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Fluid and Amylase Secretion by Perfused Parotid Gland: Physio-Morphological Approach

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Whole gland perfusion technique was applied to rat parotid glands to assess whether amylase affects fluid secretion. Control perfusion without any secretagogue evoked no spontaneous secretion. Carbachol (CCh 1 μM) induced both amylase and fluid secretion with distinctive kinetics. Fluid secretion occurred constantly around 60 μL/g-min, whereas amylase secretion exhibited an initial peak, followed by a rapid decrease to reach a plateau. Isoproterenol (Isop 1 μM) alone did not induce fluid secretion although it evoked amylase secretion as measured in isolated perfused acini. Addition of Isop during CCh stimulation evoked a rapid and large rise in amylase secretion accompanied by small increase in oxygen consumption. Morphological observations carried out by HR SEM and TEM revealed exocytotic profiles following Isop stimulation. CCh stimulation alone seldom showed exocytotic profiles, suggesting a low incidence of amylase secretion during copious fluid secretion. Combined stimulation of CCh and Isop induced both vacuolation and exocytosis along intercellular canaliculi. These findings suggest that control of salivary fluid secretion is independent of the amylase secretion system induced by CCh and/or Isop.

Under sympathetic and parasympathetic control, the major salivary glands secrete saliva with various components; sympathetic nerve excitation elicits protein-rich saliva, whereas parasympathetic stimulation induces fluid secretion with a low concentration of protein. The intercellular canaliculi in the human salivary glands (1) were observed as a pathway into which amylase and fluid enter across the luminal plasma membrane and the junctions among secretory endpiece cells. Yoshimura (2) classified three patterns of amylase secretion by isolated parotid acinar cells of the rat. 1) An initial transient increase of amylase secretion followed by low plateau level, caused by intracellular Ca^{2+}-mobilizing secretagogues. 2) A slow increase in amylase secretion and followed by high plateau level, stimulated by cyclic AMP (cAMP)-mobilizing agents. 3) A massive and sustained increase of amylase secretion, stimulated by combination of Ca^{2+}- and cAMP-mobilizing agents. It is likely that amylase release somehow affects fluid secretion. However, there has been no report on the relationship between these two phenomena in rat parotid glands. In addition, there are no morphological observations during above three modes of amylase secretion at the interface between cytoplasm and intercellular canaliculi. In the present study, we tried to correlate physiological measurement on amylase and fluid secretion with morphological observation, using the isolated perfused gland (3).

Male Wistar strain rats were anesthetized with pentobarbitone sodium (50 mg/kg body weight) and parotid glands were surgically isolated and arterially perfused. The rate of oxygen consumption (QO_2) was calculated from the arteriovenous difference in oxygen content and the perfusion rate. The rate of fluid secretion was calculated from time-differentiation of the cumulative volume of saliva. Amylase activity in saliva was assayed by the method of Bernfeld (4), expressed as the maltose digestion rate. For light- and electron-microscopic observation, small pieces of the gland were excised at fixed times during the experiments. Intercellular canaliculi were observed by scanning electron microscope (HR-SEM) using an osmium maceration method (5).

Amylase and fluid secretion

The fluid secretion increased rapidly at the start of carbachol (CCh 1 μM) administration and gradually declined during stimulation. Addition of isoproterenol (Isop, 1 μM) did not potentiate the fluid secretion at 5 min from start of single CCh stimulation. Single Isop stimulation did not induce fluid secretion, so that we could not measure amylase from the gland. This indicates that the amylase secreted by single Isop stimulation cannot reach the oral cavity. The amylase secretion induced by CCh includes an initial increase and a sustained plateau

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level. The initial peak was 10 mg maltose/30 sec per g wet weight of the gland and the plateau level was 1 mg maltose/30 sec. Combined stimulation of CCh and Isop increased amylase secretion rapidly to the plateau level of 15 mg maltose/30 sec.

Oxygen consumption

Oxygen consumption increased by single CCh stimulation and reached a plateau level. During sustained stimulation of CCh, the plateau level remained at the same level. Overloading of Isop during CCh stimulation gradually increased oxygen consumption. This suggests that the relative energy requirement for fluid secretion is much larger than that of protein secretion in response to secretagogue. Mucin secretion was also measured in perfused mandibular gland during stimulation of CCh and/or Isop. Time course of mucin secretion was similar to that of amylase, whereas the fluid secretion and oxygen consumption was not influenced by addition of Isop. These observations suggest that energy requirement for the activation of protein secretion is too small to be detected from oxygen consumption.

Morphological observation

Light microscope

Single CCh stimulation seldom showed exocytotic profiles in parotid acinar cells from control resting gland at 1 min after the start of stimulation. At 3 min, the intercellular canaliculi enlarged slightly and a few vacuoles appeared. Combined stimulation of CCh and Isop increased the number of such profiles which looked as vacuoles at the level of intercellular canaliculi. During washout of secretagogues, the vacuoles became smaller and the number of exocytotic profiles decreased.

TEM

Single Isop stimulation produced exocytotic profiles along intercellular canaliculi. Combined stimulation of CCh and Isop greatly increased the number of such profiles. When these two secretagogues were washed out from the perfusion, the "vacuolar" profiles diminished and abundant lysosomes containing membranous structures appeared in the apical cytoplasm.

SEM

Qualitatively, the overall morphology of intercellular canaliculi seen by SEM from the interior of the cell appeared unchanged. However, morphometric analysis showed that the diameter of intercellular canaliculi enlarged from 0.74-0.77 μM in the resting control to 0.9 μM at 1 min from start of CCh stimulation. Then the size decreased slightly to 0.6-0.8 μM at 3 min. Under combined stimulation, intercellular canaliculi showed both abundant vacuolation and exocytotic profiles.

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