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pH-modulated Ion Channels in Acinar Cells

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The mechanism for the H^+ interaction with Ca^{2+}-activated channels in acinar cells is not clear. In the present work, the effects of intracellular pH(pH_i) on Ca^{2+}-activated Cl^- and K^+ currents in rat submaxillary and lacrimal acinar cells were examined, respectively. Cl^- currents were recorded by conventional whole cell patch-clamp methods using micropipettes filled with NMDG-Cl (K^+-free solution) in NMDG-Cl(Na^+-free) bath solutions. Intracellular pH was varied by using electrode solutions with pH at 6.8, 7.3 and 7.8. At pH 6.8, there was no obvious time-dependent activation or inactivation. At pH 7.3, there also was no time-dependent activation or inactivation, except at the large depolarizing potential at 80 and 100 mV. However, at pH 7.8, large inward currents inactivated with time at hyperpolarizing potentials and the time-dependent outward currents at depolarizing potentials were observed. At this pH, I-V relationship displayed outward rectification, which is a result of the time-dependent activation of currents at depolarizing potentials. Increasing pH from 6.8 to 7.8 was also found to increase the open probability(Po) of K^+ channel in inside-out mode. Our results suggest that an increase in pH_i help sustain both Cl^- and K^+ channel activities during prolonged secretory activities.

Key word : Ca^{2+}-activated channels, acinar cells, intracellular pH, patch-clamp

Introduction

Salivary or lacrimal gland fluid secretion is normally at low basal rates. When mammalian acinar cells are activated by the cholinergic or α-adrenergic agonist, secretion can be rapidly enhanced, which is associated with a rapid rise in the intracellular free calcium concentration($[Ca^{2+}]_i$) (Dissing *et al.*, 1990; Merritt and Rink, 1987; Nauntofte and Dissing, 1987). Consequently, $[Ca^{2+}]_i$ activate Ca^{2+}-dependent K^+ channels and Cl^- channels at the basolateral and luminal membrane, respectively (Maruyama *et al.*, 1983; Petersen, 1992; Nauntofte, 1992). Intracellular pH also changes in secretagogue-induced salivary fluid secretion (Melvin *et al.*, 1988; Lau *et al.*, 1989; Pirani *et al.*, 1987; Steward *et al.*, 1989) and several ion transport pathways, including Cl^--HCO_3^- and Na^+-H^+ exchangers, are involved in pH_i regulation (Sardet *et al.*, 1989; Kopito, 1990). Activation of the Na^+-H^+ exchangers buffers the initial intracellular acidification by extruding protons in exchanges for extracellular Na^+ and shifts the pH to a more alkaline pH above the resting pH_i. Paired exchangers therefore participate in maintaining the driving force for anion-dependent fluid secretion as well as regulating pH_i.

It's known that changes in pH_i modulate the activity of ion channels in many cell types (Cook *et al.*, 1984; Christensen and Zuthen, 1987; Klaerke *et al.*, 1993; Park and Brown, 1995). However, it's unclear whether these changes in pH_i have a functional role or not, especially in salivary and lacrimal secretion. The stimulus-induced loss of Cl^- is dependent on Ca^{2+}. The loss of Cl^- can also be induced by the addition of Ca^{2+} ionophores to acini in a Ca^{2+}-containing medium (Martinez and Cassity, 1986; Nauntofte and Dissing, 1987; Soltoff *et al.*, 1989). This shows indirect evidence of existence of Ca^{2+}-activated Cl^- channels in salivary gland. In the present work, we identified Cl^- currents by using the whole cell patch clamp technique: the properties of Ca^{2+}-activated Cl^- currents in salivary gland and K^+ currents in lacrimal acinar cells were examined, with particular emphasis on the effects of pH_i.

Materials and Methods

Cell preparation

Adult Sprague-Dawley rats(about 200 g in body weight) were sacrificed by cervical dislocation and the submaxillary or lacrimal glands were removed.

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The glands were finely minced and incubated in a Ca^{2+}-free saline solution containing(mM): 140 NaCl, 5 KCl, 1 $MgCl_2$, 5 glucose, 5 N-2-hydroxyethylpiperazine-N'-2-ethansulfonic acid (HEPES)-NaOH (pH 7.2), supplemented with bovine serum albumin (5 mg/ml, Sigma), for 10 min at 37°C. The tissue was then incubated in the above solution supplemented with trypsin (0.4 mg/ml, type XI. Sigma) for 10 min. After washing with Ca^{2+}-free saline, the tissue was then incubated in the solution supplemented with collagenase (0.15 mg/ml, type-II. Sigma) and trypsin inhibitor (2 mg/ml, type-II. Sigma) for 15 min. The tissue could then be dissociated mechanically by repeated pipetting through a glass pipette tip. The cell suspension obtained was filtered through a nylon mesh, and filtrates were centrifuged to collect dispersed acinar cells. The resulting single acinar cells and small clusters were resuspended in M-199 media (Sigma) and stored in CO_2 incubator at 37°C. Throughout the dissociation procedure, the solutions were gassed with 100% O_2.

Patch-clamp recording

Isolated cells were placed in a small chamber on the stage of an Olympus IMT-2 inverted microscope on isolation-free table and studied by standard gigaseal whole-cell to record Cl^- chnnel activity. Inside-out patch clamp recording methods were used to record K^+ channel activity. The electrodes used were manufactured from hamatocrit tubing (Chase, U.S.A) and had a tip resistance of 2~5 M Ω. To make a whole cell, the cell membrane under the patch pipette was ruptured by gentle suction. The currents were recorded at least 2~3 min after establishing the whole cell configuration to allow the complete equilibration of the electrode solution with the cytoplasm of the cell. The reference electrode was Ag/AgCl electrode connected to bath solution.

Solutions

In the recording of Cl^- currents, the bath solution contained(in mM) 145 N-methyl-D-glucamine (NMDG)-Cl, 1 $MgCl_2$, 1 $CaCl_2$ and 5 HEPES which were adjusted to pH 7.3 with NMDG base. The electrode solution contained 120 NMDG-Cl, 1 $MgCl_2$ and 5 HEPES which were adjusted to pH 6.8 or 7.3 with HCl and pH 7.8 with NMDG base, respectively. Ca^{2+} activity was buffered at 100 nM with ethylene glycol-bis (β-aminoethyl ether)-N, N, N', N'-tetraacetic acid (EGTA). K^+ channel activity was measured in inside-out patches. HEPES which wereusted to pH 7.3 with KOH. The bath solution contained 120 KCl, 1 $MgCl_2$, and 5 HEPES which were adjusted to pH 6.8 or 7.8 with KOH. The activity of Ca^{2+} in the bath solution was also maintained at 100 nM: buffered using 10 mM EGTA plus 1.44 mM or 8.92 mM $CaCl_2$ at pH 6.8 and 7.8 respectively. The bath solution was perfused at a rate of about 2.5 ml/min and all experiments were performed at room temperature.

Anaysis of Cl^- and K^+ currents

Voltage pulse protocols were generated by computer using pCLAMP 6.0 software(Axon Instruments, U.S.A.). An axopatch 1-C patch clamp amplifier (Axon Instruments, Foster city, CA) was used to record whole cell currents of Cl^- filtered at 1 KHz and digitized at 10~20 KHz with a digital data recorder (PCM, Biomedical). Then data for current profiles were stored and analysed using the above software. Single channel activity in inside-out patches for K^+ channels were also recorded. The patches were clamped at a 40 or 60 mV membrane potential to get optimal Po. All the resulting currents were stored on VCR (44 KHz) via PCM module (Medical system Co, U.S.A.), then later analyzed by the pCLAMP software.

Results

To examine the effect of pH_i on Ca^{2+}-activated Cl^- currents, we prepared electrode solution adjusted to pH at 6.8, 7.3, 7.8 respectively by suppressing the K^+ currents using the NMDG-Cl solution in both bath and pipette. With whole-cell variant of the patch clamp technique, typical Ca^{2+}-activated Cl^- currents could be recorded in salivary acinar cells. Fig. 1 shows the profiles of the Cl^- currents evoked using a stepped voltage-pulse protocol (250 msec voltage steps at 20 mV increments over the range of -100 to 100 mV from a holding potential of 0 mV) when the pH of pipette solution was 6.8, 7.3 and 7.8, respectively. At pH 6.8 (Fig. 1A), very small sustained currents were observed. They did not show voltage-dependent activation or inactivation and there was neither activation nor inactivation with time. At pH 7.3 (Fig. 1B), there also was neither voltage-dependent nor time-dependent activation/or inactivation during hyperpolarizing and small depolarizing command potentials except at the large depolarizing potentials of 80 mV and 100 mV. Only at the large depolarizing potential of 80 and 100 mV,

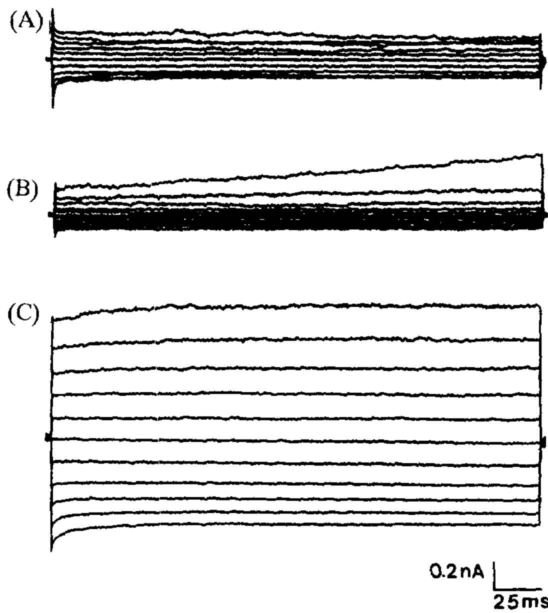

Fig. 1. Increasing intracellular pH(pH_i) increases whole cell Cl^- currents. Typical currents from three different cells using electrode solutions containing 100 nM Ca^{2+}, with pH buffered at 6.8(A), 7.3(B), and 7.8(C). Voltage steps (250-ms duration) were applied over the range of -100 to 100 mV at 20-mV increments from a holding potential of 0 mV. Recording was done by using NMDG-Cl solution in both bath and pipette.

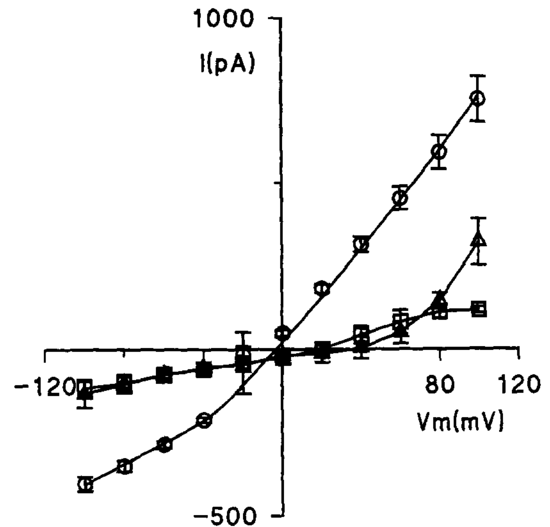

Fig. 2. Current-voltage relationships of Cl^- channel activated by an increase in pH_i. Data (mean±SE) are maximum current(I) recorded during each 250-ms voltage step from 5 experiments at pH 6.8(□) and 7.3(△) or from 6 experiments at pH 7.8(○). The lines through the data were fit by eye. Vm, membrane potential.

were small time-dependent outward currents measured. In contrast, much larger current were observed at the pH of 7.8 (Fig. 1C). Initial large inward currents were inactivated rapidly with time at hyperpolarizing command potentials. At depolarizing command potentials, large outward currents activated slowly.

In Fig. 2, the maximum currents measured at the end of each voltage step were plotted against the membrane potential. The currents at pH 7.8 (n=6) were much larger than at pH 7.3 (n=5) at all membrane potentials and showed outward rectification in the current-voltage relationship. However, the currents at pH 7.3 were not quite different from those at pH 6.8, except at the command potentials of 100 mV. At pH 6.8 (n=5), the current-voltage relationship was almost linear and showed very small inward and outward currents. The reversal currents of pH 7.8 were observed at about -3 mV (Fig. 2), which is a value close to E_{Cl}.

Fig. 3 A shows increased K^+ channel activity induced by increasing the pH of the bath solution from 6.8 to 7.8 in continuous inside-out recording at 60 mV membrane potential. Increasing pH was found to increase open probability (Po) of K^+ channels

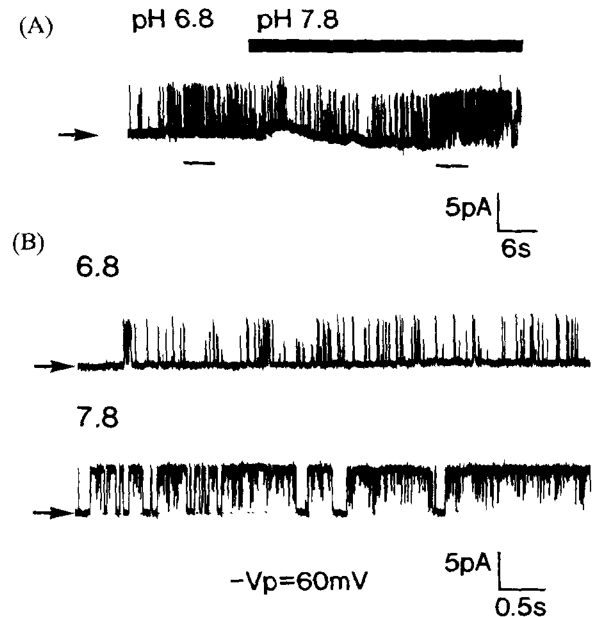

Fig. 3. K^+ channel activity increased by increasing pH of bath solution from 6.8 to 7.8 in inside-out patch clamp recording. Pipette potential was maintained at -60 mV. Increasing pH was found to increase Po of K^+ channel from 0.11 at pH 6.8 to 0.80 at pH 7.8. B. Expanded time scale at pH 6.8 and 7.8 respectively. Each trace was indicated by small horizontal bar in Fig. 3A.

from 0.11 at pH 6.8 to 0.80 at pH 7.8. Such increase of Po was also found in 3 other cells in 40 mV or 60 mV potentials (data not shown). Fig. 3B shows that expanded time scale at pH 6.8 and 7.8

respectively. Each trace, which is at the peak channel activity, is indicated by horizontal bar in Fig. 3A. Contrary to pH effect on the Po, the conductances of the channels were not effected by the change in pH. Average conductance at pH 6.8 was 156±9 pS (n=4) and that of pH 7.8 was 153±4, which showed no significant difference. Our data indicate that K^+ channel activation by the change in pH_i was not the result from the increasing of conductance but from the increasing of Po of the channel.

Discussion

It is generally known that intracellular pH changes during secretion in salivary acinar (Melvin *et al.*, 1988; Pirani *et al.*, 1987; Steward *et al.*, 1989) and several ion transport pathways are involved in pH_i regulation, including Cl^--HCO_3^- and Na^+-H^+ exchangers (Sardet *et al.*, 1989; Kopito, 1990). In salivary acinar cells similar to other exocrine acinar cells (Saito, 1988), biphasic changes in pH_i have also been observed during stimulated fluid secretion. Stimulated Cl^- efflux is accompanied by an initial transient acidification due to HCO_3^- efflux, possibly mediated by Ca^{2+}-dependent Cl^- channels (Melvin *et al.*, 1988; Lau *et al.*, 1989). And then, pH_i shifts to ~0.1 pH unit above the resting pH_i by the activation of the Na^+-H^+ exchangers. This shift to alkaline pH is sustained once intracellular Ca^{2+} has lowered to almost the initial value after the initial increase of secretory conditions such as activation by the cholinergic or α-adrenergic agonist (Bird, 1992; Marty and Tan, 1989). However, functional role of pH_i changes in secretion is unclear. Therefore, we examined the effects of pH_i on the activity of Ca^{2+}-activated Cl^- currents in submaxillary gland and Ca^{2+}-activated K^+ currents in lacrimal gland, since both channels are mainly involved in fluid and electrolyte secretion. We were able to record typical Ca^{2+}-activated Cl^- currents which were activated by an increase of pH_i, which was similar to those of other exocrine glands evoked by acetylcholine (Evans and Marty, 1986).

Intracellular pH is critical for many normal cell functions such as fluid and electrolyte absorption and secretion, enzyme activity, and cell growth and proliferation (Aronson, 1985; Boron, 1986; Moolenaar, 1986). In this study, we could find that pH_i can influence exocrine secretion mechanism by modulation of Ca^{2+}-activated Cl^- and K^+ channels. Ca^{2+}-activated K^+ channel was modulated by pH_i, as known by the previous studies (Cook, 1984). Acidification of intracellular fluid in choroid plexus epithelial cells reduced the channel opening probability of Ca^{2+}-activated K^+ channels mainly by increasing the channel closed time (Christensen and Zeuthen, 1987; Brown *et al.*, 1988). It was suggested that H^+ can compete with Ca^{2+} in binding to the same site of Ca^{2+}-activated K^+ channels, thereby preventing channel opening. However, in a recent study (Laurido *et al.*, 1990), Ca^{2+} activations were not affected by changes in pH, and it was suggested that proton exert their effect allosterically and not by competing with Ca^{2+} for the ion binding site.

The profile of Ca^{2+}-activated Cl^- currents was similar to that of lacrimal acinar cells in maximum current amplitude and time course, which were also activated by an increase of pH_i (Park and Brown, 1995). If Ca^{2+} binds to the similar site on Ca^{2+}-activated Cl^- channels as Ca^{2+}-activated K^+ channels, the modulation of Ca^{2+}-activated Cl^- channels by pH_i can be explained in the similar mechanism. Without verification by single channel studies, it is difficult to confirm the effect of pH_i on Cl^- channels. However, single channel studies on Cl^- channels are not easy to perform because Cl^- channels have low conductance (1-2 pS; Marty and Tan, 1984) and are mainly located on the hardly accessible apical membrane of acinar cell (Peterson, 1992).

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