ULTRASTRUCTURAL OBSERVATIONS ON EFFECTS OF DIFFERENT CONCENTRATIONS OF CALCIUM AND THYROXINE IN VITRO ON LARVAL EPIDERMAL CELLS OF RANA CATESBEIANA TADPOLES

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SUMMARY

During anuran metamorphosis dramatic changes in morphogenesis and differentiation of epidermis occur under the influence of thyroid hormones. Modification of ionic calcium concentration also markedly alters the pattern of proliferation and differentiation in amphibian epidermal cells in vitro. The present study was designed to determine the direct effect of low (0.05 mM) and high (0.5 mM) calcium (Ca\(^{2+}\)) in the absence or presence of thyroxine (10^{-7} M) on epidermal cells of the body and tail tissue in vitro. When tail fin and body skin explants were maintained in low (0.05 mM) calcium for 48 h, normal ultrastructural morphology and integrity of the cells was observed in both the tissue types. When tissues were exposed to high levels of calcium (0.5 mM) in culture medium, tail epidermis showed stratification, and skein cells exhibited apoptosis, both in the presence or absence of thyroid hormones. Under high calcium conditions, the body epidermis showed keratinization of apical cells, apoptosis of skein cells, and increased desmosome formation. These results suggest that (1) optimal Ca\(^{2+}\) concentration for larval epidermal cells is quite low (0.05 mM), (2) high Ca\(^{2+}\) leads to keratinization only in body epidermis, and (3) apoptosis occurred in skein cells of both the tissues at high Ca\(^{2+}\) concentrations (0.5 mM). The present study therefore suggests that the extracellular calcium concentration regulates the process of cell death and differentiation in Rana catesbeiana larval epidermis, and this effect may be similar to the effect of calcium on mammalian epidermal cells.

Key words: calcium; apoptosis; differentiation; anuran; epidermis; amphibians.

INTRODUCTION

Development, function, remodeling, and senescence of multicellular organisms depend on the coordinated occurrence of actively induced cell death in two major patterns: terminal differentiation—often considered as physiological cell death (difftoposis) and programmed cell death or apoptosis (Paas et al., 1993). Amphibian metamorphosis provides an excellent model for studying these two types of cell death. Keratinization or terminal differentiation of body epidermis can serve as a useful model of physiological cell death and tail tissue regression serves as a model of apoptosis.

Additionally, amphibian metamorphosing skin is one of the best studied examples of an organ that remodels in response to thyroid hormones. Remodeling of existing organs is accomplished by the death of larval cells and the growth and differentiation of adult stem cells (Ishizuya-Oka and Shimozawa, 1992; Yoshizato, 1992; Izutsu et al., 1993). At early stages of development, there are no differences between the tail and body epidermis. As metamorphosis progresses, basal cells appear in the body epidermis which proliferate and differentiate into germinative basal cells of the adult-type of keratinized epidermis (Robinson and Heintzelman, 1987; Izutsu et al., 1993, 1996).

Modification of ionic calcium concentration in the culture medium markedly alters the pattern of proliferation and differentiation in cultured mouse epidermal cells (Hennings et al., 1980). Similarly, changes in extracellular calcium are known to regulate growth and differentiation in isolated adult amphibian epidermal cells in vitro in a manner similar to mammalian epidermal cells (Shimizu-Nishikawa and Miller, 1991). However, an earlier study by Denefle and Lechaire (1986) using organ cultures of Rana esculenta skin have suggested that calcium concentration does not affect differentiation of epidermis.

Several reports are available on the effects of hormones on isolated tadpole epidermal cells cultured in vitro (Nishikawa et al., 1989, 1990, 1992; Shimizu-Nishikawa and Miller, 1992; Kanamori and Brown, 1993) and whole skin explants (Mathisen and Miller, 1987, 1989). However, no literature is available on the effects of low and high Ca\(^{2+}\) concentrations on whole skin explants of larval epidermal cells in terms of the role of calcium in proliferation, differentiation, and growth. The present study allowed us to study the process of apoptosis and differentiation in organ culture under physiological conditions, i.e., within an intact tissue and in the presence of mesenchymal and matrix signals. This organ culture system we developed was particularly informative, as isolated epidermal cells cannot be used for investigating cell to cell interaction.

Our previous observations using in vivo experiments have shown that Ca\(^{2+}\) accelerates metamorphosis in Rana catesbeiana tadpoles (Menon et al., 2000), inducing apoptosis in tail and differentiation...
FIG. 1. Light micrographs of tail and body skin cultured for 48 h under different experimental conditions. Abbreviations: CL—collagen layers; CT—connective tissue; S—skein cells; Sg—gland; thick arrows—apical cells; arrowheads—basement membrane; asterisk—basal cells. A: Tail explant maintained in Ca²⁺-free medium showing complete loss of cell integrity. Note ballooning of the cells (star). The inset shows extruded chromatin material (thick short arrows) intensely stained with toluidine blue. B: Tail skin under low Ca²⁺ (0.05 mM) showing typical bilayered epidermis with apical cells (arrow), skein cells (S), and underlying collagen fibers (CL). C: Tail skin under high