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Importance of Inhibitor for Phosphodiesterase(PDE) in the Histochemistry of Adenylate cyclase (AC).

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To consider the reaction mechanism for the AC medium, the importance of inhibitor for PDE was examined.

Materials and Methods

The retina of male SD rat was fixed with glutaraldehyde. After 40μ sectioning AC was demonstrated adding theophilline (TP)(0.5-4mM) or isobutyl methylxanthine (IBMX)(2mM), as well as PDE, 5'-nucleotidase(5N), alkaline phosphatase (ALP).

Results and Discussion

In the rat retina the sites of activities of above enzymes were considerably overlapped. Without the inhibitor, the activity of AC was very much similar to 5N. The AC medium replacing AMP-PNP with cAMP showed the pattern of reaction product as addition of 5N to PDE. These activities were completely inhibited by 4mM of TP or by 2mM of IBMX. To demonstrate the AC inthe medium without inhibitor or lower concentration than 4mM of TP or 2mM of IBMX have the risk to pick up the PDE and 5N activities.

Peroxidase activity of collagen-phagocytosing cells in the postpartum rat uteri

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It is well known that there is a difference in the localization of peroxidase(PO)activity in the cytoplasm between exudate and resident macrophages (Mφs). Many researchers noted that Mφs were implicated in phagocytosis of collagen fibrils in the postpartum rat uteri. In the present study, we investigated the nature of Mφs in the same tissues especially by the reaction of PO and acid-phosphatase(ACP).

By the light microscopic cytochemistry, at two or three days postpartum, numbers of PO-positive cells were seen in the stroma. Afterwards, they decreased gradually in number and only a few PO-positive cells remained at two weeks postpartum. This change in the number of PO-positive cells was similar to that of ACP-positive cells. By the electron microscopic cytochemistry, PO-positive cells in the stroma proved to be Mφs, stromal fibroblasts and eosinophils. Mφs revealed PO-activity in cytoplasmic granules; this finding suggests that most Mφs are exudate ones. Stromal fibroblasts which phagocytosed collagen fibrils displayed PO-activity in cytoplasmic granules and vacuoles.

3,3'-DAB is photooxidized by photosystem I

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For elucidating photooxidation mechanism of DAB in photosynthetic electron flow, photooxidation of DAB was investigated on thylakoid membranes in aldehyde-fixed cells of a blue-green alga, *Nostoc muscorum*, and barley. In *N. muscorum*, DAB photooxidation was positive on vegetative cells in which both PS I and PS II are operative. Thylakoid membranes of heterocysts, in which only PS I is active, showed positive reaction. The reaction was not affected by inhibitors around PS II (NH_2OH, Tris, DCMU, HOQNO and o-phenanthroline). These results strongly indicate that DAB was photooxidized by PS I. However, KCN known as a inhibitor for plastocyanin, possible acceptor of electron from DAB, had no effect on the reaction. Therefore, DAB may donate electron to a component after plastocyanin of PS I. P700 may be the candidate, since DCIPH, which is electron donor for either plastocyanin or P700, interfered DAB photooxidation. Antimycin A, aminotriazole and catalase had no effect on the reaction. The reaction was negative in the cell treated with $HgCl_2$ or heat. In barley the results was the same as in *N. muscorum*.

A Coupled Peroxidatic Oxidation Method for Monoamine Oxidase(MAO)

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A coupled oxidation method for MAO using H_2O_2 produced by the enzyme reaction was developed to demonstrate type A and B isozymes, since this makes possible introduce the common substrates such as tyramine and also the prefered substrates for each type. Animal was perfused with 1% FA and 1% GLU solution (pH 7.6) and tissue blocks were postfixed in 0.2%FA solution containing 15% sucrose overnight at 4°C. Cryostat sections were stocked and rinsed in chilled 15% sucrose solution(pH7.6) and incubated with floating for 90 min at 37°C in reaction mixture:10ml 0f 0.005-0.01% DAB in Tris-HCl buffer (pH 7.6), 10mg of tyramine, 10mg of HRP. After washing, sections were mounted on gelatin coated glass slides, dried, dehydrated, cleared and embedded in entellan.

Substitution of substrate with serotonin or benzylamine could preferentially demonstrate type A and B enzymes respectively. Each type of the enzyme was more clearly showed by systemic administration of clorgyline or pargyline. This method revealed that glial MAO consists mostly of type B enzyme.

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