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TGF-β1 induces the expression of fast inactivating K^+ (I_A) channels in rat vascular myofibroblasts

Ming-ming Wu,[a] Ping-jin Gao,[b] Song Jiao,[a] Ding-liang Zhu,[b] Zhi-hong Zang,[a] and Yan-ai Mei[a,*]

[a] *Department of Physiology and Biophysics, Research Center of Brain Science, School of Life Sciences, Fudan University, Shanghai 200433, China*
[b] *Shanghai Institute of Hypertension, Ruijin Hospital, State Key Laboratory for Medical Genomics, Shanghai Second Medical University, Shanghai 200025, China*

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Abstract

It is well established that transforming growth factor-β1 (TGF-β1) can induce the transformation of fibroblasts to myofibroblasts. The molecular mechanisms of the phenotypic change remain unknown. The effect of TGF-β1 on the expression of K^+ channels in cultured rat vascular fibroblasts was investigated by using the patch-clamp technique and quantitative RT-PCR. In fibroblasts, the only voltage-dependent outward K^+ current that can be electrophysiologically detected is non-inactivating. In myofibroblasts, induced by the treatment of fibroblasts with TGF-β1, we report the emergence of an additional transient outward K^+ current The TGF-β1-induced outward current is inhibited by 4-aminopyridine. $K_{V2.1}$, the transcript for a non-inactivating potassium channel gene, was detected by quantitative RT-PCT in both cultured fibroblasts and myofibroblasts. In contrast, the transcript of the transient I_A gene, $K_{V4.1}$, can be detected only in myofibroblasts. The results suggest that TGF-β1-induced phenotypic transformation of vascular fibroblasts to myofibroblasts is accompanied by the induction of I_A channels.

Keywords: TGF-β1; K^+ channels; Vascular fibroblasts; Myofibroblasts

Adventitial fibroblasts play an important role during arterial repair. A number of investigations showed that coronary endoluminal injury can invoke cell proliferation and migration involving adventitial fibroblasts. These responses are associated with their differentiation to myofibroblasts, manifested by a rapid induction of α-SM actin expression [1,2]. It is well established that transforming growth factor-β1 (TGF-β1) can induce the phenotypic transition of fibroblasts to myofibroblasts, which affects arterial remodeling via their mechanical and synthetic properties [3]. The spatial and temporal relationships between the expression and the formation of vascular myofibroblasts have been previously described [4]. Although exogenous TGF-β1 has been shown to induce myofibroblast phenotype in both cardiovascular and nonvascular fibroblasts [5,6], their mechanisms are not well understand.

The regulated expression of ion channels is known to serve critical roles in developmental processes in many cell types [7,8]. For examples, growth factors are capable of promoting or inhibiting cell growth by regulating the expression of ion channels [9,10]. Using patch clamp techniques and quantitative RT-PCR we seek to establish the linkage between the expression of a new set of ion channels and the TGF-β1-induced formation of myofibroblasts. Furthermore, we seek to identify the properties of ion channels on vascular fibroblasts and those regulated by TGF-β1.

Experiment procedures

Cell culture and preparation. The isolation and culture of adventitial fibroblasts from thoracic aortae of 6–8 week-old male Wistar-Kyoto rats has been previously described [11]. In brief, thoracic aortae of 3–4 rats were aseptically removed and cleaned of fat tissue and blood cells. Adventitia was cut into small pieces and placed on 60 × 5 mm tissue dishes in minimum essential medium (MEM) containing 15% fetal calf

* Corresponding author. Fax: +86-21-6565-0149.
E-mail address: yamei@fudan.edu.cn (Y.-a. Mei).

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serum (FCS). Fibroblasts grew from these explants within 10–14 days. They were then passaged and cultured in 10% newborn calf serum (NCS). In the present study, cells were used from passages 3 to 5. Myofibroblast differentiation was induced by the addition of 20 ng/ml TGFβ1 to subconfluent fibroblasts made quiescent serum free in 48 h, as described commonly. Examination of cell morphology by immunocytochemistry and scanning electron microscopy provided evidence that the culture and differentiated cells were indeed fibroblasts and myofibroblasts [13].

Patch-clamp recordings. Currents were recorded using the "whole-cell" patch-clamp technique in rat vascular fibroblasts. Prior to recordings, the culture medium was replaced by a bath solution containing (in mM): NaCl, 145; KCl, 2.5; Hepes, 10; $MgCl_2$, 1; and glucose, 10 (pH 7.4 adjusted with NaOH). For recording the delayed rectifier outward K^+ current (I_K), calcium was removed from the bath solution to eliminate Ca^{2+} activated K^+ current. The solution used to fill patch pipettes had the following composition (in mM): KCl, 140; $MgCl_2$, 4; EGTA, 10; and Hepes, 10 (pH 7.4 adjusted with KOH). In some experiments EGTA was lowered to 0.1 mM.

Data acquisition and analysis. All current signals were recorded with an EPC-7 amplifier operated in the voltage-clamp mode. Step voltage commands, data acquisition, and analysis were performed with the pClamp 6.03 software (Axon Instruments, USA) and/or Origin 5.1 analysis software (Micrococal software). Current was corrected for leak and residual capacitance transients by a P/4 protocol. Values are given as means ± SEM. Statistical analysis was conducted using Student's *t* test with paired comparisons if relevant.

Real-time quantitative reverse transcription-polymerase chain reaction (RT-PCR). RNA extract and reverse transcription (RT) were carried out essentially as previously described [13]. The primers for $K_{V2.1}$ and $K_{V4.2}$ were designed by using Primer Express software 1.0. The real time measurements were performed with the Rotor-Gene 2000 Real-time instrument (Corbett Research, Mortlake, Australia). Samples were analyzed in duplicate in four independent runs. $K_{V2.1}$ gene was amplified with the primers forward: 5′-CGG GAG TCC ACC ATT ACA TA-3′ and reverse: 5-CTC TGT GGT AGG GAG CTC AG-3′. $K_{V4.2}$ gene was amplified with the primers forward: 5-AGG ACG CTC TAA TTG TGC TG-3′ and reverse: 5-TGT CAG TGT CTG CAT CAT CC-3′.Quantitative RT-PCR was performed in 50 μl reactions consisting of 1× PCR buffer, 5.5 mmol/l $MgCL_2$, 0.025 U/μl AmpliTaq. 20 pmol primers, 2 μl SYBR Green, and 3 μl RT product. PCR cycling for $K_{V2.1}$ was carried out as follows: 95 °C for 3 min, followed by 35 cycles of 92 °C for 1 min, 63 °C for 1 min, and 72 °C 1 min. PCR cycling for $K_{V4.2}$ was carried out as follows: 95 °C for 3 min, followed by 35 cycles of 92 °C for 1 min, 60 °C for 1min, and 72 °C 1 min. Cycle threshold (Ct) values and concentrations of samples were calculated using a Rotor-Gene 2000 software.

Results

Only slow activating outward K^+ current is expressed in rat vascular fibroblasts

Before TGF-β1 treatment, the membrane currents of rat vascular fibroblasts were studied by different voltage protocols and by changing the ionic composition of the external or internal solutions. Fig. 1A shows that inward Na^+ current could not be evoked by depolarization to +55 mV from a holding potential of −95 mV ($n = 13$). Furthermore, in all 16 fibroblasts recorded, no inward Ca^{2+} current was observed (Fig. 1B), even after substituting Ba^{2+} for Ca^{2+} which often enhanced the amplitude of the current flowing through some Ca^{2+} channels. Voltage-gated K^+ current was tested in another 69 cells with normal internal and external solutions. The outward K^+ current was evoked by two sequential 200 ms depolarizing pulses to 60 mV at 1 s interval from a holding potential of −100 and −40 mV, respectively. (Fig. 1C). This protocol is designed to detect both transient activation outward K^+ current (first pulse) and slow activation outward K^+ current (second pulse). Outward K^+ current elicited by this double-pulse protocol from fibroblasts produced the current with almost identical amplitudes and kinetics. The fast activating or fast inactivating current component was not observed

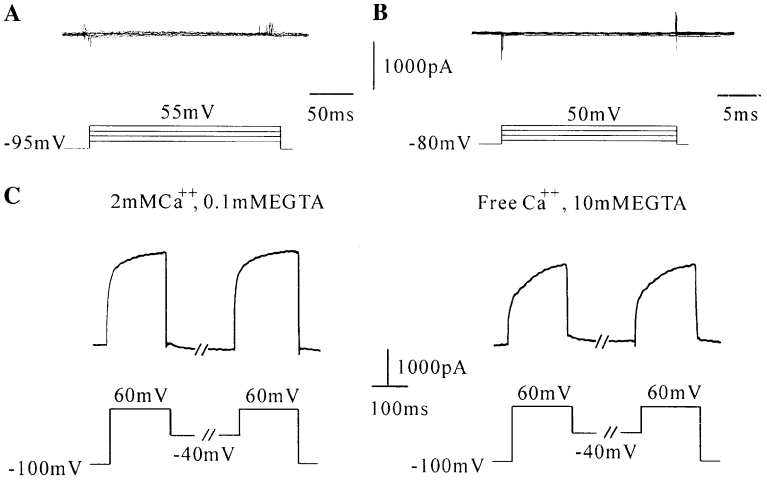

Fig. 1. The voltage-dependent membrane currents in rat vascular fibroblasts. (A) No Na^+ currents were obtained when current was evoked by a set of increment depolarizing pulses. The cells were held at −95 mV and $CsCl_2$ was added into pipette solution for eliminating the outward K^+ current. (B) No Ca^{2+} currents were recorded when the cells were held at −80 mV and depolarized by 10 mV steps from −60 to 40 mV using a high concentration of Ba^{2+} in bath solution. (C) The potassium currents were evoked by two sequential 200 ms depolarizing pulses to 60 mV at a 1 s interval. The holding potentials were set at −100 mV (first pulse) and −40 mV (second pulse). The left current traces were recorded using Ca^{2+} bath solution and internal solution containing 0.1 mM EGTA. The right current was obtained using free Ca^{2+} external solution and internal solution containing 10 mM EGTA.

with strong depolarization from a holding potential of −100 mV to the first pulse. These results suggest the absence of fast inactivating K^+ currents (I_A) in the rat vascular fibroblasts. Instead, a slow activating K^+ current was observed. Two types of outward K^+ currents, delay rectified outward K^+ current and calcium-activated K^+ current, can display this type of slow activation kinetics. To distinguish between those two types of K^+ currents, we repeated the experiment under condition, designed to dissect out the Ca^{2+}-activated K^+ current, i.e., 10 mM EGTA was added to pipette solution and Ca^{2+} was removed from the bath solution. Under the latter condition, intracellular Ca^{2+} would be lowered, thereby, decreasing the main stimulus for the gating of Ca^{2+}-activated K^+ channels. As shown in Fig. 1C (right) there was a ~20% decrease in current amplitude (from 1659.0 ± 447.6 pA to 1291.1 ± 422.7 pA for normal and for lowered Ca^{2+}), the slowly activating current component being preserved. These data are consistent with the idea that rat vascular fibroblasts express a set of slow activating K^+ channels, including Ca^{2+}-dependent and Ca^{2+}-independent K^+ channels, but do not express the fast inactivating K^+ channels (I_A). Similar results were obtained among experiments using cells from third to seventh passages (data not shown). No significant change in the type or property of membrane current was found during different passages of cultured vascular fibroblasts as long as they were not exposed to TGF-B1.

Fig. 2 shows the voltage-dependent activation and steady-state inactivation characteristic of outward K^+ in rat fibroblasts. For the voltage-dependent activation,

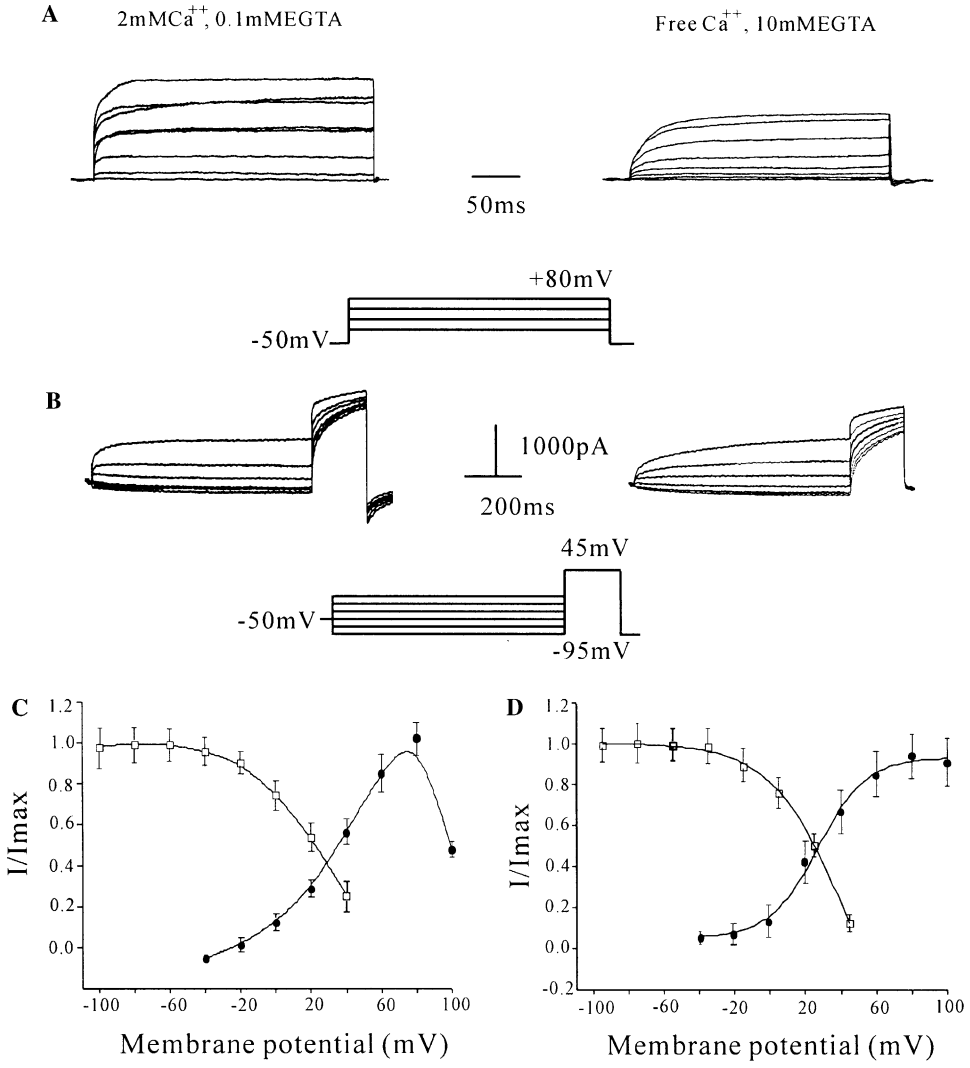

Fig. 2. The steady-state activation and inactivation properties of slowly activating K^+ current recorded in the absence (left) and presence (right) of 10 mM EGTA in the pipette solution. (A) Voltage-dependent activation evoked by depolarizing step commends between −50 and 80 mV. (B) Voltage-dependent inactivation. The membrane potential was held at −50 mV and 1-s conditioning prepulses between −100 and 40 mV were applied before the test pulse to 45 mV. (C) and (D) Voltage-dependent activation curve (•) and steady-state inactivation curve (○) resulting from the K^+ current recorded in the presence and absence of 10 mM EGTA in the internal solution. The peak current amplitude normalized to the maximal current was plotted against the command potential or prepulse potential. Data points were fitted with a Boltzmann function.

the membrane potential was held at −50 mV and the outward K^+ current was evoked by 200 ms depolarized pulses from −60 to 80 mV in steps of 20 mV at 10 s intervals (Fig. 2A). When recording in the presence of Ca^{2+} in the bath solution, the current evoked by the depolarizing pulse above 40 mV and the peak current amplitude increased no more with voltage increment, and the voltage–current relationship curve came into being in a bell form (Fig. 2C, solid circle). After removing Ca^{2+} from the bath solution and adding EGTA to the internal solution, the voltage–current relationship curve became a typical outward rectifier (Fig. 2D, solid circle). For voltage-dependence of the steady-state inactivation, the currents were elicited by using a 1 s conditioning prepulse to various potentials between −100 and 45 mV, followed by a fixed test pulse to 50 mV to evoke outward current (Fig. 2B). The current evoked from each holding potential normalized as a fraction of the maximum current is plotted to give a steady-state inactivation curve. The data shown in Fig. 2C (open circle) and Fig. 2D (open circle) from the K^+ current recorded in different external and internal solutions gave similar inactivation kinetics.

The K^+ channels expressed on rat vascular fibroblasts were then investigated by pharmacological means (Fig. 3). 4-AP, a classic blocker for fast inactivating K^+ channel, was applied to 5 cells.In response to the external application of 4-AP (5 mM), the amplitude of outward current did not change significantly (663.8 ± 155.8 and 639.8 ± 151.5 pA in the absence and presence of 4-AP, $n = 5$, $P > 0.05$). However, the outward current was significantly and reversibly inhibited by the application of ChTX, an antagonist that preferentially blocks calcium-activated K^+ channels. Addition of ChTX (10 nM) to the extracellular solution reduced the current amplitude from 1422.4 ± 168.2 to 664.6 ± 86.21 pA ($n = 27$, $P < 0.05$).

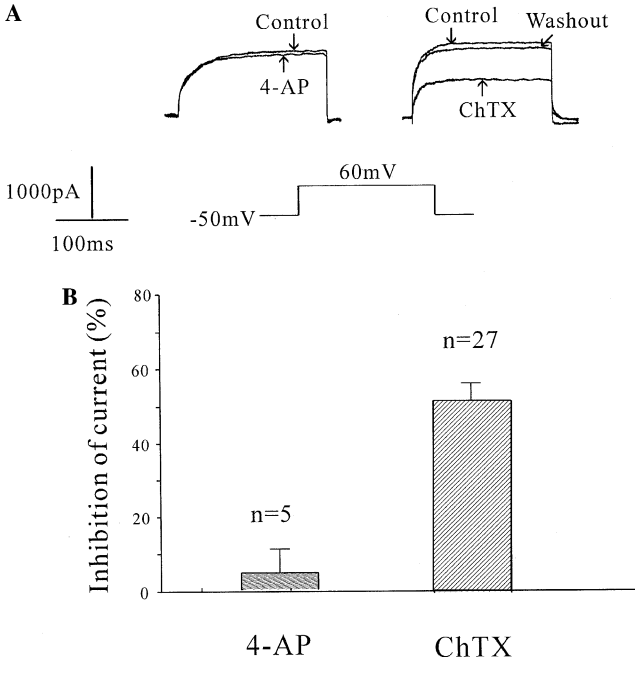

Fig. 3. The effects of different K^+ channel blockers on the K^+ current recorded from rat vascular fibroblasts. Superimposed K^+ current traces obtained during 200 ms depolarizing step pulse from −50 to 60 mV in the presence and absence of 4-AP (5 mM) and ChTX (15 nM). (B) Percentage inhibition of 4-AP and ChTX on the K^+ current. The number above each column indicated the number of cells tested.

TGF-β1 induced the expression of fast inactivating K^+ channel in rat vascular fibroblasts

Before investigating the effect of TGF-β1 on the expression of ion channel in rat vascular fibroblasts, the action of serum-free treatment on ion channel was checked. Type and kinetic property of membrane currents in rat fibroblasts cultured in serum-free medium without TGF-β1 were not modulated or changed (results not shown). After incubation with TGF-B1 for 24 h a significant change in the activation and inactivation properties of outward currents was observed in 48 out of 92 cells. When K^+ current was evoked by the same protocol used for on-treatment fibroblast shown in Fig. 2A, TGF-B1 exposure led to a K^+ current with faster activation (20–30 ms) and the appearance of channel inactivation (compare AF and MF traces of Fig. 4A). Then we repeated the experiment by using double depolarizing pulse that was used as shown in Fig. 1. When the membrane potential was held at −100 mV, the first pulse elicited a global outward current that activated rapidly (5–10 ms) and then clearly decayed with time. After 1 s interval at a conditioning potential of −40 mV, the second depolarizing step evoked only a slowly activating outward K^+ current. Subtraction of the outward K^+ current evoked at a holding potential of −40 mV from the global K^+ current elicited at a holding potential of −100 mV has isolated a fast activating and fast inactivating K^+ current (Fig. 4B). This fast transient outward current looks like an A-type K^+ current that has been described in many cell types such as myocardial cells and neuron. The steady-state activation and inactivation of outward K^+ in rat myofibroblasts were then investigated with the same voltage protocol for fibroblasts shown in Fig. 1 (Fig. 4C). The voltage-dependence of the steady-state activation and inactivation curves shown in Fig. 4D give a half-inactivation at −44.05 ± 8.65 mV ($n = 11$) and inactivation was completed at a potential positive to 20 mV. Both the transient activation and inactivation or steady-state activation and inactivation of K^+ current induced by TGF-β1 in myofibroblasts were totally changed.

Subsequently, a set of experiments were conducted for addressing the question whether the activation or inactivation kinetics of original K^+ current was modu-

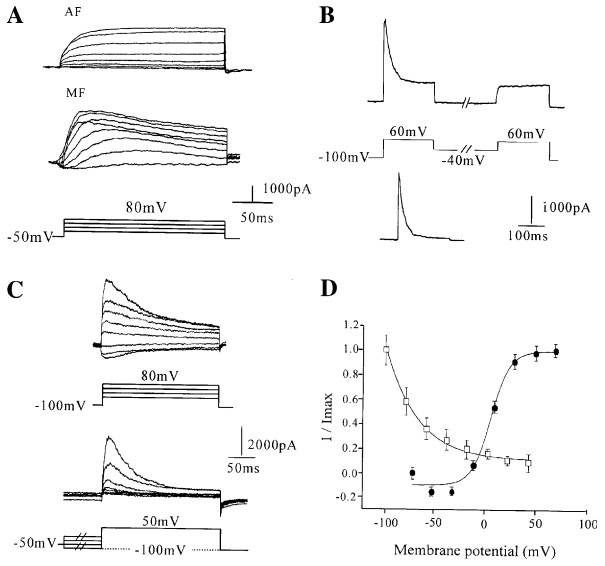

Fig. 4. The voltage-dependent K^+ currents recorded in vascular fibroblasts after 24 h of incubation with TGF-β1. (A) K^+ currents were evoked from a holding potential of −50 mV by 20 mV steps to 80 mV and were obtained in untreated fibroblasts (AF) and myofibroblasts treated by TGF-β1 (MF). (B) The K^+ currents evoked by two sequential 200 ms depolarizing pulses to 60 mV at a 1 s interval. The holding potentials were set to −100 mV (first pulse) and −40 mV (second pulse) for separating the two types of K^+ currents. Subtracting the residual sustained current elicited by second pulse from the overall currents obtained by the first pulse isolates a fast activating and fast inactivating K^+ current shown at below. (C) Voltage-dependent steady-state activation and inactivation of outward K^+ currents obtained in fibroblasts treated by TGF-β1. For the activation, the membrane potential was held at −100 mV and the outward K^+ current was evoked by 200 ms depolarized pulses from −60 to 80 mV in steps of 20 mV at 10 s intervals. For voltage-dependence of the steady-state inactivation, the membrane potential was held at −50 mV and 1-s conditioning prepulses between −100 and 40 mV were applied before the test pulse to 50 mV. (D) Voltage-dependent activation (•) and steady-state inactivation (○) curves of K^+ current in myofibroblasts. Data points were obtained from 11 independent cells and were fitted with a Boltzmann function.

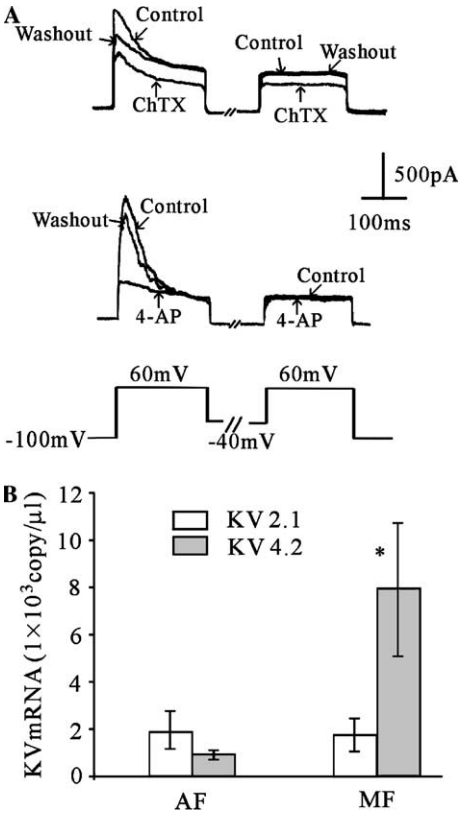

Fig. 5. Pharmacological characters of fast inactivating K^+ current and quantification of K_V gene expression in the fibroblasts induced by TGF-β1. (A) The K^+ currents evoked by double voltage protocol. 4-AP markedly inhibits the transient activated current evoked by first depolarizing pulse, hardly affecting the current elicited by second pulse. ChTX parallely inhibited the overall K^+ current and the current evoked by second pulse. (B) $K_{V2.1}$ and $K_{V4.2}$ mRNA expression was measured in rat adventitial fibroblasts (AF, open bars) and in fibroblasts treated with TGF β1 (20 ng/ml) for 24 h (MF, solid bars). Quantification of K_V gene expression was made in 4 experiments. $^*P < 0.05$, compared with untreated fibroblasts.

lated, or an additional fast transient activated K^+ current was induced by TGF-β1 in rat vascular fibroblasts. We first tested the effect of 4-AP on the two K^+ current components evoked by two sequential 200 ms depolarizing pulses. While the current was elicited by a depolarizing pulse from a holding potential of −100 mV, the addition of 4-AP (5 mM) to the extracellular solution produced a reversible decrease of overall outward current with a predominant inhibitory effect on the early inactivating component. It is evident that 4-AP hardly affected the latter current evoked by the second depolarizing pulse. In contrast, ChTX parallely inhibited the residual sustained component of K^+ current elicited by the first depolarizing pulse and the K^+ current evoked by the second depolarizing pulse (Fig. 5A). These results suggested that TGF-β1 induced a new fast inactivating outward K^+ current in myofibroblasts and which might be a 4-AP sensitive A-type current.

For supporting the hypothesis that TGF-β1 can induce the expression of I_A channel, the mRNA expression of K_V genes $K_{V2.1}$ and $K_{V4.2}$ in fibroblasts and myofirbroblasts was measured using a real-time quantitative RT-PCR technique. As shown in Fig. 5B, $K_{V4.2}$ mRNA expression at fibroblast was very low and was significantly increased in fibroblasts treated by TGF-β1 compared with untreated fibroblasts ($P < 0.05$). In contrast, $K_{V2.1}$ mRNA expression was not significantly different between TGF-β1 treated- or untreated-fibroblasts ($P > 0.05$).

Discussion

The physiological function of K^+ channels and their roles in nerve system and cardiovascular diseases has been extensively investigated [14]. To date, at least four types of K^+ channel currents were identified in peripheral vascular smooth muscle cells, i.e., voltage-

dependent outward K_V channel, calcium-activated K_{Ca} channels, ATP-sensitive K_{ATP} channel, and inward rectifier K_{ir} channel [15]. The voltage-dependent outward K_V channel current could be separated into a transient outward K^+ current (I_A) and a delayed rectifier K^+ current (I_K) by different voltage protocols and the specific blocker of K^+ channel [16]. In non-excitable cells such as human T-lymphocytes, endothelial cells, and a variety of fibroblast cell lines, functional I_K and I_{KCa} have been detected and were considered as a more dominant K^+ channel current [17,18]. However, the membrane current including K^+ channel current in vascular fibroblasts has not been systemically identified in previously studies. In our results, neither calcium current nor sodium current can be found in the rat ventricular fibroblast. The global outward K^+ current amplitude in cell dialyzed with 0.1 mM EGTA was a little different from that measured in cell dialyzed with 10 mM EGTA. The remaining current amplitude was held at about 60–70% of overall current, and furthermore, *I–V* current became a typical outward rectifier phenomenon. The steady-state activation and inactivation kinetic properties show a window current at −50–60 mV and were similar to those described for I_K in several other cell types [19,20]. Therefore, our data might suggest that slow activating and non-activating K^+ current is only one of the membrane currents that existed in rat ventricular fibroblast and moreover, at least two types of K^+ channels, Ca^{2+}-dependent and Ca^{2+}-independent K^+ channels, were involved.

Exogenous TGF-β1 has been shown to induce adventitial differentiation from fibroblasts to myofibroblasts in arteries [21,22]. The most important finding in the present study is that TGF-β1 induced the expression of an I_A channel during the differentiation of rat vascular fibroblasts to myofibroblasts. In myofibroblasts, the same voltage protocol at a holding potential of −50 mV evoked a K^+ current with faster activated and inactivated kinetics. However our results suggest that the new current is an I_A other than the I_K and K_{Ca} with modulated kinetics. We have proposed that TGF-β1 does not modulate the existing I_K channel, leading to alterations in the electrophysiological and pharmacological properties of the I_K. By contrast, the expression of a new I_A channel takes place in TGF-β1-induced myofibroblasts, since: (1) the transient activation was accelerated whereas inactivation was removed by hyperpolarization, a typical character of I_A; (2) the steady-state activation and inactivation curves plotted by the early component of current have shifted to a more negative, voltage range similar to I_A, (3) 4-AP, an I_A channel blocker in vascular smooth muscle cells and brain, does completely abolish the transiently activated component and the original current remains. Except the pharmacological and electrophysiological data, the expression of I_A channel induced by TGF-β1 in vascular myofibroblasts was further confirmed by molecular biological data. The expression of K_V genes at mRNA levels from the fibroblasts and myofirbroblasts was observed using a quantitative RT-PCR technique in our study. The transcriptional expression of many subunits encoding either I_K or I_A has been demonstrated in many tissues. It was reported that the mRNA of $K_{V1.2}$, $K_{V1.3}$, $K_{V2.1}$, and $K_{V2.2}$ transcribed from K_V genes encoding delayed rectifier I_K channel and the mRNA of $K_{V4.1}$ and $K_{V4.2}$ transcribed from the gene encoding I_A channel were more frequently detected in cardiovascular tissue and brain [23–25]. Thereby, $K_{V2.1}$ as a major component of delayed rectifier channel and $K_{V4.2}$ as an α-subunit of I_A channel were selectively chosen in our experiment (see Fig. 5). The RT-PCR results demonstrated that $K_{V2.1}$ expression could be observed in fibroblasts and myofibroblasts. Moreover, $K_{V4.2}$ was detected only in vascular fibroblasts after incubating with TGF-β1 for 24 h. These results indicated the transcriptional expression of specific K_V channel gene induced by TGF-β1 corresponding to the I_A current recording by patch-clamp technique in rat vascular myofibroblasts.

Four separate classes of K^+ channel, L-type Ca^{2+} channel, and Na^+ channel have so far been identified in vascular smooth muscle cells [26,27]. Although I_K and I_A still are two major types of K_V channels current in these cells, I_K or Kca does coexist with I_A in a few types of vascular smooth muscle cells, including those from rat pulmonary and rabbit pulmonary arteries [28,29]. Our results indicated that the types and characteristics of membrane ion channels in myofibroblasts induced by TGF-β1 are different from those of the vascular fibroblasts. As neither a Ca^{2+} current nor a Na^+ current has been recorded after incubating cells with TGF-β1, the ion channels in myofibroblasts are also different from the vascular smooth muscle cells even though I_A channel is present in the cells. It has been suggested that TGF-β1 may play a role in inducing the differentiation from fibroblasts to myofibroblasts, but TGF-β1 could not transform the vascular fibroblasts to vascular smooth muscles cells. Furthermore, it may be argued that in our experiment when a −100 mV of holding potential was used to record I_A, an inward current was evoked in myofibroblasts. Applying Ca^{2+} channel and Na^+ channel blockers, the current is still present in all experiments (results not shown). Whether anyother inward cation channel passed by K^+ was involved, further investigation needs to be conducted.

Acknowledgments

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References

[1] Y. Shi, M. Pieniek, A. Fard, J. O'Brien, J.D. Mannion, A. Zalewski, Adventitial remodeling after coronary arterial injury, Circulation 93 (1996) 340–348.

[2] N.A. Scott, G.D. Cipolla, C.E. Ross, B. Dunn, F.H. Martin, L. Simonet, J. N.Wilcox, Identification of a potential role for the adventitia in vascular lesion formation after balloon overstretch injury of porcine coronary arteries, Circulation 93 (1996) 2178–2187.

[3] S. Yi, E. James Jr., O'Brien, F. Ali, Z. Andrew, Transforming growth factor-β1 expression and myofibroblast formation during arterial repair arteriosclerosis, Thromb. Vasc. Biol. 16 (1996) 1298–1305.

[4] S. Hashimoto, Y. Gon, I. Takeshita, K. Matsumoto, S. Maruoka, T. Horie, Transforming growth factor-βl induces phenotypic modulation of human lung fibroblasts to myofibroblast through a c-jun-NH2-terminal kinase-dependent pathway, Am. J. Respir. Crit. Care. Med. 163 (2001) 152–157.

[5] I.M. Khouw, P.B. van Wachem, J.A. Plantinga, Z. Vujaskovic, M.J. Wissink, L.F. De Leij, M.J. van Luyn, TGF-β and FGF affect the differentiation of proliferating porcine fibroblasts into myofibroblasts in vitro, Biomaterial 20 (1999) 1815–1822.

[6] M. Eghball, R. Tomek, C. Woods, B. Bhambi, Cardiac fibroblasts are predisposed to convert into myocyte phenotype: specific effect of transforming growth factor β, Proc. Natl. Acad. Sci. USA 88 (1991) 795–797.

[7] S.G. Rane, Potassium channels as targets for modulating cell growth and differentiation, in: H. Darbon, J.M. Sabatier (Eds.), Perspectives in Drug Discovery and Design, Kluwer Academic Publishers, Dordrecht, 1999, pp. 295–311.

[8] C. Kopta, J.H. Steinbath, Comparison of mammalian adult and fetal nicotinic acetylcholine receptors stably expressed in fibroblasts, J. Neurosci. 14 (1994) 3922–3933.

[9] S.G. Rane, Ion channels as physiological effectors for growth factor receptor and ras/ERK signal pathways, Adc. Second. Messenger. Phosphoprotein. Res. 33 (1999) 107–127.

[10] T.L. Pena, S.H. Chen, S.F. Konieczny, S.G. Rane, Ras/MEK/ERK up-regulation of the fibroblast K_{Ca} channel FIK is a common mechanism for basic fibroblast growth factor and transforming growth factor-β suppression of myogenesis, J. Biol. Chem. 275 (2000) 13677–13682.

[11] D.L. Zhu, T. Herembert, P. Marche, Increased proliferation of adventitial fibroblasts from spontaneously hypertensive rat aorta, J. Hypertens. 9 (1991) 1161–1168.

[13] P.J. Gao, D.L. Zhu, Y.Y. Chen, J. Yi, Phenotypic transition of vascular smooth muscle cells and protein kinase Cα, Chin. J. Cardiol. 27 (1999) 453–455.

[14] C.G. Sobey, Potassium channel function in vascular disease, Arterioscler. Thromb. Vasc. Biol. 21 (2001) 28–38.

[15] Y. Lu, J. Zhang, G. Tang, R. Wang, Modulation of voltage-dependent K^+ channel current in vascular smooth muscle cells from rat mesenteric arteries, J. Membr. Biol. 180 (2001) 163–175.

[16] Y.A. Mei, E. Louiset, H. Vaudry, L. Cazin, A-type potassium current modulated by A1-adenosine receptor in frog melanotrophs, J. Physiol. 489 (1995) 431–442.

[17] S. Cai, L. Garneau, R. Sauve, Single-channel characterization of the pharmacological properties of the K(Ca^{2+}) channel of intermediate conductance in bovine aortic endothelia cells, J. Membr. Biol. 163 (1998) 147–158.

[18] R.S. Lewis, M.D. Cahalan, Potassium and calcium channels in lymphocytes, Annu. Rev. Immunol. 13 (1995) 623–653.

[19] N. Leblanc, X. Wan, P.M. Leung, Physiological role of $Ca^{(2+)}$-activated and voltage-dependent K^+ currents in rabbit coronary myocytes, Am. J. Physiol. 266 (1994) C1523–C1537.

[20] C.L. Huan, M.O. Zhou, M.M. Wu, Z.H. Zhang, Y.A. Mei, Activation of melatonin receptor increases a delayed rectifier K^+ current in rat cerebellar granule cells, Brain Res. 917 (2001) 182–190.

[21] M.B. Vaughan, E.W. Howard, J.J. Tomasek, Transforming growth factor-β1 promotes the morphological and functional differentiation of the myofibroblast, Exp. Cell Res. 257 (2000) 180–189.

[22] P.A. Folger, D. Zekaria, G. Grotendorst, S.K. Masur, Transforming growth factor-β-stimulated connective tissue growth factor expression during corneal myofibroblast differentiation, Invest. Ophthalmol. Vis. Sci. 42 (2001) 2534–2541.

[23] H. Murakoshi, J.S. Trimmer, Identification of the $K_{V2.1}$ K^+ channel as a major component of the delayed rectifier K^+ current in rat hippocampal neurons, J. Neurosci. 19 (1999) 1728–1735.

[24] C.X. Xu, Y.J. Lu, G.H. Tang, R. Wang, Expression of voltage-dependent K^+ channel genes in mesenteric artery smooth muscle cells, Am. J. Physiol. (1999) G1055–G1063.

[25] S.L. Archer, E. Souil, A.T. Dinh-Xuan, B. Schremmer, J.C. Mercier, A. Ei Yaagoubi, L. Nguyen-Huu, H.L. Reeve, V. Hampl, Molecular identification of role of voltage-gated K^+ channels KV1.5 and Kv2.1, in hypoxic pulmonary vasoconstriction and control of resting membrane in rat pulmonary artery myocytes, J. Clin. Invest. 101 (1998) 2319–2330.

[26] G.V. Perkov, F. Fusi, S. Saponara, H.S. Gagov, G.P. Sgaragli, K.K. Boev, Characterization of voltage-gated calcium currents in freshly isolated smooth muscle cells from rat tail main artery, Acta Physiol. Scand. 173 (2001) 257–265.

[27] A.E. Belevych, A.V. Zima, I.A. Vladimirova, H. Hirata, A. Jurkiewicz, N.H. Jurkiewicz, M.F. Shuba, TTX-sensitive $Na^{(+)}$ and nifedipine-sensitive $Ca^{(2+)}$ channels in rat vas deferens smooth muscle cells, Biochim. Biophys. Acta 1419 (1999) 343–352.

[28] S.V. Smirnov, P.I. Aaronson, Inhibition of vascular smooth muscle cell K^+ currents by tyrosine kinase inhibitors genistein and ST 638, Circ. Res. 76 (1995) 310–316.

[29] X.J. Yuan, M.L. Tod, L.J. Rubin, M.P. Blaustein, Inhibition of cytochrome p-450 reduces voltage-gated K^+ current in pulmonary arterial myocytes, Am. J. Physiol. 268 (1995) C259–C270.