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Morphological analysis of olfactory receptor cells using whole-mount preparations

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The distribution and entire shape of olfactory receptor cells were investigated by means of whole-mount preparations of the nasal mucosa. Whole mucosa isolated from the nasal septum of rats was processed, as "a free-floating section", and examined by the avidin-biotin complex (ABC) method using antisera against protein gene product 9.5 (PGP 9.5) and calbindin. Essentially all receptor cells were immunolabeled with the PGP 9.5 antiserum, but only half of PGP 9.5-immunoreactive cells were calbindin-immunoreactive. In the immunostaining of whole-mount preparations, pretreatment of tissues by freeze-thawing and dipping in ethanol and xylene greatly improved the permeability of antibodies. Overview of the nasal septum showed that the dorsal and ventral portions of the rostral olfactory area extended deeply into the

respiratory area, making a "semi-lunar" shape. The boundary between the two areas was clearly demarcated, although several receptor cells were scattered in the respiratory area near the boundary. Observation at higher magnification clearly demonstrated that several axons derived from perikarya gathered to form nerve bundles showing a dendritic pattern. Proximal axons close to perikarya displayed beaded structures with intense immunoreactivity. They were electron-microscopically identified as swollen portions of axons which might be formed in association with the axonal flow. The present study showed that whole-mount preparation of the nasal mucosa for immunohistochemistry is a useful tool to analyze the morphology of olfactory receptor cells and axons.

Observation of type B intercalated cells in the rat kidney by a monoclonal antibody

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The collecting duct in the kidney consists of three structurally and functionally distinct cell types: principal cells, type A intercalated cells and type B intercalated cells. Intercalated cells are engaged in the regulation of acid-base balance. It is believed that type A intercalated cells secrete H^+ , whereas type B intercalated

cells secrete HCO_3^- . Although some histochemical methods have been reported to distinguish two types of intercalated cells on tissue sections, cell-specific markers of type B cells have not been found.

Recently, Oriksa et al. (1996) reported that a great number of macrophagic cells were

proliferated from cultured rat glomeruli. In the course of study, they raised monoclonal antibodies against these macrophagic cells for further analysis. One of the antibodies, named OS-3, was found to selectively detect a cell population scattered in the collecting duct of rat kidney as well as interstitial macrophages in various tissues and Kupffer cells in the liver.

To define distribution and type of the OS-3-immunopositive cells in the rat collecting duct, we performed double staining using antisera against calbindin and cytokeratin. OS-3-immunopositive cells existed in both the connecting segment and cortical collecting duct, and were different from calbindin-positive or cytokeratin-positive epithelial cells.

Definite identification of OS-3-immunopositive cells was performed by the electron microscopic observation of immunostained sections. The positive cells were spherical or triangle in shape with few microprojections on the narrow apical surface. Their dark cytoplasm contained numerous mitochondria, and a number of vesicles

developed near the apical membrane. The immunopositive cells exhibited complicated and shallow infoldings of the basolateral membrane. Distribution and morphological properties of the OS-3-immunopositive cells showed that they are type B intercalated cells. Immunoblotting of extracts from the kidney demonstrated an immunoreactive band at a molecular weight of 43 kDa.

Out of the kidney, the author could detect specific and intense immunoreactivity with OS-3 in several tissues. The immunoreactivity was found in the epithelium of pancreatic excretory duct, and glandular cells in the duodenal gland, salivary glands and pyloric gland, which are all HCO_3^- -secreting cells. These immunohistochemical findings imply that OS-3 recognizes a common molecule involved in the HCO_3^- synthesis and secretion. The selective localization of the immunoreactivity on the basolateral membrane of cells suggests that the antigen for OS-3 is an ion transporter which is engaged in the HCO_3^- synthesis.

Effect of cholecystinin on cytosolic Ca^{2+} dynamics in rat pancreatic B cells

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1. Cholecystinin (CCK) has been immunohistochemically identified in nerve fibers innervated to rat pancreatic islets but its physiological role has not fully been understood. The present study was thus carried out to explore the role of CCK in pancreatic endocrine regulation. To begin with, effects of CCK on cytosolic Ca^{2+} dynamics were examined in detail by using pharmacological tools, i.e., various Ca^{2+} antagonists. Cytosolic Ca^{2+} concentration

$([\text{Ca}^{2+}]_c)$ was measured with microfluorometry by using fura-2, a fluorescent Ca^{2+} probe, in isolated rat pancreatic islets.

2. Perfusion of the islets with solution containing CCK-octapeptide (CCK-8; 10^{-9} , 10^{-8} , 10^{-7} M) for 30 seconds caused an abrupt increase in $[\text{Ca}^{2+}]_c$ followed by a rapid decrease to the basal concentration (100–150 nM). Repetitive stimulation with CCK-8 at a higher concentration (10^{-7} M) caused a repetitive increase in $[\text{Ca}^{2+}]_c$