Spatiotemporal dynamics of cytosolic ions measured with optical methods in isolated cells of salivary and other secretory glands of the rat

Yoshiaki Habara and Tomio Kanno
National Institute for Physiological Sciences and Hokkaido University

Spatiotemporal dynamics of $[Na^+]_i$ and $[K^+]_i$ were studied by microfluororatiometry using fluorescence indicators, SBFI and PBFI, in acinar cells isolated from the salivary glands and from the exocrine pancreas and in adrenal chromaffin cells isolated from the rat. $[Na^+]_i$ of unstimulated cells was 3-7 mM in all cell types. Continuous stimulation of submandibular acinar cells with carbachol (1-100 μM) or noradrenaline (1-100 μM) increased $[Na^+]_i$ to several tens mM in a dose-dependent manner. The stimulation less increased $[Na^+]_i$ of parotid and sublingual acinar cells. The secretagogue-induced increase in $[Na^+]_i$ depended on $[Na^+]_o$. Digitized image analysis showed that the increase in $[Na^+]_i$ occurred from the basolateral region. A beta-agonist, isoproterenol (100 μM), was without effect. $[K^+]_i$ did not change by stimulation in all cell types examined. An inhibitor of Na^+-H^+ antiporter, amiloride (100 μM), decreased $[Na^+]_i$ in unstimulated submandibular acinar cells. Ouabain (1 mM) slightly increased $[Na^+]_i$ and inhibitors of Na^+-K^+-$2Cl^-$ cotransporter, frusemide (1 mM) and bumetanide (100 μM), increased $[Na^+]_i$. Ouabain caused a faint effect on secretagogues-induced increase in $[Na^+]_i$, whereas a further increase in $[Na^+]_i$ was observed with frusemide and bumetanide. Amiloride significantly decreased $[Na^+]_i$ of submandibular acinar cells stimulated with secretagogues. Stimulation with 10 μM carbachol or noradrenaline under Ca^{2+}-deficient condition in the absence or the presence of BAPTA/AM unchanged $[K^+]_i$. TEA and apamin were also without effect in stimulated submandibular acini. No changes in $[Na^+]_i$ or $[K^+]_i$ was detected in pancreatic acinar cells upon stimulation with the peptide CCK-8 (100 pM), carbachol (3 μM), the peptide secretin (100 pM), and the peptide hVIP (1 nM). Carbachol (3 μM) was without effect in $[Na^+]_i$ and $[K^+]_i$ of adrenal chromaffin cells. These results indicate that submandibular acinar cells are unique in terms of $[Na^+]_i$ dynamics. $[Na^+]_i$ of submandibular acinar cells changes much more drastically by Na^+-H^+ antiporter and Na^+-K^+-$2Cl^-$ activities during stimulation. The effect of frusemide and bumetanide on $[Na^+]_i$ suggests that Na^+-K^+ ATPase is functionally associated with Na^+-K^+-$2Cl^-$ cotransporter. Unidirectional Cl^- flow via Na^+-K^+-$2Cl^-$ cotransporter as well as paracellular Na^+ flow appear to be involved in creating large secretory pressure for salivary extrusion in submandibular acinar cells. This driving force may be weak in parotid and sublingual acinar cells and may not be very active in pancreatic acinar cell and adrenal chromaffin cell.

Present address: Laboratory of Physiology, Department of Biomedical Sciences, Graduate School of Veterinary Medicine, Hokkaido University