Some Ultrastructural Effects of Testosterone and Insulin on the Ventral Prostate of Rats in Organ Culture

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Summary. The fine structure of the secretory epithelial cells of rat's ventral prostate has been studied following organ culture. Culturing with either testosterone or insulin alone, and with the two hormones combined, were carried out to investigate how insulin modifies the action of testosterone on the maintenance of cellular integrity. After 4 days in hormone-free culture, the secretory epithelial cells showed signs of cellular atrophy and regression, involving loss of the apical microvilli, absence of the apical secretory vacuoles, atrophy of the Golgi apparatus, decrease in rough endoplasmic reticulum and the appearance of autophagic vacuoles. The presence in the medium of either testosterone or insulin alone, or combined, prevented cellular atrophy and regression. The best maintenance of cellular integrity was obtained in a culture containing both hormones. The effects of insulin was approximately equivalent to those of testosterone in the maintenance of cellular integrity.

Key words: Prostate — Tissue culture — Testosterone — Insulin — Ultrastructure.

Introduction

It is well known that in most adult mammals testosterone is mainly responsible for the maintenance of tissue and cellular integrity in the prostate. This subject has recently been investigated electron-microscopically by several investigators using organ culture techniques (Gittinger and Lasnitzki, 1972; Ichihara, Santti and Pelliniemi, 1973) with or without testosterone supplementation. Although the central role of testosterone can hardly be questioned, several other hormones (prolactin, growth hormone, adrenal steroids and insulin) have been claimed to be involved in the maintenance and function of the gland (Chase et al., 1957; Grayhack, 1963; Angervall et al., 1967; Klaiber et al., 1968; Tisell, 1970; Ichihara et al., 1973; Surfin and Prutkin, 1974).

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In our previous study (Ichihara et al., 1973), it was noted that the regressive changes in fine structure of rat's ventral prostate epithelium proceeded much faster in culture than in vivo in castrated animals (Szirmai and Van der Linde, 1965; Brandes, 1966; Helminen and Ericsson, 1970, 1971, 1972), suggesting that, in addition to testosterone, other factors are also involved in maintaining the fine structure of the prostatic epithelium. Further the treatment of the explants with insulin showed a positive effect on the maintenance of the fine structure.

The aim of the present study was firstly to investigate the effects of either testosterone or insulin alone, and in combination, on the fine structure of the prostatic epithelium cultured on a chemically defined medium; and secondly to determine how insulin modifies the effect.

**Material and Methods**

The ventral prostate obtained from Dawley-Sprague rats of 10- to 11-weeks were used for the experiments and the organ culture method of Trowell (1959) was followed with minor modifications. Ventral prostates were also fixed in vivo by perfusion (Ichihara and Pelliniemi, 1975) for studying the fine structure of the organ before culture. The explants were cultured on medium 199 with Earle's salts (GIBCO) containing sodium G-penicillin (100 IU/ml) and streptomycin (200 µg/ml) for 96 h (4 days). Testosterone (Organon Holland) was dissolved in propylene glycol before adding to the culture medium. Insulin (Nordisk Insulinlaboratorium, Denmark) was added in an aqueous solution. The final concentrations of the hormones in the culture medium were 10^-7 mol/l for testosterone and 0.081U/ml for insulin. The hormone containing medium was not renewed during the culture period. Full details of the culture system have been published elsewhere (Santti and Johansson, 1973).

After cultivation, pieces of the explants, as well as tissue pieces fixed in vivo, were cut into small pieces of less than 1 mm size and fixed in 3% glutaraldehyde and 0.044% calcium chloride in 0.1 mol/l sodium cacodylate buffer of pH 7.4 for 3 h between 0 and 4°C. The tissue pieces were washed in 7.5% sucrose and 0.044% calcium chloride in the same buffer for 45 min, postfixed for 2 h in 1% osmium tetroxide in the same buffer containing 0.044% calcium chloride and 6.85% sucrose, dehydrated in a graded strengths of ethanol, and infiltrated and embedded in Epon 812 (Luft, 1961). The resin was polymerized for 48 h at 60°C. Ultrathin sections were cut on Porter Blum MT-1 and Porter Blum MT-2B ultramicrotomes, mounted on unsupported nickel grids, stained with aqueous uranyl acetate (Watson, 1958) and lead citrate (Venable and Coggeshall, 1965), and viewed in a Hitachi HU-12A and in a JEOL JEM-T8 electron microscopes.

**Results**

**Prostatic Secretory Epithelial Cells before Culture**

The fine structure of the rat's ventral prostatic secretory epithelium has been described previously (Szirmai and Van der Linde, 1965; Brandes, 1966; Sakurai, 1969; Helminen and Ericsson, 1970; Didio, 1971; Dahl et al., 1973; Flickinger, 1975; Ichihara and Pelliniemi, 1975) and only the most characteristic features will be mentioned here. The luminal border of these cells is thickly populated with microvilli and has sharply localized pittings (Fig. 1). Many, irregularly round and oval secretory vacuoles are observed in the apical cytoplasm and their content is either of moderately electron dense, homogeneous material, or flocculent material. Well-developed rough endoplasmic reticulum is permeated through-