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K^+ Channels in Mucous Cells Isolated from the Rat Sublingual Gland

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The basolateral, voltage dependent K^+ channels of mucous cells dissociated from rat sublingual gland were studied using the patch-clamp technique. In the cell-attached mode, two types of K^+ channels could be observed in these cells. One had a large conductance of 222±13 pS (mean±SEM, n=4), and the other had a small conductance of 43±7 pS (n=3). Acetylcholine (ACh; 10^{-5} M) activated both K^+ channels by increasing the open probability (Po). Open probability for the large conductance channel increased from 0.18 in the control solution to a maximum value of 0.85 after addition of Ach. Whereas, Po for the small conductance channels increased from 0.002 in the control solution to a maximum value of 0.31. K^+ channel activation by 10^{-5} M Ach was observed in 2 other mucous cells. When the osmolarity of bath solution was reduced to 60% of control, both large and small conductance K^+ channels were activated. Open probability for the large and small conductance K^+ channels increased significantly ($P < 0.05$) from 0.006±0.001 (n=4) and 0.002±0.001 (n=4) in the control solution to a maximum value of 0.30±0.04 and 0.12±0.03 in the hypotonic solution, respectively. It is concluded that both K^+ channels may play a crucial role in secretion induced by muscarinic receptor activation and in regulatory volume decrease (RVD).

Key words : sublingual gland, mucous cells, patch clamp, K^+ channels, acetylcholine, RVD

Introduction

The viscosity of saliva produced by the sublingual glands is higher than that of other major salivary glands, such as the submandibular or parotid gland. This is because there is a high concentration of mucins in the saliva from the sublingual gland (Tabak *et al.*, 1982). The structure of the sublingual gland is also different from the other major salivary glands. The parotid gland is primarily composed of serous cells, while the submandibular gland is composed of both serous and mucous cells. In contrast, the sublingual gland is mainly composed of mucous cells with relatively few serous demilune cells (Pinkstaff, 1980).

The mechanism of mucous cell secretion is unique in that there is a concomittent discharge of secretory granules and fluid transport upon muscarinic stimulation. Both α_1- and β-adrenergic stimulation could not evoke protein secretion in sublingual mucous acini (Putney *et al.*, 1978; Nieuw Amerongen *et al.*, 1980; Compton *et al.*, 1981). In response to carbachol, cell aggregates are synthesized and mucins are secreted in sublingual gland mucous cells (Culp *et al.*, 1991). Carbachol also induces K^+ efflux (Larsson *et al.*, 1990), and it has been reported that there are sustained losses of K^+ and Cl^- content, together with a sustained increase in Na^+ content and the intracellular free Ca^{2+} concentration during muscarinic stimulation (Melvin *et al.*, 1991). Mucin discharge coupled with water movement is desirable to flush the thick, mucin-containing saliva to the oral cavity. However, there is no direct patch clamp evidence for the effects of muscarinic agonists on the ionic channels which are involved in fluid and electrolyte secretion. Thus, contrary to the many studies of either serous cell secretion in the parotid gland or seromucous cell secretion in submandibular gland, little is known of the mechanisms in mucous cell secretion of the sublingual gland.

Many patch clamp studies of exocrine acinar cells have shown the presence of K^+ channels at the basolateral membrane, e.g, rat and mouse pancreatic acini (Maruyama and Petersen, 1982); pig pancreatic acini (Maruyama *et al.*, 1983a);

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mouse parotid, submandibular and rat parotid (Maruyama *et al.*, 1983b); lacrimal gland (Marty *et al.*, 1984) and human submandibular gland (Morris *et al.*, 1987). These K^+ channels are thought to play a crucial role in the primary secretion of saliva. The aim of this study was to identify K^+ channels in mucous cells in the sublingual gland, and examine the possible role of this channel in secretion induced by muscarinic agonists, and in the process of regulatory volume decrease (RVD).

Materials and Methods

Materials

Trypsin, collagenase, trysin inhibitor, bovine serum albumin and M199 medium were purchased from Sigma (St. Louis, MO). Acetylcholine was from RBI (Natick, USA).

Cell preparation

Adult Sprague-Dawley rats (about 200 g body weight) were sacrificed by cervical dislocation after ether anesthesia. Excised bilateral sublingual glands were finely minced and incubated in oxygenated Ca^{2+} -free saline solution containg (mM): 140 NaCl, 5 KCl, 1 $MgCl_2$, 5 Glucose, 5 HEPES (pH adjusted to 7.2 with HCl), supplemented with bovine serum albumin (5 mg/ml), for 10 min at 37°C. Single isolated sublingual gland mucous cells were prepared using a method described by Kotera and Brown (1993). In brief, the tissue was incubated in the above solution supplemented with trypsin (0.4 mg/ml) for 10 min. After washing with Ca^{2+} -free saline, the tissue was incubated in the same solution supplemented with collagenase (0.15 mg/ml) and trypsin inhibitor (2 mg/ml) for 15 min. The tissue was then dissociated by repeated pipetting. Throughout the dissociation procedure the solutions were gassed with 100% O_2. The resulting single mucous cells were resuspended in Medium 199, and stored in a CO_2 incubator at 37°C until use.

Patch-clamp recording

Cells were allowed to settle at the bottom of a recording chamber (volume 400 μl), and were viewed with an Olympus IMT-2 inverted microscope. The cells were superfused (2~3 $ml.min^{-1}$) with a NaCl-rich control solution (mM): 140 NaCl, 5 KCl, 1 $MgCl_2$, 1 $CaCl_2$, 5 HEPES, pH 7.4 (pH adjusted with NaOH). Electrodes were manufactured from hematocrit tubes (Chase, U.S.A.). These were filled with a K^+ -rich solution containing (mM): 140 KCl, 2 $MgCl_2$, 1 $CaCl_2$, 5 HEPES, pH 7.2 (pH adjusted with KOH), and had tip resistances of between 3 to 5 MΩ. Single channel activity was monitored with an Axopatch-1C amplifier (Axon Instruments, Foster City, CA). The patches were clamped at a series of potentials and the resultant records were stored as a digital signal on videotape (44 KHz) using a modified digital audio processor (PCM, Biomedical). The control level of activity in cell-attached patches was established by recording single channel currents from cells bathed in the control solution for 1 min. The bath solution was then changed to a test solution containing 10^{-5} M Ach or 60% hypotonic solution. The hypotonic solution (60%) was produced by diluting the control bath solution with distilled water.

Data analysis

Single channel current amplitudes were measured using either the cursors on a digital storage oscilloscope (Philips PM3350A), or by a computer using the pClamp 6.0 software package (Axon Instruments). Open probabilities (Po) were determined for 20 sec periods of data using the pClamp program, before and after stimulation of cells. The usual conventions of current flow were observed throughout, i.e., positive charge moving out of the cell (into the electrode) is a positive current. The potential applied to the electrode in cell-attached patches (-Vp) does not include the contribution of the membrane potential of the cell. Values are expressed as mean±SEM. Students *t* test was used for statistical analysis of data and P values less than 0.05 were considered significant.

Results

Conductances of K^+ channel

We found that there are two types of K^+ channels in mucous cell which have different conductances. Fig. 1A shows one of the typical tracings of large conductance K^+ channels from a cell-attached patch. At the more depolarized potentials (-Vp=60 and 80 mV), the frequency and duration of channel openings were increased, demonstrating that the channel is voltage dependent. The current-voltage (I-V) relationship for this K^+ channel is shown in Fig. 1B. The

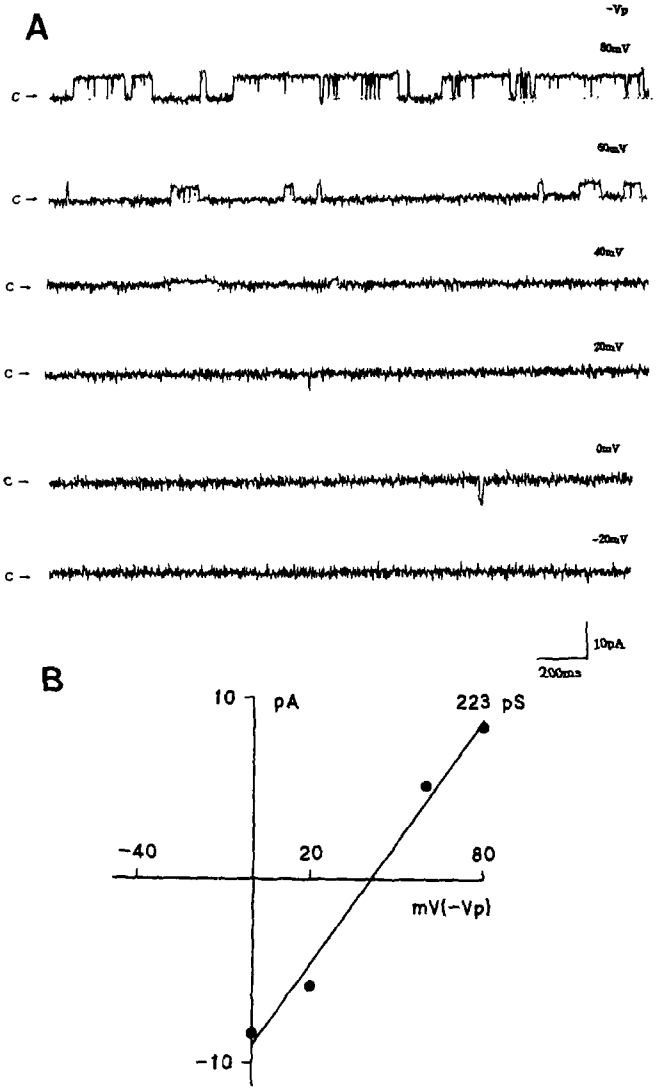

Fig. 1. (A) Single channel records for a large conductance K^+ channel in a cell-attached patch. Recordings were made at different holding voltages (-Vp). The letter "C" on the left hand side indicates the closed state of the channel. The patch pipette contained a K^+-rich solution. (B) The current-voltage (I-V) relationships for large conductance. The conductance was 223 pS with current reversal at -45 mV of -Vp. The line was drawn by linear regression.

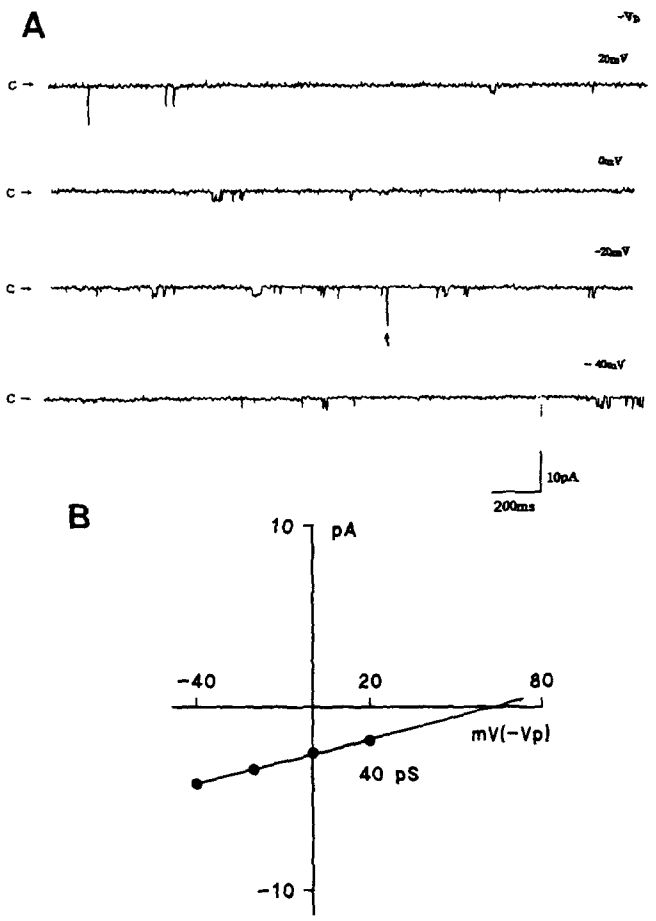

Fig. 2. (A) Single channel recordings for a small conductance K^+ channel in a cell-attached patch at different -Vp. The arrow in the tracing at -Vp=-20 mV indicates the opening of a large conductance K^+ channel. The patch pipette contained a K^+-rich solution. (B) The current-voltage (I-V) relationships for the small conductance K^+ channel. Conductance was 40 pS with current reversal at -61 mV of -Vp. The line was drawn by linear regression and reversal current of the small conductance K^+ channel was estimated by extrapolation.

conductance was 223 pS, with current reversal at Vp=-45 mV. The line through the data was drawn by linear regression. The average conductance of these channels was 222±13 pS (mean±SEM, n=4) with current reversal at Vp=-51±17 mV.

Fig. 2A shows one of the typical tracings of small conductance K^+ channels. In this Figure, the current of large conductance K^+ channels are also observed (indicated by arrow at -20 mV of -Vp). The conductance of the small K^+ channel was 40 pS with current reversal at -61 mV of -Vp (Fig. 2B). The average conductance of these channels was 43±7 pS (n=3) with with current reversal at Vp=-56±5 mV. The line through the data was drawn by linear regression and reversal current of these channels was estimated by extrapolation. The similar value of current reversal for the large and small conductance channels suggest that these channels are both K^+ channels. In contrast to the large conductance K^+ channels, the small conductance K^+ channels did not appear to be voltage dependent.

Effects of ACh on the K+ channel activity

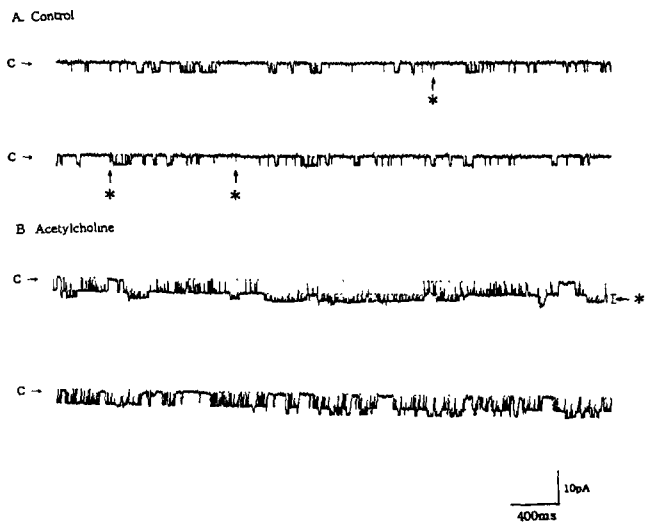

Fig. 3. K^+ channel activity from the patch before (A) and during (B) superfusion of the cell with a bath solution containing 10^{-5} M Ach. The applied potential (-Vp) was -40 mV and the downward current deflections (inward current) represent channel openings. Openings of the small conductance K^+ channel are indicated by*.

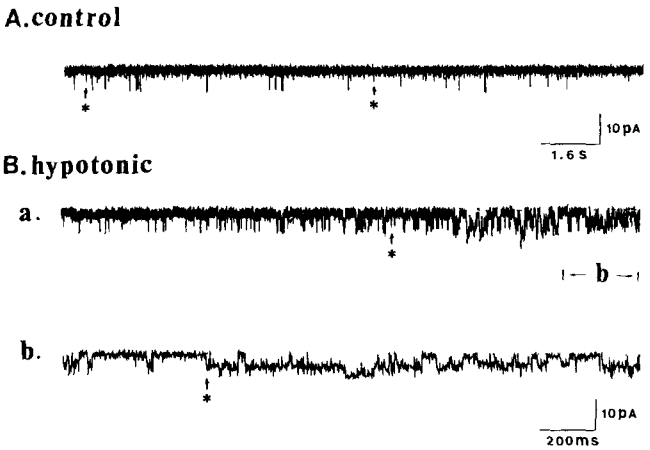

Fig. 4. K^+ channel activation by hypotonic solutions. (A) Single channel records from a cell-attached patch at the resting state. (B)a. The same cell was superfused with hypotonic solution (60% of control) and (B)b. a 2.1s period of channel acitivity on an expanded time scale.

In this experiment, we studied the effects of Ach (a muscarinic agonist) on the K^+ channels in sublingual mucous cells. The open probability (Po) for the large conductance K^+ channels increased from 0.18 in the control solution (Fig. 3A) to a maximum value of 0.85 after addition of 10^{-5} M Ach in the bath (Fig. 3B). In this figure, the patch was held with -40 mV of pipette potential (-Vp=-40 mV) and channel openings were observed as downward current steps (current amplitude=-4 pA). These inward currents are probably carried by K^+ moving from the electrode into the cell. K^+ channel activation by 10^{-5} M Ach was observed in 2 other mucous cells. Interestingly, the small conductance K^+ channels were also activated by 10^{-5} M Ach. These channels were open very little in the control condition (indicated by* in Fig. 3A). However, Po increased from 0.002 in the control solution (Fig. 3A) to a maximum value of 0.31 after addition of 10^{-5} M Ach (Fig. 3B). Current amplitude of the large conductance K^+ channels shown in Fig. 3B was slightly increased over that of Fig. 3A, which suggests that mucous cells might be hyperpolarized during Ach stimulation.

Activation of channels by hypotonic stress

Fig. 4 shows single channel currents from a cell-attached patch in a sublingual mucous cell. The trace shows continuous activity from the patch before and during superfusion of the cell with a hypotonic solution (60% of control). The patch was held with no applied pipette potential throughout the experiment (-Vp=0), and openings for the large conductance channel were observed as downward current steps (current amplitude=-7 to -8 pA). The small conductance K^+ channel was also observed in this experiment (indicated by*). Channel openings were very infrequent in the control solution (Fig. 4A). However, a sudden increase in channel activity was observed within 20 sec of exposing the cell to the hypotonic solution (Fig. 4B). Individual channel openings can be seen more clearly in Fig. 4B.b which shows the channel activity of Fig. 4B.a on an expanded time scale. Activation of both large and small conductance K^+ channels was observed and the average Po for the large conductance channels significantly increased from 0.006 ± 0.001 (n=4) in the control solution to a maximum value of 0.30 ± 0.04 ($P<0.05$) in the 60% hypotonic solution. The average Po for the small conductance channels also increased from 0.002 ± 0.001 (n=4) in the control solution to a maximum value of 0.12 ± 0.03 ($P<0.05$).

Discussion

Conductances of K^+ channels

There were two distinct types of potassium channels in sublingual gland mucous cells, differing in their voltage dependence and conductance. One type had a large conductance of 222 ± 13 pS and

opened at depolarizing potentials. These properties are similar to those for "maxi" Ca^{2+}-activated K^+ channels which have been previously described in other major salivary gland acinar cells (Maruyama *et al.*, 1983b; Petersen and Maruyama, 1984), and lacrimal gland cells (Findlay, 1984; Trautman and Marty, 1984; Lechleiter *et al.*, 1988; Park *et al.*, 1994). The other type, the small K^+ channel, had a small conductance of 43±7 pS and was not sensitive to voltage. Marty *et al.* (1984) reported a similar unit conductance of 25 pS channel in lacrimal gland, but it does not correspond with our small conductance K^+ channel. Our channel was K^+ selective based on reversal potential of -56±5 mV, but 25 pS channel of lacrimal gland was nonselective cation channels which have 0 mV reversal potential.

Effects of ACh on the K^+ channel activity

Addition of 10^{-5} M Ach in the bath solution activated both K^+ channels by increasing Po of the channels from 0.18 and 0.002 in the control solution, to a maximum value of 0.85 and 0.31 in the presence of 10^{-5} M Ach for the large and small conductance K^+ channels respectively. The current amplitude of Fig. 3B was slightly increased over Fig. 3A, which suggests that mucous cells might be hyperpolarized during muscarinic stimulation. Such hyperpolarization of acinar cells by Ach stimulation also reported in mouse salivary gland cells in the case of the resting potential was less negative than -50 mV (Petersen, 1973). Activation of K^+ channels by Ach is probably due to an increase in intracellular Ca^{2+}, since an increase in intracellular Ca^{2+} is induced by muscarinic agonists in sublingual mucous cells (Melvin *et al.*, 1991). Our data therefore suggest that both types of K^+ channels of sublingual mucous cells may be Ca^{2+}-activated K^+ channels.

Activation of K^+ channels by hypotonic stress

In many types of cells, volume regulation in response to hypotonic stress occurs by Cl^- and K^+ loss through channels which are activated as a result of cell swelling (Hoffman and Simonsen, 1989). The simultaneous opening of Cl^- and K^+ channels presumably facilitates KCl loss from the cytosol, leading to RVD regulation. Exposure of sublingual mucous cells to a hypotonic solution (60% of control) was found to activate both types of K^+ channels in cell-attached patches on the basolateral membrane (see Fig. 4). The activation of these channels by hypotonic stress has not been previously observed in mucous cells from sublingual glands. However, there are a number of reports of similar phenomena for "maxi" K^+ channels in other epithelia, e.g., the choroid plexus (Christensen, 1987), kidney proximal tubule (Dube *et al.*, 1990), kidney thick ascending limb cells (Taniguchi and Guggino, 1989) and lacrimal gland acinar cells (Park *et al.*, 1994). Volume regulation also shown that "maxi" K^+ channels contribute to RVD in these cells. The activation of the small conductance by hypotonic solution suggests that this channel may also contribute to RVD in sublingual mucous cells.

In summary, there are two types of K^+ channels in sublingual mucous cells; large and small conductance K^+ channels. Both channels are activated by Ach, probably by increasing intracellular Ca^{2+} in sublingual gland mucous cells. These channels are also activated during RVD induced by hypotonic solutions.

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