

Expression of Ca^{2+} -activated K^+ Channels and Their Role in Proliferation of Rat Cardiac Fibroblasts

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Cardiac fibroblasts constitute one of the largest cell populations in the heart, and contribute to structural, biochemical, mechanical and electrical properties of the myocardium. Nonetheless, their cardiac functions, especially electrophysiological properties, have often been disregarded in studies. Ca^{2+} -activated K^+ (K_{Ca}) channels can control Ca^{2+} influx as well as a number of Ca^{2+} -dependent physiological processes. We, therefore, attempted to identify and characterize K_{Ca} channels in rat Cardiac fibroblasts. First, we showed that the cells cultured from the rat ventricle were cardiac fibroblasts by immunostaining for discoidin domain receptor 2 (DDR-2), a specific fibroblast marker. Secondly, we detected the expression of various K_{Ca} channels by reverse transcription polymerase chain reaction (RT-PCR), and found all three family members of K_{Ca} channels, including large conductance K_{Ca} (BK- α 1- and - β 1~4 subunits), intermediate conductance K_{Ca} (IK), and small conductance K_{Ca} (SK1~4 subunits) channels. Thirdly, we recorded BK, IK, and SK channels by whole cell mode patch clamp technique using their specific blockers. Finally, we performed cell proliferation assay to evaluate the effects of the channels on cell proliferation, and found that the inhibition of IK channel increased the cell proliferation. These results showed the existence of BK, IK, and SK channels in rat ventricular fibroblasts and involvement of IK channel in cell proliferation.

Key Words: Cardiac fibroblasts, Ca^{2+} -activated K^+ channels, Proliferation

INTRODUCTION

Cardiac fibroblasts constitute more than 90% of the interstitial cells of the myocardium (Eghbali et al, 1988), and have been suggested to be important determinants of both structure and function of the myocardium (Kohl et al, 1999; Gaudesius et al, 2003; Kizana et al, 2005), and contribute to structural, biochemical, mechanical and electrical properties (Mackenna et al, 2000; Sun and Weber, 2000; Camelliti et al, 2004). However, the ionic basis for their membrane potential has not been fully understood.

Ca^{2+} -activated K^+ (K_{Ca}) channels are highly selective for K^+ as charge carrier, prominent in mammalian cells, and require intracellular Ca^{2+} for channel gating. They have a unique ability to translate changes in the level of intracellular second messenger, Ca^{2+} , to changes in membrane K^+ conductance and resting membrane potential (Stocker, 2004). K_{Ca} channels can be divided into three main subfamilies: the large-conductance K_{Ca} (BK) channels, the intermediate-conductance K_{Ca} (IK) channels, and the small-conductance K_{Ca} (SK) channels (Xu et al, 2003).

Cell proliferation is very important in wound healing processes such as inflammation, matrix deposition, and tissue remodeling (Luo and Chen, 2005), and ion channels have recently been shown to contribute to their regulation:

inhibition of K^+ channel function leads to a decrease of proliferation in both physiological and pathological conditions (Wonderlin and Strobl, 1996). Nevertheless, only a few types of K^+ channels have been identified to correlate with cell proliferation; for example, voltage-dependent K^+ channel, *Herg* channel, *Eag1*, and TASK-3 channel (Pardo, 2004). The proliferation of the cardiac fibroblasts is a critical determinant of cardiac fibrosis which is a hallmark of heart disease and is the result of a variety of structural changes that occur after pathological stimuli to the cardiovascular system (Judgutt, 2003). The progression of cardiac dysfunction and failure in hypertensive heart disease, which is resulted from myocardial infarction, depends greatly on the degree of cardiac fibrosis. Despite the important roles played by cardiac fibroblasts, the relationship between K_{Ca} channels and cardiac fibroblast proliferation has not yet been studied. Therefore, we investigated the presence of K_{Ca} channels and the effect of the channels on proliferation of rat ventricular fibroblasts.

METHODS

Cell preparation and culture

Cardiac fibroblasts were obtained by enzymatic dispersion of ventricles of Sprague-Dawley young rats (2 to 6 days old).

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ABBREVIATIONS: K_{Ca} channel, Ca^{2+} -activated K^+ channel; IBTX, iberiotoxin; CLT, Clotrimazole.

In brief, the ventricles were removed from the heart, cut into small segments, and then digested by shaking in phosphate-buffered saline solution (PBS, GIBCO, Grand Island, NY), containing collagenase type II (1000 U/ml, Sigma, St. Louis, MO), at 37°C for 12 min. Dissociated cells were gently centrifuged, suspended in Dulbecco's modified Eagle's medium (DMEM, GIBCO) supplemented with penicillin (100 U/ml, Sigma), streptomycin (100 µg/ml, Sigma) and 10% fetal bovine serum (FBS, GIBCO). They were then plated onto culture plates and incubated at 37°C in a humidified environment, containing 5% CO₂ and 95% air state, for 2 hr. Following incubation, the non-adherent cells (mostly myocytes and endothelial cells) were removed, the plates were washed, and the attached cells were then cultured in DMEM, containing 10% FBS, at 5% CO₂ incubator. At confluence, the resulting fibroblasts were detached with trypsin (0.05%, Sigma) and EDTA (0.02%, Sigma) in DMEM, and then serially passed into 100 mm culture dishes. Cells at the 2nd passage of culture were frozen in cell culture freezing medium and stored in liquid nitrogen. Before experiments, the cells were thawed and cultured in DMEM with 10% FBS. When the 3rd passage cells achieved confluence, the cells were washed with DMEM and treated with trypsin/EDTA in DMEM. After 3 ~ 5 min of incubation in a 5% CO₂ incubator (37°C), the cells were suspended in fresh DMEM and centrifuged again. The supernatant was then discarded, and the diluted cell suspension (1 : 10 with DMEM) was seeded onto poly-L-lysine coated cover slips for experiments. Human dermal fibroblast cell line (CRL-1474, ATCC, Manassas, VA) was used as positive cell line and rat skeletal myoblast cell line (CRL-1458, ATCC) as negative cell line for confirmation of fibroblast in our cultured cell preparation.

Immunocytochemistry

Cells cultured on chamber slides were processed according to standard protocols. Briefly, cells were washed twice with ice-cold PBS, fixed in ice-cold methanol (100%) for 10 min, permeabilized in PBS with 0.2% Triton X-100 plus 1% normal rabbit serum (Vector laboratories, CA) for 5 min, and washed with PBS. They were then blocked in PBS containing 2% normal rabbit serum and 0.3% Triton X-100 for 30 min and then incubated with primary antibody raised against discoidin domain receptor-2 (DDR-2, goat polyclonal IgG, Santa Cruz Biotechnology, CA) for 2 hr at room temperature. Cells were washed four times for 10 min each with PBS and incubated with secondary antibody conjugated with fluorescein isothiocyanate (FITC, Vector Laboratories, CA) for 1 hr. After washing four times with PBS, cells were mounted and stained on slides with vectashield mounting medium containing propidium iodide (PI, Vector laboratories). Propidium iodide is a fluorescent vital dye that stains DNA and is used in two-color immunocytochemistry to distinguish cells (Fawcett et al., 1998).

Isolation of RNA and reverse transcription polymerase chain reaction (RT-PCR)

Total RNA was extracted from isolated rat cardiac fibroblast using Total RNA isolation kit (Qiagen, Hilden, Germany). First-strand cDNA was prepared with the Super Script kit (Invitrogen, Tokyo, Japan), according to the

manufacture's instructions. PCR was performed with 1 µl of RT product (cDNA) with specific primers designed from nucleotide sequence retrieved from the GenBank using perfect premix (Takara, Kyoto, Japan) in GeneAmp PCR System 9700 (Applied Biosystems, Foster city, CA). The thermocycler program used for PCR amplification included a hot start (94°C for 5 min) followed by 34 cycles of denaturation at 94°C for 40 sec, annealing at 50°C to 60°C for 40 sec, and extension at 72°C for 40 sec. And then, reactions were completed by additional 7 min extension at 72°C. RT-PCR reaction products (cDNA) were resolved on 2% agarose gel electrophoresis, and stained with ethidium bromide for visualization under ultraviolet light. The primer sequences were designed using Entrez (NCBI, NIH) and Primer 3 (Whitehead Institute for Biomedical Research, Cambridge, MA) based on known mRNA sequences (NCBI, GeneBank). T_m of the primer was 55°C and GC concentration was 45% to 55%. Primers are listed in Table 1.

Electrophysiological recordings

Electrophysiological measurements were taken using the tight-seal patch clamp method with an Axopatch 200B patch clamp amplifier (Axon Instruments, Union City, CA). pCLAMP 9.0 (Axon Instruments) software was used for data acquisition as well as analysis of the currents. Recording pipettes were pulled from borosilicate glass capillaries (TW150F-4, World Precision Instruments, Sarasota, FL) using a microelectrode puller (PP-83, Narishige, Tokyo, Japan), and then fire polished on a microforge (MF-83, Narishige). After fire polished, the pipettes exhibited a resistance of 3~4 MΩ when filled with pipette solution. All experiments were carried out at 21~23°C. Whole-cell currents were filtered at 2 kHz and digitized at 10 kHz. Cells were clamped at -50 mV, and depolarizing pulses were applied in 10 mV steps to evoke outward K⁺ currents. The patch pipette solution was high K⁺ solution (in mM/L); 145 KCl, 1.652 CaCl₂ (pCa 6.0), 1.013 MgCl₂, 10 HEPES, 2 EGTA, and 2 K-ATP (pH 7.3 with KOH). The bath solution contained 145 NaCl, 5 KCl, 1 CaCl₂, 1 MgCl₂, 5 glucose, and 5 HEPES (mM/L, pH 7.35 with NaOH). Free [Ca²⁺] was calculated using a program written by Peter Graffiths (Oxford University, U.K.). DMEM and FBS were purchased from GIBCO (Grand Island, NY). Iberiotoxin (IBTX) and other chemicals were purchased from Sigma (St. Louis, MO). Apamin and clotrimazole were dissolved in dimethyl sulfoxide (DMSO), and the concentration of DMSO was less than 0.1%.

Cell proliferation assay

The confluent fibroblasts of the 3rd passage cultures were trypsinized with DMEM (containing 0.25% trypsin and 0.02% EDTA) and freshly prepared in DMEM supplemented with 10% FBS immediately before use. The cells were seeded at a density of 2,500~3,000 cells/well in 96-well plates. Cell cultures were incubated for 24 hr in a humidified atmosphere of 95% air and 5% CO₂. Ten µl of Cell Counting Kit-8 (CCK-8) solution (Dojindo, Japan) was added to each well and incubated for 2 hr in 95% air and 5% CO₂ incubator. The optical density of each well was measured at 450 nm, using a Spectra Max 340 ELISA reader (Molecular devices, Sunnyvale, CA) with a reference wavelength at 600 nm. Tetrazolium salt (WST-8) of CCK-8

Table 1. Oligonucleotide primers used for RT-PCR analysis

Gene	Genebank	Primer	Sequence (5'~3')	Size (bp)
Rat BK- α 1	NM031828	Sense	TGGCAGAGTCCTGGTTGTGT	206
		Antisense	CCAGCCAGAACCACAGCTTA	
Rat BK- β 1	NM019273	Sense	CAAATCCTCCTTCTCCTT	229
		Antisense	ATGATTGTCCAAAGAGGTTG	
Rat BK- β 2	NM176861	Sense	TTCTGACCCAGAAGGGAACC	172
		Antisense	TCCGTTGGATCCTCTCACAA	
Rat BK- β 3	XM227040	Sense	TCGTCAACCTTAGCCACTCG	144
		Antisense	AATTCCTTGATGTCCAGCGC	
Rat BK- β 4	NM023960	Sense	AAACGCAAGTTCTCTTAACG	242
		Antisense	AGCTAAGAGCTTGACCCTTT	
Rat IK	AF149250	Sense	CGGAAGCTGGAGTTCAACAA	221
		Antisense	CTTCCGATGTTTCAGCCGTA	
Rat SK1	AF000973	Sense	TCATCTCCATTACCTTCCTG	264
		Antisense	AGCCTGGTGTGTTTGTAGAT	
Rat SK2	NM019314	Sense	ACCTGCACGAGATGGACTCA	145
		Antisense	TTGTGCTCCGGCTTAGACAC	
Rat SK3	NM019315	Sense	GCCAACCCCTACAGCTCTTC	179
		Antisense	TGTTTTGGTTTTTCCGCTTG	
Rat SK4	NM023021	Sense	AGAATCAGGCTATGTACCGA	211
		Antisense	GATGGCCTATGTGTAGTGGT	
Rat GAPDH	NM017008	Sense	GAGTCTACTGGCGTCTTCAC	280
		Antisense	CCATCCACAGTCTTCTGAGT	

solution produces a formazan dye, and the amount of yellow colored formazan dye generated by dehydrogenase in the fibroblasts is directly proportional to the number of viable cells in a culture medium. The optical density of control group was taken as 100% viable cells.

Statistical Analysis

Data are expressed as means \pm S.E.M. Comparisons of measurements between groups were conducted using the Student's t-test. A significant level of difference was set at either $p < 0.05$ or $p < 0.01$.

RESULTS

Cardiac fibroblasts from neonatal rat heart were grown under conditions favorable for their proliferation, and they displayed typical fibroblast morphology; with a few cells assuming a large satellite shape and some cells being binucleated. Immunocytochemistry was used to evaluate the expression of discoidin domain receptor 2 (DDR-2), the specific marker of fibroblasts (Olaso et al, 2002), to confirm whether our cultured cells were indeed fibroblasts (Fig. 1). The cells were double immunostained with propidium iodide (PI) and antibody against DDR-2 under confocal laser scanning microscope. PI was used in two-color immunocytochemistry to distinguish cells (Fawcett et al, 1998). As shown in Fig. 1, DDR-2 was expressed in our cultured rat ventricular cells and positive cell line (CRL-1474, human dermal fibroblast cell line), however, not in negative cell line (CRL-1458, rat skeletal myoblast cell line).

To ensure that cardiac fibroblast cells retained K_{Ca} channel at RNA level, we employed RT-PCR to amplify transcripts encoding BK channel (α - and β -subunits), IK channel, and SK channel (1~4 subunits). Thus, total RNA

was isolated from rat ventricular fibroblasts and transcribed, and selective regions were amplified as described above (see Table 1). As shown in Fig. 2, RT-PCR analysis revealed that mRNA was expressed in cultured cardiac fibroblasts. Various subunits of BK channel were detected: Rat BK- α 1 subunit was amplified at 206 bp, and the auxiliary β subunits of rat BK channel were detected in 4 different forms (rat BK- β 1, rat BK- β 2, rat BK- β 3, and rat BK- β 4 subunits) and the sizes of those amplification products were 229, 172, 144, and 242 bp, respectively. Rat IK channel was also detected at 221 bp. In rat SK channels, SK1, SK2, SK3 and SK4 subunits were found: SK1 and SK2 subunits were amplified at 264 and 145 bp, while SK3 and SK4 subunits were amplified at 179 and 211 bp. GAPDH was used as a positive control and the amplification products size was 280 bp.

To further identify the presence of various K_{Ca} channels in rat ventricular fibroblasts, we carried out electrophysiological measurement by whole-cell mode patch clamp techniques. Macroscopic K⁺ currents were recorded in normal Ringer bath solution and K⁺-internal pipette solution (pCa 6.0). Outward K⁺ currents were generated by incremental 10 mV depolarizing steps from -60 mV to 50 mV for 500 ms and the holding potential was -50 mV. Outward currents were activated at about -40 mV and well maintained throughout the test pulse.

The K⁺ currents were decreased by addition of 0.1 μ M iberiotoxin (IBTX, a specific BK channel blocker) to the bath. The current-voltage (I~V) relationship of the currents showed strong outward rectification and amplitude change (Fig. 3A). The change of current amplitude at 50 mV with IBTX was decreased by about 50% in this cell, compared with 100% control. In other cells, the K⁺ currents were decreased by clotrimazole (CLT, 2 μ M, a specific IK channel blocker) or apamin (0.5 μ M, a specific SK channel blocker), and the bar graph showed the decrease of current amplitude by about 66% (Fig. 3B) and 77% (Fig. 3C) at 50

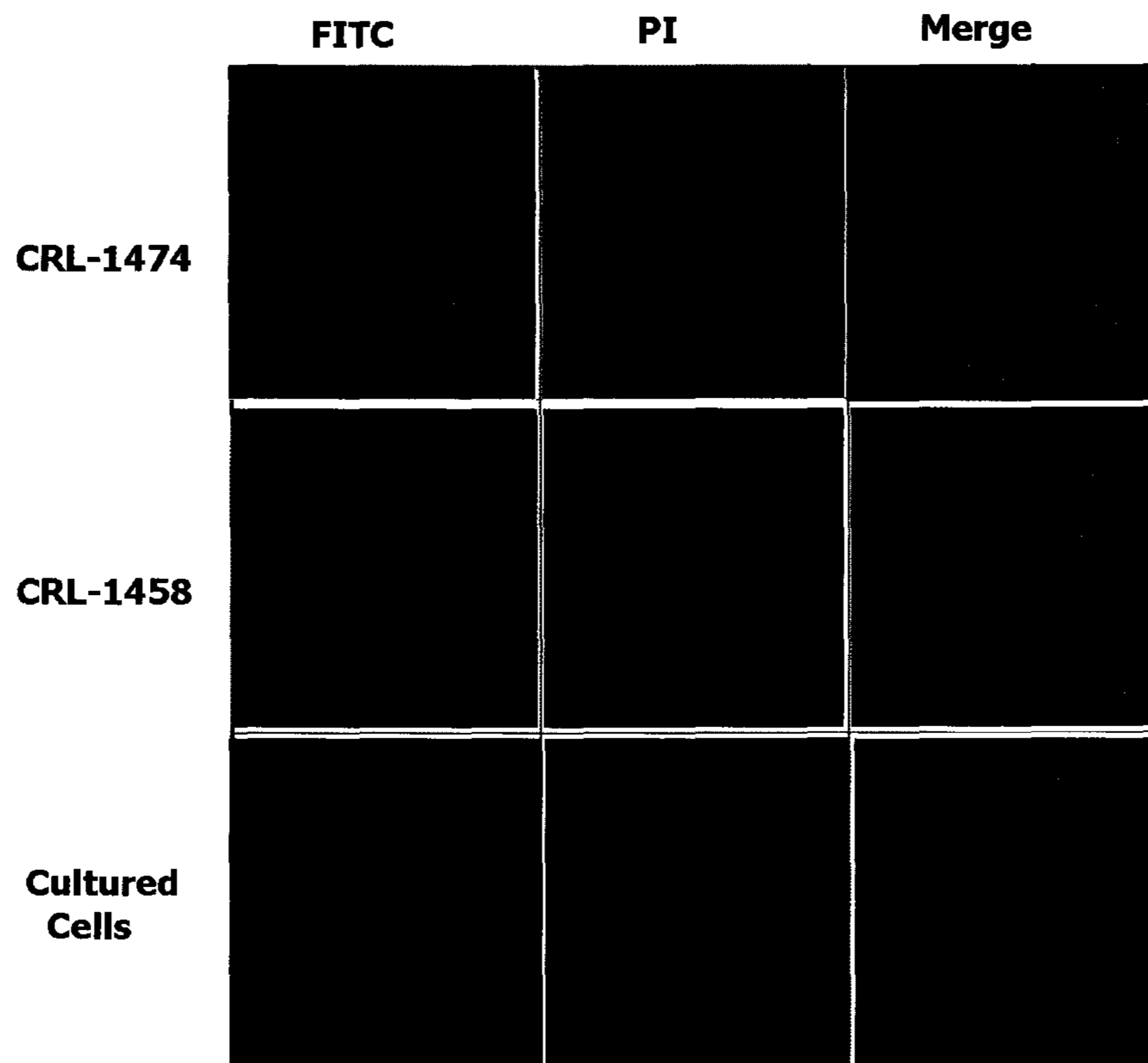


Fig. 1. The expression of DDR-2 (a specific fibroblast marker) in rat cardiac fibroblast cells. Rat cardiac cells were double stained by DDR-2 antibody and PI. DDR-2 was positive also in fibroblast cell line (CRL-1474), but not skeletal myoblast cell line (CRL-1458).

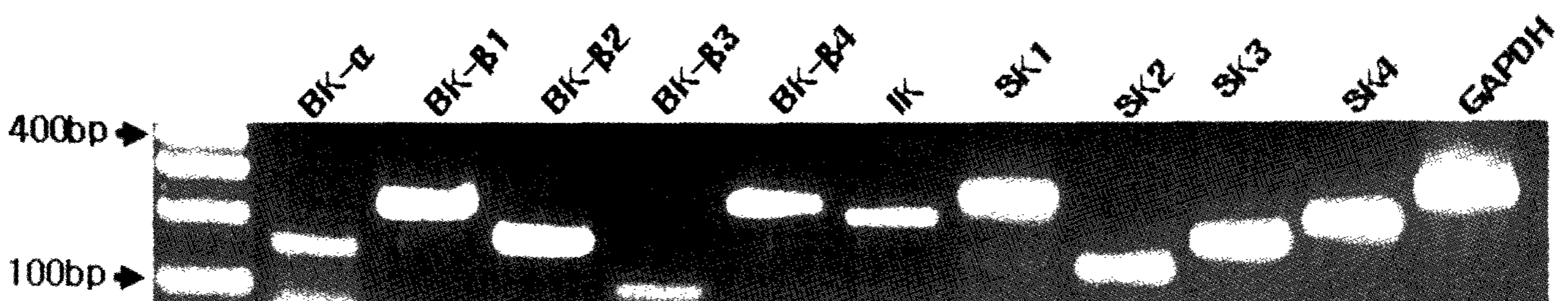


Fig. 2. Identification of various K_{Ca} channels in rat cardiac fibroblasts by RT-PCR. The size of amplification products was BK- α 1 (206 bp), BK- β 1 (229 bp), BK- β 2 (172 bp), BK- β 3 (144 bp), BK- β 4 (242 bp), IK (221 bp), SK1 (264), SK2 (145 bp), SK3 (179 bp), and SK4 (211 bp). GAPDH was used as positive control and the size of amplification products was 280 bp.

mV, compared with 100% control by the drugs. Fig. 3D shows the effects of various K_{Ca} channel blockers on the amplitude of K^+ currents of the cardiac fibroblasts at 50 mV. As shown at bar graph, IBTX ($0.1 \mu\text{M}$) in the bath solution decreased the K^+ currents to $55.1 \pm 6.8\%$ ($n=7$, $p < 0.01$, compared with 100% of control). When clotrimazole ($2 \mu\text{M}$) was added to the bath solution in other cells, current amplitude was changed to $69.1 \pm 5.84\%$, compared with 100% control ($n=5$, $p < 0.05$). Apamin ($0.5 \mu\text{M}$) also decreased the currents to $82.6 \pm 1.67\%$, compared with 100% of control ($n=5$, $p < 0.05$).

To investigate whether three types of K_{Ca} channel were expressed in one fibroblast, we recorded various K_{Ca} channels with their specific blockers in a fibroblast. Fig.

4A shows that outward K^+ currents were generated by incremental 10 mV depolarizing steps from -40 mV to 70 mV for 500 ms and the holding potential was -50 mV. The representative K^+ currents were increased with depolarizing stimulation and oscillated at strong depolarization in a control state. The amplitude of the K^+ currents was decreased by the addition of apamin ($0.5 \mu\text{M}$), and further decreased by clotrimazole ($2 \mu\text{M}$) along with apamin ($0.5 \mu\text{M}$) in bath solution. When IBTX ($0.1 \mu\text{M}$) was added to the bath solution which contained apamin and clotrimazole, the decreased currents were further inhibited. The current-voltage ($I \sim V$) relationship of the currents of the fibroblasts showed strong outward rectification and amplitude change by the various K_{Ca} channel blockers (Fig.

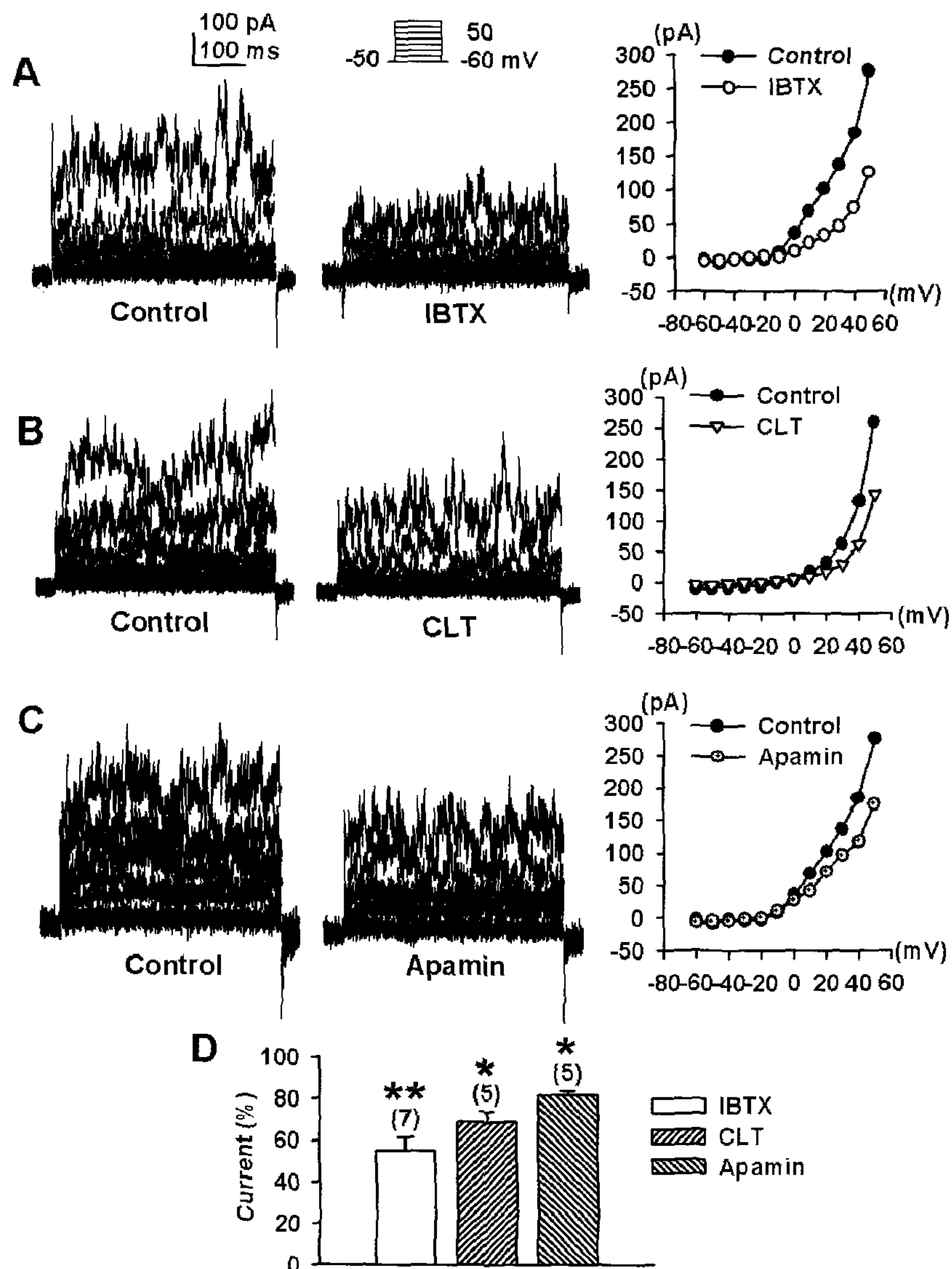


Fig. 3. Identification of BK, IK, and SK channels in rat ventricular fibroblasts by whole-cell mode patch clamp technique. Outward K⁺ currents were generated by incremental 10 mV depolarizing steps from -60 mV to 50 mV for 500 ms and the holding potential was -50 mV. (A) Representative effect of IBTX (0.1 μM) on the outward K⁺ currents in fibroblast and the I-V curve shows the mean values of steady state currents. (B) Representative effect of clotrimazole (CLT, 2 μM) and (C) apamin (0.5 μM) on the K⁺ currents in other cells, and their I-V curves are shown. (D) The effects of various K_{Ca} channel blockers on the amplitude of the K⁺ currents of rat cardiac fibroblasts at 50 mV. Data show mean±S.E.M. of indicated numbers (*p<0.05, **p<0.01, compared with 100% of control).

4B).

The role of various K_{Ca} channels in the proliferation of the cardiac fibroblasts was investigated (Fig. 5). Data presented are the average of observations in 24 wells (2,500 ~3,000 cells/well), while the control response at each experimental condition was set as 100%, which is represented by optical density of non-stimulated cells. When we tested the effects of various K_{Ca} channels blockers on cell proliferation by adding them the culture medium for 24 hr, only clotrimazole (2 μM) increased cell proliferation (116.6±0.76%, p<0.01 compared to 100% of control, Fig. 5). On the contrary, however, when IBTX (0.1 μM) or

apamin (0.5 μM) was added to the culture medium for 24 hr of incubation, cell proliferation was not significantly changed (98.3±0.63% on IBTX; 96.0±0.91% on apamin, compared to 100% of control).

DISCUSSION

In the present study, molecular and electrophysiological studies revealed that three types of K_{Ca} channels were expressed in rat ventricular fibroblasts. Cardiac fibroblast is the predominant cell type that constitutes the majority

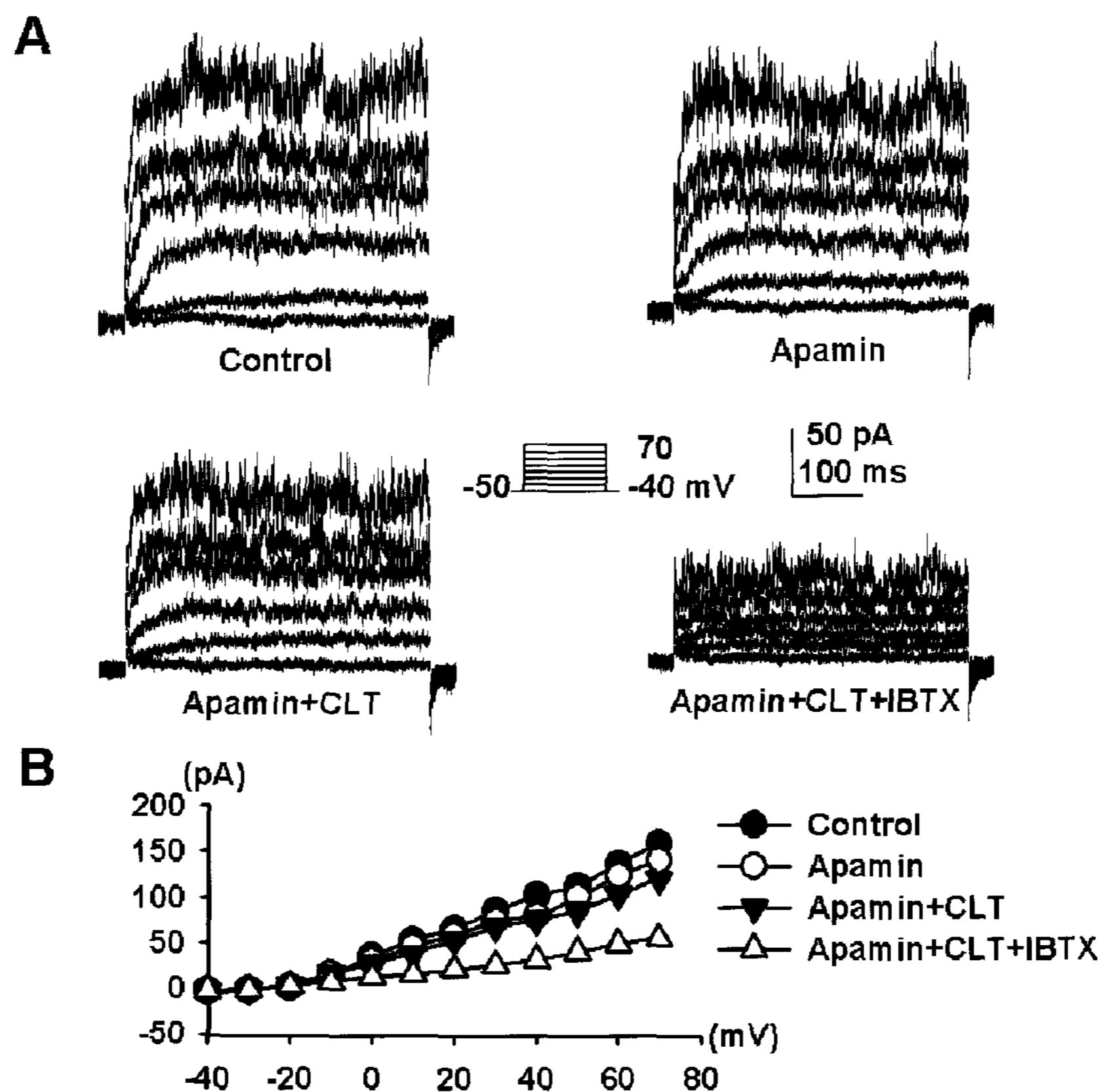


Fig. 4. Investigation for the expression of three types of K_{Ca} channels in a fibroblast of rat ventricle by whole-cell mode patch clamp technique. Currents were evoked by 500 ms step depolarization to 70 mV from -40 mV. Holding potential was -50 mV. (A) The effects of various K_{Ca} channel blockers on the outward K^+ currents in one fibroblast. Apamin ($0.5 \mu\text{M}$), clotrimazole (CLT, $2 \mu\text{M}$), or IBTX ($0.1 \mu\text{M}$) was added to the bath solution. (B) The $I \sim V$ curve shows the mean values of steady state currents in the fibroblast.

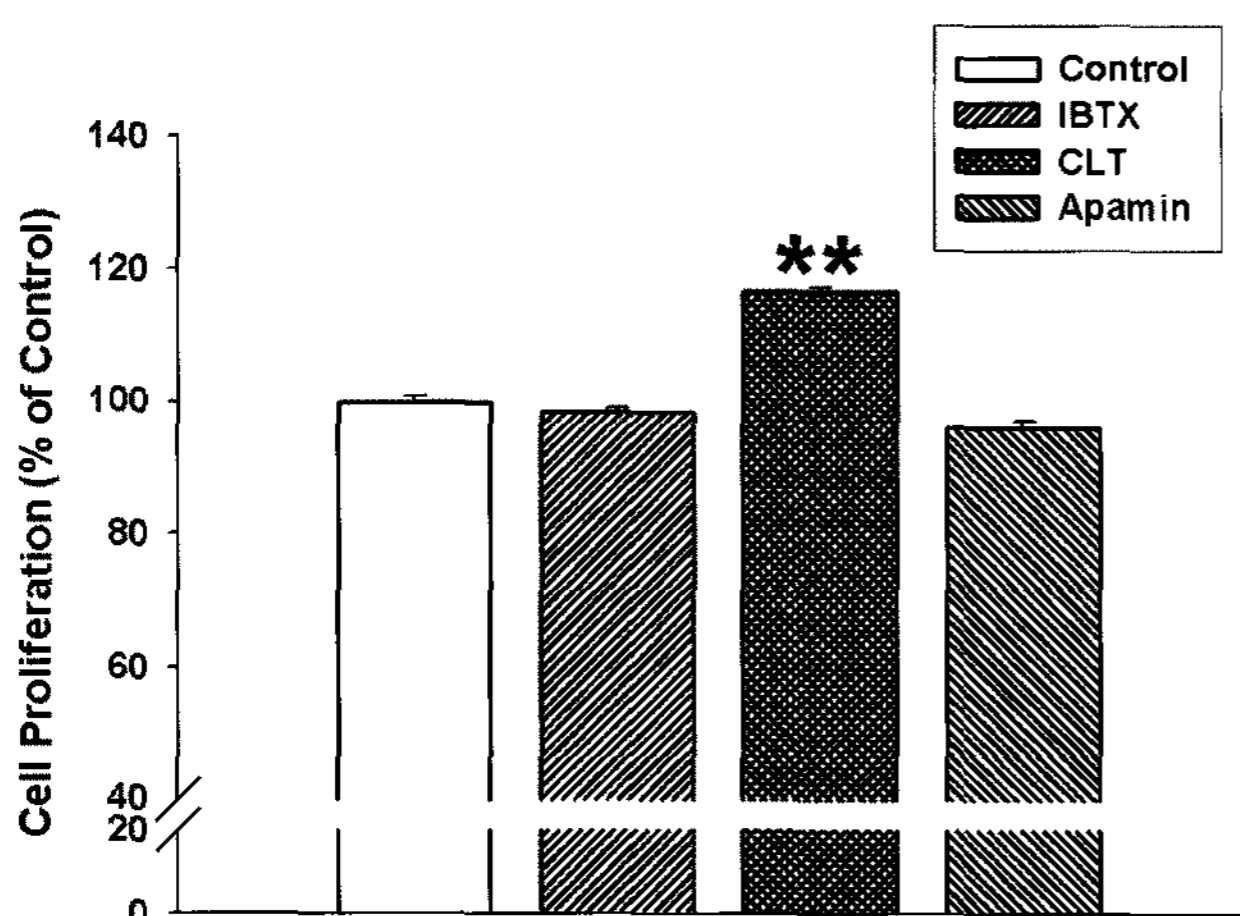


Fig. 5. The effects of various K_{Ca} channel blockers on the proliferation of the rat ventricular fibroblasts. IBTX ($0.1 \mu\text{M}$), clotrimazole (CLT, $2 \mu\text{M}$), and apamin ($0.5 \mu\text{M}$) were tested. Only clotrimazole ($2 \mu\text{M}$) significantly increased cell proliferation (** $p < 0.01$ from control at 24 hr). The control response was set as 100%, which is represented by optical density of unstimulated cells. Data show mean \pm S.E.M. of observations in 24 wells (2,500 \sim 3,000 cells/well).

of myocardial cell population in the heart, and plays a major role in the deposition of extracellular matrix. They produce mainly collagen type I which represents 80% of the total newly synthesized collagens, and deposition of excessive collagen in cardiac fibrosis results in a detrimental outcome of chronic hypertension or myocardial infarction, since it may lead to heart failure. They also release growth factors, cytokines, and other signaling molecules involved in the maintenance of myocardial tissue (Manabe et al, 2002) and play a crucial role in cardiac remodeling which induces significant changes. One of the limiting factors in studying the cardiac fibroblasts in vivo is the lack of a specific marker. Recent studies showed discoidin domain receptor (DDR-2), one of the receptor tyrosine kinase (RTK) families, as novel receptor for collagen and fibroblasts (Olaso et al, 2002). In the present study, we performed immunocytochemistry staining, using highly specific antibody for DDR-2, and found that a uniform expression of DDR-2 was localized at the cell membrane with punctuate staining pattern, suggesting that the fibroblast-like non-myocytes in our cultures were cardiac fibroblasts. It should be mentioned here that activation of DDR-2 by collagen results in up-regulation of expression of matrix metalloproteinase-1 and -2 which are important components in the turnover and remodeling of

the extracellular matrix of the heart (Vogel et al, 1997; Goldsmith et al., 2004), and that fibroblasts are responsible for production of the structural collagens, suggesting a potential role of DDR-2 in modulating collagen production/remodeling in both heart development and disease (Morales et al, 2005).

To confirm that various K_{Ca} channels are expressed in cardiac fibroblasts, we performed RT-PCR with RNA isolated from rat ventricular fibroblasts. RT-PCR products generated through the use of rat gene specific primers showed that mRNAs for K_{Ca} channels were indeed expressed in rat ventricular fibroblasts: We found various subunits of BK channel; that is, the main pore-forming α 1 subunit (rat BK- α 1) and four different auxiliary β subunits (rat BK- β 1, rat BK- β 2, rat BK- β 3, and rat BK- β 4). Rat IK channel and four SK channel subunits (rat SK1~SK4 subunits) were found. The activity of K⁺ channels in cardiac fibroblasts tends to influence the membrane potential of cardiac action potential, thereby exacerbating the propensity of cardiac arrhythmias; recent evidence indicates that ventricular fibroblasts communicate to other fibroblasts as well as cardiomyocytes via gap junctions (Goldsmith et al, 2004; Xi et al, 2004), suggesting that K⁺ conductance can regulate the membrane potential of ventricular fibroblasts from rat hearts. We confirmed in the present study that BK, IK and SK channels were expressed in cultured rat cardiac fibroblasts, evidenced with their specific blockers by electrophysiological methods. Furthermore, to elucidate the role of the BK channel in cell proliferation of ventricular fibroblasts, we carried out cell proliferation assay, and found that it was increased by clotrimazole (a specific IK channel blocker), but not by IBTX (a specific BK channel blocker) or apamin (a specific SK channel blocker) during 24 hr of incubation. Our results on IK channels are in good accordance with previous observation that 1-EBIO (a specific IK activator) suppresses mRNA expression of IK channels and cell proliferation in HaCaT keratinocytes (Koegel et al, 2003) and C6 glioma cell lines (Manaves et al, 2004). However, activation of IK channels with 1-EBIO increases T lymphocytes proliferations, whereas inhibition of the channel with clotrimazole decreases proliferation (Khanna et al, 1999).

BK channel showed opposite effects on endothelial cells proliferation; the specific BK channel activator, NS1619, is responsible for the reduction of endothelial cell growth (Kuhlmann et al, 2004), however, activation of BK channels is important in basic fibroblast growth factor (bFGF)-induced endothelial cell proliferation (Wiecha et al, 1998). Our present results of BK channel on cardiac fibroblast proliferation are different from the above-mentioned previous studies. The difference could be due to different cell types and species. SK channel also affects fundamental cellular events of proliferation and myogenic differentiation (Pena and Rane, 1999), although it plays a more significant role in mediating currents for neuronal hyperexcitability (Lappin et al, 2005). However, our results showed that SK channel did not affect the proliferation of rat cardiac fibroblasts.

Cell proliferation and apoptosis constitute two counterparts by sharing the responsibility for maintaining normal tissue homeostasis, and enhanced K⁺ efflux has been shown to be an essential mediator of both of them. Transient hyperpolarization is required for the progression of the early G₁ phase of the cell cycle (Wonderlin and Strobl, 1996) and provides an electrochemical gradient for influx of Ca²⁺,

a messenger in the mitogenic signal cascades of cells. Cell proliferation must be accompanied by increase of cell volume and is typically paralleled by activation of K⁺ channels, which is required for the maintenance of the cell membrane potential, a critical determinant of Ca²⁺ entry through Ca²⁺ channels. Sustained or excessive increase of Ca²⁺ triggers apoptotic cell death, typically paralleled by cell shrinkage due to activation of Ca²⁺-activated K⁺ channels. The K⁺ exit through K⁺ channels decreases intracellular K⁺ concentration and facilitates cell shrinkage which is typically paralleled by apoptosis. Thus, alterations of K⁺ channel activities may participate in the triggering of both cell proliferation and apoptosis. Accordingly, the same ion channel blockers may interfere with both cell proliferation and apoptosis, depending on cell type, regulatory environment and condition of the cell (Lang et al, 2000).

In summary, our present results showed that three types of K_{Ca} channel were expressed in rat cardiac fibroblasts and IK channel activation inhibited the proliferation of the cells.

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