

Cytochemical Demonstration of Adenylate Cyclase and Guanylate Cyclase Activities with Dimethyl Sulfoxide

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Elucidation of the localization of adenylate cyclase (ACLase) and guanylate cyclase (GCLase) within the cell is essential for understanding the mechanism of cell function. In this study, we assessed the effect of dimethyl sulfoxide (DMSO) added to fixatives as well as to the incubation medium, and demonstrated the localization of ACLase and GCLase in rat adrenals.

Materials and Methods: Adrenals from adult rats were fixed in 2% paraformaldehyde-0.25% glutaraldehyde in 0.1M cacodylate buffer with 5% DMSO and 8% sucrose, pH 7.2, for 30 min. at 4°C. After washing in the same buffer, fixed tissues were cut with a Vibratome. Sections were incubated for 30 min. at 37°C in a medium consisting of 80mM Tris-maleate buffer with 8% sucrose, pH 7.4, 0.5mM adenylyl imidodiphosphate (AMP-PNP), 10mM NaF, 2mM theophylline, 4mM MgSO₄, 2mM Pb(NO₃)₂, and 5% DMSO for ACLase or in a medium consisting of 80mM Tris-maleate buffer with 8% sucrose, pH 7.4, 0.5mM guanylyl imidodiphosphate (GMP-PNP), 2mM theophylline, 3mM MnCl₂, 2mM Pb(NO₃)₂, and 5% DMSO for GCLase. For biochemical estimation of enzyme activity, the cAMP or cGMP radioimmunoassay kits (Yamasa Shoyu Co., Ltd., Chiba) were used.

Results: The biochemical studies showed that 30-40% of enzyme activity was retained after fixation with DMSO; 20% of enzyme activity of fixed tissues was retained at 2mM Pb(NO₃)₂.

Enzyme activity in adrenals was cytochemically enhanced by the addition of DMSO. ACLase activity was localized in the plasma membranes of adrenal medulla cells (fig.1), and splanchnic nerve endings. After treatment with 0.4 units ACTH in vitro, the activity was detected in the adrenal cortex. GCLase activity was localized in adrenal medulla cells (fig.2).

Discussion: DMSO seems to preserve ACLase and GCLase activity during fixation, and contribute to the increased permeability of membranes for the incubation medium. The localization of ACLase in the adrenal medulla suggests that cAMP formed by ACLase and theophylline may speed up the synthesis of catecholamines.

