Effect of K^+ channel openers on K^+ channel in cultured human dermal papilla cells

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Abstract : Minoxidil sulfate and pinacidil, well-known activators of the ATP-sensitive K^+ (K_{ATP}) channel, induce hair growth in clinical studies. The opening of K^+ channels is thought to be an important mechanism in the regulation of hair follicles. In the present study, we used the patch clamp technique to characterize the K^+ channels and tested the effect of K^+ channel openers on K^+ channels in cultured human dermal papilla cells. In dermal papilla cells, the Ca^{2+}-activated K^+ (K_{Ca}) channel with large conductance (179.3±13.1 pS in symmetrical 150 mM K^+ solutions, n=9) was dominant and we could not observe K_{ATP} channels in cell-attached and inside-out patches. In addition, minoxidil and pinacidil failed to activate K_{ATP} or K_{Ca} channels. In inside-out membrane patches, the channel was blocked by 10 mM tetraethylammonium ion, 2 mM 4-aminopyridine to the cytosolic face of the membrane or by lowering Ca^{2+} using 10 mM EGTA, but not by glibenclamide. In the cell-attached patch configurations, extracellular application of 1 mM sodium nitroprusside, a nitrovasodilator, activated the K_{Ca} channel. Methylene blue (2 mM) inhibited channel activation by sodium nitroprusside. Extracellular application of 20 mM dibutyryl cGMP activated the K_{Ca} channel, suggesting that channel activation is mediated by cGMP. Nitrovasodilators, which have no effect on hair growth, now appear to activate K_{Ca} channels in dermal papilla cells. These results suggest that increased K^+ permeability itself in dermal papilla cells may not be sufficient for promotion of hair growth. J. Med. Invest. 44 : 73-77, 1997

Key Words : *ion channel ; patch clamp ; sodium nitroprusside*

INTRODUCTION

Potassium channels play important roles in the functions of cardiac muscle cells(1), pancreatic β-cells (2) and vascular smooth muscle cells (3). However, few studies have examined K^+ channels in hair follicular cells. Dermal papilla cells play an important role in hair growth and have been shown to produce a factor (or factors) that enhances the growth of follicular epithelial cells (4,5). Activators of ATP-sensitive K^+ (K_{ATP}) channels, including minoxidil and pinacidil, have been shown to induce hair growth in cultured mouse vibrissa follicles (6) as well as in clinical studies. These observations are consistent with the possibility that the opening of K^+ channels is an important mechanism in the regulation of hair follicles. However, there have been no studies which have tested the effect of K^+ channel openers on the K_{ATP} channel of dermal papilla cells. Therefore, we tested the effects of the K^+ channel openers, minoxidil and pinacidil, on K^+ channels in dermal papilla cells to clarify relation between hair growth and the K^+ channel opening.

Gelband et al. (7) found that cromakalim, another K^+ channel opener, also activated Ca^{2+}-activated K^+ (K_{Ca}) channels in the rabbit aorta. Therefore, we also tested other vasodilators with K_{Ca} channel opening. Nitroprusside, which activates K_{Ca} channels in vascular smooth muscle cells and increases circulatory flow (8,9), also activated K_{Ca} channels in dermal papilla cell. Based on these findings, we discussed the relation between the increased K^+ permeability and hair growth.

MATERIALS AND METHODS

Cell Preparation

Human dermal papilla cells were cultured as described (10). Dermal papillae were enuculated from excised hair follicles with an intact bulbous portion and then cultured in Eagle's MEM supplemented with 15% fetal bovine serum. Ca^{2+} concentration of medium was kept at 10 μM, because the cells differentiate at higher Ca^{2+} concentrations. Dermal papilla cells were subcultured after they

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had grown out from the papillae and achieved subconfluence. Cells from the second to the fifth passage on thin cover slips were used for the experiments.

Solutions and Chemicals

High K^+ solution consisted of 140 mM KCl and 10 mM K-3-(N-morpholino) propane-sulfonic acid (MOPS) buffer (pH 7.2). EGTA-Ca^{2+} buffer was used to adjust the concentration of Ca^{2+} to less than 5 μM. Free Ca^{2+} concentrations were determined with a Kd of 87 nM. Dibutyryl cGMP and methylene blue were obtained from Sigma (St. Louis, MO, USA). Sodium nitroprusside (SNP), tetraethylammonium ion (TEA) and 4-aminopyridine were from Wako (Osaka, Japan).

Electrophysiological Measurements

Membrane currents were recorded in the cell-attached configurations with a patch clamp amplifier (model EPC-7 ; List Medical Electronics, Darmstadt, FRG) as described (11). Soft-glass patch pipettes prepared with an electrode puller (PP-83 ; Narishige Scientific Institute Laboratory, Tokyo, Japan) were coated with Sylgard before use. The electrical resistance of the patch pipettes was 5 to 7 MΩ for single-channel recording. Experiments were performed at 35℃ to 37℃. Patch potentials are expressed as minus pipette potential (-Vp), as they roughly correspond to patch membrane potential (Vm) in symmetrical high K^+ solutions (Vm=-Vp). Data were stored with a PCM recorder (model PCM-501 ES ; Sony, Tokyo, Japan) with a low-pass filter (3 kHz), and single-channel currents were analyzed with Axograph (Axon Instruments, Foster City, CA, USA). Channel open probability (NPo) was determined from current amplitude histograms and the equation :

$$NPo = \sum_{n=1}^{N} (n \cdot Pn)$$

where N is the number of channels in the patch and Pn is the integrated channel opening. Results are expressed as means±SEM. Statistical analysis was performed with the Wilcoxon test. A P value of <0.05 was considered statistically significant.

RESULTS

Fig.1 a shows typical channel activity in cell-attached membrane patches of cultured human dermal papilla cells. The channel showed a large amplitude and spiky deflections, and was only active at a more negative pipette potential than -20 mV (or more positive membrane potential than +20 mV). Fig.1 b shows the current-voltage relation for this channel in the cell-attached patch configurations with both pipette and bath solutions containing 150 mM K^+. The slope conductance was 179.3±13.1 pS (n=9). The NPo of the channel increased with an increase in membrane potential (Fig.1 c).

In the inside-out patch configurations and with both pipette and bath solutions containing 150 mM K^+, the reversal potential (-Vp) of the channel was 3.7±2.2 mV (n=8) ; with a pipette solution containing 150 mM K^+ and a bath solution of 50 mM K^+, the reversal potential (-Vp) was +20.0±0.8 mV (n=8), which was close to the calculated equilibrium potential for K^+ of +29 mV, suggesting that this channel was highly K^+ selective.

In the inside-out patch configurations at a -Vp of 20 mV, mean numbers of open channel (NPo) were <0.001 (n=9) in the bath solution containing 100 nM Ca^{2+}, and NPo was 0.526±0.132 (n=9) in the bath solution containing 10 μM Ca^{2+}. In the inside-out patch configurations, the K^+ channel was blocked by the application of 10 mM EGTA to the cytosolic face of the membrane (n=7) (Fig.2 a). These results suggest that this K^+ channel was the KCa channel.

This K_{Ca} channel was blocked by the application of 10 mM TEA, a blocker of large-conductance K_{Ca} channel, to the cytosolic side (NPo 0.024±0.29 to <0.001,n=5) (Fig. 2 b). 4-AP, which blocks the Kca channel with a small conductance in vascular smooth muscle cell (12), also blocked this channel (NPo 0.012±0.021 to <0.001). But 20 μM glibenclamide, a K_{ATP} channel blocker, could not block this channel, suggesting that this channel was not the K_{ATP} channel (NPo 0.010±0.022 to 0.012±0.025, n=5).

Fig.3 shows the effect of K^+ channel openers, minoxidil sulfate (a) and pinacidil (b), on K channel activities. These drugs did not activate K_{ATP} or K_{Ca} channels. In addition,

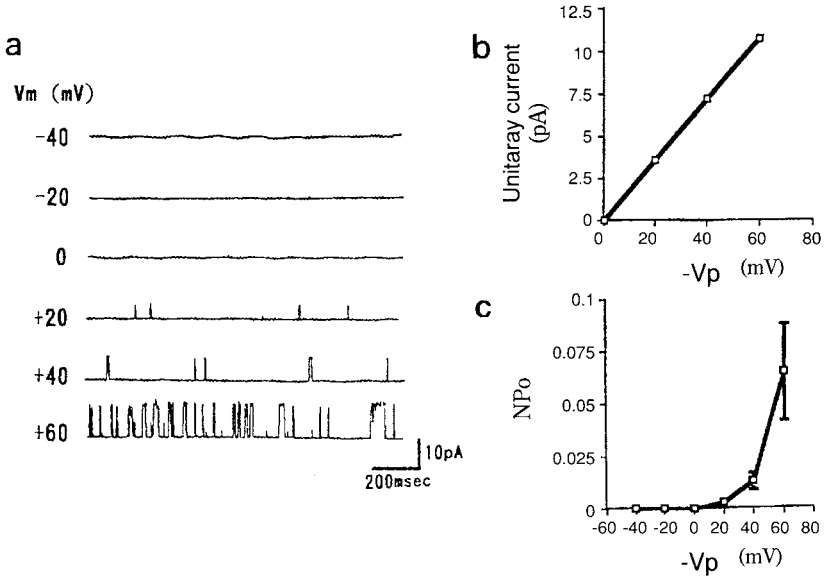

Fig. 1. (a) K_{Ca} channel currents in cell-attached patches of cultured human dermal papilla cells at various pipette potentials (-Vp). The bath solution contained 140 mM KCl, 10 mM K-MOPS, 10 mM Ca^{2+} ; the pipette solution contained 140 mM KCl, 10 mM K-MOPS, 1 mM Ca^{2+}. Upward deflections indicate outward-directed transmembrane currents and dashed lines show the zero current level in this and other Figs. (b) The current-voltage relation obtained by plotting the peak values of current amplitude against -Vp in the cell-attached patch configuartion. (c) The effect of- Vp on NPo of the K_{Ca} channel in the cell-attached patch configuration. Values are means ±SEM (n=5). NPo increased significantly (P<0.05) with the increase in Vm (-Vp).

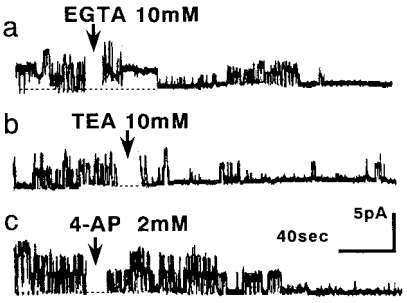

Fig. 2. Effects of EGTA (a) , TEA (b), and 4-Amino-pyridine (4-AP) (c), on the K_{Ca} channel in inside-out membrane patches at a -Vp of+10 mV. EGTA (10 mM), TEA (10 mM), and 4-AP (2 mM) were added to the cytosolic side of the membrane. The pipette and bath solutions were as in Fig.1.

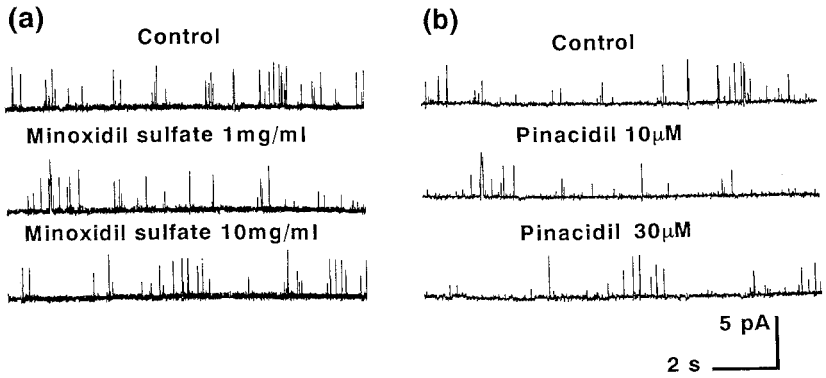

Fig. 3. Effect of minoxidil sulfate (a) and pinacidil (b) on K channels in dermal papilla cells. Minoxidil and pinacidil did not activate K_{Ca} channel. ATP-sensitive K^+ channel was not activated by these drugs either.

no channel appeared even at the highest concentration tested.

We also tested the effect of SNP, a vasodilator with K channel opening, on the K_{Ca} channel of dermal papilla cells in the cell-attached patch configurations (Fig.4 a). Infrequent single channel activities were observed under control conditions. Application of 1 mM SNP to the bath solution increased K_{Ca} channel activity ; NPo was <0.001 and 0.556±0.217 before and after the application of SNP, respectively (n=5, P<0.05).

SNP produces nitric oxide (NO), which, in turn, activates soluble guanylate cyclase and thereby increases the intracellular cGMP concentration. To clarify whether the effect of SNP on the K_{Ca} channel was mediated by cGMP, we tested the effect of dibutyryl cGMP, a membrane permeable analog of cGMP, on channel activity. In the cell-attached patch configuration, 20 μM dibutyryl cGMP activated the K_{Ca} channel (NPo increased from <0.001 to 0.258±0.112 ; n=3, P<0.05) (Fig.3 b). SNP did not activate the K_{Ca} channel in the presence of 2 μM methylene blue, an inhibitor of soluble guanylate cyclase (n=3) (Fig.3 c). These results suggest that the K_{Ca} channel of dermal papilla cells is modulated by cGMP.

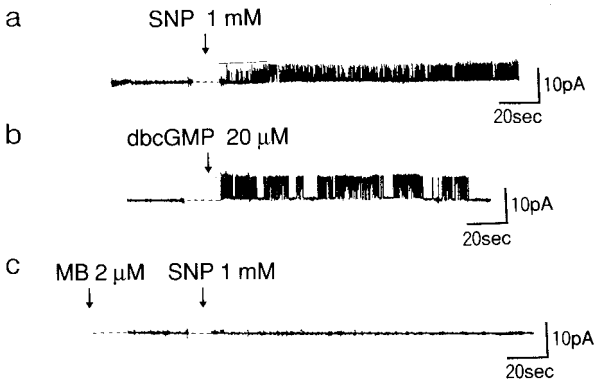

Fig. 4. Effects of SNP (a), dibutyryl cGMP (dbcGMP) (b) and methylene blue (MB) plus SNP (c) on K_{Ca} channel activity in the cell-attached patch mode at a -Vp of +20 mV. The pipette and bath solutions were as in Fig.1. The various agents were added to the bath at the indicated concentrations.

DISCUSSION

We investigated the K^+ channels in dermal papilla cells and effect of the K^+ channel openers. Under control conditions, we could not observe K_{ATP} channels and the K^+ channel openers, minoxidil and pinacidil, did not activate K^+ channels in dermal papilla cells, suggesting that either there are no K_{ATP} channels or the effects of the K^+ channel openers are absent in dermal papilla cells. The dominant K^+ channels in dermal papilla cells were large-conductance K_{Ca} channel. The K_{Ca} channel was activated by extracellular application of SNP, a nitrovasodilator, in the cell-attached patch configuration. These results do not support the possible relation between hair growth and increased K^+ permeability or peripheral circulation.

Dermal papilla cells are important in hair growth and play a crucial role in the dermal-epidermal interactions that control hair production and events of the growth cycle (13). Recently, the interaction between cultured rat dermal papilla cells and wound epidermis was examined by implanting cultured cells into small cuts in the rat ear pinna ; the implanted cells induced the growth of new follicles and also of fibers with vibrissa-type characteristics that were larger than ear hairs (14).

Minoxidil and pinacidil, activators of the K_{ATP} channel, were shown to induce hypertrichosis during early clinical trials as an antihypertensive agent (15). Minoxidil sulfate also activates the K_{ATP} channel in Madin-Darby canine kidney cells (16). Buhl, et al. (6) showed that K^+ channel activators induced hair growth in cultured mouse vibrissa follicles. These results support the hypothesis that K^+ channel activation is an important mechanism in the regulation of hair follicles.

In the present study, we could not find the K_{ATP} channel in dermal papilla cells. We do not know whether the K_{ATP} channel is present in dermal papilla cells or not. Our results, however, showed that at least two well-known K_{ATP} channel openers could not activate K_{ATP} channels in this cell. In our previous study, we also found that minoxidil sulfated and pinacidil failed to increase the $^{86}Rb^+$ efflux in dermal papilla cells, suggesting no activation of the K^+ channel (17). Thus, the effect of K_{ATP} channel activators on hair growth may be unrelated to their

opening effect on K^+ channels. More recently, K^+ channel openers have additional effects such as DNA synthesis in addition to opening of this channel (18). Moreover, sulfonylurea receptors a part of the ATP binding cassette and secrete ATP, which exerts various functions in the cell (19). These findings as well as the results of this study indicate that hair growth by K^+ channel openers might be due to the other effects than K^+ channel opening or K^+ permeability. Another possibility is that K^+ channel openers act on other cell types and not on dermal papilla cells.

Minoxidil is thought to stimulate hair growth by improving circulatory flow as a result of K^+ channel activation in vascular smooth muscle cells and by modulating K^+ channel activity in dermal papilla cells. Therefore, we studied the effect of nitrovasodilators, which have both K channel opening and vasodilating actions. SNP is known to activate both K_{Ca} and K_{ATP} channels (20), but it activated the K_{Ca} channels but not the K_{ATP} channels in this study.

TEA at high concentration (10 mM) blocked this channel almost completely, although at lower concentrations (0.1 mM) it reduced unitary conductance in vascular smooth muscle cells. K_{Ca} channels were modulated by cGMP in this study in agreement with previous studies in vascular smooth muscle cells and other cells (7, 9). Nitrovasodilators, which activate K_{ATP} channels in addition to K_{Ca} channels, have been used to treat angina pectoris. However, hypertrichosis has not been observed as a side effect (21, 22). Thus, increased permeablity of K^+ appears unrelated to hair growth. The reason why K channel openers and nitrovasodilators, i.e., activators of K_{ATP} and K_{Ca} channels, differ in their effects from each other on hair growth is unclear, although activation of both channels similarly increase K efflux. The difference may be attributable to the differences in the properties of the respective channels. Although it is difficult to conclude from our study, mechanisms different form ionic channels might be related to hair growth by K^+ channel openers.

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