The Effect of γ-Guanidinobutyric Acid on the Clotting Time of Normal Plasma and on the Euglobulin Lysis Time of Fibrinolytically Active Plasma*

LYMAN M. FISHER, PHYLLIS S. ROBERTS, AND WARNER E. BRAXTON

Division of Clinical Pathology, Department of Pathology, and Department of Medicine, Medical College of Virginia, Richmond

It has been established that ε-aminocaproic acid (EACA) inhibits the activation of human plasminogen (Ablondi *et al.*, 1959; Alkjaersig, Fletcher, and Sherry, 1959). Because of this observation, this compound has been used extensively to inhibit the pathologically occurring fibrinolytic system in patients. Recently Roberts (1965) reported that another compound, γ-guanidinobutyric acid (GGBA), like EACA, inhibits the lysis of human blood clots. Furthermore, GGBA, unlike EACA, retards the formation of these clots.

The present investigation was undertaken to determine whether GGBA inhibits clot formation in the one-stage prothrombin and in the partial thromboplastin time tests. In addition, the ability of GGBA to inhibit clot lysis was tested using blood from a patient showing active fibrinolysis.

Materials and Methods

Blood was collected in a 3.8% sodium citrate anticoagulant (9 parts blood, 1 part citrate) from 15 individuals. The blood was centrifuged at 2,500 rpm in an angle centrifuge for 10 minutes to obtain the plasma. Clotting tests were performed in duplicate in glass test tubes (10 × 75 mm. at 37° C. Solutions of EACA (Mann Research Laboratories, New York, N.Y.) and GGBA (Calbiochem, Los Angeles, Calif.) were prepared, each having a concentration of 0.1 M in physiological saline. The pH of the EACA solution was 7.02 and that of the GGBA solution was 7.03.

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The clotting tests were performed in the following manner.

1. One-stage prothrombin time: 0.1 ml of plasma; 0.1 ml of either physiological saline (control), 0.1 M EACA, or 0.1 M GGBA; 0.2 ml of equal volumes of thromboplastin and $CaCl_2$ (0.025 M). The time taken for clot formation was measured.

2. Partial thromboplastin time: 0.1 ml of plasma; 0.1 ml of either physiological saline (control), 0.1 M EACA, or 0.1 M GGBA; 0.1 ml of Thrombofax (Ortho Research Foundation, Raritan, N.J.). The mixture was incubated for 30 seconds, and then 0.1 ml of $CaCl_2$ (0.025 M) was added. The tubes were removed from the water bath after incubation for 60 seconds, wiped dry, tilted, and the time taken for clot formation was measured.

The euglobulin lysis time was performed as outlined by von Kaulla and Schultz (1958), except that 0.1 ml of either physiological saline, 0.1 M EACA, or 0.1 M GGBA was added to the euglobulin precipitate.

Results and Discussion

The effects of EACA and GGBA on the clotting systems are shown in table 1.

There was no significant difference between the values obtained with the one-stage test for the samples containing saline or EACA. The values obtained when GGBA was added, however, were significantly prolonged ($p < 0.001$) when compared with the saline control.

The findings for the partial thromboplastin test were similar to those obtained for the one-stage test. GGBA significantly prolonged the clotting time when compared with the samples containing either saline or EACA ($p < 0.01$).

Opportunity to test the usefulness of GGBA in a clinical situation presented itself when a 23-year-old Negro female, gravida 5, para 3, abortus 1, delivered unattended at home a stillborn term infant. Vaginal bleeding was profuse, and a physician was called. When the latter noted that the blood failed to clot, the patient was transferred to the Medical College of Virginia Hospital, arriving in a state of shock. Numerous blood samples were drawn and the clots lysed spontaneously within ½ hour even though considerable fresh blood and fibrinogen were administered. Following the demonstration of a lytic process, EACA was given intravenously and an almost immediate cessation of the lysis occurred. The results of euglobulin lysis times performed on the plasma samples obtained prior to and after the administration of EACA (table 2) show that GGBA was effective in inhibiting lysis, but to a lesser degree than was EACA.

GGBA is a naturally occurring compound (Pisano, Abraham, and Udenfriend, 1963) of low toxicity (Kamiya, Kiyota, and Kita, 1962). We have shown here that it, like EACA, can inhibit the lysis of a clot made from the plasma of a patient with pathological fibrinolytic activity. Therefore, it appears that GGBA, like EACA, interferes with the activation of human plasminogen by the naturally occurring activator. This work also confirms that GGBA inhibits the formation of clots when it is added to normal plasma in the one-stage prothrombin or the partial thromboplastin time tests, while EACA has no effect.

The mechanism of action of GGBA in the formation and lysis of blood clots is unknown.

TABLE 1

Effect EACA and GGBA on the One-Stage Prothrombin and Partial Thromboplastin Times

	No. of Samples	One-Stage Prothrombin Time		Partial Thromboplastin Time	
		Mean	S.D.*	Mean	S.D.*
		sec		*sec*	
Saline control	15	12.8	0.7	76	5
EACA, 0.1 M	15	13.2	0.7	76	6
GGBA, 0.1 M	15	16.0	1.0	89	14

* S.D. = standard deviation.

Summary

The addition of γ-guanidinobutyric acid (GGBA) to human plasma significantly prolonged the one-stage prothrombin and the partial thromboplastin times. On the other hand, the addition of ε-aminocaproic acid (EACA) had no significant effect on either of these tests. GGBA inhibited the lysis of a clot that was formed from the euglobulin fraction of the blood of an obstetrical patient with active fibrinolysis. EACA inhibited the lysis to a lesser degree.

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TABLE 2

Effect of EACA and GGBA on the Euglobulin Lysis Time

Test Modified with	Before EACA Administration	After EACA Administration
Saline, 0.1 ml	27 min	>10 hr
EACA, 0.1 ml	5 hr	>10 hr
GGBA, 0.1 ml	3 hr, 42 min	>10 hr

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