Confocal Microscopy with Double Immunofluorescence Staining Reveals the Functional Transient Receptor Potential Vanilloid Subtype 1 Expressed in Myoepithelial Cells of Human Submandibular Glands

QIANWEN DING,[1,2] YAN ZHANG,[3] XIN CONG,[3] ZHIGANG CAI,[1] JINGYAN HAN,[4] YUNCHAO SU,[3] LI-LING WU,[3]* AND GUAN-GYAN YU[1]*

[1]*Department of Oral and Maxillofacial Surgery, Peking University School and Hospital of Stomatology, Beijing, China*
[2]*Department of Dental Emergency, Beijing Stomatological Hospital, Capital Medical University, Beijing, China*
[3]*Department of Physiology and Pathophysiology, Peking University Health Science Center and Key Laboratory of Molecular Cardiovascular Sciences, Ministry of Education, Beijing, China*
[4]*Department of Integration of Chinese and Western Medicine, Peking University Health Science Center, Beijing, China*

KEY WORDS TRPV1; MECs; calponin; vimentin; α-SMA; p-MLC_{20}/MLC_{20}

ABSTRACT Myoepithelial cells (MECs) mainly surround acini and intercalated ducts in the human salivary glands. The contraction of MECs provides the expulsive force to promote salivation. We previously found functional transient receptor potential vanilloid subtype 1 (TRPV1) was expressed in rabbit and human submandibular glands and increased saliva secretion. However, it was unknown whether TRPV1 was expressed in MECs of submandibular glands. In this study, we observed the immunoflourescence of TRPV1 was not only located in serous acini and ducts but also surround the basal layer of the acinus and intercalated ducts of human submandibular glands. Double immunofluorescence staining revealed colocalization of TRPV1 with calponin, vimentin, and α-smooth muscle actin, which indicated the myoepithelial expression of TRPV1. Treating submandibular gland tissues with capsaicin, an agonist of TRPV1, substantially increased the phosphorylation of the 20-kDa regulatory light-chain subunit of myosin (MLC_{20}), a crucial molecule for contraction of smooth muscle cells, in MECs. Pretreatment with capsazepine, a specific TRPV1 inhibitor, blocked capsaicin-induced MLC_{20} phosphorylation. These results suggest that TRPV1 is expressed in MECs of the human submandibular gland and mediates myoepithelial contraction via a mechanism involving MLC_{20} phosphorylation. *Microsc. Res. Tech. 75:555–560, 2012.*

INTRODUCTION

Saliva, secreted by salivary glands, is vital for oral health, including maintaining the integrity of the oral mucosal surface to preserve an ecological balance and protect hard and soft tissues against infection (Amerongen and Veerman, 2002). The alteration of the quantity and quality of saliva is a considerable cause of oral diseases such as xerostomia, dysphagia, dental caries, oropharyngeal infections, and mucositis (Fox, 1998). The salivary glands primarily consist of parenchyma (acinus, duct, and myoepithelium) and mesenchyma (Tandler and Phillips, 1998). Saliva is produced by acinar cells and then collected, modified, and transported by the duct system; contraction of myoepithelial cells (MECs) is considered the contributing factor of salivation (Ogawa, 2003).

MECs are commonly found in the basal layer of the acinus and intercalated ducts of human and rat salivary glands. Electron microscopy and immunohistochemical studies have demonstrated that MECs contain cytokeratins and myofilaments, which are characteristic of epithelium and smooth muscle cells, respectively (Norberg et al., 1992). Stimulation of both parasympathetic and sympathetic nerves induces pressure changes and a secretory response in the submandibular gland of anaesthetized cats and dogs, which indicates that the contraction of MECs is controlled by both types of autonomic nerves (Emmelin et al., 1968, 1969). Investigation of dog submandibular glands suggested that the parasympathetic nerve-induced contraction of MECs is via muscarinic receptors, and the sympathetic nerve-elicited contraction is through the α_1-adrenoceptor (Lung, 2003). The contraction of MECs can shorten and widen the duct and increase pressure on the acinus, thereby lowering the outflow resistance and forcing secretory material into the duct system (Garrett, 1998). However, the exact functions of MECs during saliva secretion have not been fully investigated because of limited methodology.

Transient receptor potential vanilloid subtype 1 (TRPV1), also called vanilloid receptor 1, is a ligand-gated nonselective cation channel that can be activated

*Correspondence to: Li-ling WU, Peking University Health Science Center, Beijing, 100191, China. E-mail: pathophy@bjmu.edu.cn and Guang-yan YU, Peking University School of Stomatology, Beijing, 100081, China. E-mail: gyyu@263.net

Received 9 June 2011; accepted in revised form 11 August 2011

Contract grant sponsor: National Natural Science Foundation of China; Contract grant numbers: 30730102, 81070847; Contract grant sponsor: National Supporting Program for Science and Technology; Contract grant number: 2007BAI18B11; Contract grant sponsor: Capital Medical Development Foundation; Contract grant number: 2005-2009

DOI 10.1002/jemt.21090

Published online 29 September 2011 in Wiley Online Library (wileyonlinelibrary.com).

by heat and acid, as well as capsaicin, thus resulting in calcium influx (Caterina et al., 1997; Tominaga et al., 1998). As a multimodel nociceptor, TRPV1 is highly expressed in primary afferent fibers of the trigeminal, vagal, and dorsal root ganglia to detect noxious chemical, thermal, and high threshold mechanical stimuli (Nagy et al., 2004). Increasing evidences indicate that TRPV1 proteins are also present in various types of non-neural cells throughout the body, including urothelium, vascular endothelial, and smooth muscle cells (Lazzeri et al., 2004; Tiruppathi et al., 2006; Wang et al., 2008). We previously demonstrated TRPV1 expressed in rabbit and human submandibular glands, and activation of TRPV1 by capsaicin, a TRPV1 agonist, increased saliva secretion of the submandibular gland by regulation of aquaporin 5 (Zhang et al., 2006; Ding et al., 2010). These results suggest that TRPV1 may be a novel receptor participating in the regulation of saliva secretion. However, the expression and function of TRPV1 in MECs of submandibular glands are unknown.

This study was designed to test whether TRPV1-mediated saliva secretion involves the contraction of MECs in the human submandibular gland. We also studied the possible signaling molecule mediating the effect of TRPV1 on myoepithelial function. Our data provide new evidence that MECs-located TRPV1 acts as an accelerator to promote myoepithelial contraction, which is helpful to further understand the mechanism of saliva secretion regulated by TRPV1.

MATERIALS AND METHODS

Source of Human Submandibular Glands

Human submandibular gland samples were collected from eight patients (four males, age 34∼69 years) from the Department of Oral and Maxillofacial Surgery, Peking University School and Hospital of Stomatology. The patients had primary oral squamous cell carcinoma but not received irradiation and chemotherapy and were undergoing functional neck dissection as part of the surgical treatment for their malignancy. All glandular tissues were confirmed histopathologically normal by a pathologist. The surgical samples were transported to the laboratory within 30 min in 4°C Krebs–Ringer Hepes solution (120 mM NaCl, 5.4 mM KCl, 1 mM $CaCl_2$, 0.8 mM $MgCl_2$, 11.1 mM glucose, 20 mM Hepes, and pH 7.4) aerated with 95% O_2. The research protocol was approved by the Peking University Institutional Review Board, and the patients gave their informed consent for the collection of tissue.

Immunofluorescence

The submandibular gland tissues were fixed in 4% polyformaldehyde buffer, then frozen in Tissue-Tek OCT compound (Sakura, Zoeterwoude, The Netherlands). Microwave antigen retrieval was assayed with a 10-μm section of submandibular gland, which was then washed twice in PBS for 5 min each. The treated section was blocked with 10% horse serum for 30 min at room temperature and then incubated with primary antibody (1:200) at 4°C overnight and secondary antibody (1:400) for 2 h at 37°C. Normal goat IgG (santa cruz) was used as a negative control. Nuclei were stained with 4,6-diamidino-2-phenylindole (DAPI). Fluorescence images were captured with use of a confocal microscope (Zeiss LSM 510, Carl Zeiss, Germany). Antibody to TRPV1 (sc 12498) and rhodamine-conjugated secondary antibody were from Santa Cruz Biotechnology (Santa Cruz, CA). Antibodies to calponin (ZM 0326) and α-smooth muscle actin (α-SMA, ZM 0003) were from Beijing Zhongshan Goldenbridge Biotechnology (Beijing, China). Antibody to vimentin (M0721) was from Dako (Glostrup, Denmark). Alexa fluro-488 labeled secondary antibody was from Invitrogen (Carlsbad, CA). Mounting media with DAPI was from Vector laboratories (Burlingame, CA). Antibody to phosphorylated 20-kDa regulatory light-chain subunit of myosin (p-MLC_{20}) at serine[19] (M6068) was from Sigma-Aldrich (St. Louis, MO).

To detect the distribution of p-MLC_{20}, fresh gland samples were minced into small pieces (1 mm^3) and cultured at 37°C for 60 min in Dulbecco's modified Eagle's medium (containing 100 U/mL penicillin and 100 μg/mL streptomycin) (Gibco; Grand Island, NY) in a humidified atmosphere of 5% CO_2 and 95% air. The tissues were treated with or without carbachol (1 μM) or capsaicin (10 μM) for 10 min and capsazepine (10 μM) for 30 min, then fixed, sectioned, and incubated with the antibodies to p-MLC_{20} and α-SMA for immunofluorescence staining. Carbachol, capsaicin, and capsazepine were purchased from Sigma-Aldrich (St. Louis, MO).

RESULTS

Expression of TRPV1 in MECs of Human Submandibular Glands

We examined slides of submandibular glands from both male (n = 4) and female (n = 4) patients. TRPV1 proteins were widespread observed in both the serous acini and intercalated and striated ducts but not mucous acinar cells on immunofluorescent staining (Figs. 1A–1D) as we found previously (Ding et al., 2010). Immunofluorescence of another TRPV1 antibody from Merck (pc 547, original from calbiochem) displayed the similar results (data not shown). Moreover, TRPV1 staining was observed around the basal layer of serous and mucous acini and intercalated ducts. These results suggest the expression of TRPV1 in MECs of the human submandibular gland.

Because observing MECs, other than acini and ducts, with their shape and location is difficult, we used double immunofluorescence staining to identify the expression of TRPV1 in MECs using antibodies for calponin, vimentin, and α-SMA, three immunocytochemical markers of MECs (Ogawa, 2003). Calponin staining (red) characteristically surround the acinus and intercalated ducts but not striated ducts (Figs. 1E and 1F). Vimentin staining (red) was around the serous acini and in the mesenchyma (Fig. 1G). α-SMA staining (red) surrounded the acinus (Fig. 1H). These results were consistent with previous results of the distribution of MECs (Ogawa, 2003). The merged images revealed staining for TRPV1 partially colocalized with calponin (Figs. 1I and 1J), vimentin (Fig. 1K), and α-SMA (Fig. 1L). Interestingly, TRPV1 colocalized with calponin around the mucous acini, which showed no TRPV1 expression (Fig. 1J). Thus, in addition to acini and ducts, TRPV1 was also expressed in MECs surrounding the serous and mucous acini and intercalated ducts of the human submandibular gland.

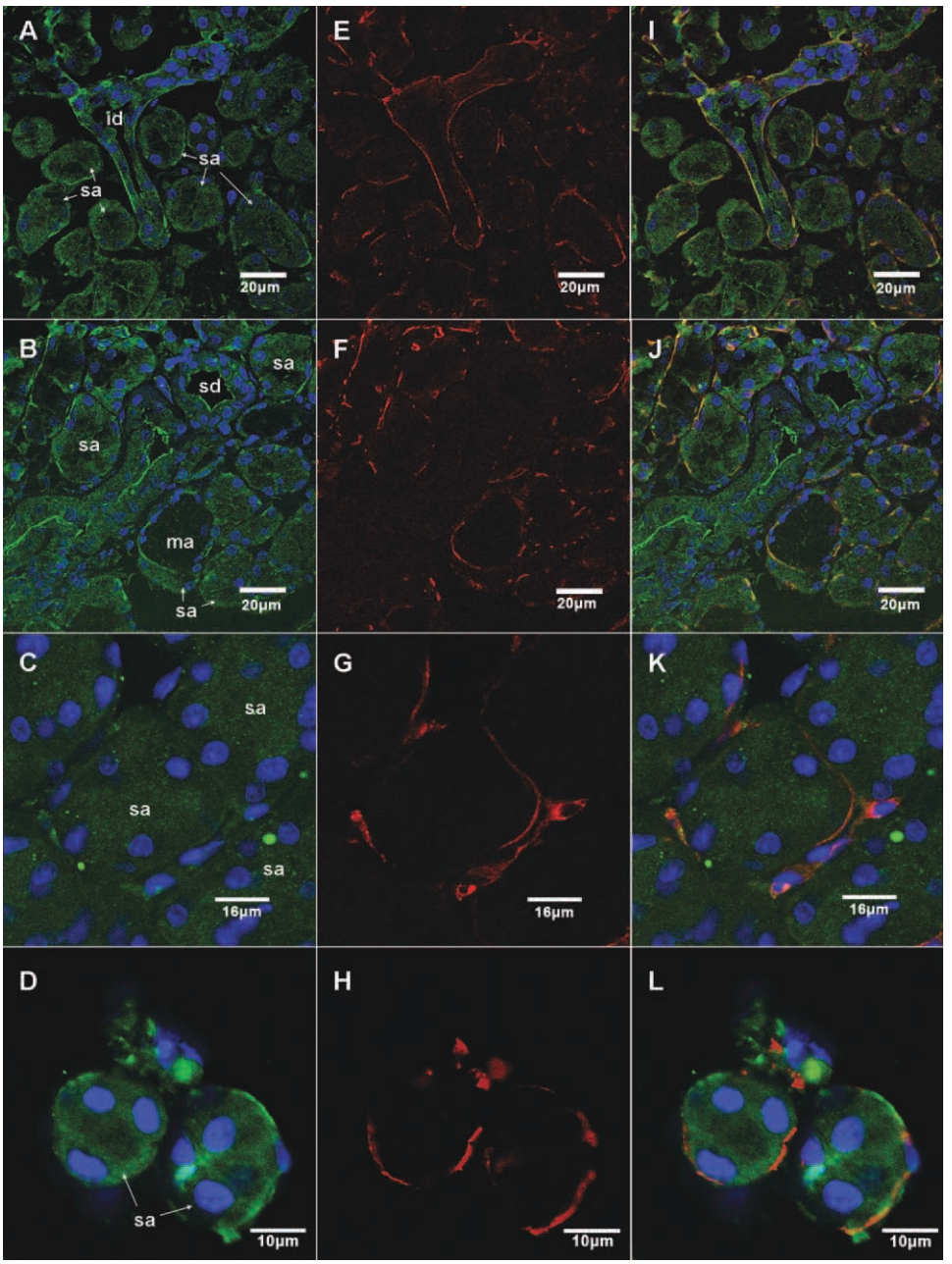

Fig. 1. Double immunofluorescence labeling of transient receptor potential vanilloid subtype 1 (TRPV1) with calponin, vimentin, and α-smooth muscle actin in human submandibular glands. **A**–**D**: Distribution of TRPV1 (green) in acini and ducts, (**E** and **F**) calponin (red), (**G**) vimentin (red), and (**H**) α-smooth muscle actin (red). Bright red lines were observed surrounding the acini and intercalated ducts but not striated ducts. **I**–**L**: Image overlay. Nuclei were stained with DAPI (blue). Serous acinus (sa), mucous acinus (ma), intercalated duct (id), and striated duct (sd).

Effect of TRPV1 Activation on Phosphorylation of MLC_{20} in MECs

Phosphorylation of MLC_{20} at serine[19] allows for activation of myosin adenosine triphosphatase by actin, which results in smooth muscle contraction, and is often used as an indicator of the contraction of smooth muscle cells (Somlyo and Somlyo, 1994). However, the phosphorylation of MLC_{20} in contracted MECs in salivary glands has not been previously examined. We observed linear, sparse, weak immunofluoresence of p-MLC_{20} in unstimulated submandibular glands (Fig. 2B). The glands treated with capsaicin (10 μM for 10 min) showed significantly increased immunofluoresence intensity of p-MLC_{20} (Fig. 2C). Pretreatment with capsazepine (10 μM for 30 min), a specific antagonist of TRPV1, abolished the capsaicin-induced p-MLC_{20} (Fig. 2D), which indicates that the response to capsaicin was the receptor mediated. Capsazepine (Fig. 2E) or DMSO (Fig. 2F), the vehicle for capsaicin, alone had no effect on MLC_{20} phosphorylation.

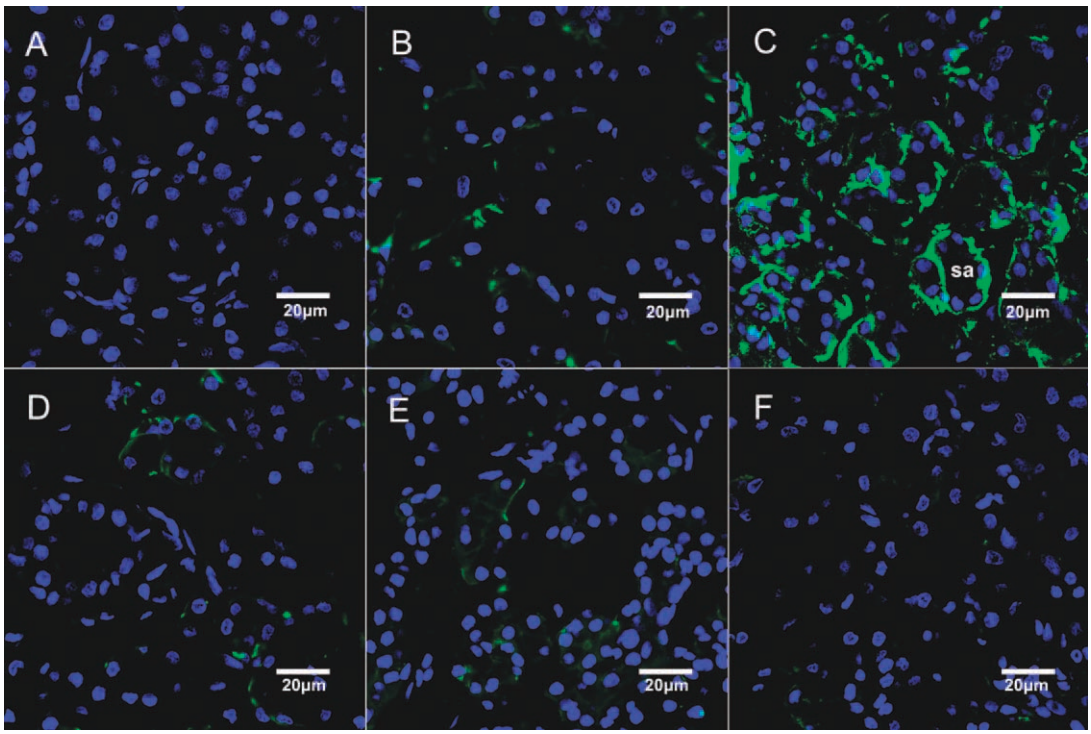

Fig. 2. Effect of activation of TRPV1 on phosphorylation of 20-kDa regulatory light chain subunit of myosin (p-MLC_{20}) in human submandibular glands. P-MLC_{20} (green) in human submandibular glands. **A**: Negative control, (**B**) untreated tissues, (**C**) treated with 10 μM capsaicin for 10 min, (**D**) pretreated with 10 μM capsazepine (30 min) then capsaicin, (**E**) treated with capsazepine alone, or (**F**) treated with DMSO (1:10,000). Nuclei were stained with DAPI (blue). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

To further investigate the possible effects of TRPV1 activation on the contraction of MECs in submandibular glands, we used 1 μM of carbachol, an agonist of muscarinic cholinergic receptor, to stimulate human submandibular gland tissues for 10 min and then used immunofluorescence staining to detect MLC_{20} phosphorylation. Compared with the untreated tissues (Fig. 2B), capsaicin and carbachol significantly increased the green immunofluorescence intensity and the area surrounding acini (Figs. 3A and 3B). P-MLC_{20} (green) colocalized with α-SMA (red) (Figs. 3D and 3E), which indicates that capsaicin, as well as carbachol, induced MLC_{20} phosphorylation mainly occurred in the MECs of the submandibular glands. Response of microvascular smooth muscle to carbachol was used as positive control (Figs. 3C, 3F, and 3I).

DISCUSSION

Although saliva is primarily secreted from acinar cells and modified by ductal cells, contraction of MECs has been suggested to provide the expulsive force to promote salivation. In this study, we showed TRPV1 proteins expressed in MECs of human submandibular glands by double immunofluorescence labeling. Furthermore, we revealed that activation of TRPV1 and the muscarinic cholinergic receptor significantly increased phosphorylation of MLC_{20} in MECs. Thus, TRPV1 was functionally expressed in MECs of human submandibular glands, and TRPV1-induced saliva secretion involved, at least in part, myoepithelial contraction via MLC_{20} phosphorylation.

MECs have structural features of both epithelial and smooth muscle cells. Although TRPV1 proteins are expressed in acini and ducts in rabbit and human submandibular glands and activation of TRPV1 increases saliva secretion (Zhang et al., 2006; Ding et al., 2010; Zhang et al., 2010), TRPV1 distribution and the physiological role in MECs of salivary glands have not been elucidated. MECs are located at the basal layer of acinus and the intercalated duct of human and rat salivary glands (Ogawa, 2003), but identifying MECs by light microscopy is difficult. The specific immunocytochemical markers of myoepithelium, including smooth muscle protein markers (calponin, α-SMA and h-caldesmon) and epithelial protein markers (keratins 14, 5, and 17, α1β1 integrin metallothionein), are often used to visualize MECs. Vimentin is the additional marker of MECs and is expressed in mesenchymal cells (Ogawa, 2003). We selected calponin, α-SMA and vimentin to distinguish MECs from acinar and ductal epithelial cells. The colocalization of TRPV1-calponin, TRPV1-vimentin and TRPV1-α-SMA surrounding the acini and intercalated ducts indicated that TRPV1 was expressed in MECs of the human submandibular gland.

TRPV1 proteins have been described in numerous tissues, including the smooth muscle cells and their roles on the contraction or relaxation of the smooth muscle cells has attracted increasing attention (Lazzeri et al., 2004; Wang et al., 2008). In the normal human urinary bladder, TRPV1 was detected in smooth muscle cells and in primary afferent fibers and

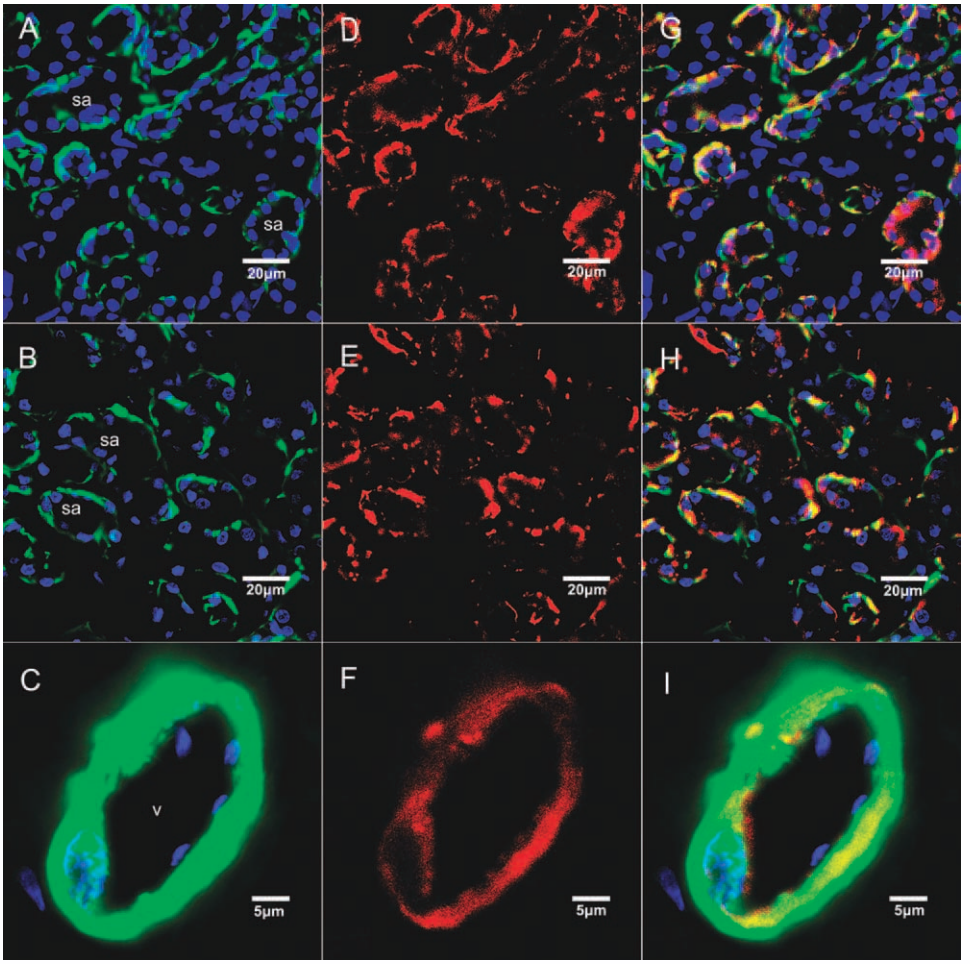

Fig. 3. Effect of capsaicin and carbachol on p-MLC_{20} in myoepithelial cells of human submandibular glands. **A** and **B**: p-MLC_{20} (green) in human submandibular glands treated with capsaicin (10 μM) and carbachol (1 μM) for 10 min, respectively. **C**: p-MLC_{20} (green) in microvascular smooth muscle treated with carbachol (1 μM for 10 min). **D**–**F**: α-Smooth muscle actin (red). **G**–**I**: Overlay of p-MLC_{20} and α-smooth muscle actin.

endothelium (Ost et al., 2002; Lazzeri et al., 2004), but its exact role was unclear. In isolated rat skeletal muscle arterioles, the functional expression of TRPV1 mediated vasoconstriction of resistant arteries (Kark et al., 2008). In guinea pigs, the activation of TRPV1 caused the contraction of tracheal smooth muscle (Kikuno et al., 2006). Matsumoto et al. reported on the capsaicin-induced contractile effect in mouse large intestine and indicated that TRPV1 channels located on the rectum and distal colon play a major role in the motor function of the lower gastrointestinal tract (Matsumoto et al., 2009). However, capsaicin but not capsazepine markedly reduced acetylcholine- and KCl-induced contraction in rat ileal longitudinal smooth muscles. Other than being involved in a TRPV1-dependent pathway, capsaicin may relax the rat ileum via its direct action on smooth muscle (Fujimoto and Mori, 2004). Thus, the biological function of TRPV1 in smooth muscle cells may be tissue and region specific. The possible role of TRPV1 in the MECs of the human submandibular gland needs to be further investigated.

Stimulation of the parasympathetic nerve elicits contraction of MECs in the dog submandibular gland (Lung, 2003). Carbachol induces significant contraction of MECs in the lacrimal gland (Satoh et al., 1997) and smooth muscle cells of vascular and visceral (Ehlert et al., 1997; Hegde and Eglen, 1999). Activation of muscarinic cholinergic receptor by acetylcholine increased the phosphorylation of MLC_{20}, a crucial molecule for contraction of smooth muscle cells, in ileal longitudinal smooth muscles of rats (Fujimoto and Mori, 2004). On the basis of these findings, we hypothesized that TRPV1 expressed in the MECs of human submandibular gland might increase saliva secretion by regulating myoepithelial contraction through a mechanism involving MLC_{20} phosphorylation. We used carbachol as a positive control to induce myoepithelial contraction in salivary glands and to illustrate the change and mechanism from the resting to contracted status of MECs. Interestingly, in carbachol-stimulated submandibular glands, a substantial increase in p-MLC_{20} surrounded the acinus other than the cytoplasm of acinar and ductal cells. Double immunofluorescence labeling

with α-SMA confirmed that the increased p-MLC_{20} was mostly located in MECs. Similar results were observed in contracted microvascular smooth muscle cells with good repeatability. More importantly, capsaicin substantially increased the level of p-MLC_{20} in MECs around the acinus, and this effect was abolished by a specific TRPV1 inhibitor, capsazepine. These findings were consistent with our hypothesis and indicated that activation of TRPV1 could induce the contraction of MECs via a mechanism involving MLC_{20} phosphorylation. Moreover, despite MECs express in different tissues and play an important role in physiological and pathophysiological conditions, their function and the underlying mechanism in vivo have not been fully evaluated due to methodology limitation. Our results suggested that the method of double immunofluorescence staining to detect the colocalization of MLC_{20} phosphorylation and the protein-markers of MECs can be used to directly assess the contraction activity and regulation of MECs, as well as smooth muscle cells, in health and disease.

In summary, the present results provide new evidence for the physiological role of TRPV1 in MECs. TRPV1 was expressed in MECs of human submandibular glands, and TRPV1-induced saliva secretion involves, at least in part, myoepithelial contraction via a mechanism involving MLC_{20} phosphorylation. Our findings might lead to a more comprehensive evaluation of TRPV1 in saliva secretion. Furthermore, because activation of TRPV1 results in increased salivation, TRPV1 may represent a novel pharmacological target for treating secretory dysfunction.

REFERENCES

Amerongen AV, Veerman EC. 2002. Saliva-the defender of the oral cavity. Oral Dis 8:12–22.

Caterina MJ, Schumacher MA, Tominaga M, Rosen TA, Levine JD, Julius D. 1997. The capsaicin receptor: A heat-activated ion channel in the pain pathway. Nature 389:816–824.

Ding QW, Zhang Y, Wang Y, Wang YN, Zhang L, Ding C, Wu LL, Yu GY. 2010. Functional vanilloid receptor-1 in human submandibular glands. J Dent Res 89:711–716.

Ehlert FJ, Ostrom RS, Sawyer GW. 1997. Subtypes of the muscarinic receptor in smooth muscle. Life Sci 61:1729–1740.

Emmelin N, Garrett JR, Ohlin P. 1969. Motor nerves of salivary myoepithelial cells in dogs. J Physiol 200:539–546.

Emmelin N, Garrett JR, Ohlin P. 1968. Neural control of salivary myoepithelial cells. J Physiol 196:381–396.

Fox PC. 1998. Acquired salivary dysfunction. Drugs and radiation. Ann N Y Acad Sci 842:132–137.

Fujimoto S, Mori M. 2004. Characterization of capsaicin-induced, capsazepine-insensitive relaxation of ileal smooth muscle of rats. Eur J Pharmacol 487:175–182.

Garrett JR. 1998. Myoepithelial activity in salivary gland. In: Garrett JR, Ekström J, Anderson LC, editors. Glandular mechanisms of salivary secretion. Front Oral Biol Basel: Karger. pp.132–152.

Hegde SS, Eglen RM. 1999. Muscarinic receptor subtypes modulating smooth muscle contractility in the urinary bladder. Life Sci 64:419–428.

Kark T, Bagi Z, Lizanecz E, Pasztor ET, Erdei N, Czikora A, Papp Z, Edes I, Porszasz R, Toth A. 2008. Tissue-specific regulation of microvascular diameter: Opposite functional roles of neuronal and smooth muscle located vanilloid receptor-1. Mol Pharmacol 73:1405–1412.

Kikuno S, Taguchi K, Iwamoto N, Yamano S, Cho AK, Froines JR, Kumagai Y. 2006. 1,2-Naphthoquinone activates vanilloid receptor 1 through increased protein tyrosine phosphorylation, leading to contraction of guinea pig trachea. Toxicol Appl Pharmacol 210:47–54.

Lazzeri M, Vannucchi MG, Zardo C, Spinelli M, Beneforti P, Turini D, Faussone-Pellegrini MS. 2004. Immunohistochemical evidence of vanilloid receptor 1 in normal human urinary bladder. Eur Urol 46:792–798.

Lung MA. 2003. Autonomic nervous control of myoepithelial cells and secretion in submandibular gland of anaesthetized dogs. J Physiol 546:837–850.

Nagy I, Santha P, Jancso G, Urban L. 2004. The role of the vanilloid (capsaicin) receptor (TRPV1) in physiology and pathology. Eur J Pharmacol 500:351–369.

Norberg L, Dardick I, Leung R, Burford-Mason AP, Rippstein P. 1992. Immunogold localization of actin and cytokeratin filaments in myoepithelium of human parotid salivary gland. Ultrastruct Pathol 16:555–568.

Ogawa Y. 2003. Immunocytochemistry of myoepithelial cells in the salivary glands. Prog Histochem Cytochem 38:343–426.

Ost D, Roskams T, Van Der Aa F, De Ridder D. 2002. Topography of the vanilloid receptor in the human bladder: More than just the nerve fibers. J Urol 168:293–297.

Satoh Y, Sano K, Habara Y, Kanno T. 1997. Effects of carbachol and catecholamines on ultrastructure and intracellular calcium-ion dynamics of acinar and myoepithelial cells of lacrimal glands. Cell Tissue Res 289:473–485.

Somlyo AP, Somlyo AV. 1994. Signal transduction and regulation in smooth muscle. Nature 372:231–236.

Tandler B, Phillips CJ. 1998. Microstructure of mammalian salivary glands and its relationship to diet. In: Garrett JR, Ekström J, Anderson LC, editors. Glandular mechanisms of salivary secretion. Front Oral Biol. Basel: Karger. pp.21–35.

Tiruppathi C, Ahmmed GU, Vogel SM, Malik AB 2006. Ca^{2+} signaling, TRP channels, and endothelial permeability. Microcirculation 13:693–708.

Tominaga M, Caterina MJ, Malmberg AB, Rosen TA, Gilbert H, Skinner K, Raumann BE, Basbaum AI, Julius D. 1998. The cloned capsaicin receptor integrates multiple pain-producing stimuli. Neuron 21:531–543.

Wang YX, Wang J, Wang C, Liu J, Shi LP, Xu M, Wang C. 2008. Functional expression of transient receptor potential vanilloid-related channels in chronically hypoxic human pulmonary arterial smooth muscle cells. J Membr Biol 223:151–159.

Zhang Y, Cong X, Shi L, Xiang B, Li YM, Ding QW, Ding C, Wu LL, Yu GY. 2010. Activation of transient receptor potential vanilloid subtype 1 increases secretion of the hypofunctional, transplanted submandibular gland. Am J Physiol Gastrointest Liver Physiol 299:G54–62.

Zhang Y, Xiang B, Li YM, Wang Y, Wang X, Wang YN, Wu LL, Yu GY. 2006. Expression and characteristics of vanilloid receptor 1 in the rabbit submandibular gland. Biochem Biophys Res Commun 345:467–473.