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Regulation of articular cell metabolism by CTAP mediators

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STUDY of human osteoarthritic chondrocytes in cell culture provides an opportunity to examine factors affecting qualitative and quantitative aspects of proteoglycan synthesis. We have been interested in characteristics and actions of autacoid mediators and drugs capable of regulating proteoglycan synthesis in vitro. Basic ideas arising in the course of this work are shown in Fig. 1. "Connective tissue activation" results from the actions of autacoid mediators, derived in large part from leucocytes or platelets, which induce increased metabolic activity (activation) in connective tissue cells. The activating substances are peptides, which we have termed "connective tissue activating peptides" or CTAP. They have been found in lymphocytes,[1] tumor cells, platelets,[2-4] and polymorphonuclear leucocytes.[5] CTAP-I, -II, and -III have been purified to homogeneity and their amino acid composition is known. CTAP-III, from platelets, has been studied further; its sequence is now known and an RIA is available. CTAP-P_2, is a second platelet-derived mediator recently identified in outdated human platelets.[6]

Dose-response curves testing CTAP-I and CTAP-III against human synovial cell strains show essentially linear dose-response kinetics (Fig. 2). Incremental isotopic incorporation was shown by enzymatic techniques to be almost entirely hyaluronic acid when synovial cell strains were target cultures. When ^{14}C-glucosamine was used as a precursor for GAG synthesis in two osteoarthritic human chondrocyte straings, CTAP-I, CTAP-III, and CTAP-P_2 all caused substantial increments in ^{14}C-GAG formation. Addition of a β-xyloside had little apparent effect on incorporation of glucosamine except when added in the presence of cycloheximide. Cycloheximide markedly reduced the incorporation of glucosamine into GAG, presumably due to inhibiting synthesis of the protein core. Inhibition of GAG synthesis was reversed when β-xyloside was added. Cortisol caused approximately 50% inhibition in glucosamine incorporation in basal cultures as well as a marked reduction in the stimulatory effects brought about by the mediators.

Stimulation by CTAP mediators and β-xyloside can be identified (Table 1) by using $^{35}SO_4^-$ as a precursor. Indomethacin modestly reduced stimulated incorporation of sulfate into sulfated GAG. The combined effects of CTAP mediators and β-xyloside were somewhat greater than would be expected if they were merely additive.

The ^{14}C-glucosamine and sulfate-labeled GAG from chondrocyte cultures were chromatographed over Sephacryl S-300 columns, and a large void volume peak was shown by enzymatic analysis to be largely hyaluronic acid with a small amount of chondroitin 4/6 sulfate (CS-4/6). When the cultures included a β-xyloside, the void volume component was markedly reduced and a substantial peak developed in the retarded volume. The large peak in the internal volume was shown enzymatically to be primarily CS-

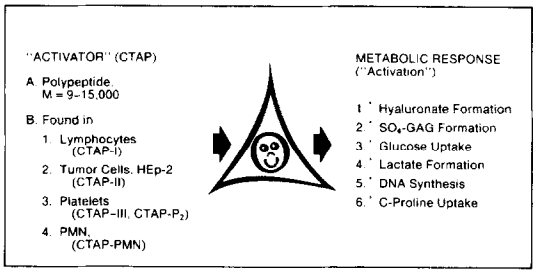

Fig. 1. Connective tissue "activation."

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Table 1. Effects of Mediators and Drugs on $^{35}SO_4$-GAG Synthesis in Human Osteoarthritic Chondrocyte Cultures

Additives	$^{35}SO_4$-GAG, CPM/20,000 Cells*	Percent Stimulation (↑) or Inhibition (↓)
0.15*M* NaCl	14,014 ± 1,016	—
0.15*M* NaCl + 10^{-3} *M* DTE	14,824 ± 1,575	—
CTAP-I (10.6 μg/ml)	20,240 ± 1,940	37% ↑
CTAP-III (10.1 μg/ml)	28,600 ± 6,670	104% ↑
CTAP-P_2 (82 μg/ml)	29,198 ± 5,276	109% ↑
Poly I:Poly C (50 μg/ml)	17,450 ± 4,589	25% ↑
Insulin (10 μg/ml)	14,428 ± 1,417	3% ↑
β-Xyloside (100 μg/ml)	43,921 ± 3,036	213% ↑
CTAP-I + β-Xyloside	58,432 ± 2,534	294% ↑
CTAP-III + β-Xyloside	84,889 ± 3,551	506% ↑
CTAP-P_2 + β-Xyloside	82,056 ± 7,005	486% ↑
Insulin + β-Xyloside	53,055 ± 5,663	279% ↑
Indomethacin (15 μg/ml)	14,476 ± 854	3% ↑
CTAP-I + Indocin	17,248 ± 1,896	16% ↑
CTAP-III + Indocin	21,190 ± 1,188	51% ↑
CTAP-P_2 + Indocin	23,998 ± 2,873	71% ↑

*Chondrocyte cell strain was EB-C, 9th passage.

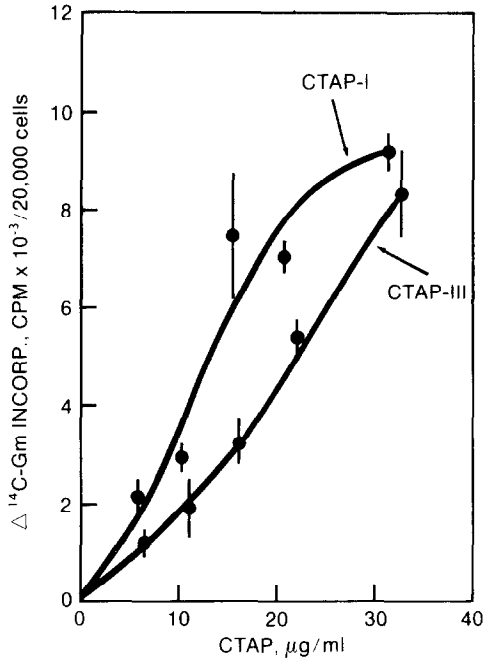

Fig. 2. Dose-response curve.

4/6. Sulfate-labeled GAG showed, in the absence of β-xyloside, a small peak of radiolabeled material of large molecular weight appearing in the void volume, which was essentially CS-4/6. With β-xyloside in the incubation mixture, a striking increase in labeled product occurred, which was the lower molecular weight CS-4/6 expected in the internal volume of the column.

The composition of ^{14}C-GAG synthesized by human chondrocytes in the presence of various test materials was examined, and it was noted that cortisol, indomethacin, and acetylsalicylic acid in clinically relevant concentrations had somewhat different effects on the qualitative composition of these glycosaminoglycans. Cortisol and indomethacin appeared to have a disproportionately greater effect in reducing hyaluronic acid formation, whereas this selective effect appeared less marked with aspirin. It is interesting to note that CTAP-I, -III and -P_2, all of which cause quantitative increase in GAG synthesis, appeared to retain the relative proportions of GAG seen in control cultures.

In summary, we believe that the quantitative aspects of proteoglycan synthesis are potentially easily subject to modulation by stimulatory and inhibitory factors in the microenvironment of the chondrocytes, and would anticipate that further work will show that the qualitative characteristics relating to the chemical anatomy of proteoglycan aggregates will in a similar manner be subject to modification at the time of synthesis.

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