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HORMONAL CONTROL OF CARTILAGE METABOLISM

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Cartilage is a target tissue for skeletal growth and among all the factors which control its development, hormones play an important role.

Clinical informations have suggested that series of hormones control the statural growth in physiological conditions as well as in pathology. All these findings were confirmed in animal studies but wether or not these hormones are acting directly on cartilage metabolism remains to be clarified for most of them.

The history of growth hormone with the discovery of its lack of direct effect on cartilage, by Salmon and Daughaday (1) was one of the most original information about hormones and cartilage so was proposed the new concept of "somatomedins" as mediating compounds. Several other in vitro approaches such as bud explants, tissue incubation, isolated or cultured chondrocytes have also shown new informations; most of them are listed on Table 1. Whereas the in vivo effect of insulin in statural development is well known, its direct effect of cartilage has been very difficult to demonstrate. In 1972, Hall and coworkers (2) failed to find any effect in chicken embryonic cartilage in vitro. Only a slight stimulating effect was shown by Salmon and coworkers on cartilage from hypophysectomized rats in 1968 (3). Since that time, several authors using different technics of cartilage incubation have shown that Insulin has a stimulating effect on cartilage sulfation similar to that obtained with Somatomedin. But this effect is obtained only with very high concentrations of Insulin.

Thyroid hormones are known to act mostly on bone formation. Its direct stimulating effect on the sulfation of embryonic chicken cartilage was shown by Audhya and Gibson (4) for the first time. But, wether or not, thyroid hormones act directly on the calcification process or on cartilage maturation as suggested by Simpson (5) and Hung (6) has not been proved until

Table 1. Direct effects of hormones on cartilage studied in vitro

HALL (1972)	Insulin	No response	Chicken embryonic cartilage
SALMON - DUVALL - THOMSON - (1968)	Insulin	↗Sulfation ↗^{3}H thymidine	Costal cartilage from hypophysectomize rats
AUDHYA - GIBSON (1975)	Triiodothyronin	↗Sulfation	Chicken embryonic cartilage
HAYASHI - SATO (1976)	Triiodothyronin	↗Cell proliferation	Cells in culture
TAKAHASHI - CORVOL (1979)	Testosterone or DHT	↗Sulfation T transformed in DHT	Rabbit cultured chondrocytes
LAYTON (1951)	Cortisone	↘Sulfation	Rat cartilage
CORVOL - GARABEDIAN (1978)	$1,25(OH)_2D_3$ $24,25(OH)_2D_3$ $25(OH)D_3$ transformed in $24,25(OH)_2D_3$	↗Sulfation	Rabbit cultured chondrocytes
CORVOL - ULMANN (1981)	$24,25(OH)_2D_3$	Specific nuclear receptors	

now. More recently, Sato and Hayashi (7) have demonstrated that triiodothyronine has a strong mitogenic activity on cultured cells and it would be of interest to study its possible growth promoting effect on cartilage in comparison by Somatomedin.

Since 1951, when described for the first time by Layton (8) the inhibitory effect of corticoids on cartilage sulfation has been well documented. In fact, its role on growth plate cartilage which seems the opposite to that of thyroid hormones during calcification and maturation, has to be more investigated.

Finally, in the last recent years a direct interaction between testosterone and cartilage cells have been described by Yoko Takahashi and Corvol (9) and since 1978, M.T. Corvol, M. Garabedian and A. Ulmann have provided evidences of direct interactions between vitamin D metabolites and cartilage cells in vitro (10, 11, 12).

It seems now difficult to speak of growth regulation in any tissue without mentioning some growth factors different by hormones, isolated from tissues and partly listed on Table 2. Their mitogenic effect has been demonstrated in different tissues. Their possible interaction with Somatomedin on cell proliferation and particularly in cartilage cells will be discussed later.

Table 2. Partial list of mitogenic factors extracted from different tissues

FACTOR	SOURCES	TARGET TISSUES
Nerve Growth F NGF	Salivary gland embryonic tissues	Sympathetic cells
Fibroblast Growth F FGF	Pituitary gland	Mesodermic derived cells
Epidermal Growth F EGF	Salivary gland human urin	Mesodermic and ectodermic derived cells
Platelet Growth F PGF	Platelets	Mesodermic derived cells
Erythropoletin	Pituitary gland liver	Erythropoletic cells

In order to better understand the hormonal control of cartilage metabolism, it is of interest not simply to screen different effects of hormones on cartilage but to ask ourselves at what cellular levels should occur the hormonal effects and if cartilage cells may contain some intrinsic factors which allow their response to certain hormones or factors.

For that purpose, several problems are present. In addition to the difficulty to be studied easily in vivo or even in vitro, growth plate cartilage has a very complex metabolism which is not even well known. It has to maintain a constant equilibrium between an intense cell division and an important specific protein synthesis. Then, a process of cell maturation occurs with the specific arrangement of extracellular matrix and the mineralization processus.

We have choosen to focus on some results recently obtained in our laboratory in a cultured system where rabbit epiphyseal growth plate chondrocytes maintain several characteristics of such metabolic differenciation and cell maturation. In this cultured system, each metabolic step has been evaluated separately by one or several markers: cell number, DNA content or DNA polymerase activities for chondrocyte proliferation; incorporation of Sulfate into proteoglycans as a major specific protein synthesis; and alkalin phosphatase activities as a reflect of extracellular degradation and mineralization.

One kind of results concerning the responsiveness of cartilage cells to Somatomedin and Insulin compared to some mitogenic factors will be developped in more details. The mitogenic factors studied were Fibroblast growth factor, Epidermal growth factor, and an Eye derived growth factor purified in Paris by Yves Courtois and Denis Barritault (13).

As was shown in many in vitro systems, cultured growth plate chondrocytes do respond to Somatomedins: Sulfated proteoglycan synthesis is stimulated after addition of a semi-purified somatomedin preparation with Insulin-like activity. This ILA preparation has been shown by Frédérique Lang and M.C. Postel-Vinay (14) to bind specifically to some specific sites in cultured chondrocytes very similarly as IGF_{II} and IGF_{I}.

The number of ILA specific binding sites measured in cultured articular and growth plate chondrocytes is always higher than the number of Insulin specific binding sites.

The effect of both hormones was studied on cell proliferation as measured as DNA content and cell number. The response to Somatomedin and Insulin is not significantly different to controls neither on DNA nor on cell number. By comparison, mitogenic factors increase strongly both parameters.

The absence of any effect of Somatomedin as well as Insulin in inducing DNA synthesis was confirmed when studied in measuring chondrocyte DNA polymerase activities. By comparison, fetal calf serum, Eye derived growth factor and Fibroblast growth factor increase significantly these enzyme activities.

It is only when chondrocytes have been pre-stimulated by 1 % serum that Somatomedin or Insulin are active.

If we look now at the morphological aspect of chondrocytes stimulated by the mitogenic factors, one can observe that on day 3 of the culture, the treated cells are more numerous with most of them in division. But, then on day 6 and 9, the treated cells become more and more elongated and look like fibroblast cells.

In addition to this morphological dedifferenciation, a great inhibition of proteoglycan synthesis is observed whereas the strong increase in DNA contain is confirmed.

In conclusion from these results, even if the cellular mechanism by which cartilage cells may respond to growth factors cannot be completely elucidated, one can suggest that they are reacting similarly to what was demonstrated by Van Wyk, and coworkers (15) in fibroblasts. Two groups of factors have been individualised: one group where growth factors stimulate preferably the cells to synthesize the highly specific proteins with a more selective activity for certain cell types (Somatomedins and Insulin should enter this group). A second group of growth factors stimulate DNA synthesis and mitosis with a lower effect or even a transitory inhibition effect on specific protein synthesis. This group should act in a wider range of cell types (FGF, EDGF, EGF will be enclosed to this group).

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