Use of Protein A-coated Colloidal Gold Particles for Immunoelectronmicroscopic Localization of ACTH on Ultrathin Sections

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Summary. ACTH was localized in dissociated porcine adenohypophysial cells using a novel indirect EM immunocytochemical technique. Incubation of ultrathin resin sections in anti-ACTH was followed by incubation with protein A-coated colloidal gold particles. Protein A binds specifically to the Fc part of the IgG molecule, and thus the ACTH-containing secretory granules became labelled with electron-dense gold particles. With this method, the dissociated porcine ACTH cell was identified as containing numerous round or ovoid 170-300 nm secretory granules.

Introduction

Protein A, a cell wall protein from Staphylococcus aureus, binds specifically to the Fc protein of mammalian immunoglobulin G (IgG) (Forsgren and Sjogquist, 1966) and has been used in detection of cellular antigens, either coupled to fluorescein isothiocyanate (Biberfeld et al., 1975) or to horseradish peroxidase (Dubois-Dalcq et al., 1977), as the second step in indirect immunocytochemical techniques. Colloidal gold particles can be labelled with various proteins (e.g., lectins and antibodies), and have been used as specific electron-dense markers for cell surface antigens in both the transmission and scanning electron microscope (Faulk and Taylor, 1971; Horisberger et al., 1975; Horisberger and Rosset, 1977; Georghegan and Ackerman, 1977). In the present study, gold particles adsorbed with protein A have been used in localization of an intracellular hormone antigen, ACTH, in dissociated pig anterior pituitary cells, on the surface of ultrathin resin sections.

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Materials and Methods

Porcine anterior pituitary cells were dissociated by an enzyme method (Walker and Hopkins, 1978) and prepared for electron microscopy as described previously (Batten and Hopkins, 1978), except that fixation in 4% paraformaldehyde was followed by postfixation in 1.0% osmium tetroxide in veronal buffer.

**Antiserum.** Antiserum to porcine ACTH was a gift from NIAMDD (Bethesda, Md., U.S.A.). Absorbed antiserum was prepared by adding 4 μg of ACTH (NIAMDD) per ml of 1:500 dilution anti-ACTH, incubating at 37°C for 1 h and storing at 4°C for 48 h before use.

**Protein A-coated Gold.** Colloidal gold particles of approximately 12 nm diameter were prepared by reduction of chloroauric acid with sodium citrate (Horisberger and Rosset, 1977). The gold particles were then coated with protein A (Pharmacia, London), stabilized with carbowax (polyethylene glycols), neutralized, centrifuged and the sedimented protein-A-coated gold particles (Au₁₂-PrA) redispersed in tris/carbowax (Horisberger and Rosset, 1977).

**Immunocytochemical Technique.** Sections on unsupported nickel grids were etched for 20 min in 10% H₂O₂, washed briefly in distilled water, and incubated in 1:500 anti-ACTH for 24 h at 4°C. After a second wash, grids were floated on drops of Au₁₂-PrA for 10 min. Sections were examined on a JEOL JEM T7 or a Philips PW6002 electron microscope with or without double staining in uranyl acetate (8% in 30% methanol, 1 min) and lead citrate (1 min).

Results and Discussion

After a double incubation in anti-ACTH followed by Au₁₂-PrA, the ACTH cells became labelled with 12 nm electron-dense gold particles. The gold particles were aggregated over the secretory granule profiles, which were electron-lucent, due to the prolonged H₂O₂ etching required to restore the antigenicity of the osmium-fixed ACTH molecules (Fig. 1a). Only a few scattered particles were observed over other areas of the cell, approximating to the level of non-specific labelling over the areas of resin between the cells; however, a higher level of non-specific labelling was sometimes observed over the nucleus. Brief double staining in uranyl acetate and lead citrate increased membrane contrast and restored the electron density of the secretory granule cores, but did not obliterate the overlying gold localization (Fig. 1b). When the primary incubation was carried out using anti-ACTH absorbed with ACTH, no aggregation of gold particles over the secretory granules resulted, and the level of non-specific labelling over the cell remained the same.

A single cell type within the dissociated cell preparation was positive with the anti-ACTH/Au-PrA method, containing densely packed round or ovoid 170–300 nm secretory granules (Fig. 1c). This corresponded to the cell previously shown to contain ACTH by the immunoperoxidase technique, after absorption of the anti-ACTH antiserum with porcine LH (Batten and Hopkins, 1978). In the present study, absorption of the anti-ACTH to remove cross-reactivity with LH, FSH or TSH was not necessary, since the antigenicity of these porcine glycoprotein hormones was destroyed by the osmium fixation. Thus, the dissociated porcine ACTH cell is characterized by the presence of secretory granules which are rather larger, and more numerous than those identified by immunoperoxidase in the cultured rat ACTH cell (Bacsy et al., 1976).