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Electrophysiological Study of Potassium Channel in Parotid Gland Acinar Cell

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When the salivary gland acinar cells are stimulated by secretagoges, its intracellular Ca^{2+} ions are increased and potassium channels are opened. The purpose of this experiment is to study the characteristics of potassium channel and its channel activity induced by Phenylephrine using dissociated acinar cells from the parotid gland. The parotid gland acinar cells were collected from Sprague-Dawley rat by trypsin and collagenase treatment. Isolated acinar cells were placed in 100% oxygenated Tyrode solution. To record channel activities, micropipette filled with HEPES-buffered high potassium solution with 1-5 MΩ resistance were used. Membrane currents were recorded by cell-attached patch clamp method. The channel activities were recorded and analyzed on pClamp soft ware. Two distinct types of potassium channels in parotid gland acinar cells were observed. The one type which had large conductance of about 170pS was voltage dependent. The other type which had small conductance was voltage independent. The potassium current was activated by phenylephrine ($1x10^{-4}$ M) which is an α-adrenergic agonist at 60 mV holding potential (Vp) by increasing open probability.

Key words : *parotid gland, intracellular Ca^{2+}, potassium channel, patch clamp, voltage dependent, Phenylephrine*

Introduction

For many years it was known that salivary glands lose K^+ to the surrounding medium (Burgen, 1956). Muscarinic and α-adrenergic agonists were observed to stimulate the efflux of K^+ or $^{86}Rb^+$ (Martinez *et al.*, 1976; Putney, 1976) and chloride (Nauntofte and Poulsen, 1986; Melvin *et al.*, 1987) from parotid. Using patch-clamp techniques to measure single channel K^+ currents in salivary acinar cells (Maruyama *et al.* 1983a), they demonstrated that the probability of the channel opening was increased by elevating Ca^{2+} at the intracellular side of the membrane, and electro-physiological studies also have demonstrated agonist-induced activation of K^+ channels and cholride channels in other secretory cells (Maruyama *et al.*, 1983b; Marty *et al.*, 1984, Findlay and Petersen, 1985).

The parotid gland cells had served as a model system to study the stimulation of phosphatidylinositol(PI) turnover by receptor-mediated agonists, which promote the production of diacylglycerol and inositol (1,4,5)-trisphosphate (IP_3) by the hydrolysis of phosphatidylinositol bisphosphate (PIP_2) via activation of phospholipase C (Aub and Putney, 1985; Downes and Stone, 1986). The link between receptor activation and the stimulation of fluid and electrolyte secretion is believed to be IP_3-induced mobilization of intracellular Ca^{2+} stores in the endoplasmic reticulum and Ca^{2+} influx across the plasma membrane (Aub and Putney, 1985; Putney, 1986; Merritt and Rink, 1987a). These dependent protein (Taylor *et al.*, 1986), and the effects of various phospholipase C-linked agonists on elevating $[Ca]_i$ in rat parotid cells has been the focus of attention in a number of laboratories (Takemura, 1985; Nauntofte and Dissing, 1986; Merritt and Rind, 1987a,b; McMillian *et al.*, 1987, 1988).

Schramm and Selinger (Schramm and Selinger, 1974, 1975) have found that muscarinic and α-adrenergic agents increase acinar cell membrane permeability to K^+ as determined by the loss of cellular K^+ into small volumes of incubation medium subsequently assayed by atomic absorption. Their data suggested that the increased membrane permeability to K^+ was sustained and required the presence of extracellular Ca^{2+}.

Phenylephrine, an α-adrenergic agonist, had a slight effect on $[Ca]_i$, raising it by 35%. Phenylephrine acted much like carbachol, but less effective, and the addition of the α-adrenergic antagonist phentolamine restored K_i. (Soltoff *et al.*, 1989).

In the present study, we investigated the nature

of K^+ channel and whether this channel is activated by phenylephrine in parotid gland acinar cell of rats.

Materials and Methods

Cell preparation

Sprague Dawley rats were sacrificed by cervical dislocation under ethyl ether anaesthesia and the parotid glands were excised. 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 HEPES pH 7.2, supplemented with bovine serum albumin (5 mg/ml; Sigma), for 10 min at 37℃. The tissue was incubated in the above solution supplemented with trypsin (0.4 mg/ml; Sigma) for 10 min. The tissue was incubated in the same solution supplemented with collagenase (0.15 mg/ml; Sigma) and trypsin inhibitor (2 mg/ml; Sigma) for 13 min. The tissue could then be dissociated mechanically by repeated pipetting through a plastic pipette tip (2 min). The cell suspension obtained was filtered through a nylon mesh. Throughout the procedure all solutions were gassed with 100% O_2. The resulting single acinar cells were resuspended in Medium 199 (Sigma) and then stored in an incubator at 37℃ with 5% CO_2.

Patch clamp recording

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 chamber on the stage of Olympus IMT-2 inverted microscope on isolation-free table. An Axopatch 1-C (Axon Instruments, U.S.A.) amplifier was employed and signal displayed on digital storage oscilloscope and simultaneously recorded for analysis on videotape. Fabrication of patch pipettes of 1-5 MΩ was pulled from microhaematocrit tubes (Chase, U.S.A.) with two stage micropipette puller (Narishige, PP-83, Japan). All experiments were carried out at 20-24℃.

Solutions

The standard extracellular solution (HEPES-buffered Tyrode solution) contained (mM) NaCl 140, KCl 5, $MgCl_2$ 1, $CaCl_2$ 1, HEPES 5, pH 7.3, 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 1×10^{-4} M Phenylephrine HCl, USP grade (RBI).

Analysis of single channel currents

Single channel current amplitudes were measured by computer using the software package pClamp 5.5 (Axon Instruments). The usual conventions of current flow were used 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.

Results

Single channel currents obtained from basolateral membranes of parotid acinar cells displayed in Fig. 1. By the depolarizing pipette potential, the inward current amplitude was increased. At near -40 mV of pipette potential, single channel currents was small to identify. At the hyperpolarizing pipette potential, we could observe outward currents. The representative current-voltage relationship of channel was showed in Fig. 2, and the relationships were well fit the linear regression of data. The conductance of channels was 170 pS. In some activated acinar cells another

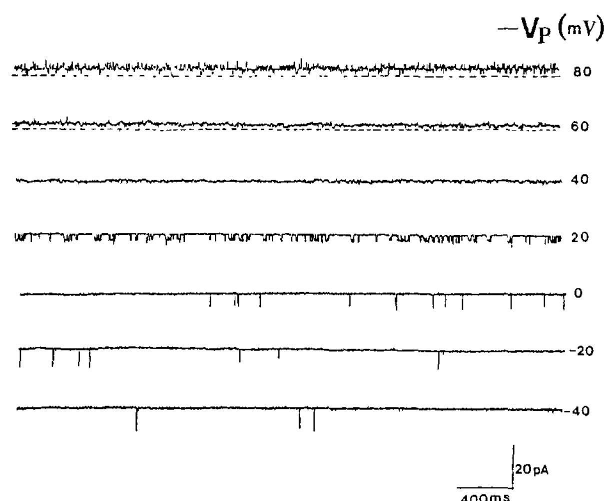

Fig. 1. Single channel recordings at different holding voltages. The potential applied to the electrode (-Vp) does not include the contribution of the membrane potential of the cell.

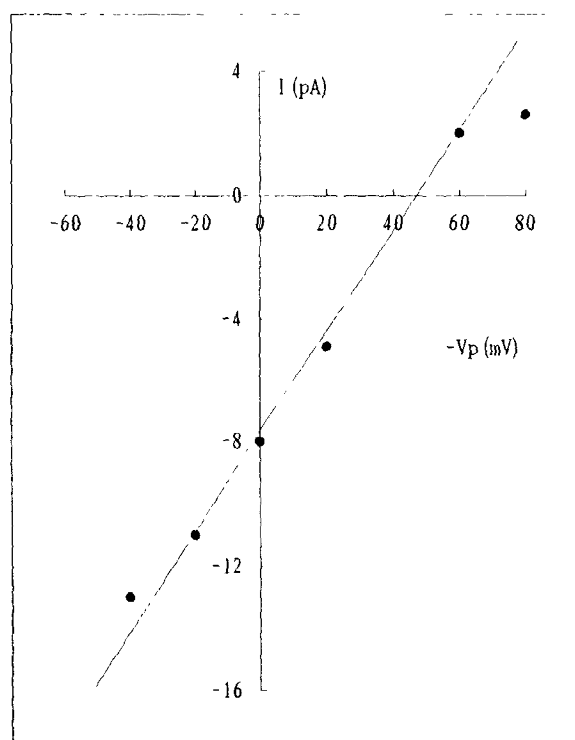

Fig. 2. The I-V plot shows the channels had a unitary conductance of 170 pS. The line through the data was fitted by linear regression.

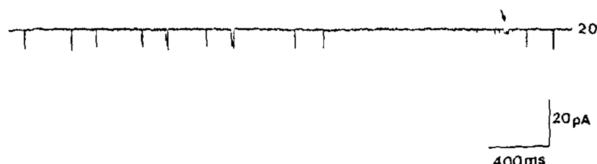

Fig. 3. Single channel current recording obtained from cell attached patch clamp at 20 mV(Vp). Arrow indicate small-K^+ current.

class of channels was observed, and the currents of those channels were not depend on pipette voltage and had small amplitude (Fig. 3). The addition of phenylephrine simultaneously evoked both K^+ channels openning after 40s application. Its open time is longer than control channels (Fig. 4). Open probability of channels in control at holding potential 60 mV(Vp) was 10.8% and Phenylephrine increased their open probability to 92.4% at 60 mV holdig pipette potatial.

Discussion

Numerous earlier studies *in vivo* have suggested that an increase in glandular potassium permeability is associated with initial steps in salivary secretion (Burgen, 1956; Burgen and Seeman, 1958; Schneyer *et al.*, 1972). Principal event of ex-

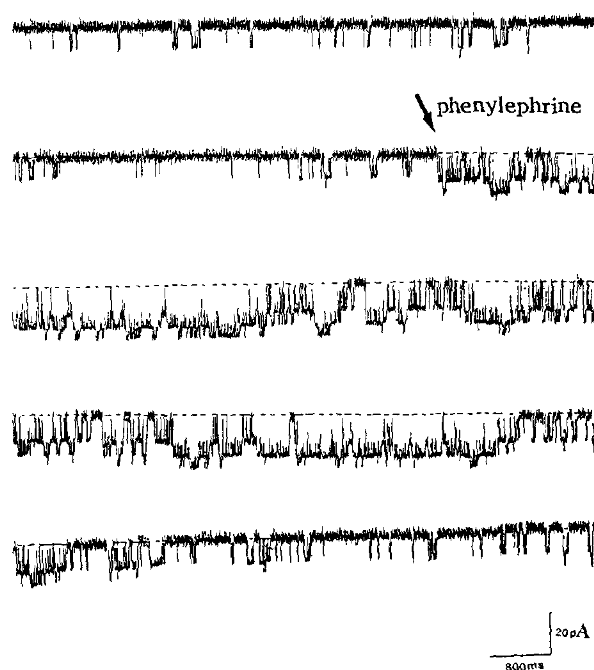

Fig. 4. K^+ channel activation by Phenylephrine (1×10^{-4} M) with 60 mV (Vp). The dotted lines in the trace mean the basal levels.

citation-secretion coupling in salivary cells was outflow of water due to loss of potassium from acinar cells (Petersen and Maruyama, 1984). Current reversal was demonstrated near the -40 mV of pipette potential, which is consistent with the fact that resting membrane potential of acinar cells is near the -40 mV, which eliminated electromotive forces for potassium ion movement, then abolished channel currents.

There are two distinct types of calcium-activated potassium channels. They differ in thier voltage dependency, calcium sensitivity, pharmacology and conductance. The one type, big K^+ (BK) or maxi K^+ channel, has large conductance and is sensitive to membrane potential, calcium ion activity, but the other one, small K^+ channel, has small conductance and is not sensitive to them (Petersen and Gallacher, 1988; Hille, 1992). In sheep parotid gland cells, there are four types of potassium channels, BK channel, 30-pS K^+ channel, Intermediate conductance K^+ (IK) channel, Nonselective cation channel (Wegman, et al. 1992). The 30-pS K^+ channels underlying inwardly rectifying K^+ conductance in sheep are highly active in unstimulated cells and have been implicated in spontaneous secretion (secretion in the absence of neural and hormonal stimulation) (Ishikawa, *et al.* 1993).

Nervous or hormonal stimulation of many ex-

ocrine glands evokes release of cellular K^+. The membrane responses to nerve stimulation in the acinar cells are thought to be mediated by a rise in internal ionized Ca^{2+} concentration (Peterson, 1980; Park *et al.* 1994). Phenylephrine is an α-adrenergic agonist which secretes from sympathetic nerve terminals. It raises intracellular Ca^{2+} like a muscarinic agonist carbacol (Soltoff *et al.* 1989). The receptors of salivary acinar cells can be distinguished two classes. The one class of receptors is coupled to adenyl cyclase, and linked to generation of cyclic AMP. The other class of receptors is coupled to phospholipase C, and linked to genertion of diacylglycerol and inositol trisphosphate (IP_3). The latter class is related in the formation of saliva via IP_3 releases calcium ion from the endoplasmic reticulum, and this evokes opening of the calcium-activated potassium channels. The raising of intracellular Ca^{2+} activated K^+ channels.

It is interesting to note that current activated by phenylephrine, a specific α-agonist, exhibited a time-dependent change in the whole-cell curent profile, first resembling Ca^{2+}-activated currents and late resembling cAMP-activated currents (Leung *et al.* 1992). And the β-effect of phenylephrine has also been observed previously in the epididymal and tracheal epithelium by the shout-circuit current measurement technique (Leung *et al.* 1992, Bainbridge *et al.*, 1989).

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