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# 17$\beta$-Estradiol Inhibits Outward Voltage-Gated $K^+$ Currents in Human Osteoblast-Like MG63 Cells

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**Abstract** Previous studies have shown that 17$\beta$-estradiol has a pivotal function by blocking voltage-gated $K^+$ (Kv) channels in several different types of cells such as cardiac myocytes and neurons. Outward Kv currents can also be measured in osteoblasts, although little is known about the effects of 17$\beta$-estradiol on these currents. In human osteoblast-like MG63 cells, we found that 17$\beta$-estradiol inhibits peak and end Kv currents, with $IC_{50}$ values of 480 and 325 nM, respectively. To elucidate the mechanism of inhibition, the kinetics of Kv currents were investigated. The half-maximum activation potential ($V_{1/2}$) was 1.3 mV and was shifted left to −4.4 mV after application of 500 nM 17$\beta$-estradiol. For steady-state inactivation, the $V_{1/2}$ was –55.0 mV and weakly shifted left to –58.2 mV. To identify the molecular basis of outward Kv currents in MG63 cells, we performed RT-PCR analyses. The expression of Kv2.1 channels appeared to dominate over that of other Kv channels in MG63 cells. In COS-7 cells with heterologously expressed Kv2.1 channels, 17$\beta$-estradiol also inhibits macroscopic currents of Kv2.1. Our data indicate that 17$\beta$-estradiol inhibits Kv currents in human osteoblast-like MG63 cells and that Kv2.1 is a potential molecular correlate of outward Kv currents in these cells.

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## Introduction

Remodeling is an important dynamic process of balance between bone formation and resorption. Osteoblasts derived from mononuclear cells of mesenchymal origin are responsible for the formation of bone and the counterbalancing of resorption (a crucial function of osteoclasts). Disturbance of this balance may lead to bone loss and osteoporosis, which frequently occurs in postmenopausal women.

Estrogen is an important factor for the development and growth of bones. Bone loss due to postmenopausal estrogen deficiency is a common cause of fractures in women. Estrogen replacement therapy reduces the incidence of bone disease and is an important preventative therapy for aging-related diseases in women; however, the actual underlying mechanism of the therapy remains unclear.

Previous work suggested that there are several $K^+$ channels in osteoblasts, including voltage-gated $K^+$ (Kv) channels (Li et al. 2005, 2006; Yellowley et al. 1998), inwardly rectifying $K^+$ (Kir) channels (Yellowley et al. 1998), ATP-sensitive $K^+$ channels (Moreau et al. 1997), calcium-activated $K^+$ channels (Henney et al. 2009; Moreau et al. 1996; Weskamp et al. 2000) and tandem-pore $K^+$ (K2P) channels (Hughes et al. 2006). Their possible functions in osteoblasts were explored by researchers in the past decade. BK channels affect both the growth and mineralization of osteoblasts (Henney et al. 2009). TREK-1, a member of the K2P channel family, contributes to the resting membrane potential of human osteoblast cells and was correlated with the proliferation of MG63 cells (Hughes et al. 2006). Although Kv channels have been shown to play crucial roles in the action potential of cardiomyocytes

(Nerbonne and Kass 2005; Nerbonne et al. 2001) and excitation transmission in neurons (Johnston et al. 2010; Rasband 2010), their function in osteoblasts is poorly understood.

Researchers reported that 17$\beta$-estradiol blocked Kv channels in several different types of cells, such as cardiac myocytes (Moller and Netzer 2006) and neurons (Druzin et al. 2011; Fatehi et al. 2005). To date, few studies have investigated whether estrogen acts on Kv channels and, thus, regulates the function of osteoblasts. In the present study, we employed the patch-clamp technique to investigate the effects of 17$\beta$-estradiol on Kv channel currents in human osteoblast-like MG63 cells, and we used RT-PCR (and an expression system) to explore the molecular basis of the measured currents. Our results present a possible alternative mechanism of the effects of estrogen on bone.

## Materials and Methods

### Cell Culture and Transfection

Human osteoblast-like MG63 cells were cultured in RPMI-1640 medium, supplemented with 10 % (v/v) newborn calf serum, 100 U/ml penicillin and 100 μg/ml streptomycin. Cells were maintained in a humidified atmosphere of 5 % $CO_2$ and 95 % air at 37 °C. The medium was changed every 2 days, and confluent cells were digested with 0.25 % trypsin and split at a rate of 1:2–1:3 every 4 days. For patch-clamp recordings, cells were resuspended and plated onto 22-mm coverslips in Petri dishes and subsequently transferred into the recording chamber.

COS-7 (kidney fibroblast cell line) cells were cultured in DMEM with 10 % (v/v) newborn calf serum, 100 U/ml penicillin and 100 μg/ml streptomycin. COS-7 cells were seeded at low density (23,000 cells/35-mm dish) for 1 day prior to transfection and then cotransfected with green fluorescent protein (GFP; Clontech, Palo Alto, CA) using Lipofectamine (Life Technologies, Bethesda, MD) according to the manufacturer's protocol.

### Expression Analysis

Total RNA was prepared from MG63 cells using RNA-Solv® Reagent (Omega Bio-tek, Norcross, GA) and further treated with DNaseI (Invitrogen, Carlsbad, CA). Total MG63 RNA (1 μg) was reverse transcribed (RT) with oligo(dT) and M-MLV reverse transcriptase (Invitrogen).

The sense and antisense PCR oligonucleotide primers chosen to amplify cDNA are presented in Table 1. One microliter of the first-strand cDNA reaction mixture was used in a 25-μl PCR consisting of 0.4 nM of each primer, 10 mM Tris–HCl, 50 mM KCl, 1.5 mM $MgCl_2$, 200 μM each dNTP and 0.625 units of Taq DNA polymerase. For semiquantitative PCR, the number of PCR cycles was selected to be within the range of the linear amplification for each transcript. The PCR conditions were 95 °C for 5 min, followed by 18–40 cycles of 95 °C for 30 s, 60 °C for 30 s, 72 °C for 30 s and finally 72 °C for 10 min. To analyze the relative amount of PCR products of the α-subunits of Kv channels, an invariant mRNA of GAPDH was used as an internal control.

### Electrophysiology

The effects of 17$\beta$-estradiol on voltage-gated $K^+$ currents in MG63 cells and Kv2.1 currents in COS-7 cells were investigated using the whole-cell patch-clamp technique. The bath solution contained (in mM) 75 Na-gluconate, 70 NaCl, 5 KCl, 1 $MgCl_2$, 5 HEPES and 5 glucose, with pH adjusted to 7.4 using NaOH; the pipette solution contained (in mM) 150 KCl, 2 $MgCl_2$, 5 HEPES, 5 EGTA, 5 glucose and 5 $Na_2ATP$, with pH adjusted to 7.3 using KOH. MG63 or COS-7 cells grown on coverslips were transferred to a chamber, which was mounted on the stage of an inverted microscope. Patch pipettes were fabricated using a P-97 micropipette puller (Sutter Instrument, Novato, CA) and the electrical resistance ranged between 1.5 and 3.0 MΩ.

### Analysis and Statistics

For analysis of the current–voltage relationship (*I–V*), peak currents at each test potential were measured as maximal amplitudes at start currents and end currents were the remaining currents at the end of test pulses. In some cases, current density was obtained for the current density–voltage relationship by normalization to cell capacitance, which was measured by applying a 10-mV ramp pulse of 5-ms duration to the cell. Membrane capacitance (*Cm*) was determined by the equation $Cm = (I \times t)/V$, where $I$ is current amplitude and the values of $t$ and $V$ for ramp pulse are 5 ms and 10 mV, respectively.

Concentration–response curves were obtained by perfusion of 1, 10, 100, 1000, 10,000 and 100,000 nM 17$\beta$-estradiol. Responses were plotted relative to the control. The response function was fitted by the Hill equation, $y = I_{min} + (I_{max}–I_{min})/[1 + (IC_{50}/x)^h]$, where $I$ is current with maximum and minimum, $IC_{50}$ is the concentration producing half-maximal inhibition and $h$ is the Hill coefficient.

For the activation and steady-state inactivation curve, the current amplitude during each test pulse was normalized to the maximal current ($I/I_{max}$) and plotted against the voltage during the conditioning prepulse. These data were fitted by a Boltzmann equation, $I/I_{Max} = 1/[1 + \exp(V_{1/2} - V_m)/k]$, where $V_{1/2}$ is the conditioning potential that gives $I/I_{max} = 0.5$, $V_m$ is the conditioning potential and $k$ describes the steepness of the curve.

**Table 1** Oligonucleotide sequences of primers used for RT-PCR

| Gene | GI | Forward primer | Reverse primer | Length (bp) |
|---|---|---|---|---|
| Kv1.1 | 119395747 | CATCGTGGAAACGCTGTGTAT | AACCCTTACCAAGCGGATGAC | 232 |
| Kv1.2 | 324021689 | GAACCGCCCTAGCTTTGATG | TCTCCCAGCTCATAAAACCGA | 120 |
| Kv1.3 | 88758564 | TTTTCTCCAGCGCGGTCTAC | CATATCGCCGTAACCCACTGT | 119 |
| Kv1.4 | 325197144 | ATGAGTGTTCCTACACGGATCT | CCTGACACATTTATCACCACACG | 106 |
| Kv1.5 | 25952086 | GTCTCCCTGGACGTGTTCG | TGCTGCTCTGCCAGTTCG | 569 |
| Kv2.1 | 27436972 | TGCAACACGCACGACTCGCT | ATGCGATGCACACGGCCTCC | 529 |
| Kv2.2 | 27436973 | CCGGGCTTAAACAGGAAGACT | AGGCTCTCGTGTGTGTTGC | 200 |
| Kv3.1 | 163792199 | GCCCCAACAAGGTAGAGTTCA | TGGCGGGTCAGCTTAAAGATG | 181 |
| Kv3.2 | 24497455 | ACCCCTACTCGTCCAGAGC | ACAACACTTGTGCCATTGATGA | 151 |
| Kv3.3 | 24497459 | CCTTCCTGACCTACGTGGAG | CGATGATGTTGAGGCTGCTTT | 117 |
| Kv3.4 | 301500673 | ATATCGACCGCAACGTGACAG | GCAGGTTCTTGACGAAGTCCA | 186 |
| Kv4.1 | 47078279 | GGAAGAATACGCTGGACCG | GCGGCATGGGATGGTCTC | 472 |
| Kv4.2 | 27436982 | TCAGCGGAGCCTTTGTCA | TGGTGGTGCTGGGTTTCA | 586 |
| Kv4.3 | 320089591 | TGGTGCCTAAGACGATTGC | CTGCTCACTGCCCTGGAT | 632 |
| Kv5.1 | 27436998 | GAGTCGTCGTGCCCGGC | GTCTCTGGATGGCTCTGCTC | 530 |
| Kv9.3 | 25952107 | GCAGTCTGTTGACCAAAGCA | CCAGTACTCGATCTCCTGGC | 244 |
| Herg1 | 325651833 | TTCGACCTGCTCATCTTCGG | CGATGCGTGAGTCCATGTGT | 229 |
| Herg2 | 27886649 | GACGGCTTCTGCGAACTCTT | CCTTGCGGTAGTAGAGGATGTC | 169 |
| Herg3 | 319918833 | CGAGACCAAGAGGCATGATATTG | GGGTGGCAGCGTTTTCATT | 206 |
| KvLQT1 | 2465514 | GGAGCCACACTCTGCTGTC | CTTACAGAACTGTCATAGCCGTC | 196 |
| KCNQ2 | 26051267 | CATCCTGGAAATCGTGACTATCG | TTCCGGGCAAACTTGAGCC | 117 |
| GAPDH | 83641890 | ACGACCACTTTGTCAAGCTC | GTGAGGAGGGGAGATTCAGT | 220 |

*Kv* voltage-gated $K^+$ channel, *Herg* Human eag-related $K^+$ channel, *GAPDH* glyceraldehyde-3-phosphate dehydrogenase

Results are expressed as mean ± SE. Statistical significance was calculated using Student's *t* test, and $P < 0.05$ was accepted for statistical difference. All experiments were carried out at room temperature (22 °C).

## Results

### 17$\beta$-Estradiol Inhibits Outward Kv Currents in MG63 Cells

At a holding potential of –80 mV, the outward Kv currents in MG63 cells were elicited by depolarization to test potentials ranging from –80 to +60 mV (Fig. 1a, b). Similar currents which are sensitive to TEA, a general blocker of Kv channels, have been described before (Yellowley et al. 1998). Application of 500 nM 17$\beta$-estradiol, a steroid sex hormone, rapidly reduced the whole-cell currents (Fig. 1a, b). In addition, two current density–voltage curves (*I–V*), which were obtained by current amplitudes measured at peak and the end of the test pulse, respectively, clearly displayed the inhibitory effects of 17$\beta$-estradiol on outward Kv currents at different potentials (Fig. 1c). At +60 mV, 500 nM 17$\beta$-estradiol significantly declined peak currents from 25.9 ± 1.7 to 18.7 ± 1.1 pA/pF and end currents from 23.4 ± 1.4 to 10.8 ± 1.1 nA/pF ($n = 5$, $p < 0.05$). Interestingly, the action of 17$\beta$-estradiol was more potent at end currents than at peak currents. A lower concentration of 17$\beta$-estradiol (10 nM) also weakly and significantly blocked these currents. However, 17$\beta$-estradiol did not affect inward currents, even at extremely high concentration (10 μM).

A concentration–response curve for the inhibitory effect of 17$\beta$-estradiol was plotted. The data were well fitted by the Hill equation (see Materials and methods) with $IC_{50}$ values of 480 nM for the peak current and 325 nM for the end current, suggesting that the latter is more sensitive to 17$\beta$-estradiol (Fig. 2a, b)

### Effects of 17$\beta$-Estradiol on Activation and Inactivation of Kv Currents in MG63 Cells

To investigate the inhibitory mechanism, further experiments were designed to test 17$\beta$-estradiol's effects on the kinetic properties of activation and inactivation. The activation data of Kv currents in MG63 cells were obtained at a test potential of –40 mV following voltage steps to potentials ranging from –90 to +60 mV. The current amplitudes at test potential after each conditioning prepulse were normalized to maximal current amplitudes. Mean normalized currents were plotted as

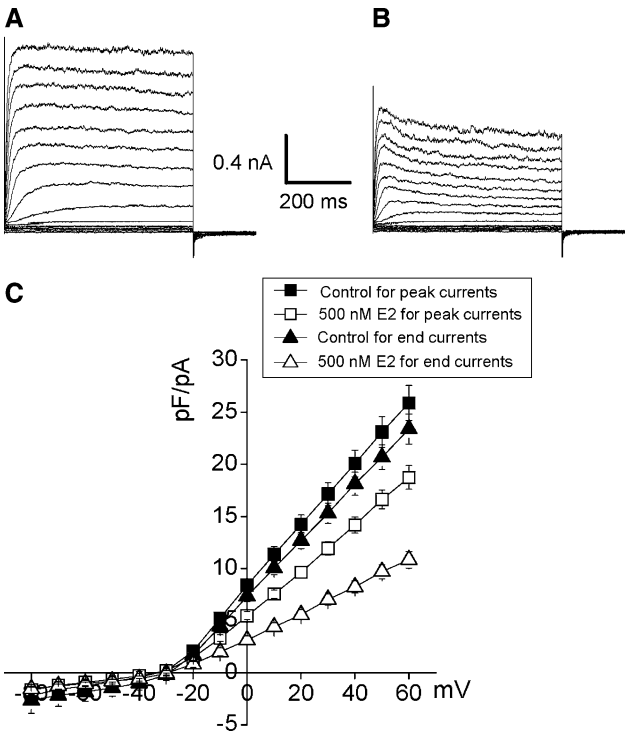


**Fig. 1** Inhibitory effects of 17β-estradiol on Kv currents in MG63 cells. Whole-cell currents from Kv channels in the absence (**a**) or presence (**b**) of 500 nM 17β-estradiol were evoked by +10-mV incremental voltage steps (600 ms) from a holding potential of –80 to +60 mV. **c** Current density–voltage relations of peak currents in the absence (control, *black square*) and presence (*white square*) of 500 nM 17β-estradiol. Current density–voltage relations of end currents for control (*black up pointing triangle*) and 500 nM 17β-estradiol (*white up pointing triangle*) conditions

a function of potential at each conditioning prepulse and fitted by a single Boltzmann equation with a $V_{1/2}$ of 1.3 mV in control. The value of $V_{1/2}$ after using 17β-estradiol of 500 nM was –4.4 mV, which is significantly different from that in control (Fig. 3a; $n = 6, p < 0.05$).

The steady-state inactivation of Kv currents was examined during voltage steps to +40 mV after 5-s conditioning prepulses to potentials between –100 and +10 mV. The amplitudes of Kv currents during each test pulse to +40 mV were measured as the difference between peak outward current and the current remaining at the end of the depolarizing pulse. The amplitudes of Kv currents evoked from each conditioning potential were then normalized to maximal current amplitudes (in the same cell). The steady-state inactivation data for outward currents in control were well described by a single Boltzmann with a $V_{1/2}$ of –55.0 mV. In the presence of 500 nM 17β-estradiol, $V_{1/2}$ was shifted left to –58.2 mV without statistical significance (Fig. 3b; $n = 4$, $p > 0.05$). Thus, 17β-estradiol only slightly affected the channel kinetics and mainly reduced the amplitudes of Kv currents in MG63 cells.

## Expression Analysis of Kv in MG63 Cells

Although it is known that outward Kv currents can be recorded in MG63 cells, it is unclear which Kv channel family members are responsible for these currents. We designed various primers (see Table 1) to detect several general α-subunits of Kv channels in MG63 cells by semiquantitative RT-PCR. The mRNA levels of Kv channels are shown in Fig. 4a, and distinct signals for Kv2.1,

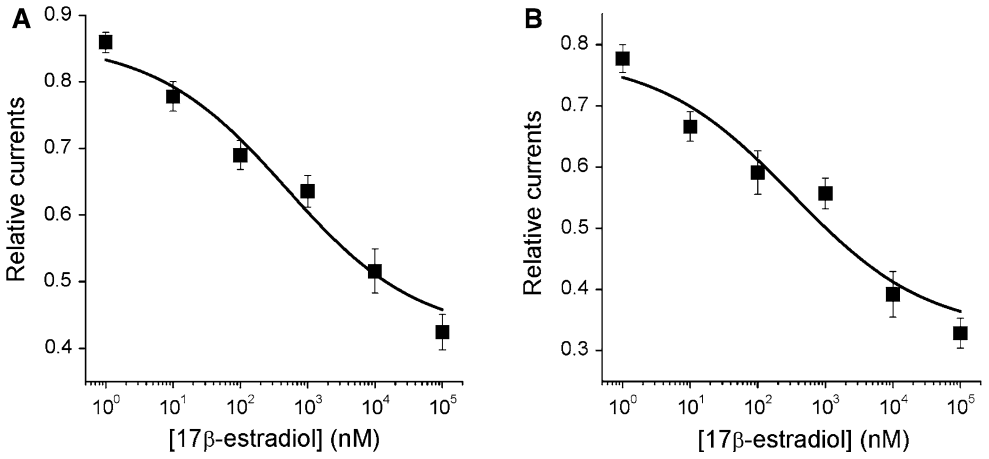


**Fig. 2** Concentration–response curves for the effects of 17β-estradiol on Kv currents in MG63 cells. The protocol of stimulation is identical to that in Fig. 1. After application of different concentrations of 17β-estradiol (1, 10, 100, 1,000, 10,000 and 100,000 nM), whole-cell currents were recorded and plotted relative to control. **a** Concentration–response curves for the inhibitory effect of 17β-estradiol on peak currents. The $IC_{50}$ value is 480 nM ($n = 6$). **b** Concentration–response curves for 17β-estradiol on end currents. The $IC_{50}$ value is 325 nM ($n = 6$)

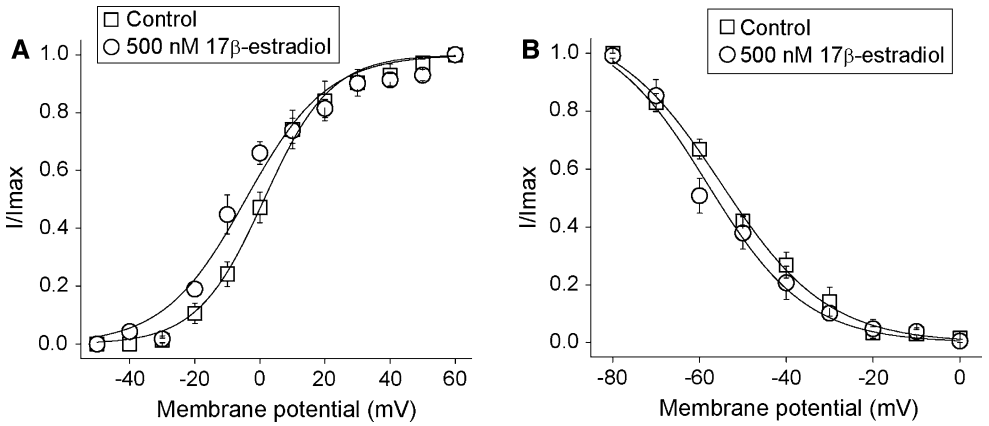


**Fig. 3** Effects of 17$\beta$-estradiol on the kinetics parameters of voltage-gated $K^+$ currents. The procedures used for activation and inactivation curves are described in the Results section. Steady-state inactivation and activation curves of Kv currents during each test pulse were normalized to maximal currents ($I/I_{max}$) and plotted against conditioning prepulse potential. Data are fitted with a Boltzmann equation. **a** Parameter values of activation are control (*white square*), $V_{1/2}$ = 1.3 mV, k = 10.2 mV; 500 nM 17$\beta$-estradiol (*white circle*), $V_{1/2}$ = –4.4 mV, k = 12.6 mV. **b** Parameter values of inactivation are control (*white square*), $V_{1/2}$ = –55.0 mV, k = 12.2 mV; 500 nM 17$\beta$-estradiol (*white circle*), $V_{1/2}$ = –58.2 mV, k = 11.5 mV. $V_{1/2}$ is the membrane potential of the half-maximal activation or inactivation and k is the slope factor

Kv2.2, Kv3.1, Kv3.3, Kv3.4, Kv4.3, Kv5.1, Kv9.3 and Herg1 suggest relatively high levels of expression for these channels ($n = 4$, $p < 0.05$). In particular, expression of Kv2.1 is higher than that of any others in MG63 cells (Fig. 4A). Weak expression (mRNA) signals for Kv1.1, Kv1.4, Kv4.1, KvLQT1 and KCNQ2 was obtained (Fig. 4A). No signals were detected for Kv 1.2, Kv1.3, Kv3.2, Kv4.2, Herg2 and Herg3.

## 17$\beta$-Estradiol Inhibits Heterologously Expressed Kv2.1 Channels

The high level of expression of Kv2.1 we detected suggests that Kv2.1channels may make an important contribution to outward voltage-gated $K^+$ currents in MG63 cells. To test this idea, we transiently transfected human Kv2.1 plasmids into COS-7 cells and measured the effects of 17$\beta$-estradiol on

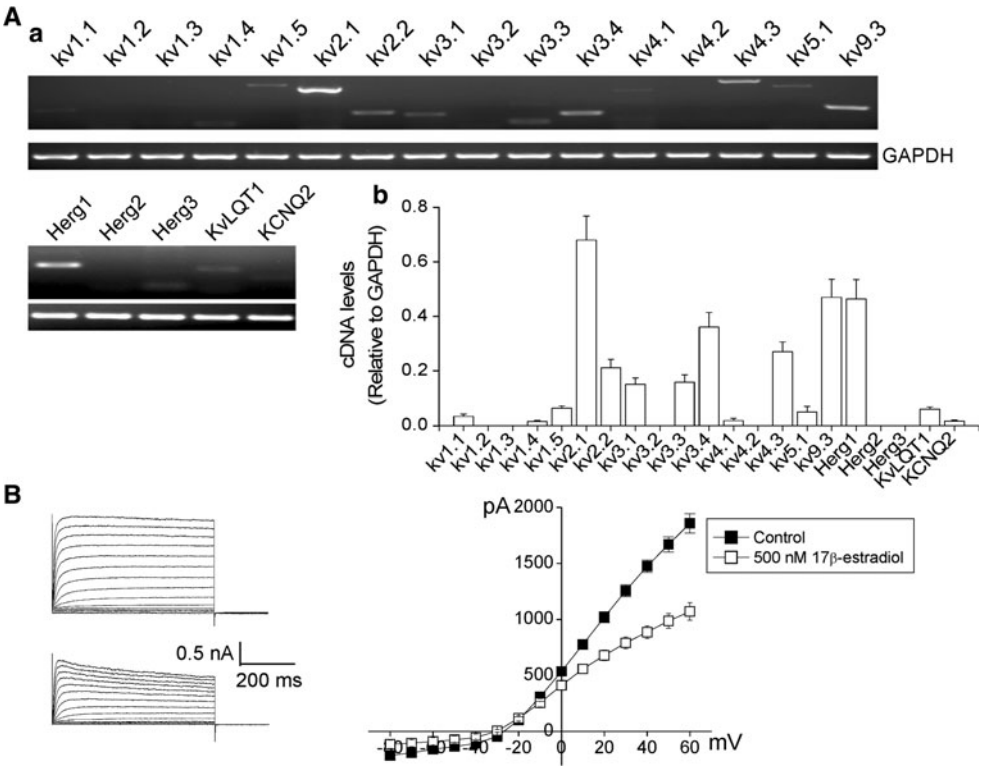


**Fig. 4** Expression pattern of Kv $\alpha$-subunits in MG63 cells and inhibitory effects of 17$\beta$-estradiol on heterologously expressed Kv2.1 channels in COS-7 cells. **A** *a* Original gel showing different levels of Kv $\alpha$-subunits (*upper panel*) and the housekeeping gene *GAPDH* (*lower panel*). *b* Summary of amplification of cDNA relative to GAPDH ($n = 5$). *Kv* voltage-gated $K^+$ channel, *GAPDH* glyceraldehyde-3-phosphate dehydrogenase. **B** 17$\beta$-Estradiol inhibited Kv2.1 channels transfected in COS-7 cells. Current–voltage relation of Kv2.1 currents for control (*black square*) and 500 nM 17$\beta$-estradiol (*white square*) conditions recorded in COS-7 cells. *Insets* show whole-cell current traces obtained from control (*upper*) and 500 nM 17$\beta$-estradiol (*lower*) conditions

whole-cell currents. The macroscopic currents from Kv2.1 were elicited by voltage steps from –80 to +60 mV. Application of 500 nM 17β-estradiol substantially inhibited elicited currents (Fig. 4B). The inhibitory effects were distinctly present in the *I–V* relationship (Fig. 4B; $n = 4, p < 0.05$). At a potential of +60 mV, 500 nM 17β-estradiol reduced Kv2.1 currents from 1.86 to 1.07 nA, a decrease of 42 % compared with control. Thus, Kv2.1 channels are good candidates for the unidentified channels mediating the estradiol-sensitive outward Kv currents in osteoblasts.

## Discussion

Yellowley et al. (1998) previously described the inward and outward ion channel currents in human osteoblast-like MG63 cells. Exposure to TEA, a classical pharmacological blocker of Kv channels, resulted in marked attenuation of the start and end outward currents in MG63 cells, suggesting that the major component of outward currents is carried by $K^+$ ions. In the present study, we found that 17β-estradiol potently inhibited outward Kv currents, with $IC_{50}$ values of 480 and 325 nM for peak and end currents, respectively. Previous studies showed that 17β-estradiol blocked Kv in several different cell types, such as cardiac myocytes (Moller and Netzer 2006) and neurons (Druzin et al. 2011; Fatehi et al. 2005). 17β-Estradiol may act on targets through two subtypes of nuclear estrogen receptors (ERs), ERα and ERβ, and initiate a signal pathway including the production of second messengers (Kelly and Levin 2001), whereas the responses through the signal pathway occurred with 10- to 20-min delays (Carrer et al. 2003). We used GTP- and $Ca^{2+}$-free pipette solutions, implying that G proteins and $Ca^{2+}$ are not involved in the inhibitory signal transduction following application of 17β-estradiol. The possible mechanism by which application of 17β-estradiol rapidly inhibits Kv currents in MG63 cells remains to be explored.

Estrogen deficiency in postmenopausal women leads to bone loss and increases the risk of fractures; thus, estrogen replacement is an important therapy against aging-related osteoporosis. Although there have been extensive research efforts, the therapeutic mechanism of action conferred by estrogen therapy remains unclear. A recent study suggested that the beneficial effects of estrogen on bone may involve the prevention of apoptosis in osteoblasts (Bradford et al. 2010). Accumulated data reveal that cytoplasmic $K^+$ efflux promotes apoptosis (Burg et al. 2006). Consistent with this notion, our data reveal that 17β-estradiol reduces $K^+$ efflux by inhibiting Kv channels, which may subsequently decrease apoptosis. This could be a possible explanation for the therapeutic effects of estrogen on bone-related disease.

Plasma concentrations of 17β-estradiol are less than 10 nM in mammals (Naftolin et al. 1990); however, the level of 17β-estradiol in women can reach 100 nM at the end of pregnancy (Runnebaum and Raube 1987). We found that 10 nM 17β-estradiol weakly and significantly inhibits Kv currents in vitro. Higher levels of estrogens may occur in vivo as a result of accumulation by target cells (Noe et al. 1992). Thus, under physiological conditions, estrogens may be important regulators of Kv currents. In order to focus on Kv channels, $Ca^{2+}$-free (EGTA-buffered) pipette solutions were used to inactivate the large-conductance, $Ca^{2+}$-activated $K^+$ channels (BK) in the current study since the BK channel is functionally expressed in MG63 cells (Henney et al. 2009). Nevertheless, acute application of 17β-estradiol can activate BK channels at nanomolar to micromolar concentrations (Valverde et al. 1999; White et al. 2005). 17β-Estradiol also induces an increase in cytosolic $[Ca^{2+}]$ (Chen et al. 2001), which could activate BK channel in MG63 cells. Therefore, estrogens may also exert acute "nongenomic" effects by regulating BK channels under pathophysiological conditions.

Besides binging to nuclear ERs and regulating gene expression, estrogen also exerts "nongenomic" (direct) effects on membrane proteins such as Kv channels. Previous studies showed that 17β-estradiol prolonged the action potential duration due to inhibition of Ito in rat ventricular myocytes (Nerbonne et al. 2001) and inhibition of Ikr and Iks in guinea pig ventricular myocytes (Pardo 2004). Estrogen suppresses A-type currents in the mouse colon (Rasband 2010). We show that 17β-estradiol, a potent estrogen, directly inhibits Kv currents in MG63 osteoblast-like cells, indicating that inhibition of $K^+$ fluxes in osteoblasts may be an important "nongenomic" action of estrogen. So far, no evidence related to the molecular basis of Kv currents in MG63 cells has been presented. To address this issue, we employed RT-PCR to examine the expression of Kv in MG63 cells. Several types of Kv channels including Kv2.1, Kv2.2, Kv3.1, Kv3.3, Kv3.4, Kv4.3, Kv5.1, Kv9.3 and Herg1, were distinctly expressed in MG63 cells. Among the various Kv channels expressed in osteoblast-like cells, our data suggest that Kv2.1 may be one of the important subtypes involved in estrogen-sensitive Kv currents.

In summary, the present study demonstrates for the first time that 17β-estradiol inhibits outward Kv currents in human osteoblast-like MG63 cells and that high-level expression of Kv2.1 substantially contributes to these currents. Our evidence provides an alternative explanation for the effects of estrogen on osteoblasts.

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