AORTIC GLYCOSAMINOGLYCANS IN AVITAMINOSIS

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In hypovitaminosis K induced by a vitamin K-deficient diet or by Pelentan (ethyl biscoumacetate) a decrease was observed in the total content of glycosaminoglycans (GAG) and a change in the relative proportions of their fractions in the aortic wall of rats. Daily administration of ascorbic acid (100 mg/kg) to the rats from the first day of their vitamin K-deficient diet prevented changes in sulfated GAG but had no effect on the decrease in the hyaluronic acid level. The results are discussed from the standpoint of the disturbance of ascorbic acid metabolism in hypovitaminosis K.

KEY WORDS: glycosaminoglycans; vitamin K; vitamin C; rat aorta.

It was shown previously that vitamin K deficiency is accompanied *in vivo* by a decrease in the total content of glycosaminoglycans (GAG), glycoproteins, and collagen in the tissues [6-8]. In the investigation described below the fractional composition of GAG in the aortic wall was studied in rats kept on a vitamin K-deficient diet and receiving various doses of vitamin K and ascorbic acid (vitamin C).

EXPERIMENTAL METHOD

Experiments were carried out on albino rats weighing 130-150 g, divided into two groups. The first group received a diet without vitamin K daily for 25 days (series II, 16 rats) or the same diet supplemented by daily administration of ascorbic acid in a dose of 100 mg/kg body weight (series III, 16 rats), or on the same diet supplemented by vikasol (synthetic vitamin K) in a dose of 10 mg/kg diet (series I, 16 rats). The second group of rats received vikasol daily by intramuscular injection in a dose of 10 mg/kg body weight for 15 days (series V, 20 rats) or the vitamin K antagonist Pelentan (ethyl biscoumacetate) perorally in a dose of 30-40 mg/kg daily for 15-20 days (series VI, 24 rats). Another 25 rats of the second group were kept under ordinary animal house conditions and served as the control for the rats of the group (series IV). Development of hypovitaminosis K was monitored by the prothrombin time. The rats were decapitated at the end of the experiments.

The aortas of four rats were pooled, cut into pieces with scissors, defatted with alcohol, ether, and acetone, and dried to constant weight in air and above paraffin wax shavings and $CaCl_2$ at 20°C. To isolate GAG the aortic tissue was subjected to proteolysis [9] with papain (from Merck, West Germany). The mixture of GAG was precipitated with cetyltrimethylammonium bromide (Cetavlon) and divided into fractions by elution with an ascending concentration of NaCl solution from Dowex columns (1 × 2, 200-400 mesh) [2]. To determine the reproducibility of the results of analysis experiments were carried out in which pure samples of GAG, obtained by analogous fractionation from tissues, or commercial preparations of GAG precipitated with alcohol, washed with alcohol-ether mixture, and dried over shavings of paraffin wax and $CaCl_2$, were added. Regeneration of GAG amounted to 89.6-101%. Quantitative analysis of the fractions was carried out by determining hexuronic acids by the carbazole reaction, with the addition of tetraborate [10]. To identify chondroitin-sulfate B (dermatan sulfate) the hexuronic acids were determined simultaneously by the orcin method [13]. The content of the individual GAG fractions was expressed in mg/100 g dry defatted tissue.

EXPERIMENTAL RESULTS

The experiments showed that keeping rats on a vitamin K-deficient diet, but with the addition of vitamin K, has virtually no effect on the content of individual GAG in the aortic

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TABLE 1. Results of Fractionation of GAG from Rat Aorta (in mg/100 g weight of dry defatted tissues; M±m)

Series of animals	Total GAG content	Hyaluronic acid	Chondroitin-sulfates A and C	Chondroitin-sulfate B	Heparitin-sulfate	Total content of sulfated GAG
I	542,3±34,7	199,3±13,4	139,6±10,0	52,3±5,4	151,1±5,8	343,0±21,1
II	377,1±26,9	111,6±24,7	103,9±6,7	41,1±4,3	120,2±14,1	258,0±21,2
P	<0,001	<0,001	<0,05	>0,05	>0,05	<0,05
III	476,7±29,1	124,1±18,0	162,7±11,0	53,9±7,4	136,7±14,0	352,6±30,1
P	>0,05	<0,001	>0,05	>0,05	>0,05	>0,05
IV	544,4±18,9	206,7±7,1	137,4±4,9	48,1±3,7	152,1±3,7	337,6±11,7
V	577,6±20,1	217,2±8,0	131,4±2,6	56,4±4,4	152,6±5,1	340,5±12,0
P	>0,05	>0,05	>0,05	>0,05	>0,05	>0,05
VI	445,3±16,9	133,4±5,8	117,9±4,7	49,8±2,8	144,1±2,6	311,9±10,2
P	<0,001	<0,001	<0,05	>0,05	>0,05	>0,05

wall (Table 1). The relative percentages of hyaluronic acid, chondroitin-sulfates A and C (together), chondroitin-sulfate B, and heparitin-sulfate were 36.7:25.7:9.6:27.8, in agreement with data in the literature [5]. Administration of vitamin K in excess to rats (series V) caused no change in the above indices of GAG metabolism.

A significant decrease in the total GAG content was observed in animals kept for a long time on a diet deficient in vitamin K, as a result of a decrease in all the fractions of biopolymers tested: hyaluronic acid (45%), chondroitin-sulfates A and C (28%), chondroitin-sulfate B (22%), and heparitin-sulfate (21%). A decrease in the GAG content in the aortic wall also was observed in the animals with secondary vitamin K deficiency, i.e., those receiving Pelentan. This points to a common mechanism of the disturbance of GAG metabolism in both cases of development of hypovitaminosis K.

In rats receiving large doses of ascorbic acid and a diet deficient in vitamin K the content of sulfated GAG in the aortic wall was restored to normal whereas the hyaluronic acid content remained abnormal (a shift of 38%). A characteristic feature of ascorbic acid deficiency is known to be a decrease in the content of collagen [1, 3] and of chondroitin-sulfates bound with this protein in the tissues [11]. In vitamin K deficiency similar changes arise in collagen [6] and chondroitin-sulfates. These facts suggest that the fall in the level of sulfated GAG in vitamin K deficiency is the result of manifestation of ascorbic acid deficiency. This explanation is not ruled out by the observed decrease in the ascorbic acid level in the tissues of animals with primary and secondary hypovitaminosis K [4].

Vitamin K deficiency *in vivo* is thus accompanied by a decrease in the content and a change in the relative proportions of the fractions of GAG in the aortic wall, one cause of which may be a disturbance of interaction between vitamins K and C.

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