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# Influences of Carbachol on Potassium Channels in Sublingual Acinar Cells of Rat

Myung-Sang Shin, Kyung-Pyo Park, Joong-Soo Kim and Jong-Heun Lee

*Department of Oral Physiology and Dental Research Institute, College of Dentistry, Seoul National University*

*The Saliva is formed by outflow of potassium ion after calcium ion influx into acinar cell which was activated by secretagogue. The channel related with the outflow of potassium ion channel is one of the channels first studied with patch clamp technique in epithelial cell. Contrary to the many studies of submandibular gland, there has been no work about the electrophysiological study of sublingual gland with patch clamp technique. The sublingual acinar cells were collected from Spraue-Dawley rat by time-scheduled trypsin and collagenase treatment. To record channel activities, micropipettes filled with HEPES-buffered high potassium solution with 2-5 MΩ resistance were used. Membrane currents were recorded by cell attached patch clamp method. 10 μM Acetylcholine(ACh) or 10 μM Carbachol(CCh) was directly added in perfusion solution. The channel activities were recorded and were evaluated with personal computer on pClamp software. The obtained results were as followed.*

1. *We could attain the isolated single acinar cells of sublingual gland.*
2. *The conductance of potassium ion channel was about 190pS and voltage dependent.*
3. *The the other type of channel was not sensitive to pipette voltage and had small conductance.*
4. *Addition of ACh or CCh increased the open probability of potassium channels without change of conductance.*

***Key word:*** *sublingual gland, potassium channel, acetylcholine, carbachol, patch clamp*

## Introduction

The sublingual glands in the rat are mixed-type salivary glands that primarily composed of tubuloacinar mucous cell and secrete organic material, ions and water in response to nerve stimulation. Contrary to other salivary glands in the rat, the acinar cells of sublingual gland secrete very viscous mucin and fluid at the same time when stimulated. Perhaps, this phenomenon seems to be very effective in flushing viscous saliva to oral cavity. The function of mucin is to protect oral epithelium from mechanical, chemical and bacterial stimuli and prevent dehydration (Melvin *et al.*, 1991).

The first intracellular microelectrode recording of membrane potential and resistance in exocrine gland was reported about forty years ago. At that time, the acetylcholine(ACh)-evoked membrane hyperpolarization was thought to be due to active chloride ion uptake into the acinar cells (Lundberg, 1958). Burgen (1956) showed that the salivary gland cells lose intracellular potassium ions to outside of cell when they are hyperpolarized, and Douglas and Poisner (1963) observed that the phenomenon of ACh evoked salivary secretion is calcium ion dependent. These findings were not linked or properly explained until finding of the presence of potassium channels in the basolateral acinar cell membrane with patch clamp technique.

The patch clamp technique had developed by Neher and Sakmann in 1976 and designed for recording single channel current. Hamil *et al.* (1981) improved the patch clamp technique more technically and then this could be applied for the recording in various cell types. The patch clamp studies of salivary acinar cells showed the presence of potassium channels in the basolateral acinar cell membranes (Maruyama *et al.*, 1983; Petersen and Maruyama, 1984).

A Number of neurotransmitters and hormones are capable of interacting specifically numerous receptors on membrane surface of salivary acinar cells. The receptors of salivary acinar cells can be distinguished into two classes, those coupled to adenylate cyclase and those coupled to phospholipase C. The latter receptors linked generation of diacylglycerol and inositol trisphosphate($IP_3$). This inositol trisphosphate releases calcium ion from the endoplasmic reticulum and this causes opening of potassium channnels. This receptor changes membrane permeability by the change of

potassium channel activity. This class of receptors is stimulated by ACh, vasoactive intestinal peptide, substance P, and norepinephrine (Petersen and Gallacher, 1988).

Potassium channels are among the first ion channels studied directly by the patch clamp technique (Sakmann and Neher, 1984). The first direct demonstration of potassium channels in acinar cells of salivary gland was made by Maruyama *et al.* (1983). There are several types of potassium channels. One of these channels has prominent, large conductance above 100pS and very selective to potassium ion. In other type of the potassium channels, they have very small conductance below 20pS (Petersen and Gallacher, 1988). The potassium channels are activated by ACh in salivary acinar cells (Gallacher and Morris, 1986).

To date few study has examined the mechanisms responsible for stimulus-secretion coupling in the rat sublingual gland (Culp *et al.*, 1991; Putney *et al.*, 1978; Zhang *et al.*, 1993, 1994) and there has been no work about eletrophysiological study of sublingual gland with patch clamp technique in spite of the uniqueness of this gland. Therefore, this study was performed to examine the existance of potassium channel in rat sublingual gland and elucidate the electrophysiological characteristic and the influences of ACh and CCh on the potassium channels.

## Materials and Methods

### Preparation of sublingual acinar cells

Adult Sprague-Dawley rats (about 200 g body weight) were sacrificed by cervical dislocation after ether anesthesia. Bilateral sublingual glands were excised and placed in 100% oxygenated Calcium-free HEPES buffered Tyrode solution ( NaCl 140, KCl 5, $MgCl_2$ 1, Glucose 5, HEPES 5, in mM, pH 7.2). Fibrous capsules of gland were removed and glands were minced. Isolated acini were prepared by 10 minutes of trypsin (0.4 mg/ml, Sigma, U.S.A.) and 15 minutes of collagenase (60 U/ml, Sigma, U.S.A.) with trypsin inhibitor (2 mg/ml, Sigma, U.S.A.) digestion, after 10 minutes bovine serum albumin (50 mg/ml, Sigma, U.S.A.) pretreatment. At the end of digestion period the digestion suspension was filtered nylon mesh, and filtrates were centrifuged at 1,000×g for 30 seconds to collect dispersed acinar cells. Isolated acinar cells transferred to culture dish containing M-199 media (Sigma, U.S.A.), and stored in $CO_2$ incubator at 37°C.

### Recording of single channel currents

Single channel currents were recorded from patches of basolateral membrane attached to isolated acini or clumps of cells. The patch clamp methods were described by Hamil *et al.* (1981). For experiments, small aliquots of acini were transferred to a 1 ml chamber on the stage of Olympus IMT-2 inverted microscope on the isolation-free table. An Axopatch 1-C (Axon instrument, U.S.A.) patch clamp amplifier was employed and signal displayed on digital storage oscilloscope and simultaneously recorded for analysis on video tape. Electrodes were pulled from microhematocrit tubes (Chase, U.S.A.) with two stage micropipette puller (Narishige, PP-83, Japan) and they had tip resistances of between 2 to 5 MΩ. All experiments were carried out at 20-24°C.

### Solutions

The standard extracellular solution (HEPES-buffered Tyrode solution) contained (mM) NaCl 140, KCl 5, $MgCl_2$ 1, $CaCl_2$ 1, HEPES 5, pH 7.4 and the standard pipette solution ( HEPES-buffered high potassium solution) contained (mM) KCl 140, $MgCl_2$ 1, $CaCl_2$ 1, HEPES 5, pH 7.2. The concentration of employed secretagogues was 10 μM ACh (Sigma, U.S.A.) and 10 μM CCh (RBI, U.S.A.).

### Analysis of single channel currents

Analysis of the single channel currents amplitude and open probability of channels were performed with pClamp (version 6.1, Axon Instrument, U.S.A.) software.

## Results

### Single channel currents

Single channel currents obtained from basolateral membranes of sublingual acinar cells displayed in Fig. 1. At resting membrane potential, inward currents of about 7.0pA amplitude were observed. The more hypopolarized membrane potential was applied, the larger inward currents

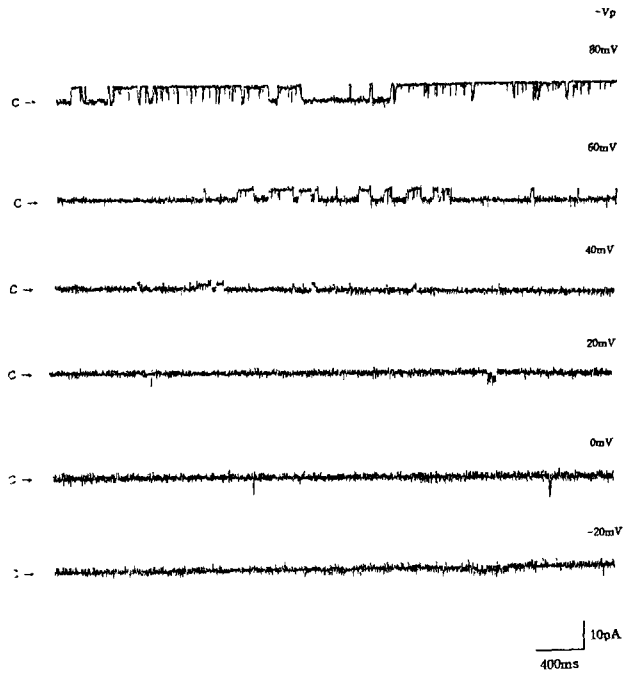


**Fig. 1.** Single channel current obtained from cell attached patch during perfusion of HEPES Tyrode solution. The numbers on the inducate negative pipette potential, and the'C'mark in the traces mean the baseal levels. Open channel inward and outward currents were recorded downward and upward, respectively.

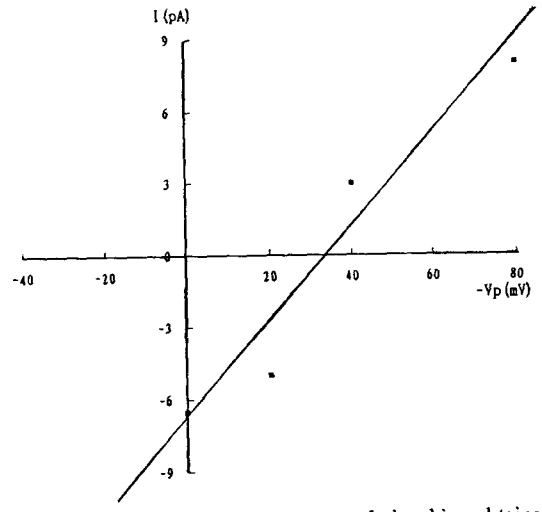


**Fig. 2.** Curresponding current-voltage relationship obtained from the single channel current from Fig. 1. The line of graph fits the linear regression of data, and it represents 186.6 pS conductance and a reversal potential about -34 mV pipette potential.

were recorded. At -40 mV of pipette potential, single channel currents were too small to identify. Applied more hyperpolarized pipette potential, we could observe outward currents. The corresponding current-voltage relationship of channel was showed in Fig 2, and that relationships were

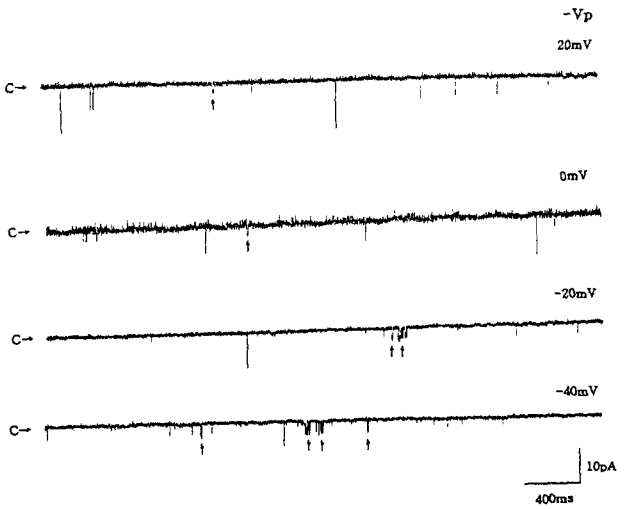


**Fig. 3.** Single channel current recording obtained from cell attached patch during perfusion of HEPES Tyrode solution. The numbers on the right inducate negative pipette potential, and the'C'mark in the traces mean the baseal levels. Open channel inward and outward currents were recorded downward and upward, respectively. Note small current amplitude (indicated by arrow).

well fit the linear regression of data. The conductance of channels was 192±17 pS (n=7, Mean ±S.E.M.). The addition of ACh or CCh could not evoke any change of amplitude of currents and conductance of channels. In some activated acinar cells another class of channels was observed, and the currents of those channels were not depended on pipette voltage and had small amplitude (Fig. 3).

### Influences of ACh and CCh on the potassium channel activity

The hypopolarizing current of membrane increased open probability of channels. Open probability of channels in HEPES-buffered Tyrode solution was 15.5±2.1% at 40 mV pipette potential. 10 μM ACh increased their open probability to 30.9±9.9% (Fig. 4. 5). At resting membrane potential from another cell, the open probability of one channel, two channels and three channels was 46.4± 2.6%, 4.0±2.7%, 0.3±0.3%, respectively. 10 μM CCh increases their open probability of one channel, two channels, three channels, four channels and five channels to 41.7±19. 2%, 24.5±1.7%, 20.6±17.6%, 5.0±1.8 %, 0.5±0. 4%, respectively.(Fig. 6.7.8).

## Discussion

The first current recording with voltage clamp

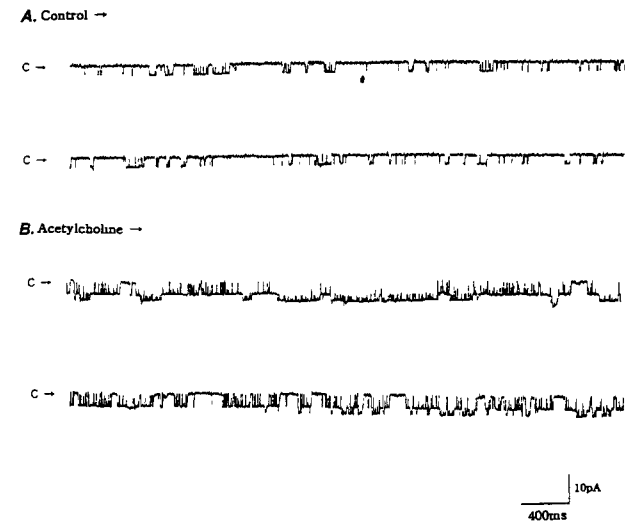


**Fig. 4.** Single channel current recorfing obtained from cell attached patch before (A) and after (B) perfusion of ACh (10 μM) in bath at 40 mV pipette potential. The 'C' mark in the traces mean the baseal levels. Open channel inward currents were recorded downward.

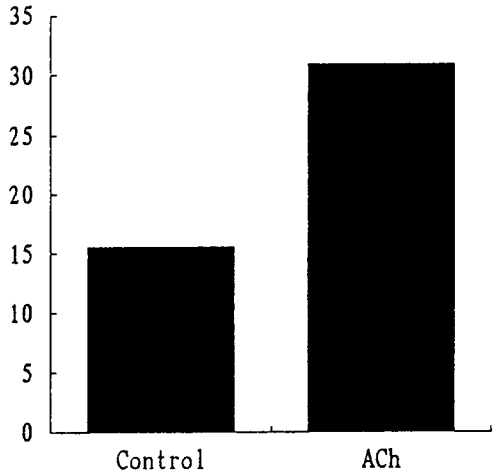


**Fig. 5.** The effects of ACh on open probability of potassium channels at 40 mV pipette potential.

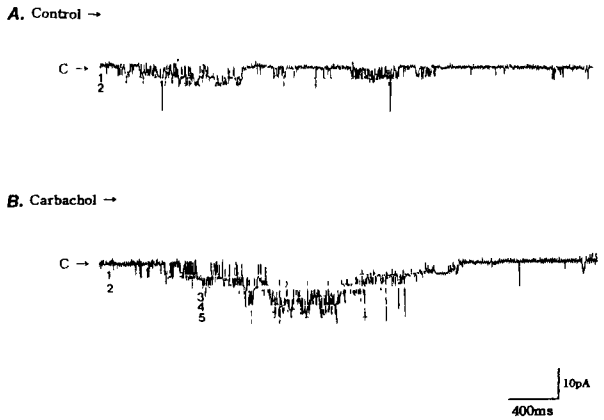


**Fig. 6.** Single channel current recorfing obtained from cell attached patch before (A) and after (B) perfusion of CCh (10 μM) in bath. The 'C' mark in the traces mean the baseal levels. Open channel inward currents were recorded downward. The figure of 1, 2, 3, 4 and 5 means different current level.

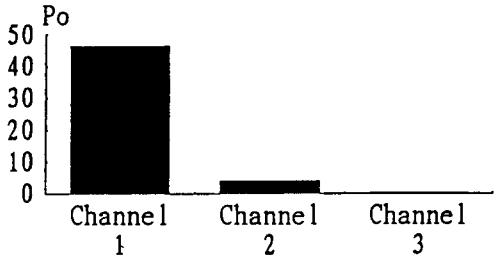


**Fig. 7.** Open probability of potassium channels at resting state.

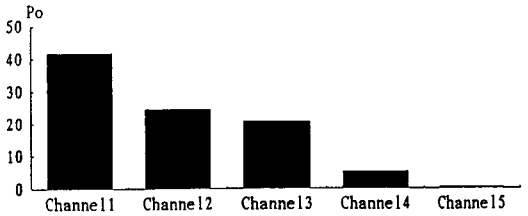


**Fig. 8.** The effects of CCh on open probability of potassium chan-

technique had performed in 1950s by Hodgkin *et al.* (1952) with giant axon of squid, but its technical limitation prevented this technique from various application on other small cells. But more advanced new technique ,patch clamp techniques, was introduced by Neher and Sakmann (1976) and then it was possible to measure of single channel currents. Electrical activation of excitable cells is a sum of ionic fluxes via various single channels. Therefore single channel recording will best elucidate the mechanisms of electrical functions of cells. Patch clamp technique was applied salivary acinar cells after more improved patch clamp techniques had been developed by Hamil *et al.* (1981).

There are several modes of patch clamp techniques. Among them, the cell-attached patch clamp mode demonstrates directly the messenger mediated effects of neurotransmitters and hormones on the activity of ion channels. ACh stimulates salivary acinar cells by increase calcium ion activity via the receptors on the membrane (Petersen, 1980), and CCh has the same effect on several exocrine gland cell (Toshio *et al.*, 1991) Therefore cell attached patch clamp method is selected for study of our experiment.

ACh is secreted by the postganglionic meurons of the parasympatheic nerve system and the salivary gland is innervated mainly by the parasym

pathetic nerve. ACh stimulation evokes a marked increase in both the frequency and duration of single channel currents in the potassium channels. This activation of potassium channels is inactivated directly by application of atropine (Gallacher and Morris, 1986; Petersen and Gallacher, 1988). In this study ACh or CCh application in bath solution increased open probability of potassium channels without any change of their conductance which coincides with earlier studies. We could identify the similarities of potassium channels among the various salivary glands.

The principal event of excitation-secretion coupling in salivary cells was outflow of water due to loss of potassium from acinar cells (Petersen and Maruyama, 1984). Inward single channel current can be obtained from influx of cation or outflux of anion. In this experiment, inward currents should be influxes of potassium ion derived from electromotive forces by membrane potential, because potassium channels prominent in basolateral membranes of acinar cells and an anion, possibly chloride ion, passes out only through apical chloride channels (Maruyama *et al.* 1983; Petersen and Gallacher, 1988) and moreover, the area is very small to that of the potassium channels. Current reversal was demonstrated near the -40mV of pipette potential, suggesting that resting membrane potential of acinar cell is near the -40 mV. Application of pipette potential of -40 mV eliminated electromotive forces for potassium influx, then abolished channel currents.

There are two distinct types of potassium channels. They differ in their voltage dependence, calcium sensitivity, pharmacology and conductance. The one type, big K or Maxi K channel, has large conductance and is sensitive to pipette voltage, calcium ion activity and tetraethyl ammonium, but the other one, small K channel, has small conductance and is not sensitive to them (Petersen and Gallacher, 1988). Both types of channels are present in salivary acinar cells (Maruyama *et al.*, 1983; Petersen and Maruyama, 1984). In this investigation we could identify the two different types of potassium channels. The first type of potassium channel had large conductance about 192 pS and exhibited voltage dependence.

In this study we could find the existance of the potassium channels on the basolateral membranes of sublingual gland in the rat and identify the electropysiological characteristics and influences of ACh and CCh on the activities of potassium channels. We could see that this results were similar to those of the submandibular and parotid gland (Maruyama *at al.* 1983)

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