THE STIMULATORY EFFECT OF CALCIUM ON THE SYNTHESIS OF CARTILAGE PROTEOGLYCAN

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SUMMARY

Calcium stimulates the uptake of $[^{35}S]$ SO_4 into cartilage at physiological concentration for ionized calcium. This effect can be blocked by puromycin, indicating calcium stimulates the synthesis of proteoglycan. It is proposed that this effect is mediated by alteration of the negative charge density of the polyanionic proteoglycan.

INTRODUCTION

In 1968, a hypothesis was proposed by Shulman and Meyer (1) to explain the aging process of cartilage in terms of cellular differentiation. There were two essential features to the hypothesis. The first was that there are three types of chondrocytes - an undifferentiated or "dedifferentiated" form which synthesizes essentially no sulfated proteoglycan, and two differentiated forms, "young" and "old" which synthesize matrix characteristic of young or old cartilage, respectively. Young cartilage contains predominantly chondroitin-4-sulfate, while old cartilage has a higher proportion of keratan sulfate and chondroitin-6-sulfate (2,3). Confirmation of the existence of the three types of chondrocytes was obtained by Kincaid et al (4) using an entirely different approach and methodology. The work of Shulman and Meyer was based on the type of matrix synthesized by cultured chondrocytes, while the work of Kincaid et al used differential histochemical staining of cartilage slices.

The second feature of the hypothesis was that the negative charge density of the polyanionic matrix surrounding the chondrocyte influenced the state of differentiation and the synthesis of cartilage matrix. It was suggested that the rise in calcium in older cartilage might lower

the pericellular negative charge which might in turn affect the synthesis of matrix.

Nevo and Dorfman (5), growing chondrocytes in suspension, found an effect of proteoglycan and other polyanions, such as dextran sulfate, on proteoglycan synthesis. The highly unphysiologic nature of suspension cultures made it unclear as to how this effect might be biologically important.

The work of Daughaday and others (6) has shown the stimulatory effect of serum, in particular a hormone called somatomedin, on synthesis of proteoglycan in rat costal cartilage. The same effect was shown in chick embryo cartilage by Hall (7), who has used this successfully as a somatomedin assay system.

The historical sequence above made it seem reasonable to test the effect of calcium on synthesis of proteoglycan in chick cartilage using a modification of the somatomedin assay system. It was hypothesized that calcium would alter the pericellular negative charge and affect the synthesis of proteoglycan. The data of Nevo and Dorfman (5) in suspension culture of chondrocytes indicated that an increase in polyanion concentration caused an increase in synthesis of proteoglycan, but only up to a concentration of 2 mg/ml. At a higher concentration, the increase in polyanion was inhibitory. If the latter situation corresponds more closely to intact cartilage, the calcium by lowering negative charge ought to have a stimulatory effect.

MATERIALS AND METHODS

12 day chick embryo vertebral columns were removed and cleaned of perichondrial material and notochord as previously described (1). Each vertebral column was placed in 1.8 ml of an amino acid solution (7). The solution was 5 mM in KCl. 2 microcuries of $[^{35}S]Na_2SO_4$ was added to each screw cap tube. $CaCl_2$, $MgCl_2$, or human serum (0.1 ml) were added to different tubes as per the protocol for each experiment. Puromycin dihydro-

TABLE 1

EFFECT OF VARYING CALCIUM CONCENTRATION ON PROTEOGLYCAN SYNTHESIS

Additions	$[^{35}S]\,SO_4$ (cpm/mg wet cartilage)
Control	1623
Ca, 0.5 mM	2557
Ca, 1 mM	4266
Ca, 1.5 mM	4650
Ca, 2 mM	4871

chloride (Nutritional Biochemical Corp.) was used in some experiments.

The tubes were incubated in a 37° shaker bath for 6 hours. No antibiotics or gas were used.

After the incubation period the cartilage was removed and placed overnight in a saturated solution of Na_2SO_4. The cartilage was then weighed after blotting off excess fluid on a paper towel, then placed in 0.6 ml formic acid in a screw cap tube. The tubes were placed in a boiling water bath for 30 minutes, then allowed to cool. 0.2 ml was removed from each tube and placed in a vial with 10 ml of Aquasol (New England Nuclear Corp.), which was then counted in a scintillation sounter.

RESULTS AND DISCUSSION

The results in Table 1 show that calcium stimulates uptake of $[^{35}S]\,SO_4$ at concentrations in the physiologic range for ionized calcium (human = 1.05 - 1.30 mM) (8). A comparison of the relative effects of calcium and magnesium (Table 2) show that calcium has a greater stimulatory effect.

The effect of calcium as well as that of somatomedin (serum) can be blocked by puromycin (Table 3), showing that calcium stimulates synthesis of proteoglycan. The puromycin effect on proteoglycan synthesis has been previously studied (9), as has the blocking of somatomedin effect (10).

TABLE 2

COMPARISON OF EFFECT OF CALCIUM AND MAGNESIUM ON PROTEOGLYCAN SYNTHESIS

Additions	$[^{35}S]SO_4$ (cpm/mg wet cartilage)
Control	1631
Mg, 2 mM	3656
Mg, 5 mM	4322
Ca, 2 mM	4870
Ca, 5 mM	5368

The calcium effect on proteoglycan synthesis may operate via a change in negative charge density of pericellular proteoglycan, though other mechanisms are also possible.

It is proposed that the chondrocyte is imbedded in an avascular cation-exchange gel filtration resin made of proteoglycan (11), which is held together by collagen fibers. Long-term changes in the affinity of cations would come about by the change in resin components (chondroitin-4-sulfate, chondroitin-6-sulfate, and keratan sulfate) as occurs with aging (2,3). These changes in properties of structural macromolecules might be thought of as analogous to changes in isoenzymes which occur with development and aging (12); in this case the changing property is the negative charge density of a connective tissue resin, instead of the kinetics of a metabolic pathway. The short-term changes in conformation and negative charge density (structural property) induced by cations (13) may be analogous to the changes caused by an allosteric effector on enzyme activity (functional property) (14). The gel filtration character of the resin is indicated by the changing volume of proteoglycan in solution with varying salt concentrations (11).

The finding of stimulation of proteoglycan synthesis by calcium at physiologic concentrations raises questions as to its importance in the homeostasis of connective tissue. For example, in malabsorption of calcium which leads to hypocalcemia and osteomalacia, the detrimental effect of hypocalcemia on bone may be in part due to a decreased synthesis of proteoglycan, as well as a lack of calcium for the formation of hydroxyapatite crystals. The question of the mechanism of action of calcium supplementation for osteomalacia is obviously in question.

The possibility of calcium therapy for osteoarthritis is intriguing and the rationale for it seems reasonable. Stimulation of proteoglycan synthesis would be in effect a stimulus to the repair process (15), which operates to compensate for cartilage destruction in osteoarthritis. The possibility of success, however, must be tempered by experience with Rumalon, a cartilage-bone marrow extract. The latter preparation stimulates the synthesis of proteoglycan in vitro (16), but has not been shown to be of definite benefit in the treatment of osteoarthritis (17). Nonetheless the possibility of calcium therapy seems worth exploring, since it is chemically defined (as compared with Rumalon) and the mechanism of action may well be different.

TABLE 3

EFFECT OF PUROMYCIN ON CALCIUM STIMULATION OF PROTEOGLYCAN SYNTHESIS

Additions	$[^{35}S]SO_4$ (cpm/mg wet cartilage)
Control	1345
Serum, 0.1 ml	2690
Ca, 1 mM	3667
Puromycin, 0.1 mM	501
Serum, 0.1 ml + Puromycin, 0.1 mM	277
Ca, 1 mM + Puromycin, o.1 mM	376

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