# RESPONSE OF ANIMALS TO THROMBIN AFTER PROLONGED EXPOSURE TO ACOUSTIC STIMULATION

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Prolonged action of an acoustic stimulus (intensity 94-96 dB, frequency 2 Hz, 5 h daily) slightly decreases the total coagulating activity of rats' blood, but weakens the intensity of the protective reaction developing after injection of thrombin into the blood stream, and this is accompanied by an increase in the mortality among the experimental animals to 72.5% (control 40.9%).

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Preliminary investigations showed that during acoustic stimulation of animals (albino rats) periodic changes are observed in the blood clotting system, marked by periodic (on the 30th and 90th days) elevation of the antithrombin activity and heparin level. These changes, an indication of a tendency toward hypocoagulation, are accompanied by changes in the opposite direction: activation of thromboplastin formation, and a lowering of the activity of antithrombins III and IV. These results suggested that during acoustic stimulation activation of coagulating agents takes place together with activation of the anticlotting system.

In order to determine which of these changes is the stronger, i.e., whether in animals subjected to prolonged acoustic stimulation there is a tendency toward hypo- or hypercoagulation, it was decided to study the survival rate of animals after intravenous injection of thrombin. The mortality rate among animals following injection of thrombin into the blood stream is known to depend on the state of the physiological anticlotting system [2-4], the level of activity of which can also be judged from the intensity of the humoral response developing after injection of thrombin [1].

## EXPERIMENTAL METHOD

Experiments were carried out on albino rats (initial weight from 80-120 g) kept on a standard diet based on the formula of the Institute of Nutrition, Academy of Medical Sciences of the USSR. The animals (41) of the control group were not exposed to stimulation of any kind. The experimental animals (60) were subjected to acoustic stimulation (94-96 dB, 2 Hz) for 5 h daily for 130 days. On the 130th day, blood samples were taken from 10 control and 11 experimental animals. The remaining animals received an injection of thrombin (0.4 ml, activity 7 sec) into the jugular vein, and 30 min later blood samples were taken from the surviving animals (10 control and 10 experimental). The clotting time of recalcified plasma, the plasma heparin tolerance, the prothrombin time, prothrombin consumption, antithrombin activity, the levels of heparin, antithrombins III and IV, and fibrinogen, and the fibrinolytic and fibrinogenolytic activity of the blood samples were determined. The methods described by Perlick [5] were used to determine these indices, with some modifications affecting the volumes of material studied. Besides biochemical tests, the survival rate of the animals after injection of thrombin and the interval between the time of injection of thrombin and death of the animals were also taken into consideration.

## EXPERIMENTAL RESULTS

A study of the survival rate of animals after injection of thrombin showed that of the 22 rats in the control group 9 (40.9%) died, and of the 40 rats subjected to acoustic stimulation, 29 (72.5%) died ($P = 0.01$).

The time elapsing from injection of thrombin to death of the animal was the same in the animals of the control and experimental groups (the animals died during the first 2-3 min). Acoustic stimulation caused a slight increase in the recalcification time, together with an increase in total antithrombin activity and the heparin level (Table 1). Meanwhile the prothrombin consumption was increased and the activity of anti-

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TABLE 1. Indices of State of Blood Clotting System in Animals Exposed to Acoustic Stimulation and Their Level After Injection of Thrombin (M ± m)

| Group of animals | | Recalcification time (in sec) | Heparin tolerance (in sec) | Prothrombin time (in sec) | Prothrombin consumption (in sec) | Fibrinogen level in (in %) | Antithrombin activity (in %) | Heparin tolerance (in sec) | Level of antithrombins III and IV (in sec) | Fibrinolytic activity (in %) | Fibrinogenolytic activity (in %) |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Control | Pre-thrombin injection | 121±5,3 | 394±16 | 14,8±0,3 | 17±1,5 | 0,5±0,03 | 107±4 | 3,6±0,7 | 51±6,3 | 16,4±4,4 | 39,4±4,1 |
| | Post-thrombin injection | 147±7,1 | 331±27 | 19,0±0,3 | 27±2,2 | 0,3±0,02 | 211±14 | 14,0±1,5 | 70±4,0 | 40,0±2,8 | 49,0±2,0 |
| | P | <0,05 | >0,05 | <0,05 | <0,05 | <0,05 | 0,05 | 0,05 | 0,05 | 0,05 | 0,05 |
| Experimental | Pre-thrombin injection | 148±8,0 | 392±13 | 15,0±0,1 | 23±1,8 | 0,5±0,02 | 150±16 | 7,5±1,3 | 28±2,4 | 10,3±2,0 | 34,5±5,3 |
| | P | <0,05 | >0,05 | >0,05 | <0,05 | >0,05 | <0,05 | <0,05 | <0,05 | >0,05 | >0,05 |
| | Post-thrombin injection | 128±6,4 | 250±18 | 18,5±0,6 | 23±1,8 | 0,4±0,01 | 169±11 | 7,1±1,0 | 44±4,4 | 19,8±2,2 | 27,3±1,5 |
| | P | >0,05 | <0,05 | <0,05 | >0,05 | <0,05 | >0,05 | >0,05 | <0,05 | <0,05 | >0,05 |

Note. Degree of significance (P) was calculated for comparison of indices obtained before injection of thrombin in animals of control and experimental groups, and also for comparison of indices obtained before and after injection of thrombin within each group (values of P are given below the corresponding mean values).

thrombins III and IV decreased. The other indices were practically identical for the animals of the two groups. Hence, besides evidence of hypocoagulation (an increase in antithrombin activity, the heparin level, and the recalcification time), other changes occurred in the opposite direction: activation of thromboplastin and a decrease in activity of antithrombins III and IV.

After injection of thrombin the recalcification time in the animals of the control group was increased, while that of the experimental animals was unchanged. The prothrombin time increased in the animals of both groups, but in the controls the increase in time was 30% compared with 20% of the initial level in the experimental animals. The fibrinogen level fell in the control animals and remained unchanged in the experimental group. Fibrinolytic activity was increased to a greater degree in the control than the experimental animals, while fibrinogenolytic activity was as a whole unchanged in the experimental animals. The antithrombin activity of the control animals was almost doubled, whereas in the experimental animals its increase was hardly perceptible. The heparin level in the control animals was increased by almost 4 times, while in the experimental animals it was unchanged. The level of antithrombins was increased almost equally in the animals of the two groups. Besides an increase in anticoagulant activity, the prothrombin consumption also was increased in the animals of the control group.

Hence, in animals exposed to acoustic stimulation the intensity of the protective anticoagulant response to injection of thrombin is less marked than in control rats. This evidently explains the higher mortality among the experimental animals after receiving injections of thrombin into the blood stream. Prolonged acoustic stimulation, although leading to a slight decrease in the total clotting power of the blood (increase in recalcification time, heparin level, and antithrombin activity), is also a factor depressing the activity of the system responsible for the protective reaction to the appearance of an excess of thromboplastic substances in the blood stream.

## LITERATURE CITED


1. A. Sh. Byshevskii, Byull. Éksperim. Biol. i Med., No. 2, 34 (1966).
2. B. A. Kudryashov and N. D. Ulitina, Dokl. Akad. Nauk SSSR, 120, No. 3, 677 (1958).
3. V. E. Pastorova, B. I. Roskin, and B. A. Kudryashov, Byull. Éksperim. Biol. i Med., No. 11, 23 (1961).
4. V. E. Pastorova and B. A. Kudryashov, Byull. Éks Éksperim. Biol. i Med., No. 9, 39 (1962).
5. E. Perlick, Anticoagulants [Russian translation], Leningrad (1965).