γ-Guanidinobutyric Acid: An Inhibitor of Clot Formation and of Clot Lysis*

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During a study of the effects of a series of guanidine compounds on the esterolytic activities of thrombin, plasmin, and streptokinase plus plasmin or plasminogen, it was found that one of these compounds, γ-guanidinobutyric acid (GGBA), acted in several ways like ε-aminocaproic acid (EACA). Neither compound had any inhibiting effects on the rate of hydrolysis of TAMe (*p*-toluenesulfonyl-L-arginine methyl ester), but both inhibited the activation of plasminogen by streptokinase. EACA was the more potent inhibitor. Since EACA has been shown to inhibit the lysis of fibrin, primarily because it inhibits the activation of plasminogen (Ablondi *et al.*, 1959, Alkjaersig, Fletcher, and Sherry, 1959), GGBA was tested to see if it, too, would inhibit the lysis of blood clots. It was found to do so. In addition, it was found that GGBA also inhibits the formation of blood clots, which EACA does not do. These preliminary results are reported here.

Materials and Methods

Fresh citrated blood from normal donors was used. Plasma was obtained by centrifuging the blood for 20 minutes at 2,500 rpm and 5°C. The euglobulin fraction of the plasma was precipitated by diluting the plasma with distilled water (1 part plasma, 14 parts water) and was brought to pH 5.35 with 0.1 N HCl. After centrifugation for 3 minutes at 1,500 rpm and 5°C, the supernatant was discarded and the precipitate was dissolved in a barbital-saline buffer, pH 7.35 (Wintrobe, 1961). It was tested immediately for clot formation and clot lysis.

The contents of a vial of thrombin, (Thrombin Topical, bovine, Parke, Davis & Co., 1,000 NIH units) were dissolved in 12.5 ml of glycerol and 12.5 ml of 1.8% NaCl and refrigerated. Just before use the stock solution was diluted with 0.9% NaCl to contain 2 or 4 NIH units per ml. Distilled water (4.0 ml) was added to a vial containing 100,000 units of streptokinase (Varidase). Immediately before use it was diluted with 0.9% NaCl to contain either 1,000 or 2,000 units per ml. EACA was purchased from Mann Research Laboratories, and GGBA from Calbiochem. A 0.1 M solution of each compound was prepared in 0.9% NaCl.

Results

With the blood and plasma from four donors, inhibition of clot lysis was shown in the following way. To test tubes containing 0.1 ml of blood or plasma from a single donor, 0.1 ml of thrombin (4 NIH units per ml) was added and the contents were mixed. A clot formed immediately in each tube. After 15 minutes at room temperature (22–25°C), the following solutions were added to duplicate tubes: (1) 0.3 ml of saline (0.9% NaCl), (2) 0.2 ml of saline plus 0.1 ml of EACA (0.1 M), (3) 0.2 ml of saline plus 0.1 ml of GGBA (0.1 M), (4) 0.2 ml of saline, 15 minutes later 0.1 ml of streptokinase (100 units per ml), (5) 0.1 ml of saline plus 0.1 ml of EACA (0.1 M), 15 minutes later 0.1 ml of streptokinase (1,000 units per ml), and (6) 0.1 ml of saline plus 0.1 ml of GGBA (0.1 M), 15 minutes later 0.1 ml of streptokinase (1,000 units per ml).

None of the clots in the first three sets of tubes lysed, even after remaining at room temperature overnight. The clots in tubes (4) were completely or partially lysed in a few hours, and were completely lysed the next morning. The clots in tubes (5) and (6) were not lysed and apparently were completely intact when examined the next morning.

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When the experiments, as outlined in (4), (5), and (6), were repeated with either double the concentration of streptokinase or double the concentration of EACA or GGBA, the same results were obtained. There was lysis in tubes (4) and no lysis in tubes (5) or (6).

Inhibition of clot formation was shown, with whole blood from two donors. To test tubes containing 0.1 ml of blood from a single donor were added: (1) 0.3 ml of saline, (2) 0.2 ml of saline plus 0.1 ml of EACA (0.1 M), or (3) 0.2 ml of saline plus 0.1 ml of GGBA (0.1 M). After 15 minutes, 0.1 ml of thrombin (4 NIH units per ml) was added to each tube. Four minutes later firm clots were present in all tubes containing saline or saline plus EACA. No clots were observed in any of the tubes containing GGBA. After 20 minutes, an additional 0.1 ml of thrombin was added to the tubes containing GGBA. Clots formed immediately. Firm clots were still present the following day in all tubes.

A combined clotting and lysing experiment was performed on the euglobulin fraction of the plasma from two donors. The euglobulin precipitate was dissolved in 0.3 ml of buffered saline. To duplicate tubes (controls), 0.1 ml of saline was added. To other tubes, 0.1 ml EACA (0.1 M) or GGBA (0.1 M) was added. Thrombin, 0.1 ml (2 units per ml) was added to each tube and the tubes were placed in a 37°C bath. After 15 minutes, firm clots were present in the control tubes and in the tubes containing EACA, but no clots were present in the tubes containing GGBA. After 60 minutes, however, the latter tubes also contained clots. No evidence of lysis of any of the clots was seen even after the tubes had remained at 37°C for 5 hours. The tubes were then left at room temperature. The next morning no clots were found in the control tubes, but clots still remained in the tubes containing either EACA or GGBA.

Discussion

Low concentrations of GGBA are widely distributed in mammalian urine, brain, liver, and other tissues (Pisano, Abraham, and Udenfriend, 1963). These authors estimate that 0.05 and 0.09 μmoles of GGBA, respectively, are present in 1 gm, fresh weight, of rat brain and liver. GGBA has been reported to be of very low toxicity in rabbits, rats, and guinea pigs (Kamiya, Kiyota, and Kita, 1962). The finding that it inhibits the formation as well as the lysis of clots in the test tube suggests that a guanidine-containing compound may be involved in the physiological regulation of blood clotting and lysing. GGBA itself is probably not the compound because it is not potent enough, judged from the preliminary experiments *in vitro*. Possibly a peptide (or peptides), which is released when fibrinogen is changed to fibrin, may be the physiological regulating compound.

From these and other data, it seems that GGBA may inhibit the hydrolysis of fibrinogen by thrombin, and in this way inhibit clot formation. EACA, on the other hand, has no effect on the action of thrombin and therefore does not inhibit clot formation. In addition, both GGBA and EACA may react reversibly with plasminogen, changing it to a compound that cannot be activated to plasmin. In this way both compounds inhibit the lysis of clots.

Experiments are now being done both *in vitro* and *in vivo* to establish quantitatively the extent of the inhibition of clot formation and lysis due to GGBA. Nagamatsu *et al.* (1963) have shown that esters of EACA are more potent inhibitors of clot lysis than is EACA itself. It is possible that esters of GGBA may also be more potent than GGBA as inhibitors of clot lysis and clot formation. These compounds or related ones may prove to be of value in preventing the formation of blood clots as well as controlling excessive fibrinolytic activity *in vivo*.

Summary

γ-Guanidinobutyric acid inhibited the formation and the lysis of clots made from whole blood, plasma, or the euglobulin fraction of the plasma from several donors. ϵ-Amino-caproic acid inhibited only the lysis of these clots, not their formation.

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