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Characterization of Intermediate Conductance K^+ Channels in Submandibular Gland Acinar Cells

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There are some evidences that K^+ efflux evoked by muscarinic stimulation is not mainly mediated by large conductance K^+ (BK) channels in salivary gland. In this experiment, we therefore characterised non BK channels in rat submandibular gland acinar cells and examined the possibility of agonist effect on this channel using a patch clamp technique. Two types of K^+ channels were observed in these cells. BK channels were observed in 3 cells from total 6 cells and its average conductance was 152±7 pS (n=3). The conductance of the another types of K^+ channel was estimated as 71±7 pS (n=6). On the basis of the conductance of this channel, we defined this channel as intermediate conductance K^+ (IK) channels, which were observed from all 6 cells we studied. When we increased Ca^{2+} concentration of the bath solution in inside-out mode, the IK channel activity was greatly increased, suggesting this channel is Ca^{2+} sensitive. We next examined the effect of carbachol (CCh) and isoproterenol on the activity of the IK channels. 10^{-5} M isoproterenol significantly increased the open probability (Po) from 0.08±0.02 to 0.21±0.03 (n=4, $P<0.05$). Application of 10^{-5} M CCh also increased Po from 0.048±0.03 to 0.55±0.33 (n=5, $P<0.05$) at the maximum channel activity. The degree of BK channel activation induced by the same concentration of CCh was lower than that of IK channels; Po value was 0.011±0.003 and 0.027±0.005 in control and during CCh stimulation (n=3), respectively. The result suggests that IK channels exist in salivary acinar cells and its channel activity is regulated by muscaricinic and β-adrenergic agonist. We conclude that IK channels also play a putative role in secretion as well as the BK channels in rat submandibular gland acinar cells.

Key Words: Submandibular, Patch clamp, IK channels, Carbachol, Isoproterenol

INTRODUCTION

Ca^+- activated K^+ channels are almost ubiquitously distributed in mammalian cells and it has diverse roles among the different cell types. In mammalian exocrine cells, Ca^+- activated K^+ channels has a critical role in secretion (Maruyama et al, 1987; Petersen, 1992). Activation of K^+ channels in basolateral membrane cause hyperpolarisation of the cell and therefore increase the electrical driving force for Cl^- extrusion. An increased extracellular K^+ also provides electromotive force for Na^+- K^+-2 Cl^- cotransport, by which Cl^- could enter and accumulated in the cell. All K^+ channel blockers, i.e., barium, TEA and decamethonium caused marked reduction in secretory rate (Young et al, 1987; Wright & Blair-West, 1990).

The types of K^+ channels and its role involve in secretion is, however, different from gland to gland. For example, K^+ channels in rat and mouse pancreatic acini are not Ca^{2+} dependent and have a 48 pS conductance (Schmid & Schulz, 1995). Neither acetylcholine nor secretin changed the activity of the channel. But in the other types of exocrine glands including rat parotid (Nauntofte & Dissing, 1988; Soltoff et al, 1990) and mouse submandibular acini (Gallacher & Morris, 1986), there are large conductance, Ca^+-activated K^+ (BK) channel, which was activated by secretagogues, acetylcholine. BK channels were also involved in volume regulation in salivary gland (Park et al, 1994). However, there is some evidence that K^+ efflux evoked by muscarinic stimulation is not mainly mediated by BK channels in salivary acinar cells (Ishikawa et al, 1994; Ishikawa & Murakami, 1995; Hyashi et al, 1995). Therefore, we examined the existence of the non BK channels and the possibility of agonist effect on this channel in rat submandibular gland acinar cells. We found that the intermediate conductance K^+ (IK) channels are exist in these cells and it's under the muscarinic and adrenergic regulation, suggesting that IK channels play a putative role in salivary secretion.

METHODS

Cell preparation

The submandibular gland tissue was isolated from about 200 g Sprague-Dawley rat after cervical dislocation. The

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ABBREVIATIONS: BK, large conductance K^+ channel; IK, intermediate conductance K^- channel; CCh, carbachol; Po, open probability; SK, small conductance K^+ channel.

isolated submandibular gland tissue was minced in a Ca^{2+}-free incubation solution (CFS) on ice. The CFS contained (mM): 130 NaCl, 4.5 KCl, 1 $NaH_2PO_4.2H_2O$, 1 $MgCl_2$, 10 D-glucose, 10 HEPES, 10 HEPES-Na at pH 7.4. For the patch clamp experiments, the minced tissue was incubated for 20 min at 37°C with CFS containing 0.4 $mg.ml^{-1}$ trypsin (Sigma, Type II-S). This was followed by a second incubation for 60 min in CFS, which contained 2 $mg.ml^{-1}$ of the trypsin inhibitor (Sigma), 100 $Units.ml^{-1}$ of collagenase (Worthington, Lakewood, UK) and 1% BSA. After the enzyme treatment, all cells were resuspended in a bath solution containing (mM): 140 NaCl, 5 KCl, 1 $MgCl_2$, 1 $CaCl_2$, 10 glucose, 5 HEPES-NaOH, at pH 7.4, and stored on ice until required.

Patch clamp recording

Channel activity was measured using single channel recording for K^+ currents in a cell-attached or inside-out mode. Electrodes were manufactured from haematocrit capillaries (Oxford Labware, St Louis, MO, USA). They had a tip resistance of 3~5 MΩ. Cells in the experimental chamber (volume=400 μl), were superfused at a rate of 2 $ml.min^{-1}$, with a bath solution. Carbachol and isoproterenol were dissolved in bath solution, containing: 140 NaCl, 5 KCl, 1 $CaCl_2$, 1 $MgCl_2$, 10 glucose, 5 HEPES, at pH 7.4. The pipette solution for recording K^+ currents contained: 140 KCl, 1 $MgCl_2$, 0.5 EGTA, 5 HEPES. pH was adjusted 7.3 with HCl. 100 nM or 10 nM Ca^{2+} of bath solutions were made by adjusting Ca^{2+}-EGTA buffer concentration.

Single channel currents were monitored using an Axopatch 200 amplifier (Axon Instruments, CA, USA). Command potentials were generated by Axopatch 200 amplifier for single channel recording. The resultant single channel currents were recorded on DAT tape using a modified DAT recorder (DTR1204, Bio-logic, France). Open probabilities (Po) were determined for 10 s periods of data using the pClamp program (Version 6.0). The Po for a patch containing two channels was estimated, assuming that the channels opened independently of each other, using Equation 1. The total number of ion channels were confirmed by exposing bath solution containing 1 mM Ca^{2+} in inside-out mode at the end of experiments.

$$Po = \frac{Time\ at\ level\ 1 + Time\ at\ level\ 2}{Total\ time \times 2} \quad (Equation\ 1)$$

The bath solution was connected to ground using a Ag-AgCl pellet electrode. All experiments were performed at room temperature (18~22°C).

RESULTS

Characteristics of the intermediate conductance K^+ (IK) channel in rat submandibular gland acinar cells

Fig. 1 shows a typical single channel recordings from a cell-attached patch in rat submandibular gland acinar cells. The channel showed a large conductance K^+ (BK) channels.

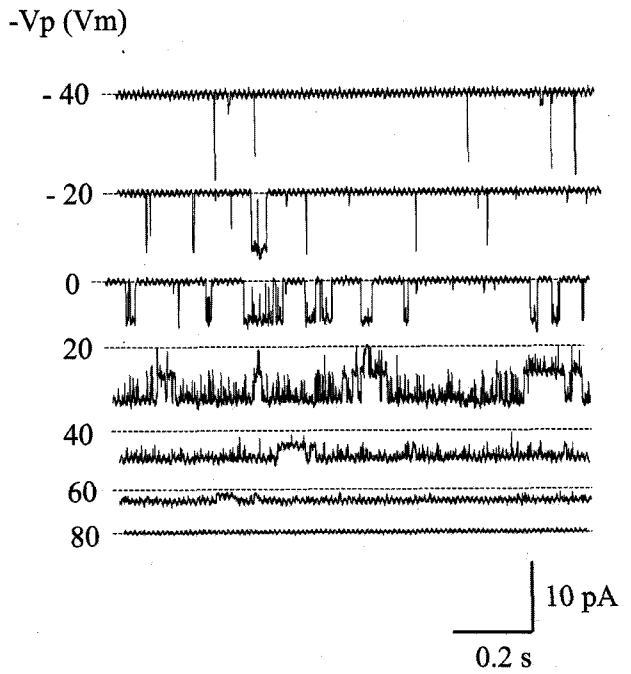

Fig. 1. Single channel recordings for large conductance K^+ (BK) channels in a cell-attached patch from rat submandibular gland acinar cells. Dotted lines indicate a closed state of the channel. Number on the left hand side indicates a reverse of pipette potential (−Vp).

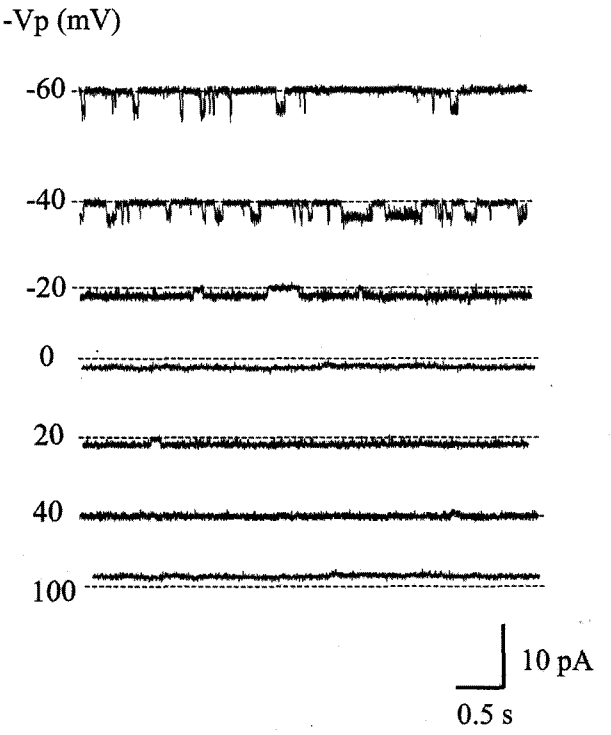

Fig. 2. Single channel recordings for intermediate conductance K^+ (IK) channels in a cell-attached patch from rat submandibular gland acinar cells.

Opening of non BK channels with small conductances also could be seen between BK channel openings. Fig. 2 shows a typical single channel recordings of an intermediate conductance K^+ (IK) channels at the range of $-$Vp between -60 mV to 100 mV. The open probability (Po) of the channel was voltage dependent, i.e., Po values were increased by depolarisation of the patch membrane. Fig. 3 shows average current-voltage (I~V) relationships of the two types of K^+ channels shown in Fig. 1 and Fig. 2. The

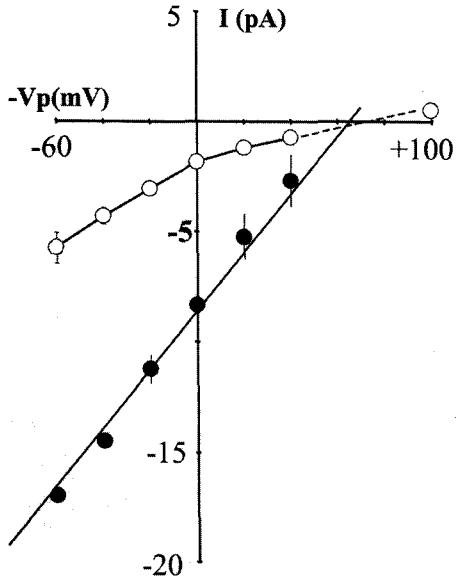

Fig. 3. Current-voltage (I~V) relationships of the two types of K^+ channels in these cells. The average conductances were 152 ± 7 pS (BK channels, dark circles) and 71 ± 7 pS (IK channels, white circles). The I~V relationship of IK channel was slightly inward rectifying.

large conductance, Ca^+- activated K^+ channels (BK) channels were observed in 3 cells from total 6 cells we studied. The average conductance of the BK channels were 152 ± 7 pS (n=3,dark circles). The I-V relationship of another types of K^+ channel (as shown in Fig. 1) was slightly inward rectifying and its conductance was estimated as 71 ± 7 pS at the range of $-$Vp between -60 mV and 0 mV (n=6, white circles). On the basis of the conductance of this channel, we defined this channel as intermediate conductance K^+ (IK) channels rather than SK channels. IK channels were observed from all 6 cells we studied. The average reversal potentials of the BK channels was -64 ± 7 mV (n=3). While, that of IK channels were estimated as -70 ± 8 mV (n=6) by extrapolation, which is not significantly different ($P>0.1$) to those of BK channels.

The channel activity of IK channel depends on intracellular Ca^{2+} concentration

We next compared the single channel activity of IK channel using two different Ca^{2+} concentration in inside-out mode. Fig. 4A and B shows single channel recordings in 5 nM and 100 nM Ca^{2+} in KCl rich bath solutions. The Po value was much higher in 100 nM Ca^{2+} than 5 nM Ca^{2+} bath under the same holding potentials, suggesting this channel activity is dependent on the intracellular Ca^{2+} concentration.

Effects of carbachol (CCh) and isoproterenol on the IK channel activity

It has been well known that salivary secretion is under the autonomic regulation. Therefore, we examined two kinds of agonists, CCh as muscarinic cholinergic agonist and isoproterenol as β-adrenergic agonist, to study a physiological role of this channel. The IK channel was greatly activated by CCh and isoproterenol. Fig. 5 shows increased channel activity by application of isoproterenol

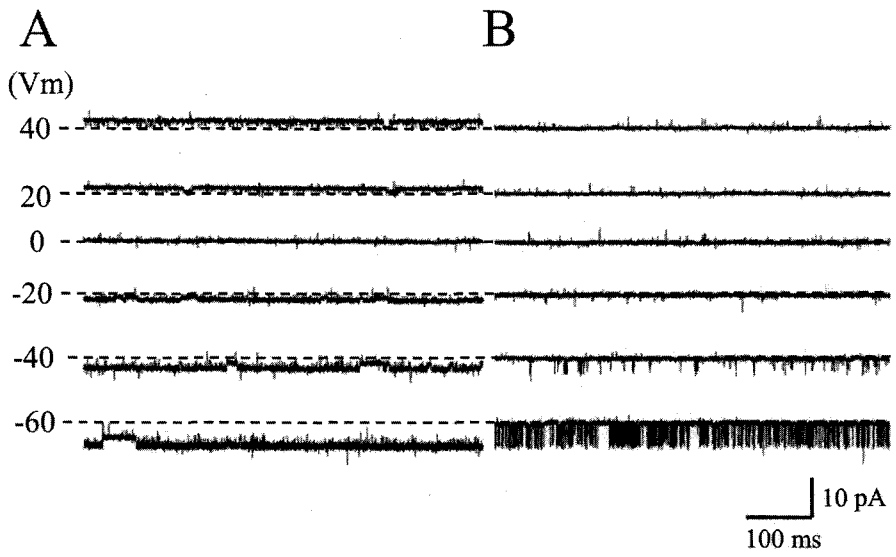

Fig. 4. Ca^{2+} dependency of the IK channel. Single channel recordings in 100 nM (A) and 5 nM (B) Ca^{2+} in KCl rich bath solutions. The Po value was much higher in 100 nM Ca^{2+} than 5 nM Ca^{2+} bath under the same holding potentials.

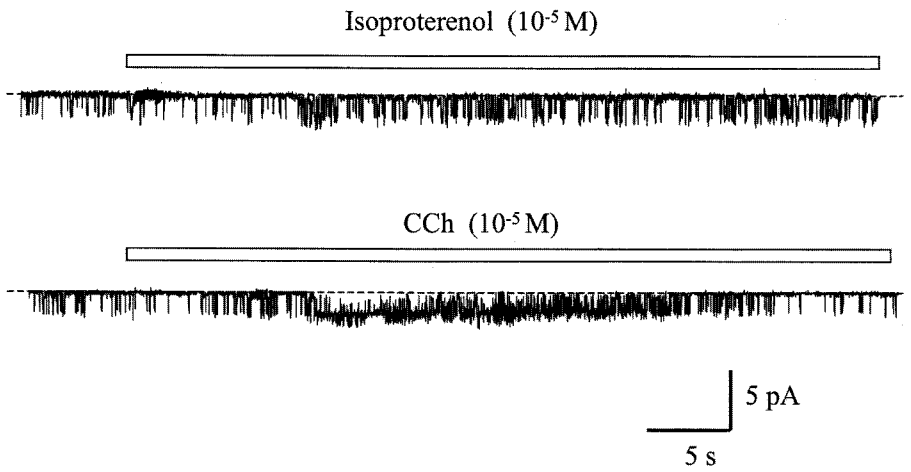

Fig. 5. Effects of isoproterenol and carbachol (CCh) and on the IK channel activity. (A) 10^{-5} M isoproterenol significantly increased Po from 0.08 ± 0.02 to 0.21 ± 0.03 (n=4, $P<0.05$) (B) Application of 10^{-5} M CCh increased from 0.048 ± 0.03 to 0.55 ± 0.33 (n=5, $P<0.05$).

(upper panel) and CCh (lower panel) at $-Vp=-60$ mV. 10^{-5} M isoproterenol significantly increased Po from 0.08 ± 0.02 to 0.21 ± 0.03 (n= 4, $P<0.05$). While, application of 10^{-5} M CCh increased from 0.048 ± 0.03 to 0.55 ± 0.33 (n= 5, $P<0.05$) at the maximum channel activity. BK channel was also activated by same concentration of CCh (data not shown), but the degree of activation was lower than IK channels; Po value was 0.011 ± 0.003 and 0.027 ± 0.005 in control and during CCh stimulation (n=3).

DISCUSSION

Ca^+-activated K^+ channels can be divided into 3 classes on the basis of their electro physiological characteristics: voltage-dependent, large-conductance channels (BK), voltage-independent, small-conductance channels (SK) and inwardly rectifying, intermediate-conductance channels (IK). BK and SK channels are widely distributed in excitable cells as well as in some none excitable cells. In our experiments, two types of Ca^+-activated K^+ channels were observed; BK and IK channels. The BK channels in our experiment has shown similar characteristics of other BK channels reported in mammalian salivary glands. They showed large conductance of more than 150 pS and the channel was activated by intracellular Ca^+ and membrane depolarisation (Maruyama et al, 1987; Gallacher & Morris, 1986; Cook et al, 1990).

There seems to be difference in regulation of secretion in species and the type of gland. In rat parotid acinar cell, most of K^+ conductance in the unstimulated and stimulated state was mediated by BK channels (Nountofte & Dissing, 1988; Soltoff et al, 1990). Recently, the role of SK channel in secretion was revaluated. The channel type responsible for carrying the K^+ current induced by muscarinic stimulation was SK channels rather than BK channels in mouse mandibular gland (Ishikawa et al, 1994; Ishikawa & Murakami, 1995). Hyashi et al. (1996a) reported that Ach-evoked whole cell K^+ current is carried by a small conductance K^+ channel rather than BK channels in mouse mandibular secretory cells. They showed that Ach evoked K^+ current does not have the blocker sensitivity pattern that would be expected if it were being carried by the BK channel (Hyashi et al, 1996b).

IK channels are apparently absent in excitable tissues but are present, endothelial cells (Olesen & Bundgaard, 1993; Sauve et al, 1988), and cell lines of epithelial origin (Christensen & Hoffmann, 1992; Devor & Frizzell, 1996). IK channels are now cloned in human and RNA dot blot analysis showed a wide spread tissue expression, with the highest levels in salivary gland, placenta, trachea and lung (Jensen et al, 1998). Physiologically, IK channels are strongly activated by release of intracellularly stored Ca^+ induced by agonists such as ATP, bradykinin, and histamine. The activation of IK channel is followed by long-lasting or oscillatory hyperpolarisations of the cell membrane, which closely reflect the intracellular Ca^{2+} activity. Interestingly, the IK channel, which has not been rigorously studied in salivary gland, showed a dominant role both in unstimulated and stimulated state in our experiment. The BK channel was observed only from an half of cells we studied. In contrast, IK channels were found in all of the cells we studied. The IK channels we identified, appears to play a functional role in secretion, since two kinds of agonists, cholinergic and β-adrenergic agonist greatly increased channel activity. Agonists-induced IK channel activation in our experiment seems to be mediated by Ca^{2+} which corresponds to the previous result (Jensen et al, 1998), since the IK channels in our experiment was also Ca^{2+} sensitive. Furthermore, the degree of activation of IK channels was even more higher than that of BK channels with the same concentration of CCh. All our results strongly suggest that IK channels coexist with BK and SK channels in salivary gland and plays a putative role in secretion as well as other type of Ca^+- activated K^+ channels, BK and SK channels.

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