this paper, the concentration selected was 50 mmoles per L., and the relative antifibrinolysin activity of ω-aminocarboxylic acids at this concentration was then studied.

Materials Used.—The biological materials used in the present studies were all commercial products.

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Human fibrinogen used was Parenogen of Cutter Laboratories and the fibrinolysin used was Actase of Ortho Pharmaceutical Corp. Thrombin was obtained from Upjohn Co. All of the ω-aminocarboxylic acids used were commercial preparations and were recrystallized from either water or ethanol four times and dried in a vacuum desiccator over phosphorus pentoxide.

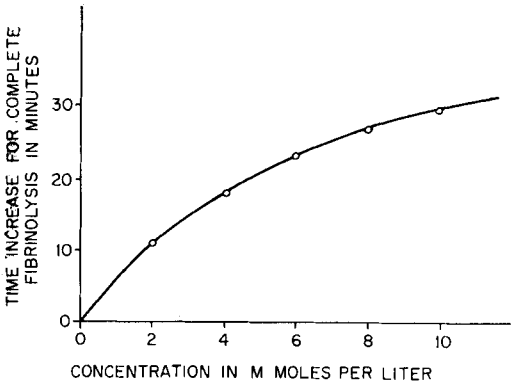

Fig. 1.—Antiplasmin activity of ε-aminocaproic acid.

RESULTS AND DISCUSSION

The inhibitory effect on fibrinolysis of each of the ω-aminocarboxylic acids at graded concentrations was studied. Only the results of the study on ε-aminocaproic acid are presented in Fig. 1 as representative of these studies. The relative antifibrinolysin activity of the ω-amino acids at 50 mmoles per L. concentration was studied in four experiments and the results obtained, in general, were essentially the same. Representative results of these studies are shown in Fig. 2. The results indicated that ε-aminocaproic acid was the most active compound. The shape of the curve in Fig. 2 suggests a sharp, geometrical fitness of the ε-aminocaproic acid molecule at the site of inhibition. The natural amino acid lysine is α,ε-diaminocaproic acid. It is a known fact that plasmin, like trypsin, can specifically hydrolyze the lysinyl bond of lysinyl peptides or lysinyl esters. These facts raised the question as to whether or not ε-aminocaproic acid exerts its inhibitory action by competing with the lysine moiety in the fibrin molecule. It was unexpected that L-lysine did not inhibit plasmin activity even at higher concentrations. At very high concentration of lysine solution,

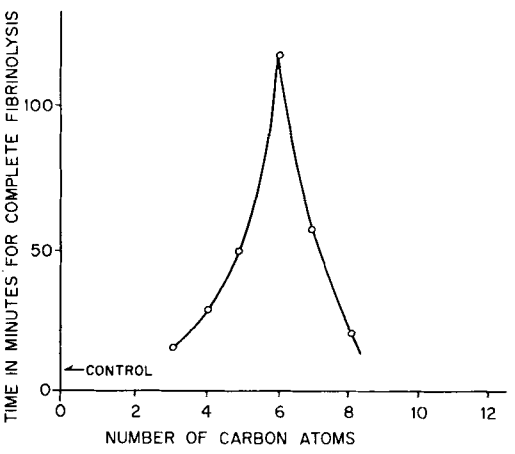

Fig. 2.—Relative antiplasmin activity of ω-aminocarboxylic acids at 50 mmoles per L.

fibrin was soluble. D-Lysine or L-arginine showed no inhibitory effect. We also found that lysine exerted no effect on the antiplasmin effect of ε-aminocaproic acid. It is interesting to point out that γ-aminobutyric acid also has considerable antiplasmin activity. This finding may be of significance since no other clue has been found on the physiological or biochemical function or functions of the large amount of γ-aminobutyric acid generally present in the normal central nervous system.

The low toxicity (3, 7) and high activity of ε-aminocaproic acid are the desirable properties of a useful therapeutic agent; however, its rapid rate of excretion (3) reduces its practical use. Further studies on these problems are under way.

SUMMARY

An enzyme system for assaying antifibrinolytic activity is described. The relative antifibrinolysin activity of the homologous series of ω-aminocarboxylic acids has been studied. It has been found that ε-aminocaproic acid has the highest antifibrinolytic activity.

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