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EFFECT OF VITAMIN D AND PLASMA CALCIUM UPON PROTEOGLYCAN SIZE IN CHICK GROWTH CARTILAGE

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SUMMARY

Proteoglycan subunit isolated from the growth cartilage of rachitic chicks is of much smaller size than the corresponding preparation from vitamin D-treated chicks. The size change can be related to differences in plasma calcium concentration and not to the vitamin D status of the chicks.

INTRODUCTION

Vitamin D deficiency results in a disorder of bone formation. In the skeletally immature animal endochondral ossification, the process by which bone grows in length, is affected as well as appositional bone formation; as a consequence the cartilage growth plate becomes abnormally wide. The two main structural components of this cartilage are collagen and proteoglycan. Collagen of the rachitic growth cartilage appears to be of normal composition and constitutional type (1) but we have found evidence that the other major structural component, proteoglycan, differs from normal (2). Proteoglycan subunits, isolated from the growth cartilage of chicks raised on a vitamin D-replete diet, is of relatively large hydrodynamic size compared with proteoglycan isolated from the growth cartilage of chicks raised on a vitamin D-deficient diet as shown by measurements of viscosity, wide pore gel electrophoresis and chromatography on columns of Sepharose 2B (2). A large proportion of these molecules from the vitamin D-replete chick elute in the void volume of a column of Sepharose Cl-2B even after reduction and alkylation or in the presence of 4M guanidinium chloride in the eluting buffer (3). It is therefore apparent that the

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proteoglycan monomer units are large and the results observed are not an artefact resulting from in vitro aggregates formed in the presence of traces of hyaluronic acid. The average size of proteoglycan subunits from rachitic chick growth cartilage is smaller than that of bovine nasal cartilage proteoglycan.

We considered initially that the large reduction in proteoglycan monomer size in the rachitic state was more consistent with a hypothesis of limited proteolytic degradation since in the rachitic state the increased size of the growth cartilage plate makes it probable that the life of proteoglycan molecules, and therefore the chance of proteolytic attack, is considerably increased. However, in vivo labelling studies with radioactive isotopes of the core protein and the glycosaminoglycan side chains of growth cartilage proteoglycan in rachitic and normal chicks showed that newly synthesised molecules were of similar size to the whole population of proteoglycan molecules in the tissue (3). There was no evidence that in the rachitic state large proteoglycan molecules were being formed and then rapidly degraded. It therefore seemed more likely that some factor relating to vitamin D-deficiency was influencing the size of the proteoglycan molecules being formed. Vitamin D-deficiency in the chick is accompanied by a marked hypocalcaemia due to the failure of the vitamin D-stimulated intestinal calcium transport. This report provides evidence that the size of proteoglycan formed in chick growth cartilage can be related to the level of plasma calcium and not to the vitamin D status of the chick.

MATERIALS AND METHODS

One day old Ranger cockerels (Ross Poultry, Lincs.) were divided into four groups of six. Two groups were raised on the standard vitamin D-deficient diet (5) containing 1.2% calcium and 0.7% phosphorous: one of these groups was given 5µg cholecalciferol orally each week. A third group was raised on a similar diet modified to reduce the calcium content to below 0.2% and each chick was given 5µg cholecalciferol orally each week. In the fourth group the hypocalcaemia of vitamin D-deficiency was compensated for by feeding the chicks the standard diet supplemented with 2% by weight of calcium as calcium carbonate. At four weeks of age chicks were bled, plasma being separated for analysis for calcium and inorganic phosphate, and then killed.

The cartilage growth plate was dissected from the proximal end of the tibiae, minced finely with a scalpel blade and extracted for 48h. at 5° with

4M guanidinium chloride (30 ml/g wet weight of tissue) containing proteinase inhibitors (1 mM iodoacetic acid, 1mM phenylmethyl sulphonyl fluoride and 2 mM ethylene diaminetetra-acetic acid). After centrifugation (20,000 r.p.m., 30 min) the specific gravity of the supernatant solution was adjusted with caesium chloride to 1.70 ± 0.01 and the solution centrifuged (40,000 r.p.m., 110,000g_{av} 48h, 10°C. A stainless steel probe was inserted to the bottom of the tube and the contents pumped out and fractions (0.6 ml) collected; those fractions containing the most hexuronic acid (approximately the lower 25-30% of the contents of the tube) were pooled, dialysed against water, then saturated sodium chloride and finally, exhaustively against water before lyophilisation. For chromatography approximately 1-2 mg of this lyophilised proteoglycan was dissolved in 0.4 ml sodium acetate buffer (0.2M acetate, pH 5.5) and applied to a column of Sepharose Cl-2B (1.6 x 27 cm) and eluted with the same buffer at a flow rate of 9.8 ml/hr. Fractions (1.8 ml) were collected and analysed for hexuronic acid.

RESULTS AND DISCUSSION

Growth cartilage proteoglycan was chromatographed on a column of Sepharose Cl-2B. The hexuronic acid elution profile was similar for proteoglycan specimens from the same group. Representative elution profiles for each group are shown in figure 1. The corresponding values of plasma calcium (mg/dl) for the chicks were: vitamin D-treated, 10.0 and 5.9; vitamin D-deficient, 11.6 and 6.6 respectively.

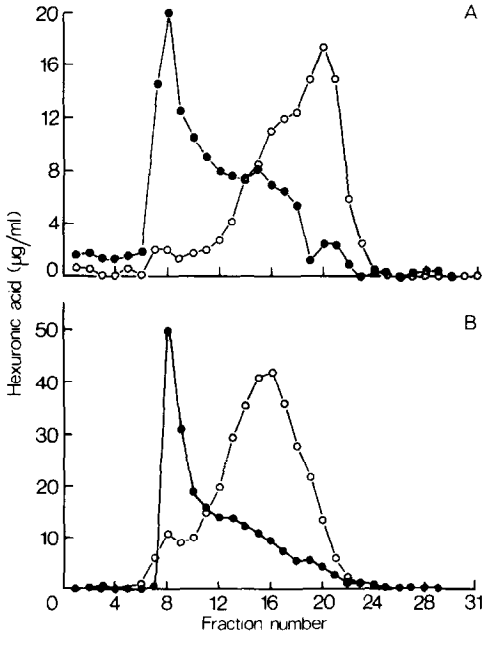

Fig 1. Representative hexuronic acid elution profile from a Sepharose Cl-2B column for growth cartilage proteoglycan from (a) vitamin D-treated chicks and (b) vitamin D-deficient chicks. Profiles are shown for normocalcaemic (●-●) and hypocalcaemic (O-O) chicks.

The size of proteoglycan isolated from the growth cartilage of vitamin D-replete, normocalcaemic chicks and vitamin D-deficient, hypocalcaemic chicks (figure 1) was similar to that of preparations studied previously (2) from pooled normal and rachitic tissue respectively. By raising the calcium concentration of the vitamin D-deficient diet to a level of 3.2% calcium we were able to correct the hypocalcaemia in vitamin D-deficient four week old chicks fed on the diet from hatching. Despite the normocalcaemia the proximal tibial growth plate showed the enlargement characteristic of rickets. Proteoglycan isolated from this tissue was similar in size (Figure 1b) to that from vitamin D-replete, normocalcaemic chicks. Chicks raised on a low calcium but vitamin D-replete diet showed pronounced hypocalcaemia at four weeks, and the proximal tibial growth cartilage plate was widened as in vitamin D-deficiency. The size of the proteoglycan isolated from these chicks resembled that from vitamin D-deficient hypocalcaemic chicks (figure 1a).

The differences in size in chick growth cartilage appear to be related to plasma calcium concentration and neither to vitamin D status nor to plasma inorganic phosphate concentration (results not shown). In the chick this phenomenon may be confined to growth cartilage since articular cartilage proteoglycan from rachitic and normal chicks was similar in average size and resembled the smaller rather than the larger growth cartilage proteoglycan (I. Dickson, unpublished work). Little is known of the proteoglycan size in mammalian growth cartilage but the size of proteoglycan-hyaluronic acid aggregates in aspirates from rachitic rat growth cartilage has been reported to be much higher than that of aggregates from other cartilagenous tissues (4) - it should be noted that the characteristic lesions of rickets in the rat are only induced when vitamin D-deficiency is accompanied by a hypophosphataemia and normocalcaemia.

There is evidence that the level of extracellular calcium can affect the biosynthesis of the structural components of cartilage matrix. The type of

collagen synthesised by rabbit articular chondrocytes in suspension culture can be varied by altering the calcium concentration of the medium (5) and proteoglycan synthesis by chick vertebral cartilage is reduced when the calcium concentration in the culture medium is below physiologically normal levels (6). The level of plasma calcium can also influence the degree of post-translational hydroxylation of bone collagen lysine residues whereas skin collagen of similar constitutional type is relatively unaffected (7).

The physiological significance of these changes is not yet clear. If the formation of small proteoglycan does have an inhibitory effect upon the process of endochondral ossification it is not the only factor that can do so since the process is still affected when large proteoglycan is formed in the normocalcaemic but vitamin D-deficient chick. A more detailed investigation of this phenomenon should give us a clearer idea of the relationship between vitamin D and calcium in the physiology of bone formation.

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