BLOOD CLOTTING SYSTEM AND HEMORRHAGE INTO THE BRAIN UNDER CONDITIONS OF NERVOUS TRAUMA

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It is known from the literature that experimental reflex epilepsy of rats, evoked by prolonged auditory stimulation, leads in some cases to the death of the animal, from brain hemorrhage [2,3].

It has also been shown that previous administration of thyroid extract causes a marked rise in mortality of the epileptic rats, with a corresponding rise in the incidence of brain hemorrhage, while previous prolonged treatment with methylthiouracil has the opposite effect of lowering the mortality rate [4].

According to L. V. Krushinsky, L. P. Pushkarskaya, and L. N. Molodkina [2] the basic cause of brain hemorrhages in nervous trauma lies in a profound disturbance of the interaction of stimulatory and inhibitory processes.

It is evident that possible pathological changes in capillary fragility and permeability, blood pressure, blood coagulation, etc., may lead to the inception of hemorrhage.

In this connection, we thought it would be of interest to examine some factors of the blood clotting system, and to ascertain whether changes in their content is related to formation of hemorrhages.

EXPERIMENTAL METHODS

We subjected rats to acoustic stimulation, as in our experiments [2]. The experimental animals were sensitive to acoustic stimuli, i.e., they reacted to the sound of a bell by violent motor excitation, leading in most cases to epileptiform fits. The rats were subjected to intermittent acoustic stimulation for a period of 15 minutes, with intervals of 10 seconds every 10 seconds, and this was followed by a three minute break, after which the bell was rung again for $1\frac{1}{2}$ minutes (if fits appeared before the completion of the schedule, the ringing was stopped earlier).

The excitation developing after the pause was found to be more intense than before the pause. In many cases (about 12% on the average) it terminated in the death of the animal. Death occurred in some cases 20-30 minutes after completing the bell-ringing schedule, and in others after several hours, or even 1-2 days. Brain hemorrhage was found at autopsy in most (90%) of the animals in question.

Blood samples were taken from the jugular vein before the experiment and in different phases of the pathological process. We determined the prothrombin, thrombotropin, and heparin content, by the methods developed in B. A. Kudryashov's laboratory (MGU) [5,6]. The present research is part of a program being conducted in this laboratory.

We have shown [2] that a marked hypothrombinemia is present in rats suffering from experimental reflex epilepsy (with the usual $1\frac{1}{2}$ minute duration of the acoustic stimulus). The prothrombin level fell by 22%, on the average, 15 minutes after exposure to sound, while the thrombotropin and heparin activities did not vary. In view of these findings, it seemed reasonable to suppose that intensification of the nervous trauma

by exposure to prolonged intermittent ringing, causing fatal cerebral hemorrhage in many of the animals, should cause a greater lowering of blood prothrombin, and possibly also of other components of the blood clotting system.

In the first series of experiments we examined the prothrombin and thrombotropin contents of the blood of 38 rats which were subjected to prolonged acoustic stimulation. Blood was sampled before the bell started ringing, after 15 minutes exposure (during the 3 minute pause), after 30 minutes, 45 minutes, 4 hours, and 24 hours. The results obtained are presented in Table 1.

TABLE 1

Changes in Blood Prothrombin and Thrombotropin of Rats Subjected to Prolonged Acoustic Stimulation Means

Component of blood clotting system	Number of experi. animals	% Mortality	Before exposure	After 15 minutes	After 30 minutes	After 45 minutes	After 4 hours	After 24 hours
Prothrombin (%)	38	10.5	94.4	68.9	68.0	70.3	85.2	100.0
Thrombotropin (%)	30	10.0	93.7	90.2	91.4	90.0	93.9	90.7

EXPERIMENTAL RESULTS

As is evident from Table 1, rats displaying an epileptiform reaction to prolonged acoustic stimulation manifested a marked hypoprothrombinemia. The prothrombin level fell by an average of 25.5% within 15 minutes of the commencement of the exposure. The prothrombin level had risen from the low level within 4 hours, and was back to normal within 24 hours.

It is worthy of note that prolongation of the duration of exposure did not lead to any further lowering of prothrombin content below the values found after a $1\frac{1}{2}$ minute exposure [1]. The organism evidently possesses some protective mechanism, which prevents any further lowering of prothrombin level by more than 30-40%, whatever the intensity of the nervous stimulation, since this would lead to a hemorrhagic condition of the animal.

Of the 38 experimental rats 4 died. Death took place within 30 minutes of commencement of exposure to sound. At autopsy, 3 of these rats had macroscopic hemorrhages into the lateral ventricles of the brain. The prothrombin level found in rats shortly before their death was not lowered more than in the survivors (decrease of 36%, 45%, 38%, and 21%). The average fall in prothrombin level is no greater than in those which survived. It may hence be concluded that the lowering of the prothrombin level of the blood of rats subjected to prolonged acoustic stimulation is not a determining factor in the development of the observed hemorrhages, but it may contribute to the action of other factors known to be concerned in the development of cerebral hemorrhage (changes in fragility and permeability of capillaries, in blood pressure, and in other components of the blood clotting system).

A study of blood thrombotropin in 30 rats, under conditions of experimental epilepsy caused by prolonged acoustic stimulation, showed no significant changes. An exception to this finding was provided by two rats which died of cerebral hemorrhage, and which had low thrombotropin levels before death (33% and 39%). We cannot, however, claim that this fall in thrombotropin content contributed in any way to the development of hemorrhage, because of the small number of cases, and because of the possibility of experimental error (blood was taken from moribund animals, and for this reason entered the syringe very slowly). The subject requires further experimental investigation.

The second series of experiments was devoted to a study of blood clotting factors in hyperthyroid rats in conditions of experimental epilepsy, and of rats previously treated with methylthiouracil.

We first examined the effect of thyroid treatment on prothrombin, thrombotropin, and heparin activities of rat blood.

Our experiments showed that in experimental thyrotoxicosis caused by prolonged administration of thyroid preparation (100-150 mg per kg body weight per diem for 10-12 days) the prothrombin level underwent

considerable individual fluctuations. Nevertheless, most of the rats (22 out 39) showed a raised level of prothrombin (in some rats up to 150-160%), on the average on the 7th or 8th day of administration, followed by a fall (in some animals down to 50%). We found no changes in thrombotropin or heparin activities of thyroid-treated rats.

The blood clotting mechanism in experimental thyrotoxicosis of animals has been very little studied. Most of the work on this subject has been done on human subjects with thyroid gland disease. It has been noted, for example, that in various hyperthyroid conditions there is a considerable retardation of clotting, amounting in some cases to a hemorrhagic state, and associated with a fall in prothrombin content [7, 8, 9, 10]. It should, however, be borne in mind that hyperthyroid patients, particularly acute cases, usually display degenerative changes of the liver cells, as a result of which production of the basic components of the blood clotting system is interfered with. It is for this reason that a hemorrhagic diathesis is encountered in severe thyrotoxicosis.

TABLE 2

Changes in Prothrombin Content and Heparin Activity of the Blood of Hyperthyroid Rats, and of Rats after Prolonged Methylthiouracil Treatment, under Conditions of Experimental Epilepsy Caused by Prolonged Acoustic Stimulation
(Mean values)

	Component of the blood clotting system	Number of experimental animals	Percentage mortality	Before thyroid administration	Before acoustic stimulation	30-40 minutes after stimulation
Hyperthyroid rats	Prothrombin, %	16	25.0	91.8	83.4	61.2
	Heparin (seconds)	16	25.0		43.2	44.3
Control	Prothrombin, %	38	10.5		94.4	68.0
Methylthiouracil rats	Prothrombin, %	14	28.6		100.7	73.4
Control	Prothrombin, %	12	58.3		93.2	62.5

A study of the content of basic components of the blood clotting system of hyperthyroid rats under conditions of experimental epilepsy showed that prolonged acoustic stimulation caused pronounced hypoprothrombinemia, observed 30-40 minutes after exposure to the stimulus (Table 2). Blood prothrombin falls by an average of 22%. This fall is, however, no greater than that found in normal "sensitive" rats (controls).

This is evidence that the observed changes in prothrombin level are not determining factors in raising the mortality of hyperthyroid rats, due to cerebral hemorrhage following prolonged acoustic stimulation.

The heparin activity of the blood of hyperthyroid rats is unaffected by prolonged acoustic stimulation.

Four of the 16 experimental rats died after exposure to sound, and 3 of them were found at autopsy to have massive hemorrhages into the lateral ventricles. Shortly before death, these rats had prothrombin levels of 63, 62, 60, and 71%, whereas the surviving rats included some with lower levels (49, 48, 81, 51, 40%).

A pronounced fall in prothrombin content was observed in some rats on the 9-10th day of thyroid treatment (down to 50% in some cases). Prolonged exposure to sound did not cause any change in their prothrombin content, a marked decrease of which was observed in rats whose prothrombin level was close to the normal. This observation also supports the view that animals possess a protective mechanism which prevents the prothrombin level from falling by more than 30-40% on exposure to this type of nervous trauma.

After prolonged methylthiouracil administration* (30 mg per 100 g body weight daily for 10 days) the fall in prothrombin content of the blood after prolonged acoustic stimulation was 30.7%, on the average; this is about the same as for the control group (27.3%). This is evidence that the changes in prothrombin content observed in the methylthiouracil group of rats were not a determining factor in reducing mortality from cerebral hemorrhage, in this group.

Summing up our experimental results, we have established that a pronounced fall in prothrombin level (by an average of 23%) takes place in the blood of rats exhibiting an epileptiform reaction to prolonged acoustic stimulation. The fall in prothrombin content is not, however, so great as to account entirely for the incidence of cerebral hemorrhage in experimental epilepsy.

Certain authors, who have endeavored to elucidate the cause of intracranial hemorrhages of new-born children, have also come to the conclusion that such subarachnoid hemorrhages are not determined by lowering of prothrombin activity [11].

The changes in blood prothrombin of rats due to impairment of nervous regulation are of short duration (the effect of acoustic stimulation appears after a few minutes, and normal levels are again found within a day). It may be supposed that the nervous system exerts a regulatory, compensatory action. This is one of the aspects of the trophic role of the nervous system.

The absence of any change in the thrombotropin content, and the observed changes in prothrombin content of the blood, suggest the existence of different regulatory mechanisms for these two thrombogenic components, which are synthesized basically in the liver. The considerable stability of the thrombotropin level under conditions of nervous trauma can, apparently, be related to the important role played by thrombotropin in the living organism as an initiator of the process of blood coagulation.

SUMMARY

Experimental reflex epilepsy in rats due to prolonged sound stimulation caused death in certain animals due to hemorrhage into the brain. The possible pathological changes in permeability and stability of capillaries, in the blood pressure and in coagulation, etc. may promote the appearance of hemorrhages. It was demonstrated experimentally that in condition of epileptiform reaction in rats caused by prolonged sound stimulation, prothrombin value of the blood shows marked reduction (by 23% on the average). The activity of thrombotropin and heparin does not change materially. Decrease in the level of prothrombin is not large enough to be the fundamental cause of appearance of hemorrhages in rats.

LITERATURE CITED

[1] N. V. Bogoyavlenskaya, Role of the Nervous System in the Regulation of the Prothrombin, Thrombotropin, and Heparin Levels of the Blood.** Thesis. Moscow, 1955.

[2] L. V. Krushinsky, L. P. Pushkarskaya and L. N. Molodkina, Vestnik Mosk. Univ., 12, 25-44, 1953.

[3] L. V. Krushinsky, Uspekhi Sovremennoi Biol., 37, No. 1, 74-93, 1954.

[4] L. V. Krushinsky and L. P. Dobrokhotova, Byull. Eksptl. Biol. i Med., 44, No. 8, 1957.***

[5] B. A. Kufryashov, P. D. Ulitina and A. A. Pugacheva, Byull. Eksptl. Biol. i Med., 11, No. 2, 99-101, 1941.

[6] B. A. Kudryashov, Doklady Akad. Nauk SSSR, 60, No. 8, 1469, 1948.

[7] G. G. Bazazyan, In Print.

[8] J. Lord, and W. D. Andrus, Arch. Surg. 1941, Vol. 42, No. 4, pp. 643-660.

[9] G. Bickel and S. Dicker, Schweiz. med. Wschr., 1942, Bd. 72, No. 15, S. 411-413.

[10] J. Goldstein, Med. Clin. North Amer., 1951, Vol. 35, No. 2, pp. 615-619.

* The rats used in this series of experiments were supplied from L. V. Krushinsky's laboratory. We determined only the prothrombin content of the blood of these animals.

** In Russian.

*** Original pagination. See C. B. Translation.

[11] C. Forattini and E. Fradelli, Folia Endocrinol., 1951, N. 4, pp. 643-649.

[12] H. P. Smith and J. E. Flinn, Ann. Rev. Physiol., 1948, N. 10, pp. 417-444.