

THROMBIN FORMATION AND NEUTRALIZATION BY ANTITHROMBIN III IN ANIMALS  
RECEIVING REPEATED INTRAVENOUS INJECTIONS OF TISSUE THROMBOPLASTIN

V. E. Pastorova

UDC 615.273.5.015.4:612.115.12

KEY WORDS: thrombin; antithrombin III; tissue thromboplastin.

It was shown previously that after a single intravenous injection of tissue thromboplastin thrombin generation develops like an avalanche in the animals' blood stream. However, the action of the enzyme is inactivated during the first 5-15 min after injection [4]. Under these circumstances a decrease in activity of the principal thrombin inhibitor (antithrombin III) is observed in the blood, accompanied by activation of function of the ant clotting system [2, 3]. The lowering of activity of antithrombin III is evidence of its direct and immediate participation as a highly important agent of the ant clotting system in neutralization of the generated clotting enzyme.

It was also shown that in response to repeated intravenous injections of tissue thromboplastin into animals the protective response of the ant clotting system is initially manifested completely and repeatedly. However, as the duration and frequency of the injections increased, this response weakened and considerable exhaustion of the function of the ant clotting system developed [4].

The dynamics of thrombin generation in the blood stream and changes in antithrombin III activity during repeated injections of tissue thromboplastin into animals were studied in the investigation described below.

#### EXPERIMENTAL METHODS

Male albino rats weighing 170-180 g were used. The animals were divided into two groups: Group 1 (experimental) received 7 intravenous injections, each of 0.3 ml tissue thromboplastin, with intervals of 30-40 min between injections; group 2 (control) received injections of 0.3 ml of 0.85% sodium chloride solution at the corresponding times. Tissue thromboplastin for intravenous injections were prepared from rat brain tissue. A suspension of thromboplastin with clotting activity of 15-16 sec (the clotting test on rat plasma) was centrifuged before use to sediment solid particles, and the supernatant was decanted and diluted 1:3 with physiological NaCl solution. Intravenous injections were given into the jugular vein. Blood was taken in the volume of 1.0 ml for analysis from the same vein into a syringe with sodium citrate (ratio 9:1). Thrombin [6] and antithrombin III [7] activity in the blood plasma and fibrinolytic activity of the euglobulin fraction of plasma [5] were determined.

#### RESULTS

In animals of group 1 receiving tissue thromboplastin intravenously, blood was taken for analysis at the following times: before and 1, 5, and 15 min after the first injection; before the 5th injection and 1, 5, and 15 min thereafter; and before the 7th injection and 1, 5, and 15 min thereafter. In animals of group 2 (control), which received injections of physiological saline, blood was taken for analysis at the same times.

As Table 1 shows, after the first injection of tissue thromboplastin (group 1A) thrombin activity in the blood rose to a maximum 1 min after injection and was completely neutralized by the 15th minute of the experiment, as was shown previously [4]. Antithrombin III activity fell to its lowest level 5 min after injection of thromboplastin, evidence of its participation in neutralization of thrombin, but by the 15th minute of the experiment it was largely restored.

---

Laboratory of Physiology and Biochemistry of Blood Clotting, M. V. Lomonosov Moscow University. (Presented by Academician of the Academy of Medical Sciences of the USSR, S. E. Severin.) Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 101, No. 5, pp. 527-529, May, 1986. Original article submitted May 20, 1985.

TABLE 1. Activity of Thrombin and Antithrombin III (AT-III) and Fibrinolytic Activity (FA) of Euglobulin Fraction of Rat Blood Plasma after 1st, 5th, and 7th Injections of Tissue Thromboplastin

| Group of animals and experimental conditions   | Number of animals | Number of injections | Thrombin activity, sec |                                |                          | AT III activity, %       |                                |                           | FA, %                     |                                |                         |                            |   |                             |                          |
|--|-------------------|----------------------|------------------------|--------------------------------|--------------------------|--------------------------|--------------------------------|---------------------------|---------------------------|--------------------------------|-------------------------|----------------------------|---|-----------------------------|--------------------------|
|  |                   |                      | before last injection  | time after last injection, min |                          | before last injection    | time after last injection, min |                           | before last injection     | time after last injection, min |                         |                            |   |                             |                          |
|  |                   |                      |                        | 1                              | 5                        |                          | 15                             | 1                         |                           | 5                              | 15                      | 1                          | 5 | 15                          |                          |
| 1 (experimental) — injection of thromboplastin |                   |                      |                        |                                |                          |                          |                                |                           |                           |                                |                         |                            |   |                             |                          |
| A  | 18                | 1                    | 480 ± 24               | 133 ± 8 <sup>a, b</sup>        | 201 ± 17 <sup>a, b</sup> | 441 ± 21 <sup>b</sup>    | 113 ± 4                        | 91 ± 3, 2 <sup>b</sup>    | 71 ± 3, 3 <sup>a, b</sup> | 96 ± 3, 4 <sup>b</sup>         | 100 ± 5, 3              | 92 ± 4, 3                  | b | 153 ± 10, 4 <sup>a, b</sup> | 200 ± 13 <sup>a, b</sup> |
| B  | 15                | 5                    | 385 ± 17 <sup>a</sup>  | 130 ± 9 <sup>a, b</sup>        | 198 ± 16 <sup>a, b</sup> | 289 ± 16 <sup>a, b</sup> | 116 ± 4                        | 73 ± 2, 9 <sup>a, b</sup> | 84 ± 3, 2 <sup>b</sup>    | 89 ± 3, 5 <sup>a, b</sup>      | 120 ± 6, 1 <sup>a</sup> | 145 ± 4, 8 <sup>a, b</sup> | b | 185 ± 11, 3 <sup>a, b</sup> | 225 ± 14 <sup>a, b</sup> |
| C  | 16                | 7                    | 322 ± 15 <sup>a</sup>  | 121 ± 7 <sup>a, b</sup>        | 169 ± 11 <sup>a, b</sup> | 227 ± 13 <sup>a, b</sup> | 99 ± 3, 7 <sup>a</sup>         | 90 ± 3, 0 <sup>b</sup>    | 81 ± 2, 2 <sup>a, b</sup> | 61 ± 3, 0 <sup>a, b</sup>      | 36 ± 2, 5 <sup>a</sup>  | 30 ± 2, 3 <sup>a, b</sup>  | b | 27 ± 4, 1 <sup>a, b</sup>   | 105 ± 5, 8 <sup>b</sup>  |
| 2 (control) — injection of 0.85% NaCl solution | 13                | 1—7                  | 485 ± 25               | 462 ± 23                       | 453 ± 23                 | 478 ± 21                 | 112 ± 4                        | 98 ± 4, 1                 | 94 ± 4, 0                 | 105 ± 3, 8                     | 100 ± 4, 5              | 102 ± 5, 1                 |   | 107 ± 5, 3                  | 122 ± 4, 3               |

**Note.** Since no difference in the parameters was found for the control animals depending on the number of injections of 0.85% NaCl solution, mean data are given in this table, disregarding the number of injections. The statistical index was calculated by comparison with the control (a) and with indices before the last injection of thromboplastin (b).

Thrombin activity, measured before the 5th injection, showed a tendency to rise a little (as was shown by shortening of the clotting time compared with group 1A at the same time of the experiment). Later during the experiment this increase became significant. By contrast with levels of thrombin activity in group 1A (a single injection of thromboplastin), complete neutralization of activity of the enzyme was not observed in the animals of group 1B by the 15th minute after the 5th routine injection. Antithrombin III activity in the animals of this group, which was reduced by the greatest degree 1 min after injection of thromboplastin, also points to its rapid participation in neutralization of the clotting enzyme. However, the period of recovery of activity of the inhibitor was more protracted and the degree of recovery was lower than in animals receiving a single injection of thromboplastin.

The absence of complete neutralization of thrombin enzyme was confirmed most clearly in animals of group 1C before and after the 7th injection of thromboplastin. It will be clear from Table 1 that immediately before the 7th injection of thromboplastin, significantly increased quantities of thrombin circulated in the animals' blood stream ( $P < 0.01$ ). Consequently, in the experimental animals, thrombin generated in the blood stream only after the previous injection of thromboplastin was not neutralized in the circulation in the course of 30-40 min (the interval between injections). Thenext, 7th injection of thromboplastin caused an even greater increase in thrombin activity 1 min after injection. The newly generated amounts of the enzyme were neutralized by the 15th minute of the experiment to an even lesser degree than in animals after the 5th injection (group 1B). The character of the change in antithrombin III activity in the animals after the 7th injection of thromboplastin differed from changes in activity of the inhibitor observed after a single injection. Antithrombin III activity was already rather lower in the period before the 7th injection than initially (group 1A, and the control group before injection). During subsequent observations antithrombin III activity fell more slowly than at the same times after the 1st and 5th injection, and its greatest fall was not observed until 15 min after the beginning of the experiment, at a time when thrombin activity was still high. Meanwhile, after a single injection of thromboplastin, a fall in antithrombin III activity was observed more rapidly (5 min after injection) and it led to rapid and complete neutralization of the clotting enzyme.

This is evidence of a slower response of the inhibitor in neutralization of the clotting enzyme with an increase in the duration of injections of the thromboplastic agent.

Besides thrombin and antithrombin III activity, activity of certain other parameters of function of the anticlotting system and, in particular, the fibrinolytic activity of the blood, was measured in these experiments.

In the experimental animals (group 1A) increased fibrinolytic activity of the plasma euglobulin fraction (Table 1) was observed after the first injection of tissue thromboplastin, as was also observed previously [1]. After the 5th injection of thromboplastin, fibrinolytic activity was high, as before. However, before the injections and 5 min after the 7th injection it was considerably depressed, and did not return to normal until the 15th minute. Under these circumstances no activation of fibrinolysis was observed at any time during the period of observation (15 min), evidence of exhaustion of the defensive mechanisms of function of the anticlotting system.

With an increase in the duration of injections of tissue thromboplastin into animals exhaustion of function of the anticlotting system is thus observed, features of which include lowering of the fibrinolytic activity of the blood and the heparin level [4], and delay of neutralization of the generated thrombin by antithrombin III. This is shown by the later appearance of the maximal response of antithrombin III in thrombin neutralization in the animals after the 7th injection of thromboplastin, so that the conditions are created for longer circulation of the clotting enzyme in the blood stream.

#### LITERATURE CITED

1. B. A. Kudryashov, G. V. Andreenko, G. G. Bazaz'yan, et al., Vest. Mosk. Univ. Ser. Biol. Pochv., No. 2, 3 (1981).
2. V. E. Pastorova, Vest. Mosk. Univ. Ser. Biol. Pochv., No. 1, 18 (1980).
3. V. E. Pastorova and S. V. Kovalev, Vopr. Med. Khimii, No. 2, 240 (1976).
4. V. E. Pastorova and B. A. Kudryashov, Probl. Gematol., No. 6, 16 (1968).
5. T. Astrup and S. Müllertz, Arch. Biochem., 40, 346 (1952).
6. E. Glueck, J. Appl. Physiol., 6, 650 (1954).
7. E. Kaulla and K. N. Kaulla, Amer. J. Clin. Path., 48, 69 (1967).