



Title	Effect of cholecystokinin on cytosolic Ca ²⁺ dynamics in rat pancreatic B cells
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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 $[\text{Ca}^{2+}]_c$

but their peak levels decreased by about 18%.

3. Removal of CaCl_2 from the perfusing solution almost completely abolished CCK-8-induced increases in $[\text{Ca}^{2+}]_c$. This result suggests that the presence of CaCl_2 in extracellular space of the islet cells is essential for $[\text{Ca}^{2+}]_c$ dynamics induced by CCK-8. This phenomenon may be explained by one of two separate hypothesis; 1) Ca^{2+} influx is important for CCK-8-induced $[\text{Ca}^{2+}]_c$ increase or 2) refilling of Ca^{2+} in intracellular Ca^{2+} stores is mandatory for $[\text{Ca}^{2+}]_c$ increase.

4. Addition of low concentration of NiCl_2 which is shown to selectively block T-type Ca^{2+} channel strongly inhibited CCK-8-induced $[\text{Ca}^{2+}]_c$ increase, suggesting that Ca^{2+} influx via T-type Ca^{2+} channel is occurring during CCK stimulation in this type of cells.

5. This idea was further supported by following

evidence. Nifedipine, a selective L-type Ca^{2+} channel blocker, ω -conotoxin GVIA, a selective N-type Ca^{2+} channel blocker, ω -conotoxin MVIIC, a selective Q-type Ca^{2+} channel blocker, and ω -agatoxin IVA, a selective P-type Ca^{2+} channel blocker, were all without effect on CCK-8-induced $[\text{Ca}^{2+}]_c$ increase.

6. Possible involvement of Ca^{2+} influx by CCK-8 stimulation was also supported by following evidence. U73122, a PLC inhibitor, had no effect on CCK-8-induced $[\text{Ca}^{2+}]_c$ increase, suggesting that PLC- IP_3 - Ca^{2+} release cascade is not functioning.

7. It was concluded that CCK may physiologically participate in regulation of pancreatic endocrine secretion by modulating cytosolic Ca^{2+} dynamics which are brought about by possible activation of T-type Ca^{2+} channel but not by L-type, N-type, Q-type, and P-type Ca^{2+} channels.

Effects of nitric oxide on cytosolic Ca^{2+} dynamics in mouse pancreatic islets.

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1. The purpose of the present study is to explore effects of nitric oxide (NO) on changes in cytosolic Ca^{2+} concentration ($[\text{Ca}^{2+}]_c$) and to clarify possible sites of action of NO in mouse pancreatic β cells. A microfluorometric method was applied by using Fura-2, a fluorescent Ca^{2+} indicator, in the isolated perfused preparations of mouse pancreatic islets.

2. Increasing glucose concentration of perfusing solution from 3 mM to 10 mM caused biphasic increases in $[\text{Ca}^{2+}]_c$, the first transient rise (first phase) followed by a continuous $[\text{Ca}^{2+}]_c$ increase on which oscillatory fluctuation was often superimposed (second phase). The first $[\text{Ca}^{2+}]_c$ increase was completely abolished by the addition

of 400 μM NOR3, a spontaneous NO donor. The second phase was also calmed by NOR3 (200 μM). This inhibitory effect by NOR3 on glucose-induced $[\text{Ca}^{2+}]_c$ rises was restored by pretreatment with 10 μM oxyhemoglobin, a NO scavenger.

3. The addition of SIN-1, which is known to produce NO and O_2^- , and resultant peroxynitrite tended to reduce the second phase of $[\text{Ca}^{2+}]_c$ increase induced by 15 mM glucose. Superoxide dismutase which scavenges produced O_2^- and thus reduces peroxynitrite production amplified the inhibitory effect by SIN-1. Based on these results, it is suggested that NO but not peroxynitrite plays a major role in the inhibition