CYCLIC 3′,5′-AMP CHANGES IN CHONDROCYTES OF THE PROTEOGLYCAN-DEFICIENT CHICK EMBRYONIC MUTANT, NANOMELIA

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Received 4 October 1979

1. Introduction

Nanomelia, a lethal recessive trait in the chick embryo is characterized by a severe reduction in size of all cartilaginous structures [1]. It has been shown that the cartilage of the mutant has reduced levels of chondroitin sulfate [2,3] which are reflected in an almost complete absence of cartilage-specific proteoglycan [4,5] whereas the cartilage-specific collagen is unaffected by the mutation [5,6]. Ultrastructurally, the extracellular matrix is deficient of proteoglycan granules and the collagen fibrils have a normal morphology. The chondrocytes in the mutant cartilage are closer to each other than in normal cartilage [6].

Since it has been shown that pressure of physiological magnitude alters cyclic 3′,5′-adenosine monophosphate (cAMP) levels in epiphyseal cartilage [7–9] the nanomelic mutation was used to examine the role of the matrix in the transduction of mechanical stimuli into cellular bone remodeling signals. As reported here, it was found that nanomelic cartilage responds to pressure like normal cartilage but the overall cAMP content and the effect of somatomedin on cAMP accumulation were significantly different in the mutant.

2. Materials and methods

Tibiae were dissected from day 16 chick embryos and cartilage cells were isolated from the epiphyses by collagenase/hyaluronidase digestion as in [10]. Cells were counted in a hemocytometer and viability was assessed by trypan blue extrusion. The same procedure was used for the isolation of cells from the sternum. Tibiae were exposed for 12 min at 37°C to compressive forces [8] oscillating at 3 Hz [9].

Cells were incubated for 6 h in serum free MCDB culture medium in 17 × 100 mm polypropylene culture tubes prior to experiments. Cyclic AMP was measured 20 min after addition of somatomedin. Cellular or tissue cAMP was determined in neutralized 10% trichloroacetic acid extracts as in [9] by radioimmunoassay [11]. DNA was determined by the method in [12].

The cell culture medium was manufactured by Pacific Biologicals, Richmond, CA. Bacterial collagenase and hyaluronidase were purchased from Worthington Biochemicals, Freehold, NJ. Nanomelic embryos were obtained from matings between parents heterozygous for the mutation. Normal sibs segregating from the matings were used as a source of normal cartilage. cAMP RIA kits were obtained from New England Nuclear, Boston, MA. Somatomedin was prepared according to [13].

3. Results

Table 1 shows that intermittent pressure increases the cAMP content (per DNA) in nanomelic chick tibia in the same proportion as in the normal controls. These experiments also revealed that the cAMP content in nanomelia cartilage was significantly lower than in matched controls.

To investigate this difference further, cells dis-

Table 1
Cyclic AMP content in epiphyseal cartilage of normal and nanomelic chick embryos under control and pressure conditions

Experimental condition	Normal (pmol cAMP/μg DNA)	Nanomelic (pmol cAMP/μg DNA)
Control	6.37 ± 0.63	3.38 ± 0.83
Intermittent compression	10.56 ± 1.40	5.52 ± 1.36

Chick embryo tibiae were dissected and exposed for 12 min at 37°C in vitro to intermittent compression, and cAMP content was measured as in section 2. Results are the mean ± SEM of 15 samples each

persed by collagenase/hyaluronidase digestion from tibiae and sterna were incubated under culture conditions in the presence and absence of somatomedin, a hormone reported to stimulate cartilage cAMP accumulation [14]. As seen in table 2 the cAMP content of dispersed nanomelic sternal cells in culture was significantly lower than that of matched control. Moreover, substantial differences were observed in cAMP response to somatomedin, a serum factor known to affect proteoglycan sulfation. Somatomedin strongly stimulated cAMP accumulation in nanomelic cells and had no effect on matched controls under the same conditions. Figure 1 shows a dose–response curve for the effect of somatomedin on cAMP accumulation in nanomelic and control sternal cells. An approximately linear log dose–response relationship was observed between 8 and 4000 ng/ml.

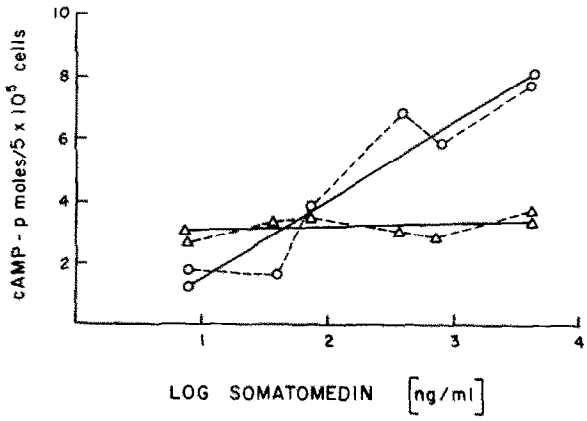

Fig.1. Dose–response curve of somatomedin on cAMP accumulation in nanomelic (○–○) and normal (△–△) sternal cells Cells were isolated, incubated with somatomedin and cAMP was measured as in section 2 on duplicate samples. The broken lines represent the actual values and the solid line, a regression analysis.

4. Discussion

The present results indicate that nanomelic cartilage

Table 2
The effect of somatomedin and insulin on cAMP accumulation in normal and nanomelic sternal chick cartilage cells

	Normal (pmol cAMP/10^6 cells)	Nanomelic (pmol cAMP/10^6 cells)
Control	2.50 ± 0.28	1.14 ± 0.13
Somatomedin (4 μg/ml)	2.68 ± 0.34	12.20 ± 2.54
Control	1.83 ± 0.45	0.95 ± 0.28
Insulin (2 μg/ml)	1.68 ± 0.35	0.74 ± 0.23

Cells were isolated, incubated, exposed to hormones, and cAMP was measured as in section 2. Somatomedin results are the mean ± SEM of 6 samples from 2 independent experiments. Insulin results are mean ± SEM of 6 samples from 3 experiments

responds to intermittent pressure by increasing its cAMP levels in the same proportion as normal cartilage. Since this mutant cartilage lacks cartilage specific proteoglycan, these results suggest that this matrix component may not play an important role in the transduction of mechanical stimuli into cellular bone remodeling signals. Of particular interest was the finding that chondrocytes from nanomelic chick embryos had lower steady state levels of cAMP which could rise substantially in response to somatomedin. Cyclic AMP has been implicated in the past in the action of somatomedin as well as in proteoglycan synthesis and in chondrogenesis. Those findings, however, are derived from various systems and do not fit into a coherent picture. Positive effects of cAMP on chondrogenesis were found in [16] where butyrylate cAMP and theophylline stimulated macromolecular synthesis in the pelvic cartilage of day 10–12 chick embryos. Growth hormone-dependent serum factors also increased cAMP levels in this tissue [17]. Cyclic AMP analogues enhanced sulfate incorporation into matrix proteoglycans of fetal rat chondrocytes [15]. However somatomedin was found to reduce the activity of cartilage adenylate cyclase [18] and in rabbit articular chondrocytes dibutyryl cAMP to promote the synthesis of type 1 collagen [19], characteristic of the fibroblast phenotype rather than the cartilage one. Dibutyryl cAMP added to cloned rat cell myoblast cultures caused morphological changes suggestive of chondrogenesis but at the biochemical level promoted the synthesis of type 1 collagen [20]. Similarly, in chick embryo somites cAMP analogues inhibited sulfated glycosaminoglycan accumulation [21], an expression of chondrogenesis. However, in limb bud cell cultures cAMP derivatives stimulate chondrogenesis [22].

These observations do not explain the differences between the reported findings but they throw new light on the effect of the nanomelic mutation and lend support to the hypothesis that cAMP is involved in the regulation of development and in the control of proteoglycan synthesis. It is suggested that the incomplete expression of the cartilage phenotype in nanomelic embryos may be related to the reduced levels of cAMP in the mutant chondrocytes and to their abnormal response to somatomedin. These preliminary findings are reported since it appears that the nanomelic mutant can serve as a powerful experimental tool for:

(i) Studying the complex relationship between somatomedin cAMP and cartilage differentiation;
(ii) Somatomedin receptor investigations;
(iii) Developing an expedient sensitive bioassay for somatomedin.

Acknowledgements

This work was supported by grant HD 09174 to P.F.G. and by grant DE 04327 to G.A.R.

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