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Inhibitory action of ICI-182,780, an estrogen receptor antagonist, on BK_{Ca} channel activity in cultured endothelial cells of human coronary artery

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Abstract

ICI-182,780 is known to be a selective inhibitor of the intracellular estrogen receptors. The effect of ICI-182,780 on ion currents was studied in cultured endothelial cells of human coronary artery. In whole-cell current recordings, ICI-182,780 reversibly decreased the amplitude of K^+ outward currents. The decrease in outward current caused by ICI-182,780 could be counteracted by further application of magnolol or nordihydroguaiaretic acid, yet not by 17β-estradiol. Under current-clamp condition, ICI-182,780 (3 μM) depolarized the membrane potentials of the cells, and magnolol (10 μM) or nordihydroguaiaretic acid (10 μM) reversed ICI-182,780-induced depolarization. In inside-out patches, ICI-182,780 added to the bath did not alter single-channel conductance of large-conductance Ca^{2+}-activated K^+ channels (BK_{Ca} channels), but decreased their open probability. ICI-182,780 reduced channel activity in a concentration-dependent manner with an IC_{50} value of 3 μM. After BK_{Ca} channel activity was suppressed by 2-methoxyestradiol (3 μM), subsequent application of ICI-182,780 (3 μM) did not further reduce the channel activity. The application of ICI-182,780 shifted the activation curve of BK_{Ca} channels to positive potentials. Its decrease in the open probability primarily involved a reduction in channel open duration. ICI-182,780 also suppressed the proliferation of these endothelial cells with an IC_{50} value of 2 μM. However, in coronary smooth muscle cells, a bell-shaped concentration–response curve for the ICI-182,780 effect on BK_{Ca} channel activity was observed. This study provides evidence that ICI-182,780 can inhibit BK_{Ca} channels in vascular endothelial cells in a mechanism unlikely to be linked to its anti-estrogen activity. The inhibitory effects on these channels may partly contribute to the underlying mechanisms by which ICI-182,780 affects endothelial function.

Keywords: BK_{Ca} channels; ICI-182,780; Coronary artery endothelial cells; Cell proliferation

1. Introduction

ICI-182,780 is known to be a specific antagonist of estrogen receptors [1–3]. ICI-182,780 could block the inhibitory effect of estrogen on the migration of adventitial fibroblasts [4]. ICI-182,780 has been reported to inhibit an increase in the activity of endothelial nitric oxide synthase caused by 17β-estradiol [5,6]. It could block the neuroprotective effect of 17β-estradiol against glutamate-induced neurotoxicity [7,8]. The decrease in intracellular Ca^{2+} induced by estradiol was also reversed by ICI-182,780 in coronary arterial smooth muscle cells [9]. In addition, evidence is growing that this compound may exert a variety of actions in a manner independent of the binding to intracellular estrogen receptors. These effects include the stimulation of the expression of quinone reductase [10] and the inhibition of cell viability in human umbilical vascular endothelial cells [11]. ICI-182,780 has also been found to suppress Ca^{2+} current and stimulate the activity of BK_{Ca} channels in vascular smooth muscle cells [12,13].

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Abbreviations: BK_{Ca} channel, large-conductance Ca^{2+}-activated K^+ channel; DCEBIO, 5,6-dichloro-1-ethyl-1,3-dihydro-2*H*-benzimidazol-2-one; HCAEC, human coronary artery endothelial cells; HCASMC, human coronary artery smooth muscle cells; I_K, K^+ outward current; *I–V*, current–voltage; NDGA, nordihydroguaiaretic acid.

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The BK_{Ca} channels, that are products of a nearly ubiquitous, alternatively spliced gene (*Slo*) [14], differ from most of other K^+ channels in that their activation is under dual control, i.e. switched on either by membrane depolarization or by an increase in intracellular Ca^{2+}. These channels present in smooth muscle cells comprise two noncovalently linked subunits, i.e. a pore-forming α-subunit and a regulatory β-subunit that forms a regulatory site sensitive to intracellular Ca^{2+} [15]. Previous studies have also demonstrated that the major type of BK_{Ca} channels in vascular endothelial cells and in the brain did not contain the β-subunit [16–18].

The activity of BK_{Ca} channels expressed in endothelial cells plays a role in modulating some of the endothelial functions in coronary circulation [19]. The increased activity of these channels may facilitate Ca^{2+} influx by hyperpolarizing the cell and thus increasing the electrochemical driving force for Ca^{2+} influx [20]. The activity of these channels may control K^+ efflux and affect the K^+ concentration in myo-endothelial gap junctions [21,22]. The enhanced activity of BK_{Ca} channels could facilitate the release of nitric oxide from endothelial cells that have been stimulated by application of shear stress on cell membrane. Membrane hyperpolarization of endothelial cells caused by activation of their BK_{Ca} channels can also readily be transmitted from endothelial cells to smooth muscle cells [23]. More interestingly, there appears to be impairment in the expression pattern of Ca^{2+}-activated K^+ channels in regenerated epithelium after balloon catheter injury [24]. However, none of the studies have thus far demonstrated the underlying mechanism of action of ICI-182,780 on these channels in endothelial cells.

Therefore, the main objective of this study was to address the question of whether ICI-182,780 could affect K^+ currents in human coronary artery endothelial cells (HCAEC), and to examine the effect of ICI-182,780 on the activity of BK_{Ca} channels expressed in these cells. In addition, its effect on cell proliferation was studied and compared. HCAEC are known to be ideal candidates for the studies of endothelial cell metabolism and other functional vasodilators (e.g. K^+ ions, nitric oxide, epoxyeicosatrienoic acids, or endothelium-dependent hyperpolarizing factor) [21,22,25]. The results presented here indicate that the inhibition by ICI-182,780 of endothelial BK_{Ca} channels may partially, if not entirely, contribute to its effect on the cellular function in these cells.

2. Materials and methods

2.1. Cell preparation

HCAEC and human coronary artery smooth muscle cells (HCASMC), originally obtained from normal human coronary arteries, were obtained from Cell Applications, Inc. HCAEC and HCASMC were, respectively, maintained in growth medium for endothelial cells and smooth muscle cells, and equilibrated in a humidified atmosphere of 5% CO_2/95% air at 37°. Cells were subcultured weekly after detachment by using culture medium containing 1% trypsin. The experiments were performed using cells obtained passages 2 and 4.

2.2. Measurement of cell proliferation

HCAEC (5×10^4 per mL) were cultured at 37° in a 96-well microplate and treated with various concentrations of ICI-182,780 (0.3–30 μM). The rate of cell growth was calculated among day 0, day 1, and day 2 of culture. A colorimetric method was used in quantifying cell densities in microplates. The method is based on the reduction of a tetrazolium salt (4-[3-(4-iodophenyl)-2-(4-nitrophenyl)-2*H*-5-tetrazolio]-1,3-benzene disulfonate; WST) by mitochondrial dehydrogenase. Only viable cells convert the dye to an orange formazan. At the end of the incubation period, WST (10 μL) was added to each well and incubated for 2 hr. The optical density of each well was measured at 450 and 650 nm using an ELISA reader. Conversion of optical density to cell density was done using a standard curve of serial diluted cells at the time of assay [26].

2.3. Electrophysiological measurements

Immediately before each experiment, HCAEC or HCASMC were dissociated and an aliquot of cell suspension was transferred to a recording chamber positioned on the stage of an inverted microscope (Diaphot-200; Nikon). Cells were bathed at room temperature (20–25°) in normal Tyrode's solution containing 1.8 mM $CaCl_2$. Patch pipettes were pulled from thin-walled borosilicate glass capillaries (Kimax-51) using a vertical two-stage electrode puller (PB-7, Narishige) and the tips were fire-polished with a microforge (MF-83; Narishige). Patch pipette had a tip resistance of 3–5 MΩ when immersed in normal Tyrode's solution. Ion currents were recorded in the cell-attached, inside-out, and whole-cell configurations of the patch-clamp technique, using an RK-400 patch-clamp amplifier (Bio-Logic) [27,28]. All potentials were corrected for liquid junction potential, a value that would develop at the tip of the pipette when the composition of the pipette solution was different from that in the bath.

2.4. Data recording and analysis

The signals consisting of voltage and current tracings were displayed with a storage oscilloscope (model HM507, Hameg Instruments). Currents were low-passed filtered at 1 kHz. A Digidata 1322A interface (Axon Instruments) was used for the analog-to-digital/digital-to-analog conversion. To minimize electrical noise, this interface device was connected to a Pentium III-based laptop computer (Slimnote VX_3, Lemel) through a universal serial bus port, and was

then controlled with the aid of Clampex subroutine in the pCLAMP 9.0 software (Axon Instruments). Ion currents recorded during whole-cell experiments were stored without leakage correction and analyzed using Clampfit subroutine (Axon Instruments), the Origin 6.0 software (Microcal Software, Inc.) or custom-made macros in Excel (Microsoft).

Single BK_{Ca} channel amplitudes were determined by fitting Gaussian distributions to the amplitude histograms of the closed and the open state, respectively. Channel dwell times were determined by applying a standard half-amplitude crossing protocol using the pCLAMP 9.0 software (Axon Instruments). The activity of the channel in a patch was expressed as NP_o, which can be estimated using the following equation: $NP_o = (A_1 + 2A_2 + 3A_3 + \cdots + nA_n)/(A_0 + A_1 + A_2 + A_3 + \cdots + A_n)$, where N is the number of active channels in the patch, A_0 is the area under the curve of an all-points histogram corresponding to the closed state, and A_1, ..., A_n represent the histogram areas reflecting the levels of distinct open state for 1 to n channels in the patch. The single-channel conductance was calculated by linear regression using mean current amplitudes measured at different voltages. Open lifetime distributions for the BK_{Ca} channel were fitted with logarithmically scaled bin width [28].

The concentration–response relationship for ICI-182,780-induced inhibition of BK_{Ca} channels or cell proliferation in HCAEC was determined by the Hill equation using a nonlinear regression analysis. That is, percentage inhibition: $(E_{max} \times [C]^{n_h})/(IC_{50}^{n_h} + [C]^{n_h})$, where $[C]$ is the concentration of ICI-182,780; IC_{50} and n_h are the concentration required for a 50% inhibition and the Hill coefficient, respectively; and E_{max} is the maximal inhibition of BK_{Ca} channels or cell proliferation caused by ICI-182,780.

The relationships between membrane potentials and relative open probability of BK_{Ca} channels obtained before and after the application of ICI-182,780 (10 μM) were fitted with a Boltzmann function of the form: relative open probability: $n_P/\{1 + \exp[-(V - V_{1/2})/k]\}$, where n_P: the maximal open probability, V: the membrane potential in mV, $V_{1/2}$: the voltage at which there is half-maximal activation, k: the slope factor of the activation curve (i.e. the voltage dependence of the activation process in mV per e-fold change). Curve fitting to data presented here was performed with the aid of the Origin 6.0 software (Microcal).

All values are reported as mean values ± SEM. Student's paired or unpaired t test was used for the statistical analyses. To clarify the statistical differences among the two or four treatment groups, analyses of variance with Duncan's multiple-range test for multiple comparisons were also performed. Differences between values were considered significant when $P < 0.05$.

2.5. Drugs and solutions

7α-[9[(4,4,5,5,5-Pentafluoropentyl)sulfinyl]nonyl]-estra-1,3,5(10)-triene-3,17β-diol (ICI-182,780; Faslodex™) and 5,6-dichloro-1-ethyl-1,3-dihydro-2*H*-benzimidazol-2-one (DCEBIO) was obtained from Tocris. 17β-Estradiol was obtained from Sigma Chemical and paxilline from Biomol Research Laboratories, Inc. Nordihydroguaiaretic acid (NDGA) was purchased from Sigma/RBI, and iberiotoxin was from Alomone Labs. Magnolol was kindly provided by Dr. Chien-Chich Chen (National Institute of Chinese Medicine, Taipei, Taiwan). Squamocin was a gift from Dr. Yang-Chang Wu (Graduate Institute of Natural Products, Kaohsiung Medical University, Kaohsiung City, Taiwan). All other chemicals were commercially available and of reagent grade. The twice-distilled water that had been de-ionized through a Millipore-Q system (Millipore) was used in all experiments.

The composition of normal Tyrode's solution was as follows (in mM): NaCl 136.5, KCl 5.4, $CaCl_2$ 1.8, $MgCl_2$ 0.53, glucose 5.5, and HEPES–NaOH buffer 5 (pH 7.4). To record K^+ currents or membrane potentials, the patch pipette was filled with solution (in mM): KCl 140, $MgCl_2$ 1, Na_2ATP 3, Na_2GTP 0.1, EGTA 0.1, and HEPES–KOH buffer 5 (pH 7.2). To measure Ca^{2+} current, KCl inside the pipette solution was replaced with equimolar CsCl, and pH was adjusted to 7.2 with CsOH. For single-channel current recordings, high-K^+ bathing solution was composed of (in mM): KCl 145, $MgCl_2$ 0.53, and HEPES–KOH buffer 5 (pH 7.4), and pipette solution contained (in mM): KCl 145, $MgCl_2$ 2, and HEPES–KOH buffer 5 (pH 7.2). The pipette solution was filtered on the day of use with a 0.22-μm pore size syringe filter (Millipore).

3. Results

3.1. Inhibitory effect of ICI-182,780 on K+ outward current (I_K) in HCAEC

In the first series of experiments, the whole-cell configuration of the patch-clamp technique was performed to investigate the effect of ICI-182,780 on ion currents in these cells. Cells were bathed in normal Tyrode's solution that contained 1.8 mM $CaCl_2$, and pipettes were filled with a pipette solution containing 3 mM ATP and 0.1 mM EGTA. As shown in Fig. 1, when the cell was held at the level of −40 mV, the voltage pulses from −30 to +60 mV in 10-mV increments elicited a family of outward currents. The amplitudes of these outward currents were increased with greater depolarization. Within 1 min of exposing the cells to ICI-182,780, current amplitude was reduced throughout the entire range of voltage-clamp steps (Fig. 1). For example, with a depolarizing step from −40 to +50 mV, ICI-182,780 (10 μM) significantly decreased the amplitude to 102 ± 13 pA from a control value of 158 ± 15 pA (N = 10). This inhibitory effect was readily reversed on the washout of ICI-182,780. These results suggest that ICI-182,780 has a depressant effect on I_K in these cells.

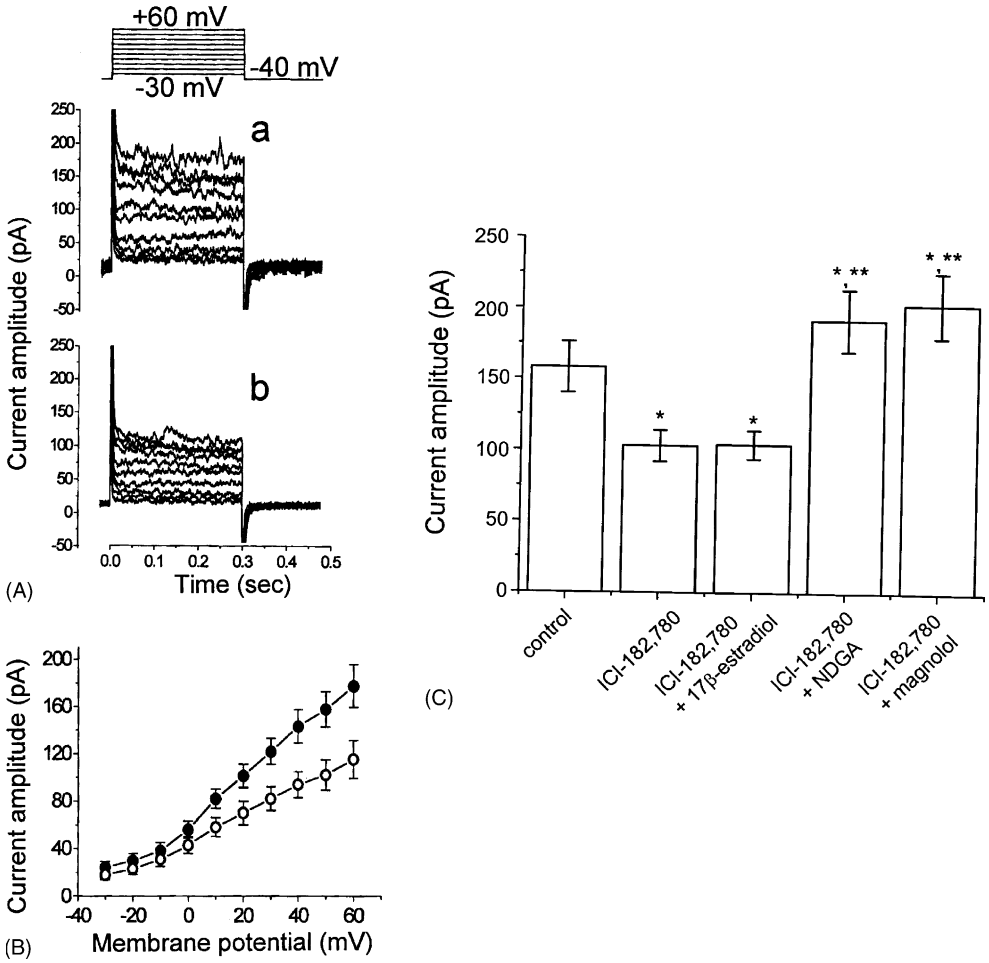

Fig. 1. Inhibitory effect of ICI-182,780 on K^+ outward current (I_K) in HCAEC. In these experiments, cells were bathed in normal Tyrode's solution that contained 1.8 mM $CaCl_2$. (A) Superimposed current traces in control and during the exposure to ICI-182,780 (1 μM). The cells, bathed in normal Tyrode's solution containing 1.8 mM $CaCl_2$, were depolarized from a holding potential of −40 mV to various potentials ranging from −30 to +60 mV in 10-mV increments. The uppermost part indicates the voltage protocol. a: control; b: 1 μM ICI-182,780. (B) Averaged *I–V* relations of I_K measured at the end of depolarizing pulses in control (●) and during exposure to 1 μM ICI-182,780 (○). Each point represents the mean ± SEM (N = 6–8). (C) Effect of ICI-182,780 (1 μM) on the amplitude of I_K in the absence and presence of 17β-estradiol (10 μM), NDGA (10 μM), and magnolol (10 μM). Currents, the amplitudes of which were measured at the end of voltage pulses, were elicited by depolarizing pulses to +50 mV from a holding potential of −40 mV. Each point represents the mean ± SEM (N = 6–10). NDGA: nordihydroguaiaretic acid (10 μM). *Significantly different from control. **Significantly different from ICI-182,780 alone group.

3.2. Comparison of the effect of ICI-182,780 on the amplitude of I_K in the absence and presence of 17β-estradiol, NDGA, or magnolol

We further examined whether the inhibitory effect of ICI-182,780 on I_K can be altered by subsequent application of 17β-estradiol, NDGA, or magnolol in HCAEC. NDGA and magnolol were reported to enhance BK_{Ca} channel activity in smooth muscle cells [29,30]. 17β-Estradiol has been found to interact with the β-subunits of the BK_{Ca} channel to increase I_K [31,32]. When the cells were depolarized from −40 to +50 mV, ICI-182,780 (3 μM) or paxilline (1 μM) significantly suppressed the amplitude of I_K; however, 17β-estradiol (10 μM) alone had no effect on I_K. NDGA (10 μM) or magnolol (10 μM) significantly reversed ICI-182,780-mediated inhibition of I_K; however, 17β-estradiol (10 μM) had little or no effect on it in HCAEC. These results are summarized and illustrated in Fig. 1C.

3.3. Characterization of the effect of ICI-182,780 on membrane potential in HCAEC

To determine whether ICI-182,780 affects the membrane potential of coronary endothelial cells, the next series of experiments was made under current-clamp condition. Cells, bathed in normal Tyrode's solution containing 1.8 mM $CaCl_2$, had a resting membrane potential of −44 ± 5 mV (N = 15). When cells were exposed to ICI-182,780 (3 μM), the membrane potential significantly depolarized to −38 ± 5 mV (N = 6). In continued presence of ICI-182,780, the application of magnolol (10 μM) or NDGA (10 μM) could hyperpolarize the cells. For example, resting potential was hyperpolarized back to

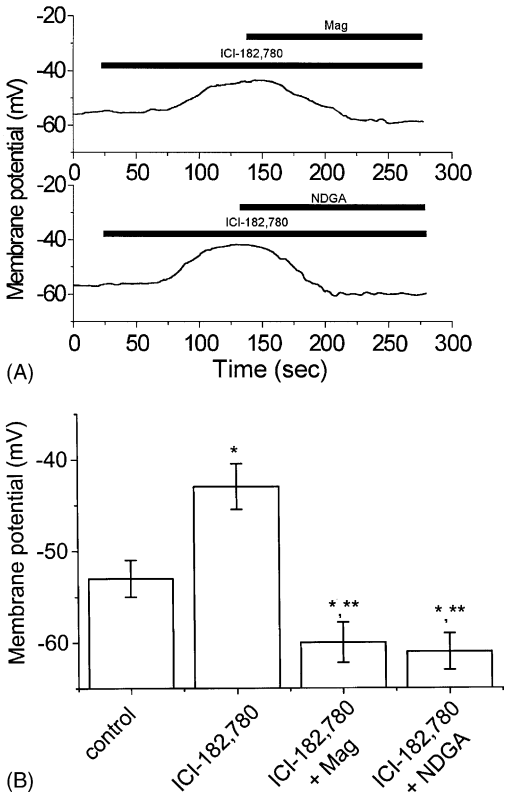

Fig. 2. Effect of magnolol and nordihydroguaiaretic acid on ICI-182, 780-induced change in membrane potential in HCAEC. The cells were bathed in normal Tyrode's solution containing 1.8 mM $CaCl_2$. The patch pipettes were filled with K^+-containing solution. (A) Changes in resting membrane potential measured under current-clamp condition. Horizontal bars shown in each panel denote the application of ICI-182,780 (3 μM), magnolol (Mag; 10 μM), or nordihydroguaiaretic acid (NDGA; 10 μM). (B) Bar graph showing effect of magnolol (Mag; 10 μM) and nordihydroguaiaretic acid (NDGA; 10 μM) on membrane potential in these cells. Each point represents the mean ± SEM (N = 5–9). *Significantly different from control group. **Significantly different from ICI-182,780 alone group.

-42 ± 5 mV (N = 5) in the presence of ICI-182,780 plus magnolol (10 μM). The typical effect of ICI-182,780 with and without subsequent application of magnolol or NDGA on membrane potential in coronary endothelial cells is illustrated in Fig. 2. Thus, the effect of ICI-182,780 on changes in membrane potential could be closely associated with the closing of a K^+ conductance.

3.4. Inhibitory effect of ICI-182,780 on BK_{Ca} channels in HCAEC

To elucidate the effect of ICI-182,780 on ion currents, its effects on the activity of single BK_{Ca} channel were further investigated in these cells. The single-channel recordings with inside-out configuration were performed in symmetrical K^+ (145 mM) concentration. The bath solution contained 1 μM Ca^{2+} and the potential was held at +60 mV. As shown in Fig. 3, in symmetrical K^+ concentrations (145 mM), the activity of BK_{Ca} channels can be readily observed in excised patches. The channel

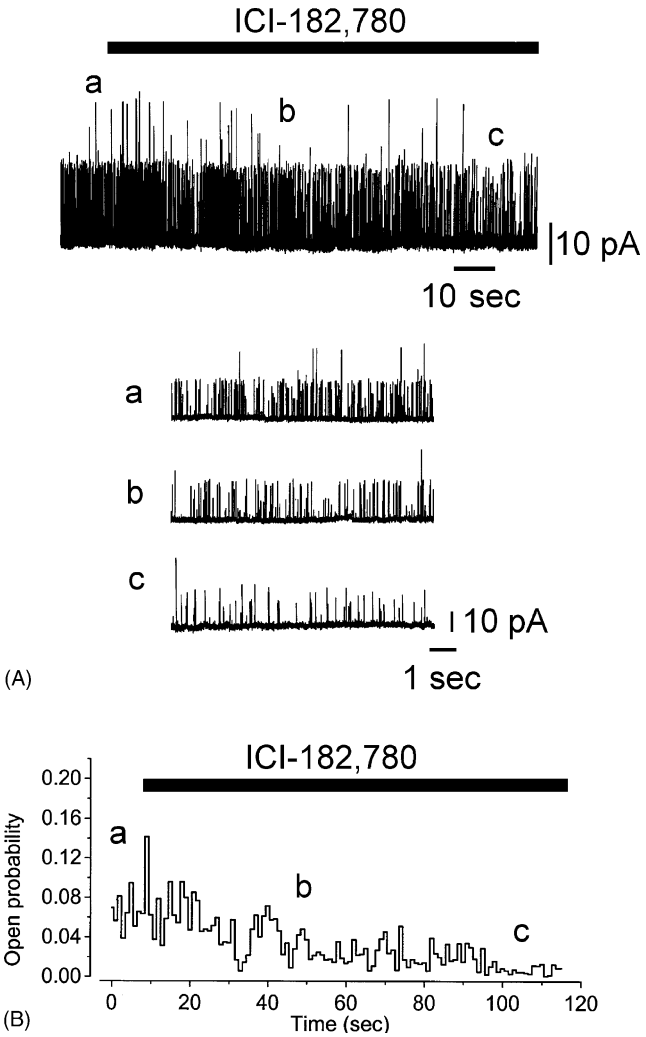

Fig. 3. Effect of ICI-182,780 on the activity of BK_{Ca} channels in HCAEC. The experiments were conducted with symmetrical K^+ concentration (145 mM). Under inside-out configuration, the holding potential was set at +60 mV and the bath solution contained 1 μM Ca^{2+}. (A) The activity of BK_{Ca} channels recorded before and during exposure to 10 μM ICI-182,780. The lower part in trace A shows current traces obtained in an expanded time scale corresponding to those labeled a, b, and c in graph B and in the upper part of trace A. Upward deflections shown in this and the following figures indicate the opening events of the channel. (B) The time course of change in open probability (1-s bin width) of unitary outward currents before and during the application of ICI-182,780 (10 μM) to intracellular surface of the channel in an excised patch. Horizontal bar shown in trace A and graph B indicates the time and duration of ICI-182,780 application into the bath.

activity was increased at Ca^{2+} concentration in bath medium was increased. An increase in channel activity could also be obtained in cell-attached patches when cells were exposed to ionomycin (10 μM) or squamocin (10 μM). These two agents are known to be Ca^{2+} ionophores [26,33]. When ICI-182,780 (10 μM) was applied to the intracellular surface of detached patches, the probability of channel openings was significantly decreased (Fig. 3). The open probability of the channel at +60 mV obtained in the absence of ICI-182,780 was found to be 0.076 ± 0.012 (N = 7). The addition of ICI-182,780

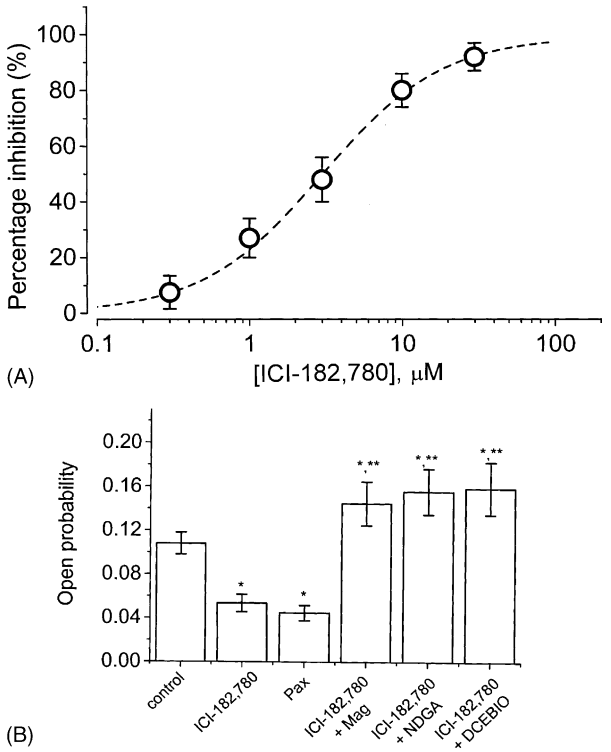

Fig. 4. Concentration–response curve for ICI-182,780-induced inhibition of BK_{Ca} channel activity (A) and effects of various inhibitors and openers of K^+ channels on the activity of this channel (B) in HCAEC. Each detached patch was held at +60 mV. Bath medium contained 1 μM Ca^{2+}. In graph A, the probability of channel openings obtained after application of ICI-182,780 into the bath was compared with the control value, i.e. in the absence of ICI-182,780 (mean ± SEM; N = 6–9 for each point). The smooth line is the fit of the data with a Hill function. The values for IC_{50}, maximally inhibited percentage of BK_{Ca} channel activity, and the Hill coefficient were 3 μM, 99%, and 1.1, respectively. In graph B, the effect of ICI-182,780 (10 μM) on BK_{Ca} channel activity in the absence and presence of magnolol, NDGA, and DCEBIO is shown. Each point represents the mean ± SEM (N = 5–9). Pax: paxilline (1 μM); Mag: magnolol (10 μM); NDGA: nordihydroguaiaretic acid (10 μM); DCEBIO: 5,6-dichloro-1-ethyl-1,3-dihydro-2*H*-benzimidazol-2-one (10 μM). *Significantly different from control group. **Significantly different from ICI-182,780 alone group.

(10 μM) significantly decreased the activity to 0.004 ± 0.001 (N = 7). When ICI-182,780 was washed out, the channel activity returned to the control level. However, the amplitude of single BK_{Ca} channel current remained unaltered in the presence of ICI-182,780. The relationship between the channel activity and the concentration of ICI-182,780 was constructed and plotted (Fig. 4A). After the curve was fitted to Hill equation, the half-maximal concentration (i.e. IC_{50}) required for the inhibitory effect of ICI-182,780 on BK_{Ca} channel activity was calculated to be 3 μM. ICI-182,780 at a concentration of 30 μM almost completely suppressed the activity of BK_{Ca} channels. Thus, the results showed that ICI-182,780 (0.3–30 μM) applied to the cytosolic face of the channel could reduce the activity of BK_{Ca} channels in a concentration-dependent manner without a change in single-channel amplitude.

3.5. Effect of magnolol, NDGA, and DCEBIO on ICI-182,780-induced inhibition of BK_{Ca} channel activity

The response to other known regulators of BK_{Ca} channels was also examined in HCAEC. Application of paxilline (1 μM) to the intracellular surface of inside-out patch resulted in a considerable decrease in the channel activity (Fig. 4B). The BK_{Ca} channel activity observed in these cells was thus sensitive to inhibition by ICI-182,780 and paxilline. In addition, a subsequent application of magnolol (10 μM), NDGA (10 μM), or DCEBIO (10 μM) was found to reverse the channel activity suppressed by ICI-182,780 (3 μM) significantly. Magnolol and NDGA have been reported to enhance the activity of BK_{Ca} channels in

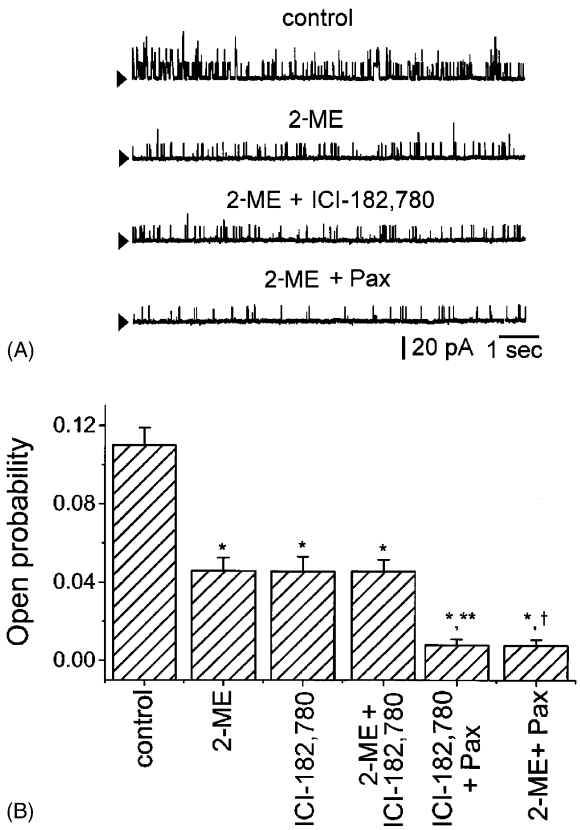

Fig. 5. Inhibitory effect of ICI-182,780, 2-methoxyestradiol, and paxilline on the activity of BK_{Ca} channels recorded from inside-out patches of HCAEC. Potential was held at +60 mV and the compound tested was applied to the bath containing 1.0 μM Ca^{2+}. (A) Original current traces recorded in control, or in the presence of 3 μM 2-methoxyestradiol (2-ME), 3 μM 2-methoxyestradiol plus 3 μM ICI-182,780, or 3 μM 2-methoxyestradiol plus 1 μM paxilline (Pax). Arrowheads indicate the zero current level. (B) Bar graph showing the effect of 2-methoxyestradol, 2-methoxyestradiol plus ICI-182,780 (3 μM), ICI-182,780 (3 μM) plus paxilline, and 2-methoxyestradiol plus paxilline on the activity of BK_{Ca} channels. 2-ME: 2-methoxyestradiol (3 μM); Pax: paxilline (1 μM). In the experiments with 2-methoxyestradiol plus each compound (e.g. ICI-182,780 and paxilline), each compound was applied after the addition of 2-methoxyestradiol. In those with ICI-182,780 plus paxilline, paxilline was subsequently applied in continued presence of ICI-182,780. Each point represents the mean ± SEM (N = 5–8). *Significantly different from control group. **Significantly different from ICI-182,780 alone group. †Significantly different from 2-methoxyestradiol alone group.

smooth muscle cells [29,30], whereas DCEBIO is an opener of intermediate-conductance Ca^{2+}-activated K^+ channels [34].

3.6. Effect of 2-methoxyestradiol on BK_{Ca} channels in HCAEC

2-Methoxyestradiol was previously reported to suppress BK_{Ca} channels in human umbilical vascular endothelial cells [35]. We also testified whether the effects of ICI-182,780 and 2-methoxyestradiol on BK_{Ca} channels are additive. Interestingly, as shown in Fig. 5, 2-methoxyestradiol (3 μM) significantly decreased the probability of channel openings; however, a subsequent application of ICI-182,780 (3 μM) did not reduce channel activity further. 2-Methoxyestradiol (3 μM) significantly decreased the open probability from a control value of 0.11 ± 0.009 to 0.046 ± 0.007 ($N = 7$). There was no significant difference in the channel activity between the presence of 2-methoxyestradiol alone and 2-methoxyestradiol plus ICI-182,780 (0.046 ± 0.007 [$N = 7$] vs. 0.046 ± 0.006 [$N = 6$]). In contrast, the addition of paxilline (1 μM) was able to decrease the open probability further in continued presence of ICI-182,780 or 2-methoxyestradiol. Taken together, the results indicate that inhibitory effects of 2-methoxyestradiol and ICI-182,780 on single BK_{Ca} channel are not additive in HCAEC.

3.7. Lack of effect of ICI-182,780 on single-channel conductance of BK_{Ca} channels

In the next series of experiments, the effect of ICI-182,780 on BK_{Ca} channels at different membrane potentials was studied. In inside-out configuration, cells were bathed in symmetrical K^+ concentrations (145 mM) and bath solution contained 1 μM Ca^{2+}. Figure 6 illustrates current–voltage (*I–V*) relations of BK_{Ca} channels obtained in the absence and presence of ICI-182,780 (10 μM). The single BK_{Ca} channel conductance calculated from a linear *I–V* relationship in control was 208 ± 13 pS ($N = 10$) with a reversal potential of 0 ± 2 mV ($N = 10$). The value of single-channel conductance for these channels was found to be similar to that described previously [35,36]; however, it did not significantly differ from that (207 ± 13 pS, $N = 9$)

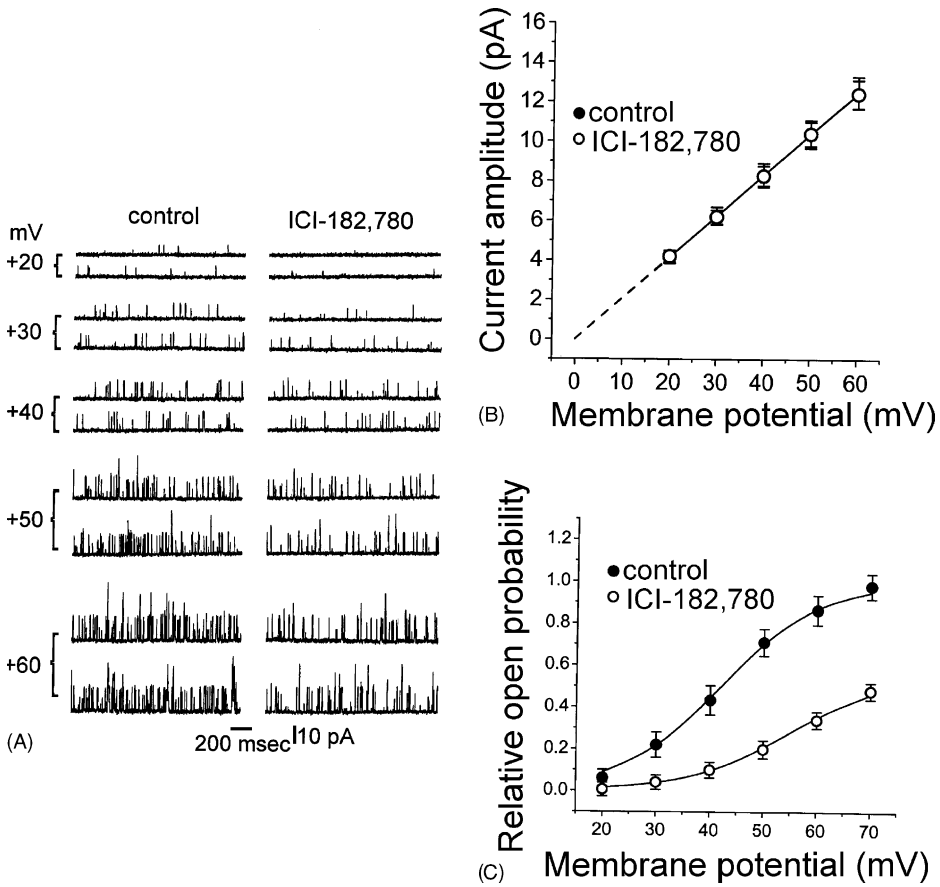

Fig. 6. Effect of ICI-182,780 on *I–V* relation of BK_{Ca} channels in HCAEC. The experiments in excised membrane patches were conducted in symmetrical K^+ concentration. Bath medium contained 1 μM Ca^{2+}. (A) The activity of BK_{Ca} channels before (left) and after (right) bath application of 10 μM ICI-182,780. Single BK_{Ca} channel currents were recorded at different membrane potentials denoted by the left side of each current trace. Upward deflections are the opening events of the channel. (B) Averaged *I–V* relations of BK_{Ca} channels obtained in the absence (filled circles) and presence (open circles) of ICI-182,780 (10 μM). Each point represents the mean ± SEM ($N = 5$–8). Notably, single-channel conductance obtained in the absence and presence of ICI-182,780 is nearly identical. (C) The relationship between relative open probability of the channel and membrane potential in the absence (●) and presence (○) of ICI-182,780 (10 μM).

measured in the presence of ICI-182,780 (10 μM). These results indicate that ICI-182,780 causes no change in single-channel conductance, although it significantly reduces the probability of channel openings in HCAEC.

3.8. Effect of ICI-182,780 on the activation curve of BK_{Ca} channels

Figure 6C shows the activation curve of BK_{Ca} channels in the absence and presence of ICI-182,780 (10 μM). The plot of relative open probability of BK_{Ca} channels as a function of membrane potential was constructed and fitted with a Boltzmann function as described in Section 2. In control, $n_P = 0.99 \pm 0.04$, $V_{1/2} = 42.2 \pm 2.1$ mV, and $k = 9.4 \pm 0.7$ mV (N = 6). In the presence of ICI-182,780 (10 μM), $n_P = 0.56 \pm 0.03$, $V_{1/2} = 54.8 \pm 2.3$ mV, and $k = 9.6 \pm 1.1$ mV (N = 6). Thus, the presence of ICI-182,780 (10 μM) caused a decrease in the maximal open probability of BK_{Ca} channels. In addition, the activation was shifted along the voltage axis to more positive potentials after ICI-182,780 was applied. However, no significant change on the slope (i.e. k value) of the activation curve was detected in the presence of ICI-182,780. Taken together, these results indicate that ICI-182,780 is capable of suppressing the activity of BK_{Ca} channels in a voltage-dependent fashion in HCAEC.

3.9. Effect of ICI-182,780 on kinetic behavior of BK_{Ca} channels in HCAEC

The effect of ICI-182,780 on mean open time of BK_{Ca} channels was examined and analyzed during recordings from patches showing only single-channel openings. As shown in Fig. 7, in control cells (i.e. in the absence of ICI-182,780), the open-time histogram of BK_{Ca} channels at +60 mV can be fitted by a two-exponential curve with a mean open time of 2.5 ± 0.4 ms and 8.5 ± 1.4 ms (N = 6). The presence of ICI-182,780 (3 μM) decreased the lifetime of the open state to 1.8 ± 0.3 ms (N = 6). Thus, the data clearly demonstrate that the inhibitory effect of ICI-182,780 on BK_{Ca} channel activity in HCAEC is primarily due to a decrease in open time, because of lack of change in single-channel conductance.

3.10. Effect of ICI-182,780 on the proliferation of HCAEC

Previous studies have shown the ability of ICI-182,780 to suppress the growth of pituitary tumor cells [37]. The effect of ICI-182,780 on cell growth in these cells was also examined. Interestingly, when ICI-182,780 was added to the culture medium of proliferating endothelial cells, the rate of cell growth was significantly reduced (Fig. 8). The IC_{50} value for the inhibition of cell proliferation was about 2 μM. At a concentration of 30 μM, ICI-182,780 almost completely suppressed cell growth. The concentration of

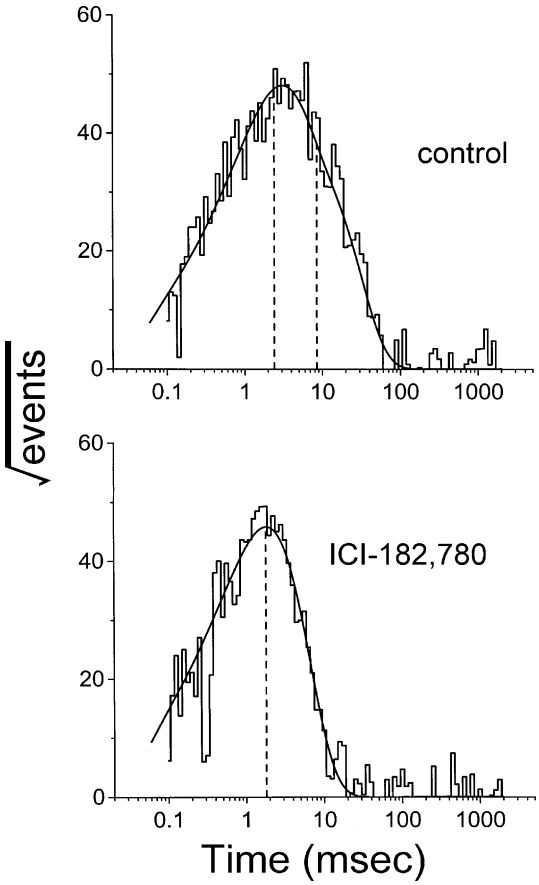

Fig. 7. Effect of ICI-182,780 on mean open time of BK_{Ca} channels in HCAEC. Under symmetrical K^+ condition, the cells were held at +60 mV in inside-out configuration. Open-time histogram in control (upper part) was fitted by a two-exponential function with a mean open time of 2.4 and 8.5 ms. The open-time histogram obtained after application of ICI-182,780 (10 μM) to the bath (lower part) was fitted by a single-exponential function with a mean open time of 1.8 ms. Data were obtained from a measurement of 366 channel openings with a total recording time of 1 min in the control, whereas data obtained during the exposure to 10 μM ICI-182,780 were measured from 298 channel openings with a total recording time of 2 min. The abscissa and ordinate show the logarithm of the open time (ms) and the square root of the number of events ($n^{1/2}$), respectively. The dashed lines shown in each lifetime distribution are placed at the value of the time constant in open state.

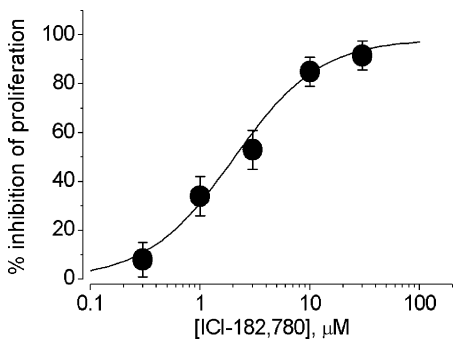

Fig. 8. Concentration-dependent inhibition of cell proliferation by ICI-182,780 in HCAEC. Smooth line represents best fit to the Hill equation. The values of IC_{50}, maximally inhibited percentage of cell proliferation and Hill coefficient were 2 μM, 99%, and 1.1, respectively. Each point represents the mean ± SEM of four experiments.

ICI-182,780 for effective inhibition of BK_{Ca} channels, which ranged between 0.3 and 30 μM, was comparable to that used to inhibit cell proliferation in these endothelial cells. However, the maximal inhibitory effect of ICI-182,780 on BK_{Ca} channel activity and cell growth did not significantly differ. Thus, these results can be interpreted to mean that growth inhibition of these cells caused by ICI-182,780 is proportional to its blockade of the BK_{Ca} channel.

3.11. Effect of ICI-182,780 on the activity of BK_{Ca} channels in HCASMC

BK_{Ca} channels, that were functionally expressed in smooth muscle cells, was recently reported to be stimulated by ICI-182,780 [13]. In a final series of experiments, we investigated whether ICI-182,780 has any effect on BK_{Ca} channels in HCASMC. As illustrated in Fig. 9, after ICI-182,780 at concentrations below 3 μM was applied into the cytosolic face of the channel, the open probability was significantly increased without a change in single-channel amplitude. For example, the addition of ICI-182-780 (3 μM) enhanced the channel activity from 0.11 ± 0.03 to 0.34 ± 0.04 ($N = 7$). However, ICI-182,780 at higher concentrations resulted in a decrease in channel openings. Figure 9B shows the relationship of BK_{Ca} channel activity and the concentration of ICI-182,780. Unlike that in human coronary endothelial cells, the BK_{Ca} channel in coronary smooth muscle was subject to stimulation by ICI-182,780 at concentrations below 3 μM.

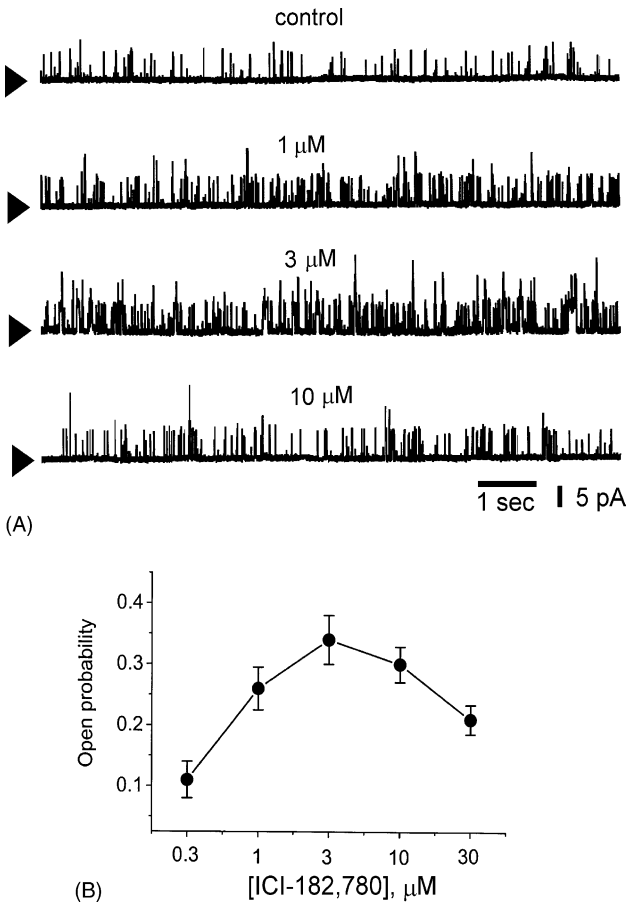

Fig. 9. Effect of ICI-182,780 on BK_{Ca} channel activity in cultured smooth muscle cells of human coronary artery (HCASMC). In these experiments, inside-out configuration was performed with symmetrical K^+ concentration (145 mM), and bath medium contained 0.1 μM Ca^{2+}. The holding potential was set at +60 mV. The different concentrations of ICI-182,780 were applied to the bath. (A) Original current traces obtained in control, and during exposure to 1, 3, and 10 μM ICI-182,780. Arrowheads indicate the zero current level. (B) Concentration–response curve for BK_{Ca} channel activity measured at +60 mV. The relationship between channel openings and the concentrations of ICI-182,780 is illustrated. Each point represents the mean ± SEM ($N = 6$–9).

4. Discussion

The results presented here show that (a) in HCAEC, ICI-182,780 can reduce the amplitude of I_K; (b) ICI-182,780 decreased the activity of BK_{Ca} channels without modifying the single-channel conductance; (c) ICI-182,780 suppressed the channel activity in a voltage-dependent manner; (d) the ICI-182,780-induced decrease in the probability of channel openings is primarily caused by a decrease in the number of long-lived openings; and (e) ICI-182-780 produced an inhibitory effect on cell proliferation in HCAEC. The inhibitory action on single BK_{Ca} channel may cause membrane depolarization, thus affecting functional activity of endothelial cells, if this action occurring in native endothelial cells is the same as that shown in this study.

The IC_{50} value for ICI-182,780-induced inhibition of BK_{Ca} channels in HCAEC was 3 μM. This value appears to be higher than the concentration (e.g. 1 μM) that was used to induce an inhibition of estrogen receptors [38,39]. However, its inhibition of BK_{Ca} channels in HCAEC was found to exhibit voltage dependence. In addition, the ICI-182,780 concentration found to suppress the growth of HCAEC was found to be close to the IC_{50} value for inhibition of BK_{Ca} channels in the same type of cells and in estrogen receptor negative cancer cells [40]. Thus, there seems to be a link between the effects of ICI-182,780 on endothelial cells and its inhibitory effect on BK_{Ca} channel activity. Indeed, it has been reported that there is a high variability in the plasma concentrations of ICI-182,780 between individual patients [41].

The single-channel conductance of the BK_{Ca} channel measured with the use of 145 mM K^+ on both sides of the membrane in HCAEC was 208 ± 13 pS ($N = 10$). This value is similar to those of typical BK_{Ca} channels previously described in vascular endothelial cells [35], but much greater than that of small-conductance Ca^{2+}-activated K^+ channels [26]. The channel activity presented here is sensitive to stimulation by membrane depolarization and/or intracellular Ca^{2+}, and can be blocked by iberiotoxin or paxilline [42]. More importantly, we provide evidence that the ICI-182,780-mediated reduction in outward currents in these cells is not due to a decrease in unitary amplitude of

the channel, because there was no significant difference in single-channel conductance of BK_{Ca} channels between the presence and absence of ICI-182,780. Kinetic analysis showed that ICI-182,780 could interact with the channel primarily by reducing the mean open time of BK_{Ca} channels. Furthermore, the experimental results showing that the inhibitory effect of ICI-182,780 and 2-methoxyestradiol on the BK_{Ca} channel is not additive, suggest that 2-methoxyestradiol and ICI-182,780, which are structurally related, may interact with the same binding site in the channel.

In our study, the ability of ICI-182,780 to produce a shift of 13 mV to a positive potential in the activation curve of BK_{Ca} channels expressed in HCAEC, indicates that this compound can suppress channel activity in a voltage-dependent manner. Therefore, its interaction with the BK_{Ca} channel would rely not only on the concentration of ICI-182,780 used, but also on the pre-existing level of resting potential. In addition, the sensitivity of cell proliferation to ICI-182,780 was found to be comparable to that of BK_{Ca} channels in HCAEC. However, neither magnolol nor NDGA could reverse the inhibitory effect of ICI-182,780 on cell growth (data not shown). Unlike ICI-182,780, iberiotoxin (200 nM) alone had little or no effect on the growth of HCAEC. Previous studies have demonstrated that oscillating activity of BK_{Ca} channels appeared to be important for the migration of transformed epithelial cells or glioma cells [43,44]. Unlike 17β-estradiol, ICI-182,780 is potent in exerting anti-angiogenetic activity in vascular endothelial cells [11]. Thus, further studies are required to determine to what extent the ICI-182,780-mediated modulation of BK_{Ca} channels is associated with its inhibitory effects on the growth of tumor vessels *in vivo*. Indeed, it has been previously reported that the enhanced activity of BK_{Ca} channels could alter the proliferation of astrocytoma or glioma [45,46].

The stimulatory effect of BK_{Ca} channels observed in HCASMC is consistent with previous studies showing the ability of ICI-182,780 to enhance the open probability of BK_{Ca} channels in smooth muscle cells [13]. The mechanism of the stimulatory action is presently unclear. It is likely, however, that ICI-182,780 molecule binds either to the β-subunits of the channel, or to the tail domain of the α-subunits, because the S9–S10 tail region in the α-subunits was recently noted to alter the β1-subunit-induced increase in apparent Ca^{2+} sensitivity of the BK_{Ca} channel [47]. On the other hand, the ability of ICI-182,780 to reduce the probability of BK_{Ca} channels to open in HCAEC appears to result primarily from the interaction with the α-subunits, because endothelial BK_{Ca} channels were reported to have little expression of β-subunits [17,18]. However, whether such an effect occurs in other types of cells remains to be determined, because of multiple splice variants of the α-subunits in the channel [48].

Another noteworthy finding in this study was that in HCASMC, ICI-182,780 exerted a concentration-related dual effect (i.e. stimulatory and inhibitory) on BK_{Ca} channel activity. It seems that this effect resembles previous observations showing a dual effect of NS1608, an opener of BK_{Ca} channels, on the activity of this channel present in smooth muscle cells [49]. Whether the effects of ICI-182,780 represent two separate domains of drug interaction on the BK_{Ca} channel or two different affinity states of the same site remains to be clarified. In addition, because vascular endothelial cells have not been found to express the β-subunits of the channel [18], it remains to be determined whether the BK_{Ca} channel where no β-subunits were co-expressed is readily subject to inhibition by ICI-182,780.

This study indicates the direct effect of ICI-182,780 on the BK_{Ca} channel present in HCAEC and HCASMC. Such an effect may influence the functional activities of these cells, despite the ability of this drug to bind to intracellular estrogen receptors. Furthermore, ICI-182,780 and other structurally related compounds seem to be intriguing pharmacological tools used to characterize the properties of BK_{Ca} channels. Elucidation of the structure of the binding site for ICI-182,780 or other structurally related compounds may provide a structural basis for the pharmacological modulation of BK_{Ca} channels.

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References

[1] DeFriend DJ, Anderson E, Bell J, Wilks DP, West CM, Mansel RE, Howell A. Effects of 4-hydroxytamoxifen and a novel pure antioestrogen (ICI 182780) on the clonogenic growth of human breast cancer cells in vitro. Br J Cancer 1994;70:204–11.

[2] Howell A, DeFriend D, Robertson J, Blamey R, Walton P. Response to a specific antioestrogen (ICI 182780) in tamoxifen-resistance breast cancer. Lancet 1995;345:29–30.

[3] Kim-Schulze S, McGowan KA, Hubchak SC, Cid MC, Martin MB, Kleinman HK, Greene GL, Schnaper HW. Expression of an estrogen receptor by human coronary artery and umbilical vein endothelial cells. Circulation 1996;94:1402–7.

[4] Li G, Chen YF, Greene GL, Oparil S, Thompson JA. Estrogen inhibits vascular smooth muscle cell dependent adventitial fibroblast migration in vitro. Circulation 1999;100:1639–45.

[5] Hayashi T, Yamada K, Esaki T, Kuzuya M, Satake S, Ishikawa T, Hidaka H, Iguchi A. Estrogen increases endothelial nitric oxide by a receptor-mediated system. Biochem Biophys Res Commun 1995;214:847–55.

[6] Xiao S, Gillespie DG, Baylis C, Jackson EK, Dubey RK. Effects of estradiol and its metabolites on glomerular endothelial nitric oxide synthesis and mesangial cell growth. Hypertension 2001;37:645–50.

[7] Honda K, Sawada H, Kihara T, Urushitani M, Nakamizo T, Akaike A, Shimohama S. Phosphatidylinositol 3-kinase mediates neuroprotection by estrogen in cultured cortical neurons. J Neurosci Res 2000;60:321–7.

[8] Honda K, Shimohama S, Sawada H, Kihara T, Nakamizo T, Shibasaki H, Akaike A. Nongenomic antiapoptotic signal transduction by estrogen in cultured cortical neurons. J Neurosci Res 2001;64:466–75.

[9] Prakash YS, Togaibayeva AA, Kannan MS, Miller VM, Fitzpatrick LA, Sieck GC. Estrogen increases Ca^{2+} efflux from female porcine

coronary arterial smooth muscle. Am J Physiol 1999;276:H926–34.
[10] Montano MM, Katzenellenbogen BS. The quinone reductase gene: a unique estrogen receptor-regulated gene that is activated by antiestrogens. Proc Natl Acad Sci USA 1997;94:2581–6.
[11] Soares R, Guo S, Russo J, Schmitt F. Role of the estrogen antagonist ICI 182,780 in vessel assembly and apoptosis of endothelial cells. Ultrastruct Pathol 2003;27:33–9.
[12] Ruehlmann DO, Steinert JR, Valverde MA, Jackob R, Mann GE. Environmental estrogenic pollutants induce acute vascular relaxation by inhibiting L-type Ca^{2+} channels in smooth muscle cells. FASEB J 1998;646:613–9.
[13] Dick GM. The pure anti-oestrogen ICI 182,780 (Faslodex) activates large conductance Ca^{2+}-activated K^+ channels in smooth muscle. Br J Pharmacol 2002;36:961–4.
[14] Butler A, Tsunoda S, McCobb DP, Wei A, Salkoff L. mSlo, a complex mouse gene encoding ''maxi'' calcium-activated potassium channels. Science 1993;261:221–4.
[15] Knaus HG, Garcia-Calvo M, Kaczorowski GJ, Garcia ML. Subunit composition of the high conductance calcium-activated potassium channel from smooth muscle, a representative of the mSlo and slowpoke family of potassium channels. J Biol Chem 1994;269:3921–4.
[16] Tseng-Crank J, Godinot N, Johansen TE, Ahring PK, Strøbæk D, Mertz R, Foster CD, Reinhart PH. Cloning, expression, and distribution of a Ca^{2+}-activated K^+ channel beta-subunit from human brain. Proc Natl Acad Sci USA 1996;93:9200–5.
[17] Chang CP, Dworetzky SI, Wang J, Goldstein ME. Differential expression of the alpha and beta subunits of the large-conductance calcium-activated potassium channel: implication for channel activity. Mol Brain Res 1997;45:33–40.
[18] Papassotiriou J, Kohler R, Prenen J, Krause H, Akbar M, Eaggermont J, Paul M, Distler A, Nilius B, Hoyer J. Endothelial K^+ channel lacks the Ca^{2+} sensitivity-regulating β subunit. FASEB J 2000;14:885–94.
[19] He GW. Nitric oxide release, EDHF, and the role of potassium channels in coronary circulation. Drug Dev Res 2003;58:23–7.
[20] Nilius B, Viana F, Droogmans G. Ion channels in vascular endothelium. Annu Rev Physiol 1997;59:145–70.
[21] Edwards G, Dora KA, Gardener MJ, Garland CJ, Weston AH. K^+ is an endothelium-derived hyperpolarizing factor in rat arteries. Nature 1998;396:269–72.
[22] Félétou M, Vanhoutte PM. Third pathway: endothelium-dependent hyperpolarization. Drug Dev Res 2003;58:18–22.
[23] Chaytor AY, Evens WH, Griffith TM. Central role of heterocellular gap junction of rabbit arteries. J Physiol (Lond) 1998;508:561–73.
[24] Kohler R, Brakemeier S, Kuhn M, Behrens C, Real R, Degenhardt C, Orzechowski HD, Pries AR, Paul M, Hoyer J. Impaired hyperpolarization in regenerated endothelium after balloon catheter injury. Circ Res 2001;89:174–9.
[25] Ruschitzka FT, Noll G, Luscher TF. The endothelium in coronary artery disease. Cardiology 1997;68(Suppl 3):3–19.
[26] Wu SN, Yu HS, Jan CR, Li HF, Yu CL. Inhibitory effects of berberine on voltage- and calcium-activated potassium currents in human myeloma cells. Life Sci 1998;62:2283–94.
[27] Hamill OP, Marty A, Neher E, Sakmann B, Sigworth FJ. Improved patch-clamp techniques for high-resolution current recording from cells and cell-free membrane patches. Pfluegers Arch 1981;391:85–100.
[28] Wu SN, Li HF, Chiang HT. Stimulatory effects of δ-hexachlorocyclohexane on Ca^{2+}-activated K^+ currents in GH_3 lactotrophs. Mol Pharmacol 2000;57:865–73.
[29] Yamamura H, Nagano N, Hirano M, Muraki K, Watanabe M, Imaizumi Y. Activation of Ca^{2+}-dependent K^+ current by nordihydroguaiaretic acid in porcine coronary arterial smooth muscle cells. J Pharmacol Exp Ther 1999;291:140–6.
[30] Wu SN, Chen CC, Li HF, Lo YK, Chen SA, Chiang HT. Stimulation of the BK_{Ca} channel in cultured smooth muscle of human trachea by magnolol. Thorax 2002;57:67–74.
[31] Valverde MA, Rojas P, Amigo J, Cosmelli D, Orio P, Bahamonde MI, Mann GE, Vergara C, Latorre R. Acute activation of Maxi-K^+ channels (hslo) by estradiol binding to the beta subunit. Science 1999;285:1929–31.
[32] Dick GM, Sanders KM. (Xeno)estrogen sensitivity of smooth muscle BK channels conferred by the regulatory β1 subunit: a study of β1 knockout mice. J Biol Chem 2001;276:44835–40.
[33] Wu SN, Chiang HT, Chang FR, Liaw CC, Wu YC. Stimulatory effects of squamocin, an Annonaceous acetogenin, on Ca^{2+}-activated K^+ current in cultured smooth muscle cells of human coronary artery. Chem Res Toxicol 2003;16:15–22.
[34] Singh S, Syme CA, Singh AK, Devor DC, Bridges RJ. Benzimidazolone activators of chloride secretion: potential therapeutics for cystic fibrosis and chronic obstructive pulmonary disease. J Pharmacol Exp Ther 2001;296:600–11.
[35] Chiang HT, Wu SN. Inhibition of large-conductance calcium-activated potassium channel by 2-methoxyestradiol in cultured vascular endothelial (HUV-EC-C) cells. J Membr Biol 2001;182:203–12.
[36] Wu SN, Lin PH, Hsieh KS, Li YC, Chiang HT. Behavior of nonselective cation channels and large-conductance Ca^{2+}-activated K^+ channels induced by dynamic changes in membrane stretch in cultured smooth muscle cells of human coronary artery. J Cardiovasc Electrophysiol 2003;14:44–51.
[37] Newton CJ, Trapp T, Pagotto U, Renner U, Buric R, Stalla GK. The oestrogen receptor modulates growth of pituitary tumour cells in the absence of exogenous oestrogen. J Mol Endocrinol 1994;12:303–12.
[38] Kandouz M, Lombet A, Perrot J-Y, Jacob D, Carvajal S, Kazem A, Rostene W, Therwath A, Gompel A. Proapoptotic effects of antiestrogens, progestins and androgen in breast cancer cells. J Steroid Biochem Mol Biol 1999;69:463–71.
[39] Honda K, Shimohama S, Sawada H, Kihara T, Nakamizo T, Shibasaki H, Akaike A. Nongenomic antiapoptotic signal transduction by estrogen in cultured cortical neurons. J Neurosci Res 2001;64:466–75.
[40] Ercoli A, Scambia G, Fattorossi A, Raspaglio G, Battaglia A, Cicchillitti L, Malorni W, Rainaldi G, Benedetti Panici P, Mancuso S. Comparative study on the induction of cytostasis and apoptosis by ICI 182,780 and tamoxifen in an estrogen receptor-negative ovarian cancer cell line. Int J Cancer 1998;76:47–54.
[41] DeFriend DJ, Howell A, Nicholson RI, Anderson E, Dowsett M, Mansel RE, Blamey RW, Bundred NJ, Robertson JF, Saunders C. Investigation of a new pure antiestrogen (ICI 182780) in women with primary breast cancer. Cancer Res 1994;54:408–14.
[42] Li HF, Chen SA, Wu SN. Evidence for the stimulatory effect of resveratrol on Ca^{2+}-activated K^+ current in vascular endothelial cells. Cardiovasc Res 2000;45:1035–45.
[43] Bordey A, Sontheimer H, Trousland J. Muscarinic activation of BK channels induces membrane oscillations in glioma cells and leads to inhibition of cell migration. J Membr Biol 2000;176:31–40.
[44] Jin M, Defoe DM, Wondergem R. Hepatocyte growth factor/scatter factor stimulates Ca^{2+}-activated membrane K^+ current and migration of MDCK II cells. J Membr Biol 2003;191:77–86.
[45] Ransom CB, Sontheimer H. BK channels in human glioma cells. J Neurophysiol 2001;85:790–803.
[46] Basrai D, Kraft R, Bollensdorff C, Liebmann L, Benndorf K, Patt S. BK channel blockers inhibit potassium-induced proliferation of human astrocytoma cells. Neuroreport 2002;13:403–7.
[47] Qian X, Nimigean CM, Niu X, Moss BL, Magleby KL. Slo1 tail domains, but not the Ca^{2+} bowl, are required for the β1 subunit to increase the apparent Ca^{2+} sensitivity of BK channels. J Gen Physiol 2002;120:829–43.
[48] Zarei MM, Zhu N, Alioua A, Eghbali M, Stefani E, Toro L. A novel MaxiK splice variant exhibits dominant-negative properties for surface expression. J Biol Chem 2001;276:16232–9.
[49] Hu S, Kim HS. On the mechanism of the differential effects of NS004 and NS1608 in smooth muscle cells from guinea pig bladder. Eur J Pharmacol 1996;318:461–8.