The Localization of Thiamine Pyrophosphatase Activity in Meckel's Cartilage Cells during Endochondral Ossification

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Summary. The cytochemical distribution of thiamine pyrophosphatase (TPPase) activity in Meckel's cartilage cells of the mouse embryo has been studied during the endochondral ossification. All the cartilage cells contain reaction product within the Golgi apparatus. In immature chondrocytes, at the reserve cell zone, TPPase activity is restricted to several inner cisternae of independent Golgi apparatus. In mature cells at the proliferative cell zone, several Golgi complexes form a Golgi network connecting with each other by the TPPase positive tubular stalks. Golgi cisternae, condensing vacuoles and vesicles also contain reaction product. In the hypertrophic chondrocytes located in the calcifying zone, their disorganized Golgi apparatus still retain reaction product. Some chondrocytes, even those located within calcified or opened lacunae, exhibit intact structures and normal cytochemical enzyme distribution. These data indicate the possibility that some chondrocytes may survive and contribute the formation of mandible.

Introduction

It is well known that Meckel's cartilage, consisting of a pair of chondroid bars and a rostral process, forms the lower jaw during the embryonic stage in the mouse. There are, however, still a few disagreements on whether Meckel's cartilage makes direct contributions to the formation of the mandible (Bhaskar et al. 1953; Bernick and Patek 1969; Frommer and Margolies 1971). The chondroid bars show a typical cartilaginous degeneration similar to that of other cartilage in developing long bones. During a degenerative process such as an endochondral ossification, Meckel's cartilage cells undergo striking changes in both their morphological and functional aspects. Their morphological modifications correlate inevitably with the organization of cellular organelles and are responsible for cytochemically detectable enzyme activities.
The localization of TPPase activity has been demonstrated mainly in the Golgi apparatus. Cytochemically detectable TPPase activity is considered to be the best marker enzyme for the Golgi apparatus. It permits us to visualize the behavior of the Golgi complex of various cells (Novikoff and Goldfischer 1961; Holtzman and Dominitz 1968; Friend 1971; Goldfischer et al. 1971; Hand 1971; Kim et al. 1976; Fujita and Okamoto 1979). The behavior of the Golgi complex during endochondral ossification of Meckel’s cartilage is poorly understood. The main purpose of the present study was to clarify changes in the distribution of TPPase activity associated with cellular modifications during endochondral ossification of Meckel’s cartilage in mouse embryos.

Materials and Methods

The ICR strain of mice was used in this study. Embryos varying in insemination age from 16 to 19 days were sacrificed. After decapitation, the lower jaws were dissected out and immersed in cold 2% paraformaldehyde and 2.5% glutaraldehyde buffered with 0.1 M cacodylate containing 4% sucrose for 30-90 min. After washing with buffer solution, tissues were cut into thick sections, at 40-60 μm, using a Vibratome (Oxford Co.). To demonstrate TPPase activity, the incubation medium was prepared according to Novikoff and Goldfischer (1961). The incubation was carried out at 0°C, room temperature, or 37°C for 30-60 min. Two types of controls were performed: (1) using the full medium without thiamine pyrophosphate chloride (obtained from Sigma) as a substrate or (2) using the full medium containing 0.1 M NaF as an inhibitor. Following incubation, the Vibratome sections were rinsed in the buffer solution and postfixed in 1% osmium tetroxide buffered with 0.1 M cacodylate. Thick sections were embedded in Spurr resin or Epon. Some ultrathin sections were doubly stained with uranyl acetate and lead citrate. Other sections, showing purple as an interference color, were observed at 200 KV of accelerating voltage.

Results

When cartilaginous degeneration in Meckel’s cartilage occurred, on about 16 days insemination age, cell shapes exhibited remarkable changes from a flattened to a spherical form at the midportion of the chondroid bars corresponding to the basal region of the developing incisor teeth. The former are referred to as the reserve or proliferative cells, and the latter as the hypertrophic ones. Following the appearance of hypertrophic cells as a sign of early cartilaginous degeneration, resorption and calcification occurred in the cartilage matrix. Similar observations were made in other developing long bones.

The flattened cells were characterized by a well-developed rough endoplasmic reticulum (r-ER) and Golgi complex, and plentiful glycogen particles. A typical Golgi complex was observed in the hypertrophic cells. It was usually identified as condensing vacuoles, vesicles, and cisternae. It was not always possible, however, to distinguish a transition form from the proliferative to the hypertrophic cells owing to the gradual nature of change. The hypertrophic cells showed a translucent appearance due to the separation of each cytoplasmic organelle, such as the dispersion of glycogen particles and free ribosomes, the reduction of Golgi complex, and dilation of cisternae of r-ER. In heavily hypertrophic cells, few cytoplasmic organelles were revealed and each Golgi component could usually be identified.