COMPARATIVE INVESTIGATION OF THE PRECIPITATING INFLUENCE OF HISTONES UPON PLASMA OF RATS WITH TUMOURS AND HEALTHY ONES

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The histones (H) which are widely distributed in the tissues of mamalians, specially in connection with certain situations of cell destructions (tumour and necrotic cell damages, large traumas, granulocyte destruction, etc.), can enter the blood-stream (1). Being already there they can form comlexes with various plasma proteins and can undoubtfully influence on certain physiological functions (2, 3).

Our previous study shew that the plasma of healthy rats can be precipitated by H (4). Having in mind that the neoplastic processes are connected with an increased cell destruction and changes in the plasma we decided to investigate the precipitating effect of H on plasma of rats with tumours.

Materials and methods

The study covers 27 rats with transplanted tumour of Walker. Parallelly with their plasma was tested the plasma of 25 healthy rats. A total H with initial concentration 300 micrograms was added to 0.5 ml plasma taken after decapitation of each rat with EDTA. After 10 min at room temperature we read the precipitation (+or—). If there was no precipitation we increased the concentration of H until obtaining it. The plasma was analysed electrophoretically in 17% PAAG, 2.5 M urea (5). H were obtained from rat livers by using 0.25 M H_2SO_4 (6) and diluted in destilled water. The protein was determined after the biuretic method (7).

Results and discussion

The added total H protein to rat plasma in vitro caused a precipitation in different degree of both normal plasma and that one from rats with tumours. The differences were considerable when small concentrations of H were used. As it can be seen from Table 1 with the results of our study, when H-concentration was 300 micrograms 63% of the rats with tumours shew a (+) reaction, while for the control group the percent was only 25.9%. With increasing of H-concentration the difference between both groups (experimental and control) was less and statistically unrerliable; at a concentration 1000 micrograms the percent of rats from both groups with an established precipitation was equal.

The electrophoretical analysis shows there are two ways of distribution of H-fractions in the supernatant of rats from both groups. The first one — H-fractions H1 and H2b+H2a are in the supernatant; second one — together with the cited H are found also the H-fractions rich in arginin H3 and H4.

Table 1

Plasma precipitation at various H-concentrations

Amount of added H-protein in micrograms/0.5 ml	Precipitation of rat plasma rats with tumours		healthy rats	
	+	−	+	−
300	**63**	37	26	74
500	**69**	31	48	52
1000	**80**	20	74	26

In both cases together with all plasma fractions in the precipitate were detected H1, H3, H2b+H2a and H4.

Our results show the different influence of H upon normal and pathological plasma (rats with tumours). This is most well expressed when small concentrations of H were applied. The more H is added the bigger the percent of (+) reaction is and with 2000 micrograms the plasmas of all rats from both groups practically precipitate. We presume, that H, being strong base proteins, form complexes with plasma proteins. Changing of their isoelectrical point requires a possible forth-coming precipitation of the formed complex (H+plasma proteins).

What is the reason of the various effect of H on normal and pathological plasma is still unknown. As for the neoplastic processes, perhaps there are certain changes of conformation, or also of aminoacid content of some plasma proteins. As a result of that they become more sensitive towards H-action and even small concentrations of a total H cause their precipitation.

The various H-fractions have different effect on the plasma. Mainly the H rich in arginin and less those rich in lysin are these H which change the stability of plasma proteins and precipitate with them. It is a probable result of the changed aminoacid content and conformation of H-fractions.

The possible diagnostic value of the stronger effect of H on pathological plasma will be the object of our future studies of patients with different diseases.

REFERENCES

1. Гурович, А. Е., С. И. Городецкий, Е. В. Сидорова. *Биохимия.* 1967, 32, 2, 302—309. — 2. Делекторская, Л. И., И. А. Сентебова. *Лаб. дело.* 1971, 8, 483—487. — 3. Daskalov, D., I. Gavazova. *Experientia,* 34, 1978, 522. — 4. Losticky, C., R. Jelinek. *Physiol. Bohemoslov.,* 25, 1976, 341—344. — 5. Daskalov, D., I. Gavazova. In: Internat. Congr. Physiol. Sci., XXIII. Budapest, 1980, Abstracs, p. 370. — 6. Panyim, S., R. Chalkley. *Arch. Biochem. Biophys.*, 130, 1969, 337. — 7. Spelsberg, T. C., L. S. Hnilica. *Biochim. Biophys. Acta,* 228, 1971, 202.

СРАВНИТЕЛЬНОЕ ИССЛЕДОВАНИЕ ПРЕЦИПИТИРУЮЩЕГО ДЕЙСТВИЯ ГИСТОНОВ НА ПЛАЗМУ ОПУХОЛЕВЫХ И ЗДОРОВЫХ КРЫС

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РЕЗЮМЕ

Добавление тотального гистона вызывает неодинаковую по своей степени преципитацию плазмы опухолевых и здоровых крыс. Различия ярче выражены при более низкой концентрации гистамина. При добавлении 300 μg в 65% плазмы опухолевых крыс получается преципитация, в то время как в контрольной группе она достигает лишь 25%. При увеличении концентрации гистонового белка эти различия уменьшаются и при 1000 μg выравниваются.