EFFECT OF SPLENIN AND ITS FRACTIONS ON ADRENOCORTICAL FUNCTION

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UDC 612.453.014.46:615.361.411

KEY WORDS: splenin; ascorbic acid; adrenocortical hormones; adenosine-5-monophosphate (AMP).

Data in the literature on functional interconnection between the spleen and adrenal glands [2, 6-8] laid the foundations for the study of the effect of splenin* on adrenocortical function. Preliminary results obtained by the present writers showed that splenin, if injected intramuscularly into rats in a single dose of 1 ml per rat weighing 120-150 g, lowered the ascrobic acid concentration in the adrenals 1 and 3 h after administration by 15-20% of its original level on average.

To determine which of the biologically active substances composing splenin is responsible for the above effect, in the investigation described below splenin was fractionated and the effect of each fraction on the ascorbic acid concentration in rat adrenals was studied.

EXPERIMENTAL METHOD

Male rats weighing 120-150 g were used. The animals were kept on the standard animal house diet. An aqueous suspension of prednisolone (1 ml) was injected subcutaneously into all animals 18 h before the experiment began in a dose of 10 mg/ml per rat. The prednisolone was first dissolved in a small volume of ethanol to obtain a more homogeneous suspension. On the day of the experiment the animals were divided into nine groups (20 rats in each group). As the control for fractions containing water-soluble substances, the solvent of splenin, namely physiological saline with the addition of 10% of ethanol (animals of group 1) was taken, and for the lipid fractions the control consisted of physiological saline with the addition of 10% ethanol and 1% Tween (group 2). Splenin was injected into the animals of group 3, fraction No. 1 of the water-soluble substances into those of group 4, fraction No. 2 of the water-soluble substances into group 5, the proteolipid fraction into group 6, the total lipid fraction into group 7, the polar lipid fraction into group 8, and the nonpolar lipid fraction into group 9. Splenin and its fractions were injected intramuscularly in a single dose: splenin in a dose of 1 ml per rat and the splenin fractions in a dose of 0.5 ml per rat, which corresponded to the content of the given fraction in 1 ml splenin. The animals were killed (with ether) 3 h after injection of splenin and its fractions, the left adrenal gland was removed, freed from adjacent tissues, and weighed. The ascorbic acid content in the adrenals was determined by the method described in the USSR State Pharmacopoeia [3].

EXPERIMENTAL RESULTS

The experiments showed (Table 1) that both splenin and its fractions containing water-soluble substances, if injected intramuscularly in a single dose, depressed the ascorbic acid concentration in the rat adrenals 3 h after injection.

The largest decrease in the ascorbic acid content in the adrenals was observed in rats of group 5 (25.8%), which received fraction No. 2 of water-soluble substances, and in the rats of group 3 (22.4%), which received splenin. A significant decrease in the ascorbic acid content in the adrenals also was observed in the animals of group 4 (16.2%), which received fraction No. 1 of the water-soluble substances.

*Splenin is a liquid therapeutic preparation obtained from bovine spleen.

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(Presented by Academician of the Academy of Medical Sciences of the USSR N. A. Fedorov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 92, No. 7, pp. 98-100, July, 1981. Original article submitted January 14, 1981.

0007-4888/81/9207-0974$07.50

TABLE 1. Changes in Ascorbic Acid Concentration in Adrenals of Rats 3 h after a Single Injection of Splenin and Its Fractions (M ± m)

Group of animals	Experimental conditions	Ascorbic acid content in adrenals, mg %	% decrease in ascorbic acid content in adrenals
1	Control		
	Physiological saline + 10% ethanol	518,0±21,0	—
3	Splenin	403,0±12,0	22,4*
4	Fraction No. 1 of water-soluble substances	435,0±21,0	16,2*
5	Fraction No. 2 of water-soluble substances	385,0±21,0	25,8*
2	Control		
	Physiological saline + 10% ethanol + 1% Tween	486,0±17,0	—
6	Proteolipids	476,0±18,0	2,1
7	Total lipids	456,0±14,0	6,2
8	Polar lipids	484,0±8,0	8,4
9	Nonpolar lipids	465,0±10,0	4,3

*P < 0.05 compared with control.

TABLE 2. Chemical Composition of Splenin Fractions Containing Water-Soluble Substances

Substance	Content in spleen fractions, mg/100 g dry residue	
	fraction No. 1	fraction No. 2
Amino acids	1686,0	10245,0
RNA	0,5	1,2
DNA	0,1	0,5
Nucleotides	120,0	560,0
Vitamins		
Thiamine	0,13	0,50
Riboflavin	0,25	1,64
Nicotinic acid	0,82	2,05
Pantothenic acid	—	2,50
Pyridoxine	0,02	0,08
Folates	0,10	0,15
Ascorbic acid	2,14	6,02

The fall in the ascorbic acid content in the adrenals of rats receiving lipid fractions of splenin (groups 7, 8, and 9) was much smaller and was not statistically significant.

It can be concluded from these results that splenin and its fractions, containing water-soluble substances, significantly lower the ascorbic acid content in the adrenals. Fractions of splenin containing lipids have no such action.

The marked decrease in the ascorbic acid content in the adrenals of rats receiving fractions of water-soluble substances served as the basis for a more detailed study of the chemical composition of these splenin fractions.

The amino-acid composition of the above-mentioned fractions has been established by thin-layer and ion-exchange chromatography, nucleic acid components have been identified by paper chromatography, and water-soluble vitamins have been determined by microbiological methods. According to data in the literature, substances of peptide nature also are present among the water-soluble components of splenin [5]. Complexes of lipids with peptides may also be present in the preparation.

Data on the chemical composition of splenin fractions containing water-soluble substances are given in Table 2. Of the water-soluble substances of splenin, AMP may have the action described above on the adrenal cortex.

Accumulation of AMP in the adrenal cortex due to the action of ACTH is known to be accompanied by activation of phosphorylase and of synthesis of steroid hormones. Furthermore, addition of AMP only to adrenocortical tissue stimulates steroid hormone synthesis, just as does the addition of ACTH only. This suggests, as other workers have stated [4], that AMP and ACTH act in accordance with the same principle.

The AMP content in fractions of water-soluble substances was determined quantitatively by a spectrophotometric method. The nucleotides were separated beforehand by paper chromatography, on Leningrad Mark M paper in a system of 15% TCA and acetone (63:35). The stains of the nucleotides were located by means of an ultrachemiscope, on the basis of their absorption in UV light. A standard preparation of AMP was applied parallel with the test sample. The stain corresponding to the position of AMP was outlined with a pencil, cut out, and eluted with 5 ml of 1 M phosphate buffer, pH 7.0, for 12 h (37°C). The eluate of an area of paper of equivalent size and location was used as the control. Spectrophotometry was carried out at 260 and 290 nm. A conversion factor [1] was used to calculate the quantity of AMP. The quantity of AMP in 1 g of fraction No. 1 of water-soluble substances was 55.4 μmoles, in 1 g of fraction No. 2 of water-soluble substances it was 14.8 μmoles, whereas the other fractions of splenin contained no AMP.

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