

## PROCEEDING

# Morphological evidence of paracellular transport in perfused rat submandibular glands

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**Abstract :** The morphological change of the paracellular route for fluid secretion is still a long-standing question. The purpose of this study was to visualize alterations in the cytoskeleton structure of tight junctions caused by carbachol (CCh) and isoproterenol (IPR) treatment of perfused rat submandibular glands (SMGs), using freeze-fracture (FF) replicas of rapidly frozen tissues. Isolated SMGs from male Wistar rats were perfused and stimulated with 1  $\mu$ M CCh and IPR. Specimens were immediately rapidly frozen with liquid helium by metal contact. After cutting and deep etching, FF replicas were obtained by rotary shadowing and were examined by transmission electron microscopy. After CCh/IPR stimulation, the strand particles of TJs rearranged with free ends and terminal loops. In the vertical fracture surface, cytoskeletal filaments beneath the plasma membrane were arranged in a thicker layer than those of the gland without stimulation. Contraction of the submembranous actin cytoskeleton during exocytosis elicited by CCh/IPR may cause rearrangement of TJ strands due to direct interactions between the TJ membrane particles and actin filaments *via* the tiny bridging structures. The rearrangement and movement of TJ membrane particles involves reconstruction of the subluminal membranous actin filament network through the intermediary of interstitial molecules and may modulate increased paracellular permeability after CCh/IPR stimulation. *J. Med. Invest.* 56 Suppl. : 395-397, December, 2009

**Keywords :** submandibular gland, paracellular pathway, fluid secretion, tight junction, cytoskeleton, freeze-fracture

## INTRODUCTION

The morphological change of the paracellular route for fluid secretion is still a long-standing question. Tight junctions (TJs) are routes for water and various molecules during saliva secretion (1, 2), and they act as a selective permeability barrier in

the paracellular pathway and, as a fence function in the structure of the plasma membrane (3). During salivary secretion, the meshwork of TJs in intercellular canaliculi are meandering and intermittent (4, 5). The purpose of this study was to visualize alterations in the cytoskeleton structure of TJs caused by carbachol (CCh) and isoproterenol (IPR) treatment of isolated, perfused rat submandibular glands (SMGs). We used freeze-fracture (FF) replicas of rapidly frozen tissues viewed by three-dimensional transmission electron microscopy.

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## MATERIALS AND METHODS

SMGs were surgically isolated from adult male Wistar rats under deep anesthesia induced by pentobarbital sodium. Isolated SMGs were cannulated and perfused with modified Krebs' solution without bicarbonate at 37°C, pH 7.4, buffered with 10 mM HEPES and saturated with 100% O<sub>2</sub> gas (1, 2), and stimulated with 1 µM CCh and IPR (Sigma).

For three-dimensional analysis, small pieces of fresh specimens were immediately transferred and rapidly frozen with liquid helium by metal contact without any chemical fixation (MF-7000/Meiwa). After cutting and deep etching at -110 to -100°C, FF replicas were obtained from the fractured surfaces of specimens by rotary shadowing with platinum and carbon coating at -100°C using FF equipment (BAF-400/Barzers). The replicas were cleaned with sodium hypochlorite solution and distilled water, and were examined using a 3D-tomography transmission

electron microscope (H-7650/Hitachi).

## RESULTS

In the resting stage, the intercellular canaliculi between acinar cells were straight with numerous microvilli. The strand particles of TJs formed 2-5 lines along the luminal margin of the lateral membrane. In the vertical fracture surface, the cytoskeletal filaments accumulated as a thick layer beneath the plasma membrane, and those microfilaments and the intermediate filaments formed a lining structure parallel to the TJ strands. Furthermore, tiny bridging structures were observed that connected the TJ membrane particles directly with these submembranous filaments. The intracellular organelles, secretory granules, mitochondria, *etc* were also connected with these cytoskeletal filaments (Fig. 1a, b).

During CCh treatment, some of the intercellular

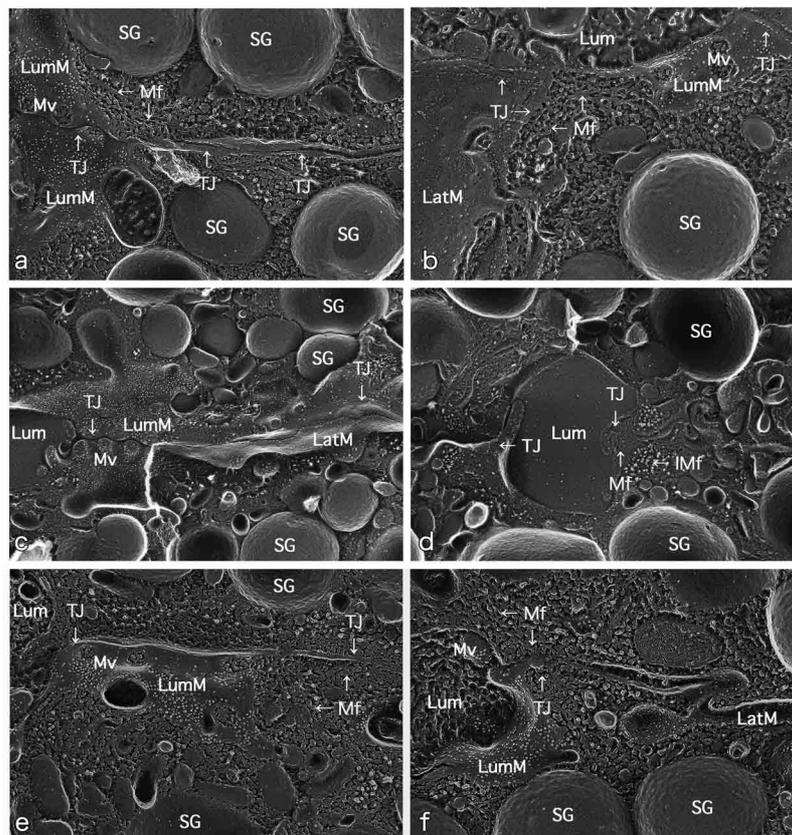


Fig 1. Freeze-fracture micrographs.

a, b, Resting stage : Intercellular canaliculi are surrounded by TJ strands, and intra-membrane particles of TJ are connected to the submembranous actin filament networks. c, d, CCh treatment : TJ structures are not remarkably changed, but cytoskeletal filaments beneath the plasma membrane are slightly thicker. e, f, CCh/IPR treatment : On the luminal membrane, the microvilli have completely disappeared except just adjacent to the cell, and cytoskeletal filaments beneath the plasma membrane are arranged in a thicker layer as compared with resting stage.

TJ : tight junction, SG : secretory granule, Lum : lumen, LumM : luminal membrane, LatM : lateral membrane, Mv : microvilli, Mf : microfilament, IMf : intermediate filament

canaliculi became dilated, and most of the microvilli remained. The TJ structures were not remarkably changed, but the arrangements of strand particles of TJs were influenced and the free ends and terminal loops could be observed. Furthermore, cytoskeletal filaments beneath the plasma membrane became slightly thicker than without the stimulation (Fig. 1c, d).

After treatment with both CCh and IPR, numerous exocytotic events occurred and the intercellular canaliculi became dilated between the acinar cells. Notable structural changes occurred on the luminal membranes; the microvilli completely disappeared except just adjacent to the cells, and numerous secretory granules were fused to the luminal membrane. The structure of TJs became more contracted, meandering, and intermittent, and the strand particles of TJs rearranged with free ends and terminal loops. In the vertical fracture surface, cytoskeletal filaments beneath the plasma membrane were arranged in a thicker layer as compared with resting stage (Fig. 1e, f).

## DISCUSSION

The structural changes in TJs noted due to treatment with CCh with or without IPR might result from rearrangements of microfilament-networks under the luminal plasma membrane. TJ strands consist of integral membrane proteins, occludin and claudin in the adjacent plasma membrane, and junction adhesion molecules, including members of the ZO family, which are the anchor strands to the actin cytoskeleton. Recent studies have demonstrated that TJs are a multifunctional complex that not only regulate the permeability barrier and have a fence function, but also are able to regulate paracellular water flux (1, 2), cell proliferation, and cell polarity (6). Contraction of the submembranous actin cytoskeleton during exocytosis by treatment with CCh/IPR may cause the rearrangement of TJ strands due to direct interactions between the TJ membrane particles and submembranous actin filaments via the tiny bridging structures.

## CONCLUSION

The rearrangement and movement of TJ membrane particles involve reconstruction of the subluminal membranous actin filament network through the intermediary of interstitial molecules which may modulate increases in paracellular permeability after treatment with CCh/IPR.

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