Effects of Irbesartan on Cloned Potassium Channels Involved in Human Cardiac Repolarization

IGNACIO MORENO,1 RICARDO CABALLERO,1 TERESA GONZÁLEZ, CRISTINA ARIAS, CARMEN VALENZUELA, ISABEL IRIEPA, ENRIQUE GÁLVEZ, JUAN TAMARGO, and EVA DELPÓN

Department of Pharmacology, School of Medicine, Universidad Complutense, Madrid, Spain (I.I., R.C., T.G., C.A., C.V., J.T., E.D.); and Department of Organic Chemistry, Universidad de Alcalá, Alcalá de Henares, Madrid, Spain (I.I., E.G.)

Received July 30, 2002; accepted October 30, 2002

ABSTRACT

We studied the effects of irbesartan, a selective angiotensin II type 1 receptor antagonist, on human ether-a-go-go-related gene (HERG), KvLQT1+minK, hKv1.5, and Kv4.3 channels using the patch-clamp technique. Irbesartan exhibited a low affinity for HERG and KvLQT1+minK channels (IC50 = 193.0 ± 49.8 and 314.6 ± 85.4 μM, respectively). In hKv1.5 channels, irbesartan produced two types of block, depending on the concentration tested. At 0.1 μM, irbesartan inhibited the current in a time-dependent manner (22 ± 3.9% at +60 mV). The blockade increased steeply with channel activation increasing at more positive potentials. However, at 10 μM, irbesartan induced a time-independent blockade that occurred in the range of potentials of channel opening, reaching its maximum at ~0 mV, and remaining unchanged at more positive potentials (24.0 ± 1.0% at +60 mV). In Kv4.3 currents, irbesartan produced a concentration-dependent block, which resulted in two IC50 values (1.0 ± 0.1 nM and 7.2 ± 0.6 μM). At 1 μM, it inhibited the peak current and accelerated the time course of inactivation, decreasing the total charge crossing the membrane (36.6 ± 7.8% at +50 mV). Irbesartan shifted the inactivation curve of Kv4.3 channels, the blockade increasing as the amount of inactivated channels increased. Molecular modeling was used to define energy-minimized dockings of irbesartan to hKv1.5 and HERG channels. In conclusion, irbesartan blocks Kv4.3 and hKv1.5 channels at therapeutic concentrations, whereas the blockade of HERG and KvLQT1+minK channels occurred only at supratherapeutic levels. In hKv1.5, a receptor site is apparent on each α-subunit of the channel, whereas in HERG channels a common binding site is present at the pore.

Irbesartan is a potent, long-acting selective AT1 receptor antagonist used widely in the treatment of hypertension (Markham et al., 2000). Recently, it has been demonstrated that irbesartan reduced heart rate and QT dispersion in hypertensive patients (Lim et al., 1999), and both actions were independent of changes in blood pressure. Moreover, patients with atrial fibrillation treated with amiodarone and irbesartan had a lower recurrence rate of persistent atrial fibrillation and a longer time to first arrhythmia recurrence than patients treated with amiodarone alone (Madrid et al., 2002).

In the human myocardium, the duration of the action potential, as well as the QT interval, is largely determined by several outward K+ currents (Nerbonne, 2000), including 1) the 4-aminopyridine-sensitive component of the transient outward current (Ito1) carried by Kv4.3 α-subunits, probably coassembled with KChIP2s auxiliary β-subunits (Wang et al., 1999; Rosati et al., 2001); 2) the rapidly activating, slowly inactivating delayed rectifier current (IKur) generated by hKv1.5 channels (Wang et al., 1993); and 3) the fast (IKs) and slow (IKr) components of the delayed rectifier current. Native IKr current is carried by channels formed by the coassembly of HERG α-subunits and MiRP1 β-subunits (Sanguinetti et al., 1995; Abbott et al., 1999), whereas coassembly of KvLQT1 α-subunits with minK β-subunits produces the IKr current (Barhanin et al., 1996; Sanguinetti et al., 1996).

Several AT1 antagonists, such as losartan and candesartan, at clinically relevant concentrations, directly modified the human cardiac repolarizing K+ currents (Caballero et al., 2000, 2001a). However, they presented marked differences in potency and blocking properties, indicating that their effects...
on K^+ channels were not related to AT1 receptor antagonism. Irbesartan is a biphenyltetrazole ring system attached to a substituted imidazole ring. It presents a spirocyclopentane ring that constitutes the hydrophobic portion of the molecule that is oriented perpendicularly to the imidazole ring. However, in losartan and candesartan this hydrophobic portion is absent or is coplanar to the imidazole ring. To analyze whether this important variation in the structure of the drug may lead to differences in the blocking properties of cardiac K^+ channels, the effects of irbesartan on HERG, KvLQT1+minK, hKv1.5, and Kv4.3 channels, expressed in mammalian cells, were studied. The present results indicated that, at therapeutic concentrations, irbesartan blocks Kv4.3 and hKv1.5 channels, whereas the blockade of HERG and KvLQT1+minK channels occurred only at supratherapeutic levels.

Materials and Methods

Cell Culture. Stably transfected Ltk^- cells expressing hKv1.5 channels were cultured in Dulbecco's modified Eagle's medium (Sigma Chemical, London, UK) supplemented with 10% horse serum and 0.25 mg/ml G418 (a neomycin analog; Invitrogen, Carlsbad, CA) in a 5% CO₂ atmosphere (Caballero et al., 2001a). The transient expression of KvLQT1+minK and Kv4.3 channels on CHO cells has been described previously (Caballero et al., 2001a). Briefly, the cells were grown in Ham's-F12 with 10% fetal bovine serum and transfected with the cDNA encoding the KvLQT1 and minK (0.8 μg, respectively) or Kv4.3 (5 μg) channels together with the cDNA encoding the CD8 antigen (0.5 μg; Invitrogen, Carlsbad, CA). The cells were then cultured in a solution containing 130 mM NaCl, 4 mM KCl, 1 mM CaCl₂, 1 mM MgCl₂, 10 mM HEPES, and 10 mM glucose (pH 7.4 with NaOH). KH₂PO₄, 5 mM MgATP, 3 mM phosphocreatine, 5 mM HEPES, and 0.25 mg/ml G418 (a neomycin analog; Invitrogen, Carlsbad, CA) supplemented with 10% horse serum and 0.2 M NaCl was used as the selection medium (Sigma Chemical, London, UK). The cells were disaggregated, and a cell suspension was obtained, bath perfusion was switched to drug-containing solution. The holding potential was maintained at −80 mV, and the cycle time for any protocol was 10 s to avoid accumulation of inactivation and/or block. The protocol to obtain current-voltage relationships consisted of 250-ms (Kv4.3), 500-ms (hKv1.5), 2000-ms (KvLQT1+minK), or 5000-ms (HERG) pulses that were imposed in 10-mV increments between −80 and +60 mV. Between −80 and −40 mV, only passive linear leak was observed, and least-squares fits to these data were used for passive leak correction. Deactivating hKv1.5, and KvLQT1+minK "tail" currents were recorded on return to −40 mV. Deactivating HERG tail currents were recorded at −60 mV. The activation curves of HERG and hKv1.5 currents were constructed by plotting tail current amplitudes as a function of the membrane potential. To obtain the inactivation curves of Kv4.3 channels a two-step voltage-clamp protocol was used. The first 250-ms-conditioning pulse from −80 to potentials between −90 and +50 mV was followed by a test pulse to +40 mV. Inactivation curves were constructed plotting the current amplitude as a function of the voltage command of the conditioning pulse. The activation and inactivation curves were fitted with a Boltzmann distribution:

\[ y = A/(1 + \exp[(V_m - V_0)/k]) \]

where A is the amplitude term, V₀ is the midpoint of activation or inactivation, V_m is the test potential, and k represents the slope factor of the curve. Voltage-dependence of Kv4.3 channel activation or conductance (G) was determined from the following relationship:

\[ G_m = I_m/(V_m - V_p) \]

To describe the time course of current activation and/or inactivation upon depolarization, as well as the tail currents upon repolarization, exponential analysis was used as an operational approach, fitting the current traces to the following equation:

\[ y = C + A_1\exp(-t/\tau_1) + A_2\exp(-t/\tau_2) + \ldots + A_n\exp(-t/\tau_n) \]

where τ₁, τ₂,τᵢ are the system time constants; A₁, A₂, and Aᵢ are the amplitudes of each component of the exponential; and C is the baseline value. The curve-fitting procedure used a nonlinear least-squares (Gauss-Newton) algorithm; results were displayed in linear and semilogarithmic format, together with the difference plot. Goodness of fit was judged by the χ² criterion and by inspection for systematic nonrandom trends in the difference plot. Fractional block was defined as follows:

\[ f = 1 - I_{\text{drug}}/I_{\text{control}} \]

where I_{CONTROL} and I_{DRUG} are the leak-corrected current amplitudes in the absence and the presence of irbesartan, respectively. To obtain the IC₅₀ (concentration of drug that produces the half-maximum blockade) and the Hill coefficient, nₜₐₜ, the fractional block obtained at various drug concentrations [D] was fitted to the following equation:

\[ f = 1/(1 + (IC_{50}/[D])^n) \]

In some cases, the fractional block was better fitted to the equation of a hyperbola:

\[ Y = B_{\text{max}} \cdot X/(IC_{50} + X) \]

obtaining the IC₅₀ and the B_{max} (maximum blockade induced by the drug) values.
Molecular Modeling. Molecular modeling studies were carried out with QUANTA/CHARMm software (Accelrys, Paris, France). The 1BL8 KcsA structure was retrieved from Protein Data Bank and used as the template for a HERG and hKv1.5 homology model. The pore region and S6 domain of HERG and hKv1.5 were modeled using an alignment between these regions of KcsA and the corresponding regions of HERG and hKv1.5 channels. Then, energy minimization was performed to eliminate close contacts. The tetramer was constructed by copying the derived monomer conformation onto the KcsA tetramer.

Irbesartan was assembled within QUANTA using standard bond lengths and bond angles; this molecule was built as an anionic species, as it exists at physiological pH (Cagigal et al., 2001). Mechanics energy minimization was done using the CHARMm force field. Irbesartan was manually docked into the Y652 and F656 region (HERG) and T507, L510, and V514 region (hKv1.5). Then, the corresponding complexes were minimized with a Newton Raphson method considering the structures as fully optimized when the energy changes between two successive iterations were less than 0.01 Kcal/mol (Morreale et al., 2002).

Statistical Methods. Data obtained after drug exposure were compared with those obtained under control conditions in a paired manner. For comparisons at a single voltage, differences were analyzed using the Student’s t test. To analyze block at multiple voltages, two-way analysis of variance was used followed by Newman-Keuls test. Results are expressed as mean ± S.E.M. A P value of less than 0.05 was considered significant.

Results

Effects of Irbesartan on HERG Currents. Fig. 1A shows current traces of HERG currents obtained when applying 5-s pulses to potentials ranging between −80 and +60 mV in 20-mV steps, in the absence and in the presence of 100 μM irbesartan. On returning to −60 mV an outward tail current was recorded. The time constant of HERG current activation at +10 mV was obtained fitting a monoexponential function to the current traces ($\tau_{\text{act}} = 647.4 \pm 91.4$ ms, $n = 4$). In contrast, two exponential components were required to describe the tail current decline ($\tau_1 = 348.4 \pm 43.7$ ms, $\tau_2 = 1580.3 \pm 121.4$ ms). Irbesartan decreased the current amplitude and accelerated the current activation, so that the time
constant of activation at +10 mV decreased to 287.9 ± 55.1 ms (n = 4, P < 0.05). Moreover, irbesartan decreased the peak tail current amplitude (48.9 ± 5.9% at +60 mV), whereas it did not modify the time course of deactivation (τd = 283.4 ± 70.7 ms and τd = 1136.4 ± 281.5 ms, n = 5, P > 0.05) (Fig. 1B). Fig. 1C represents the concentration dependence of HERG channel blockade. The blockade measured on the peak tail current, elicited upon repolarization after pulses to +60 mV was fitted to the Hill equation (eq. 5) and yielded an IC50 value of 193.0 ± 49.8 μM and a Hill coefficient of 0.7 ± 0.1.

Figure 2A represents the current-voltage relationship of HERG currents in the absence and the presence of 100 μM irbesartan. At voltages ranging between −20 and +30 mV, irbesartan decreased the current amplitude, reaching 24.1 ± 6.5% of block at 0 mV (n = 5, P < 0.05). Fig. 2B shows the effect of irbesartan on the voltage dependence of HERG channel activation. The control data were described with a Boltzmann equation and the values for V50 and k averaged −14.8 ± 2.1 and 9.5 ± 0.4 mV, respectively (n = 5). Irbesartan did not modify the V50 (−18.5 ± 3.1 mV, n = 5, P > 0.05) or the slope value of the curve (k = 9.6 ± 0.8 mV). Squares in Fig. 2B represent the fractional tail current block as a function of the membrane potential. The blockade increased steeply from −20 to 0 mV (45.9 ± 8.1%) and, thereafter, remained unchanged (48.9 ± 5.9% at +60 mV, n = 5, P > 0.05 versus data at 0 mV).

**Effects of Irbesartan on KvLQT1+minK Currents.** Fig. 3A shows current traces obtained during 2 s-pulses to +60 mV in the absence and in the presence of 500 μM irbesartan. To describe the dominant time constants of the activation process of KvLQT1+minK currents, traces to +60 mV were fitted by a biexponential function, and the fast (τa = 263.6 ± 38.0 ms) and slow (τa = 1721.6 ± 152.9 ms) time constants of activation were calculated. Irbesartan reduced the current amplitude by 61.9 ± 8.5% (n = 6, P < 0.05), without affecting the activation kinetics (τa = 244.0 ± 43.0 ms and τa = 1410.2 ± 148.5 ms, n = 6, P > 0.05). The ratio between the irbesartan-sensitive current during the depolarizing pulse, obtained by digital subtraction of the current traces, and the current in control conditions ([Ii − Ii]/Ii) is shown in the lower part of Fig. 3A. At the beginning of the depolarizing pulse no block was observed and the blockade increased during the application of the pulse. The onset of block was fitted by a monoexponential function (solid curve), to determine the time constant of development of block (τblock), which averaged 260.9 ± 42.4 ms (n = 6). The deactivating tail currents, on returning to −40 mV (Fig. 3B), declined with monoexponential kinetics (τ = 457.3 ± 48.1 ms). Irbesartan decreased the peak tail current amplitude by 64.4 ± 2.6% without modifying its time course (τ = 468.3 ± 55.4 ms, n = 6, P > 0.05). Figure 3C represents the concentration dependence of KvLQT1+minK channel blockade. The blockade measured at the end of 2-s pulses to +60 mV was fitted to the Hill equation (eq. 5) and yielded an IC50 value 314.6 ± 85.4 μM and a Hill coefficient of 1.1 ± 0.3.

**Fig. 2.** Voltage-dependent effects of irbesartan on HERG currents. A, current-voltage relationships (5-s isochronal) in the absence and in the presence of 100 μM irbesartan. B, activation curves were fitted with a Boltzmann equation (eq. 1). Squares represent the fractional block (f = Ii/Ii) from data obtained in the presence and in the absence of irbesartan. In A and B, each point represents the mean ± S.E.M of five experiments.
Fig. 3. Effects of irbesartan on KvLQT1 minK currents. A, superimposed current traces obtained by applying 2-s pulses from -80 mV to +60 mV in the absence and in the presence of 500 μM irbesartan. The lower part shows the plot of the current ratio between the irbesartan-sensitive current during the depolarizing pulse (I_C-I_B) and the current in control conditions. The continuous line represents the fit to a monoexponential function. B, effects of irbesartan on tail currents upon repolarization to -40 mV after pulses to +60 mV. The continuous lines represent the fit to a monoexponential function. In A and B, the dashed line represents the zero current level. C, concentration-dependent effects of irbesartan on KvLQT1 minK currents. The data were fitted with a Hill equation (eq. 5). Each point represents the mean ± S.E.M. of four to six experiments.

(Fig. 5A). Control tail currents were fitted by a biexponential function, the fast (τ_f) and the slow (τ_s) time constants averaging 20.8 ± 1.7 and 69.1 ± 9.3 ms, respectively. Irbesartan slowed the tail current deactivation, increasing the τ_f to 37.8 ± 6.1 ms (n = 10, P < 0.05).

Figure 4C shows the current-voltage relationship (500-ms isochronal) obtained in the absence and in the presence of 0.1 μM irbesartan. Irbesartan significantly decreased the current amplitude at potentials positive to -10 mV. The ratio I_IB/I_CON was plotted as a function of the membrane potential in Fig. 4D. The blockade increased steeply in the voltage range coinciding with that of activation of the channels (between -20 and 0 mV) and thereafter increased with a shallow voltage dependence. In fact, the blockade induced at +60 mV was significantly higher than at 0 mV (10.1 ± 1.4%, P < 0.05). Figure 4E shows the activation curves in the absence and in the presence of 0.1 μM irbesartan. Under control conditions, the activation curve yielded V_h and k values of -11.9 ± 0.9 and 4.2 ± 0.4 mV, respectively (n = 9). Irbesartan slightly decreased the tail current amplitude at potentials positive to 0 mV (9.2 ± 1.4% at +60 mV) and shifted the V_h to more negative potentials (-17.3 ± 0.9 mV, P < 0.01) without modifying the k value (4.2 ± 0.3 mV, P > 0.05).

In Fig. 5B, the blockade at the end of 500-ms pulses to +60 mV was used as an index of block and represented as a function of the irbesartan concentration. Surprisingly, at concentrations between 0.1 and 100 μM, irbesartan induced a similar amount of block (~25%). Fitting the concentration-response data to a hyperbolic function (eq. 6), an IC_{50} value of 0.02 ± 0.009 μM with a B_{max} of 26.3 ± 1.2% was obtained. Figure 5C shows the concentration-response curve derived from the reduction of the current amplitude at 0 mV. As can be observed, effects of irbesartan varied as a function of the concentration of the drug, whereas the maximum blockade was again <30%. Fitting the data to a Hill equation (eq. 5), the IC_{50} and the B_{max} value obtained were 0.2 ± 0.05 μM and 24.7 ± 0.9%, respectively. Finally, the reduction of the peak tail current amplitudes, elicited upon repolarization to -40 mV after pulses to +60 mV, was represented as a function of the irbesartan concentration in Fig. 5D. In this case, the IC_{50} and the B_{max} value averaged 0.7 ± 0.2 μM and 27.3 ± 1.4%, respectively.
Effects of High Concentrations of Irbesartan on hKv1.5 Currents. Even when the blockade elicited at +60 mV was similar across a wide range of concentrations, the characteristics of the blockade induced by concentrations of irbesartan lower than 1 μM were very different from those produced by higher concentrations. Thus, the effects of high concentrations (10 μM) of irbesartan on hKv1.5 were analyzed. Figure 6A shows hKv1.5 current traces in the absence and in the presence of 10 μM irbesartan. At this concentration, the drug did not induce a time-dependent block and the blockade at the end of pulses to +60 mV averaged 24.0 ± 1.0% (P > 0.05 versus blockade obtained with 0.1 μM irbesartan). Irbesartan significantly slowed the time course of tail current deactivation (Fig. 6B), increasing the τ_f and τ_i from 22.1 ± 3.8 and 75.6 ± 16.7 ms to 44.6 ± 11.3 and 142.3 ± 26.4 ms (P < 0.05, n = 8), respectively. Figure 6C and D, show the current-voltage curves obtained in the absence and in the presence of irbesartan, and the fractional block plotted as a function of the membrane potential, respectively. The blockade increased steeply in the voltage range of channel activation, reached a maximum at ~0 mV (31.0 ± 6.2%) and thereafter remained unchanged (24.0 ± 1.0% at +60 mV, n = 4, P > 0.05). Irbesartan also decreased the tail current amplitude at potentials positive to +20 mV (29.8 ± 5.7% at +60 mV, P < 0.05 versus blockade induced by 0.1 μM) and shifted the V_h of the curve to more negative potentials (from −14.7 ± 0.8 to −17.6 ± 1.2 mV, n = 4, P < 0.01) without modifying the slope value (Fig. 6E).

Effects of Irbesartan on Kv4.3 Currents. Fig. 7A shows superimposed Kv4.3 current traces elicited when applying 250-ms pulses to +50 mV in the absence and in the presence of 1 μM irbesartan. The currents rose rapidly to a peak (τ_act = 1.2 ± 0.2 ms, n = 20), and then inactivated following a biexponential process (τ_i = 22.5 ± 4.7 ms and τ_f = 52.3 ± 7.1 ms at +50 mV, n = 20). Irbesartan decreased the peak current by 19.4 ± 7.3% (n = 5, P > 0.05) and accelerated the time course of the inactivation process, decreasing the τ_f and τ_i values to 14.6 ± 2.2 ms (P < 0.05) and 49.4 ± 3.6 ms (n = 5, P > 0.05), respectively. The effects of irbesartan on Kv4.3 channels were reversible upon superfusion with drug-free external solution for 7 to 10 min (Fig. 7A, inset). These actions of irbesartan are suggestive of an open-channel block mechanism, in which case the reduction of peak current would not represent the steady-state block. Therefore, irbe-
sartan-induced block was measured as the reduction of the total charge crossing the membrane estimated from the integral of the current traces elicited at $V_h$.

In Fig. 7B, the decrease in charge crossing the membrane was represented as a function of irbesartan concentration. At 0.001 μM irbesartan-induced block averaged 22.7 ± 3.9% (n = 5) and between 0.001 and 10 μM, the blockade increased progressively as the concentration of the drug was augmented. Fitting the data to a hyperbolic function yielded an IC$_{50}$ and $B_{\text{max}}$ value of 1.0 ± 0.4 μM and 46.2 ± 5.9%, respectively. Surprisingly, at 50 μM the blockade did not increase but decreased and at higher concentrations the blockade progressively increased again. Thus, at concentrations between 50 and 500 μM, the IC$_{50}$ and $B_{\text{max}}$ values were 29.8 ± 12.9 μM and 34.9 ± 3.3%, respectively. Figure 7C shows the total Kv4.3 charge as a function of the potential of the test pulse in the absence and the presence of 1 μM irbesartan. Irbesartan decreased the charge crossing the membrane at potentials positive to −20 mV (n = 4, P < 0.05).

In this figure, the squares represent the fractional charge block, which reached a maximum at −10 mV (41.6 ± 6.8%), thereafter remaining unchanged (36.6 ± 7.8% at +50 mV, P > 0.05). The conductance-voltage curves of Kv4.3 channels (Fig. 7D) were described by a Boltzmann function, yielding $V_h$ and $k$ values in control conditions of 8.6 ± 0.8 and 11.8 ± 0.9 mV, respectively. Irbesartan did not significantly modify either the $V_h$ (10.9 ± 2.4 mV) or the $k$ (15.3 ± 2.2 mV) (n = 4, P > 0.05) values of the curve.

Figure 8A shows representative Kv4.3 current traces obtained with the protocol used to assess the voltage dependence of inactivation and Fig. 8B the inactivation curves in the absence and the presence of irbesartan. Under control conditions, the $V_h$ and the $k$ values averaged −34.3 ± 2.7 and 5.9 ± 0.3 mV. Irbesartan decreased the peak Kv4.3 current amplitude and shifted the $V_h$ of the curve to −37.2 ± 2.8 mV (n = 6, P < 0.05) without modifying the $k$ value (5.7 ± 0.4 mV). To relate the voltage dependence of drug-induced block to the voltage dependence of Kv4.3 inactivation, fractional block was plotted as a function of the voltage of the preceding pulse (Fig. 8B). The blockade remained unchanged at potentials between −90 mV (10.6 ± 4.5%) and −50 mV (15.2 ± 3.9%, n = 6, P > 0.05), but at more positive potentials, it augmented as the amount of inactivated channels increased, reaching a maximum at −20 mV (56.1 ± 11.8%, P < 0.05).
versus blockade at −90 mV). These results indicated that irbesartan binds to the inactivated state. Thus, the reduction of the current amplitude obtained after conditioning pulses to −20 mV was plotted as a function of the irbesartan concentration (Fig. 7B). As can be observed, between 0.001 and 1 μM, the blockade increased as the concentration of the drug was augmented. Fitting the data to a hyperbolic function, the IC50 and the Bmax values were 1.0 ± 0.1 nM and 56.0 ± 1.4%, respectively. However, between 1 and 10 μM, the blockade decreased to 40.1 ± 13.3%, but progressively increased at higher concentrations of irbesartan, allowing the calculation of IC50 and Bmax values of 7.2 ± 0.6 μM and 68.5 ± 1.1%, respectively.

**Discussion**

We have analyzed the effects of irbesartan on several K+ currents involved in human cardiac repolarization. Maximum plasma concentrations obtained after administration of therapeutic doses of irbesartan (150–300 mg/day) were 7 to 11 μM (Markham et al., 2000). Considering that irbesartan is highly bound to plasma proteins (90%), the free plasma concentration would be 0.7 to 1.1 μM. Thus, this study demonstrated that, at therapeutic concentrations, irbesartan blocked hKv1.5 and Kv4.3 channels, whereas its effects on HERG and KvLQT1+minK channels occurred only at supratherapeutic levels. Furthermore, these effects were not related to the AT1 receptor antagonism, because the experiments were carried out in the absence of angiotensin II and the effects of irbesartan on these currents differ in potency and in voltage and time dependence, in a manner that is not consistent with a common mechanism of action.

**Effects on HERG and KvLQT1+minK Channels.** Irbesartan exhibited a low affinity for HERG and KvLQT1+minK channels, the IC50 values being ~200-fold higher than the free plasma concentrations. This low affinity of irbesartan could be attributed to the spatial orientation of the most hydrophobic portion of the molecule (the spirocyclopentane ring), which is oriented perpendicularly to the imidazolone ring. Therefore, the irbesartan moiety would be rigid, preventing the proper interaction of the drug with its receptor site.

Irbesartan-induced block of HERG channels increased steeply at the voltage range of channel opening, suggesting that it preferentially blocks the open state of the channel. The drug accelerated the time course of activation and shifted the activation curve to more negative potentials, suggesting that irbesartan altered the channel gating. Because
the I_{Kr} carried by HERG channels plays a critical role in the control of the ventricular action potential repolarization and refractoriness in human (Tseng, 2001), our results suggest that effects of irbesartan at the ventricular level, if present, are not attributable to the HERG blockade.

Irbesartan also blocked KvLQT1/minK channels. The fast development of block with no block at the beginning of depolarizing pulses strongly suggested an open-state interaction. Because KvLQT1/minK currents exhibited a fast rundown, no further effort was made to determine the voltage dependence of the blockade.

**Effects on hKv1.5 and Kv4.3 Channels.** Irbesartan exhibits a high affinity for hKv1.5 and Kv4.3 channels. However, the efficacy of block was low because the maximum blockade obtained was less than 30 and 60%, respectively. Furthermore, the concentration-dependent effects of irbesartan on Kv4.3 channels, depicted using either the reduction in charge crossing the membrane at positive potentials (open-channel interaction) or the inhibition of the current elicited after conditioning pulses (interaction with the inactivated state), were biphasic. The reasons for this behavior are unknown, and experiments on site-directed hKv1.5 and Kv4.3 mutant channels would be needed to elucidate this issue. However, what cannot be excluded is that when the concentration of bulky molecules of irbesartan near the binding site increases, the steric hindrance interactions between them might decrease the efficacy of block. The importance of steric hindrance interactions in determining the blockade of quinidine at Kv1.4 channels has been demonstrated previously (Zhang et al., 1998).

Irbesartan induced a voltage-dependent block on hKv1.5 channels that increased at the voltage range of channel activation and produced crossover of the tail currents, suggesting an open-channel interaction. However, at concentrations lower than 1 μM the blockade was time-dependent, whereas at higher concentrations a time-independent block was observed. Assuming an open-state interaction, the time-dependent decline would represent the time course of relaxation.
toward a new equilibrium, whereas the effects of 10 μM irbesartan can be explained considering that the development of block is faster than the current activation, even when it cannot be excluded that irbesartan also binds to the closed state of the channel. At potentials positive to 0 mV, at which the channel opening reached saturation, the blockade induced by 0.1 μM irbesartan increased, whereas that produced by 10 μM was not modified. Irbesartan is a weak acid that predominates in its anionic form (Cagigal et al., 2001); however, the effects of the transmembrane electrical field cannot account for the voltage dependence of the blockade because it depends on the irbesartan concentration.

Irbesartan slightly decreased the peak current amplitude and accelerated the time course of current inactivation on Kv4.3 channels. Moreover, irbesartan-induced block occurred in the range of potentials of channel opening. All these effects suggest an open-channel interaction. Furthermore, the blockade significantly augmented with channel inactivation, suggesting that irbesartan also binds to the inactivated state. Affinity for both the open and the inactivated state has been previously described for flecainide and quinidine on Kv4.2 channels (Caballero et al., 2001b).

**Molecular Modeling of the Binding Site of Irbesartan.** Molecular modeling was used to define the energy minimized docking for irbesartan in hKv1.5 and HERG channels (Fig. 9). However, the following limitations of the model should be considered: 1) other ligand dockings are possible; 2) the homology model is based on the KcsA channel crystal structure, which represents the closed state conformation (Doyle et al., 1998); 3) the structure of hKv1.5 differs from that of the KcsA channels by the introduction of a sharp bend in the inner (S6) helices (Del Camino et al., 2000) that occur
Fig. 9. Molecular modeling of hKv1.5 and HERG channels. A and B, docking of the irbesartan molecule within the cavity of the homology model of the hKv1.5 channel. A, view of the S5-S6 domain of three hKv1.5 subunits with docked molecules of irbesartan between the S5 and S6 domains. B, close-up view of irbesartan in a four-subunit model of the channel. T507, L510, and V514 of the S6 domain are shown in sticks; irbesartan is shown as liquorice model. Only one of the four S5 and S6 domains is shown. C and D, docking of irbesartan molecule within the cavity of the homology model of the HERG channel. C, view of the of the S5-S6 domain of three HERG subunits with docked molecule of irbesartan. D, close-up view of irbesartan in a four-subunit model of the channel. T623 of the pore helix and Y652 and F656 of the S6 domain are shown in sticks; irbesartan is shown as liquorice model. Only one of the four S5 and S6 domains is shown.
at a Pro-X-Pro sequence, which is absent in HERG channels; and 4) the dockings for irbesartan on hKv1.5 and HERG channels have not been corroborated with experiments on site-directed mutant channels.

In hKv1.5, we have considered that irbesartan binds to the residues T507, L510, and V514 that are critical in determining quinidine affinity (Yeola et al., 1996), the stereoselectivity of bupivacaine-induced block (Franqueza et al., 1997) and the increasing or blocking effects of benzocaine (Caballero et al., 2002). The model predicted that, at least in the closed state, these amino acids are not facing the channel pore (Fig. 9B), and suggested that irbesartan blocks the hKv1.5 channels by hydrogen bonding with T507 and van der Waals interactions with L510 and V514 (Fig. 9, A and B). Thus, there exists one binding site on each of the four α-subunits that form the channel. This result might explain the concentration dependence of the effects of irbesartan, considering that the different receptors are negatively coupled (Waud, 1968), i.e., binding of one molecule at one α-subunit decreased the affinity for the binding to the other nonoccupied sites, thus decreasing the efficacy of irbesartan for blocking hKv1.5 channels. Moreover, binding to this region of the S6 segment, which comprises part of the activation gate (Rich et al., 2002), could account for the voltage dependence of the blockade.

In HERG channels, Y652 and F656 are the most important determinants of the binding of both high- and low-affinity HERG blockers (Mitcheson et al., 2000a; Sánchez-Chapula et al., 2002). These residues are located on the S6 domain and homology modeling predicted that they face the central cavity of the channel (Fig. 9C). The molecular modeling suggested that irbesartan binds by hydrogen bonding interactions with Y652, by π-stacking interactions with Y652 and F656, and by van der Waals interactions with T623 (Fig. 9D). Considering this site as the receptor binding site for irbesartan, it can be observed that one molecule of irbesartan is enough to block the potassium permeation through the pore.

In conclusion, we describe for the first time that irbesartan blocks Kv4.3 and hKv1.5 channels, the latter being present only in the atria (Wang et al., 1993). This direct effect on cardiac K⁺ channels together with the reversal of myocardial hypertrophy and fibrosis (i.e., structural remodeling) produced by irbesartan (Markham et al., 2000) may be of interest in patients with supraventricular arrhythmias with minimal risk of ventricular proarrhythmia. The effects of free plasma concentrations of irbesartan on hKv1.5 and on Kv4.3 are moderate, and we cannot rule out that these change might not be sufficient to alter the atrial repolarization. Unfortunately, the resultant effect on human atrial action potential duration is, as yet, unknown and not easily predictable. Very recently, it has been demonstrated that the addition of irbesartan to patients treated with amiodarone decreases the rate of recurrence of persistent atrial fibrillation (Madrid et al., 2002). However, further studies are needed to confirm its possible antiarrhythmic properties and the mechanisms responsible for this effect.

Acknowledgments

We thank Drs. M. M. Tamkun and D. J. Snyders for providing hKv1.5 and Kv4.3 channels, Drs. M. Weerapura, S. Nattel, and T. Hebert for providing the CHO cells stably expressing HERG channels, and M. Keating and M. Sanguinetti for providing the Kv-LQT1 and minK clones. We also thank Guadalupe Pablo for excellent technical assistance.

References


