PLASMA CORTICOSTERONE CONCENTRATION, GLUCOCORTICOID SUPPLY TO THE TISSUES, AND RESPIRATORY ENZYME ACTIVITY IN EXPERIMENTAL TETANUS

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Changes in the corticosterone content and respiratory enzyme activity in various tissues during tetanus exhibit well-defined phases: in the incubation period of ascending and hematogenous tetanus there is a definite increase in the hormone level in the heart, liver, kidneys, cerebral hemispheres, hypothalamus, and skeletal muscles. Meanwhile the activity of succinate dehydrogenase and cytochrome oxidase in these organs increases considerably. At the height of development of tetanus the corticosterone level of the blood plasma and adrenals rises sharply. In the tissues of other organs and in the CNS at this period there is a significant decrease in the corticosterone concentration and respiratory enzyme activity. A direct correlation is found between the respiratory enzyme activity of the tissues and their content of glucocorticoids in both forms of tetanus at various stages of its development.

Many aspects of the pathogenesis of tetanus have now been explained and the view that tetanus is a disease affecting several systems is well established [4-6]. However, some very important aspects of its pathogenesis, essential to the development of rational methods of combined treatment of tetanus, have still received little study. These include the state of adrenal function and the glucocorticoid supply to the tissues, closely bound up with protein and carbohydrate metabolism, and also respiratory enzyme activity, changes in which may play a special role in tetanus in connection with the hypoxia. A combined study of these problems is also interesting because of the link between the glucocorticoid level and respiratory enzyme activity [1, 3, 7-9, 12].

The object of the investigation described below was to study the state of adrenal function and the glucocorticoids supplied to the tissues and also the activity of succinate dehydrogenase (SDH) and cytochrome oxidase (CCO) in experimental tetanus.

EXPERIMENTAL METHOD

Two forms of experimental tetanus were produced: ascending tetanus, caused by injection of a lethal dose of tetanus toxin into the muscles of the hind limb while blocking the hematogenous route of spread of the toxin by means of antitoxin [4], and hematogenous tetanus caused by intravenous injection of a lethal dose of the toxin. The experiments were carried out at different stages of development of the disease: at

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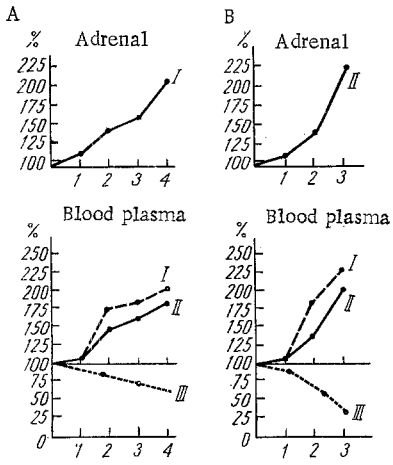

Fig. 1

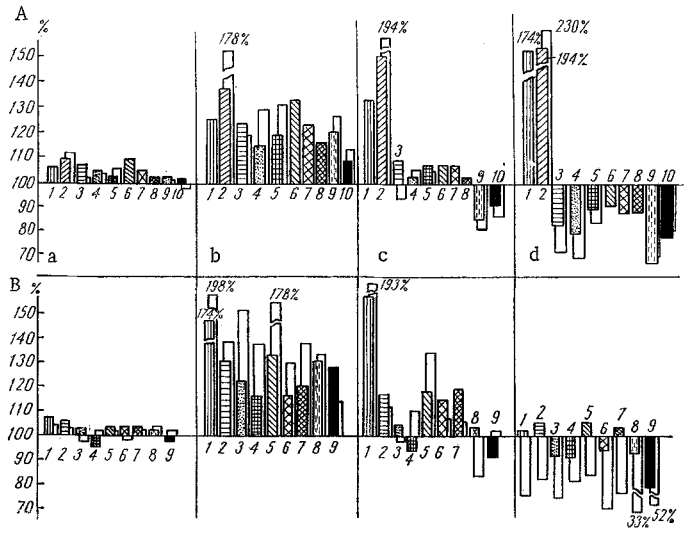

Fig. 2

Fig. 1. Dynamics of changes in corticosterone concentration in adrenals and peripheral blood in ascending (A) and hematogenous (B) tetanus. Ordinate, index studied in per cent of initial level (normal); abscissa: 1) injection of inactivated toxin, 2) incubation period, 3) stage of local tetanus (ascending) or period of clinically marked manifestation of hematogenous tetanus; 4) period of clinically marked manifestations of general ascending tetanus ("universal departure station" phenomenon). I) Free corticosterone; II) total corticosterone; III) eosinophil count.

Fig. 2. Dynamics of changes in corticosterone concentration (A) and respiratory enzyme activity (B) in experimental ascending tetanus: 1) adrenal, 2) blood plasma, 3) heart, 4) liver, 5) kidneys, 6) hypothalamus, 7) right hemisphere, 8) left hemisphere, 9) muscles of affected limb, 10) muscles of opposite limb. A: Shaded columns show level of total corticosterone, unshaded columns show level of free corticosterone; B: shaded columns show succinate dehydrogenase activity, unshaded columns show cytochrome oxidase activity. a) Injection of inactivated tetanus toxin, b) incubation period of ascending tetanus, c) local tetanus, d) period of clinically marked manifestations of ascending tetanus.

the end of the incubation period (17 h after injection of the toxin in the ascending type and 30 h after its injection in hematogenous tetanus), in the stage of local (after 24-26 h) and general ascending tetanus (on the 4th day of the disease) characterized by the "universal departure station" phenomenon [4], and in the period of clinically marked (general muscular rigidity) general hematogenous tetanus (on the 2nd day). The animals died toward the end of the 3rd day after injection of the toxin in hematogenous tetanus and on the 5th-6th day in ascending tetanus.

The corticosterone content was determined in the adrenals, blood plasma, heart, liver, kidneys, hypothalamus (both hemispheres), and the muscles of the affected and opposite limbs at intervals during the development of both forms of tetanus. Parallel tests of SDH and CCO activity were carried out on the same organs. Male albino rats were used. The total number of animals was 130, and depending on the stage and form of the disease each group contained 12-15 animals. Control experiments were carried out on intact animals and on animals receiving tetanus toxin inactivated by heat.

The total corticosterone concentration was determined by the method of De Moor et al. [13] and the content of free corticosterone by a modified method of Knigge and Hoar [14]. The intensity of fluorescence was measured with a Hitachi MPF-2A fluorescent spectrophotometer. In model experiments the spectral characteristics of standard solutions of corticosterone were determined and these agreed completely with the spectral characteristics of the corticosterone investigated in the tissues.

SDH activity was investigated by a modified method which ensures great accuracy and avoids the mistakes usually made when Thunberg's method (with methylene blue) is used. CCO activity was determined by the method described by Tret'yakova [11].

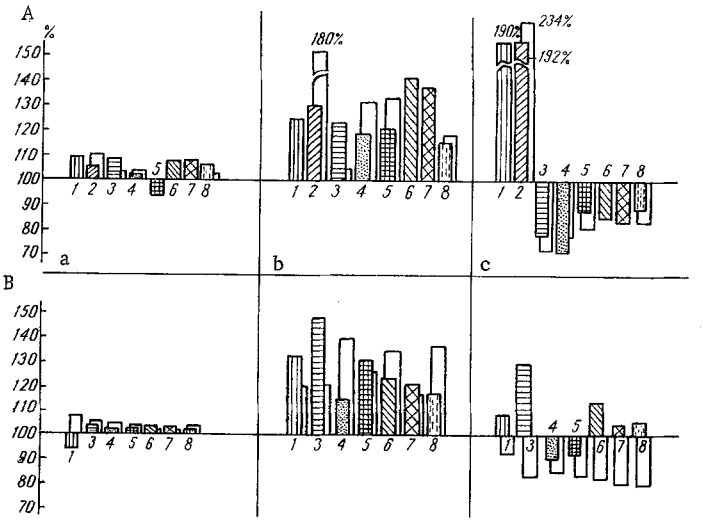

Fig. 3. Dynamics of changes in corticosterone concentration (A) and respiratory enzyme activity (B) in experimental hematogenous tetanus: 1) adrenal, 2) blood plasma, 3) heart, 4) liver, 5) kidneys, 6) cerebral hemispheres, 7) hypothalamus, 8) skeletal muscle. A: Shaded columns show total corticosterone level, unshaded columns show free corticosterone level. B) shaded columns show succinate dehydrogenase activity, unshaded columns show cytochrome oxidase activity; a) injection of inactivated tetanus toxin, b) incubation of hematogenous tetanus, c) period of clinically marked manifestations of hematogenous tetanus.

The experimental results were subjected to statistical analysis, with determination of the standard error, and to correlation analysis.

EXPERIMENTAL RESULTS

The experimental results showed that activation of adrenal function takes place during the development of experimental tetanus. This conclusion is based on the high level of corticosterone found in these experiments both in the adrenals themselves and in the blood plasma at all stages of development of ascending and hematogenous tetanus (Fig. 1). Further evidence in its support is given by the results of determination of the eosinophil count in the peripheral blood, which was significantly reduced in both forms of tetanus.

Comparison of the results of investigation of adrenal cortical function in ascending tetanus with the results obtained in the hematogenous form showed that the direction of the changes was the same in both forms of the disease, although the degree of glucocorticoid activity differed during its development. As Fig. 1 shows, in the period of clinical manifestations of hematogenous tetanus there was a more marked increase in glucocorticoid function than in ascending tetanus. This result can be explained on the assumption that the clinical course of the hematogenous tetanus was more severe.

The investigation showed that the changes in coricosterone content and respiratory enzyme activity have a well-defined phasic character in both forms of tetanus (Figs. 2 and 3).

In the incubation period of ascending and hematogenous tetanus the corticosterone concentration in the blood plasma and other organs tested rose significantly. In most organs the level of the steroid rose mainly on account of the free, biologically active fraction of the hormone, whereas the concentration of protein-bound hormone showed no significant change. Meanwhile there was a significant increase in the activity of the enzymes in these structures.

Later, with the development of both forms of tetanus and with an increase in the duration of poisoning, the concentrations of total and free corticosterone in the blood plasma increased about equally by about 80% ($P < 0.001$) and 130% ($P < 0.001$) respectively. The corticosterone concentration in the adrenals also was high at this period, being 75% above its initial level ($P < 0.001$) in ascending and 90% above ($P < 0.001$) in the hematogenous form of tetanus.

The corticosterone content in the tissues fell during the development of the disease, and in ascending tetanus this began in the stage of local tetanus. However, during this period the decrease was relative, only by comparison with the increase in the level of the steroid in the incubation period. During the development of ascending tetanus (the stage of the "universal departure station" phenomenon) and at the height of development of hematogenous tetanus an absolute decrease in the corticosterone content and, in particular, of its free fraction, was observed. In the heart muscle, for instance, the content of the free fraction fell by 27% ($P < 0.001$) in the ascending form and by 23% ($P < 0.001$) in hematogenous tetanus, while in the liver it fell by 28% ($P < 0.05$) and 24% ($P < 0.01$) respectively, and in the limb muscles by 28% ($P < 0.01$) and 16% ($P < 0.01$). These changes in the concentration of the hormone corresponded to definite changes in the activity of the respiratory enzymes; whereas the CCO activity fell significantly below its initial value, the SDH activity showed relatively little change at this period, but nevertheless, it was significantly lower than in the preceding incubation period of the disease.

It is important to note that changes in the glucocorticoid content and activity of the respiratory enzymes are interconnected. This is shown by the parallel nature of and the positive correlation between the changes in these indices.

The results of these investigations thus show that at the height of development of the disease there is relative glucocorticoid deficiency despite increased adrenocortical function. This type of situation can be found in other forms of pathology, and in particular, in states of shock [2, 3, 7, 10].

The mechanism of this phenomenon can be explained by a decrease in the content of the biologically active fraction of corticosterone in the tissues, where it is utilized intensively, while at the height of development of the disease the increased utilization of the hormone in the tissues is evidently far in excess of its supply.

Special attention must be given to the experiments in which the animals received inactivated tetanus toxin. These experiments showed that, regardless of the method of its administration (intramuscularly or into the blood stream), in the absence of tetanus the enzyme activity and corticosterone level in the organs studied remained unchanged. The results of these experiments also show that the changes observed in the glucocorticoid function of the adrenal cortex and in the respiratory enzyme activity are due to poisoning by tetanus toxin itself.

It is difficult at present to pick out the primary link in the complex chain of development of the interconnected reactions taking place in tetanus, some of which are the changes discovered in the glucocorticoid function of the adrenal cortex and the activity of the investigated respiratory enzymes. Nevertheless, knowledge of the changes in these aspects of the glucocorticoid balance and in the respiratory enzyme activity can be of great importance to the understanding of the pathogenetic structure of the disease and the development of rational pathogenetic methods of its treatment.

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