EFFECT OF PRODIGIOSAN ON SERUM GLUCOCORTICOID TO INSULIN RATIO IN ALBINO RATS

L. E. Panin,* I. I. Smolentseva,
N. V. Atuchina, and T. A. Tret'yakova

UDC 615.275.4.015.4:[616.154:577.175.53+577.175.722]-076.9

KEY WORDS: corticosteroids; insulin; hexokinase; glucose-6-phosphate dehydrogenase; steroid production.

Prodigiosan is widely used in medicine at the present time as an agent increasing the nonspecific resistance of the body, in the treatment of various diseases: of the lungs, liver, kidneys, etc. It is most effective in the treatment of chronic, indolent forms of disease. However, the mechanism of the protective action of prodigiosan is not yet clear. It may be realized through activation of the pituitary–adrenals system [1], the mononuclear phagocyte system [5], or the immunity system [2]. It is not yet known what is the primary and what the secondary components. Other bacterial polysaccharides, such as zymosan, also have a similar action.

In the investigation described below, administration of prodigiosan to experimental animals was used as the model with which to study the mechanism of the effect of bacterial polysaccharides on steroid production in the adrenals, assuming that these glands occupy a key position in formation of the nonspecific resistance of the body. Since many metabolic effects of glucocorticoids are insulin-dependent, the effect of prodigiosan on changes in the serum insulin level was studied at the same time.

EXPERIMENTAL METHOD

Male Wistar rats weighing 170-200 g were used. Prodigiosan was injected intraperitoneally in a dose of 0.25 μg/g body weight in a volume of 1 ml. Control animals were given an intraperitoneal injection of an equal volume of physiological saline. The animals were decapitated 1 day later, laparotomy performed, and the adrenals removed and weighed. A homogenate was then prepared in 0.04 M Tris-HCl buffer, pH 7.4. Total hexokinase [7] and glucose-6-phosphate dehydrogenase (G6PDH) activity [8] was determined in the supernatant fraction (20,000g). To study the rate of steroid production, slices (quarters) of the adrenals were incubated in Krebs–Ringer bicarbonate buffer, pH 7.4, containing 2% albumin, equilibrated with a CO_2:O_2 mixture in the ratio of 5:95 at 37°C, with constant shaking for 30 min. The 11-hydroxycorticosteroid (11-HCS) concentration in the incubation medium and blood serum was determined fluorometrically [6] and the serum insulin level by radioimmunoassay using standard kits from the Institute of Biorganic Chemistry, Minsk.

EXPERIMENTAL RESULTS

The weight of the adrenals was increased on average by 21% 24 h after injection of prodigiosan. This was associated with hypertrophy of the glands, which was confirmed by the results of histological investigation. Activity of hexokinase, the key enzyme of glycolysis, was increased by 34%, and that of G6PHD, the key enzyme of the pentose phosphate pathway of carbohydrate oxidation, by 47% (Table 1).

The increase in the rate of glycolysis and of the pentose phosphate pathway associated with activation of the key enzymes led to increased formation of NADH and NADPH. The latter, which is involved in reduction processes, has the ability to intensify steroid production in the adrenals. This was confirmed experimentally.

*Corresponding Member, Academy of Medical Sciences of the USSR.

Laboratory of Biochemistry, Institute of Clinical and Experimental Medicine, Siberian Branch, Academy of Medical Sciences of the USSR, Novosibirsk. Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 107, No. 3, pp. 259-260, March, 1989. Original article submitted March 25, 1988.

0007-4888/89/0003-0277$12.50

TABLE 1. Changes in Hexokinase and G6PDH Activity in Adrenals and in Serum 11-HCS and Insulin Concentrations in Rats under the Influence of Prodigiosan

Group of animals	Weight of adrenals, mg	Hexokinase (nmoles·min^{-1}·mg protein^{-1})	G6PDH (nmoles·min^{-1}·mg protein^{-1})	11-HCS, μg%	Insulin, μU/ml
Intact	40,7±1,6 (26)	22,0±1,2 (24)	256±23,4 (25)	15,8±0,9 (10)	37,6±3,7 (34)
Experimental	49,3±2,7* (18)	29,5±3,2* (15)	376±30,2* (19)	16,0±1,3 (10)	54,8±5,1* (20)

Legend. *$p < 0.05$ (number of experiments shown in parentheses).

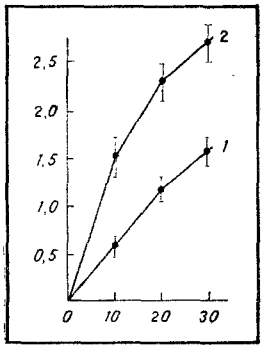

Fig. 1. 11-HCS production by adrenal slices of control rats (1) and of animals receiving prodigiosan 24 h before experiment (2). Average results of 4-5 experiments. Abscissa, incubation time (in min); ordinate, 11-HCS concentration (in μg/100 mg tissue in incubation medium).

The rate of secretion of 11-HCS by surviving adrenal slices from rats receiving prodigiosan in incubation medium was 2.5 times greater than in the control animals (Fig. 1). Hypertrophy of the adrenals, increased activity of hexokinase and G6PDH, and also steroid hormone production are evidently interconnected and involve components of the same chain. This is evidence that the primary factor in the action of prodigiosan on the adrenals is structural changes (hypertrophy). The view has been expressed that they are formed with the participation of tissue-specific resident macrophages, stimulated by bacterial polysaccharides [5].

Our results are in agreement with those obtained by other workers, showing that following injection of prodigiosan into patients, the urinary excretion of 17-hydroxysteroids, 17-ketosteroids, and dihydroepiandrosterone [1] is increased.

Despite the increased rate of steroid production in the adrenals and increased production of steroid hormones, blood levels of the latter in rats receiving prodigiosan were virtually unchanged (Table 1). Moreover, the writers showed previously that under the influence of prodigiosan the concentration of glucocorticoids in the blood may actually be reduced on account of their active reception by peripheral tissues [5]. This mechanism lies at the basis of the increase in nonspecific resistance of the body under the influence of bacterial polysaccharides.

Some of the metabolic effects of glucocorticoids in peripheral tissues are inhibited by insulin [4]. These include potentiation of their lipid-mobilizing action, inhibition of glycolysis and glycogenolysis, and others. Changes in the blood insulin level were accordingly determined simultaneously. Under the influence of prodigiosan, the blood insulin level was raised by 45% (Table 1).

Prodigiosan thus potentiates the production not only of steroid hormones but also, evidently, of insulin. This phenomenon may probably be based on activation of hypertrophic and proliferative processes in the tissues. Increased steroid hormone production must be regarded as an important instrument of this mechanism, determining the increase in nonspecific resistance of the body.

LITERATURE CITED

1. Z. V. Ermol'eva and G. E. Vaisberg, Stimulation of Nonspecific Resistance of the Body and Bacterial Polysaccharides [in Russian], Moscow (1976).
2. V. P. Kostromina, Prob. Tuberk., No. 10, 39 (1982).
3. L. E. Panin, Energetic Aspects of Adaptation [in Russian], Leningrad (1978).
4. L. E. Panin, Biochemical Mechanisms of Stress [in Russian], Novosibirsk (1983).
5. L. E. Panin and N. N. Mayanskaya, Lysosomes: Their Role in Adaptation and Recovery [in Russian], Novosibirsk (1987).
6. Yu. A. Pankov and I. Ya. Usvatova, Methods of Investigation of Some Hormones and Mediators [in Russian], Moscow (1965), pp. 137-145.
7. H. M. Katzen, D. D. Soderman, and C. E. Wiley, J. Biol. Chem., 245, No. 16, 4081 (1970).
8. J. W. Löhn and H. D. Waller, Methoden der enzymatischen Analyse, Vol. 1, Berlin (1970), pp. 599-606.

ROLE OF THE ACTIVE CENTER OF ENZYMES IN TRIGGERING THE MECHANISM OF COMPENSATORY REACTION TO PLASMIN

T. M. Kalishevskaya, M. G. Golubeva, R. B. Aisina, G. Yu. Popova, and G. V. Bashkov

UDC 612.115.2.06:612.128.015.13

KEY WORDS: plasmin; acyl-plasmin

Plasmin (EC 3.4.21.7) is a serine proteinase whose function is to maintain the fluid state of the blood. Besides hydrolysis of fibrinogen and fibrin, plasmin also causes activation of high-molecular-weight kininogen, factors XII and VII, factors C1 and C3 of complement, and prorenin, and has a controlling influence on the level of plasma components of hemostasis, namely factors II, V, VIII, and XIII, and on platelet aggregation. The proteolytic activity of the enzyme is limited by the α_2-antiplasmin of the blood and through a physiological compensatory reaction leading to release of plasmin inhibitors and procoagulants into the bloodstream [11]. Plasmin binds highly specifically with the endothelium [7] and excites the receptor appartus of the vascular wall, with consequent activation of the sympathetic division of the autonomic nervous system [2]. Vasoactive reactions evoked by catecholamines lead to the release of procoagulants and of fibrinolysis inhibitors from the vascular wall into the bloodstream. Catecholamines also activate the contact phase of blood clotting [3] and stimulate platelet aggregation [10]. The structural features of the enzyme responsible for the triggering mechanism of the compensatory reaction are not yet known.

It was decided to study the role of the active center of the enzyme in realization of the triggering mechanisms of the compensatory reaction, using plasmin with a chemically modified active center.

EXPERIMENTAL METHOD

Plasmin was obtained by activation of human plasminogen (Leningrad Research Institute of Hematology and Blood Transfusion, USSR) and streptokinase (Streptase, Behringwerke, West Germany). Activation was carried out in the proportion of 33 U of streptokinase to 1 CTA U of plasminogen. The plasminogen was homogeneous on electrophoresis in polyacrylamide gel with SDS and had a molecular weight of 87 ± 2 kilodaltons. The caseinolytic activity of the plasmin was 14-16 CTA U/mg protein. The amidase activity of the plasmin preparation was determined spectrophotometrically from the rate of hydrolysis of valine–leucine–lysine paranitroanilide (S-2251, from "Kabi Diagnostica," Sweden), by the method in [13]. The plasminogen

Department of Human and Animal Physiology, Faculty of Biology, and Department of Chemical Enzymology, Faculty of Chemistry, M. V. Lomonosov State University, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR I. P. Ashmarin.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 107, No. 3, pp. 260-264, March, 1989. Original article submitted December 18, 1987.

0007-4888/89/0003-0279$12.50