Correlation of Electron Microscopic and Secretory Response of Human Parathyroid Adenomas with Different Calcium Concentrations in Organ Culture

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Summary. Twelve parathyroid chief cell adenomas from patients with primary hyperparathyroidism were incubated in a tissue culture system in the presence of different calcium concentrations and for various time periods. The endocrine response of the tissue was examined electron microscopically and radioimmunologically.

After incubation in a medium of low calcium concentration the parathyroid adenomas showed ultrastructural signs of stimulation with proliferation of the hormone-synthesizing organelles. The development of the ultrastructural response could first be observed after four hours and increased up to several days. Radioimmunologically, an increase of the hormone secretion could be demonstrated.

Converse results were obtained after incubation of the tumor tissue under suppressive culture conditions.

To check for de-novo synthesis of the hormone released the tissue was incubated in a $^{75}$Se-methionine-containing medium. This resulted in radioactivity of the secreted parathyroid hormone, indicating de novo synthesis in our culture system.

The biological potency of the released hormone was demonstrated by comparison of the PTH out of the medium with the international human MRC standard using two different radioassays.

Key words: Human parathyroid adenomas — Electron microscopy — Parathyroid hormone release — PTH radioimmunoassay — Pathophysiology of primary hyperparathyroidism.

Introduction

Ultrastructural and radioimmunological studies on parathyroid physiology have shown an inverse relationship between the calcium concentration of the extracel-
lular fluid and the parathyroid hormone (PTH) secretion of normal human and animal parathyroid glands (Copp et al., 1961; Raisz, 1963; Care et al., 1966; Sherwood et al., 1966, 1968, 1970, 1971; Deftos et al., 1968; Hamilton and Cohn, 1969; Oldham et al., 1971; Martin et al., 1972; Black et al., 1973; Fujita et al., 1974; Feinblatt et al., 1975; Habener et al., 1975; Lee and Roth, 1975; Habener and Potts, 1976). This inverse correlation could be demonstrated in in-vivo and in-vitro experiments.

There are controversial data with regard to the autonomy of PTH secretion in primary hyperparathyroidism (PHPT). Adenoma tissue has often been said to be autonomous and therefore not sensitive to changes of the calcium concentration in the extracellular fluid (Rasmussen, 1968; Reiss et al., 1969; Buckle, 1968, 1970). After induction of severe hypercalcemia (Reiss et al., 1969; Buckle, 1970) or hypocalcemia (Buckle, 1968) in patients with parathyroid adenomas no change occurred in serum PTH concentrations. Other in vivo studies on hyperparathyroid patients produced contrary results: EDTA infusions (Potts et al., 1971; Wen Chen et al., 1972; Murray et al., 1972; Lockefeer et al., 1974) or phosphate infusions (Binswanger and Fischer, 1974) resulted in a decrease of serum calcium levels. In response to this there was an increase of serum PTH concentration exceeding the already raised serum PTH levels of the patients. Calcium chloride infusions, however, produced a sudden decrease of serum PTH (Murray et al., 1972; Wen Chen et al., 1972; Monchik et al., 1977). In vitro experiments (Chertow et al., 1977; Dietel et al., 1977; Birnbaumer et al., 1977) have supported the hypothesis that adenoma tissue is calcium sensitive.

The present study examines the in vitro influence of different calcium concentrations on the ultrasturcture and secretion of immunologically and biologically potent, de novo synthesized PTH from parathyroid adenomas. The study was performed in order to elucidate whether sensitivity of the tumor to extracellular calcium concentrations is evident. We also attempted to correlate ultrastructural changes and radioimmunological findings.

**Materials and Methods**

The adenoma tissue was obtained during operation from patients with PHPT. The diagnosis was established by histological evidence of a parathyroid chief cell or mixed cell adenoma and postoperative decrease of serum calcium. Twelve cases were examined.

The methods for preparation and incubation have been described in detail previously (Dietel et al., 1977). After transporting the tumor to the laboratory in cold medium the connective tissue was removed. Subsequently the tumor was cut into slices of about 1 mm³. For culture medium Ham's F 10 (modified) was used with an addition of 10 ml fetal bovine serum and 0.5 ml glutamine (both from Flow Lab.) per 100 ml medium. The specimens were incubated at a temperature of 37° in an atmosphere of 95% air and 5% CO₂.

We used three calcium concentrations in the media: normal (1.2 mM, corresponding to the calcium concentrations of the extracellular fluid (Rasmussen, 1970)), low (0.6 mM) and high (2.6 mM). These calcium concentration in the media were controlled by atomic absorptions spectrophotometry. The magnesium concentration was always 0.83 mM.

The parathyroid adenoma tissue was examined electron microscopically immediately after surgery and after incubation in the different calcium concentrations. The periods of incubation of the specimens were 2 h, 4 h, 6 h, 1 day and then varied up to 26 days. The tissue was fixed in 3% glutaraldehyde in cacodylate buffer for two hours, then buffered with 0.1 M cacodylate