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Assembly with the Kvβ1.3 Subunit Modulates Drug Block of hKv1.5 Channels

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ABSTRACT

The assembly of voltage-gated potassium (Kv) channels with β subunits modifies the electrophysiological characteristics of the α subunits. Kvβ1.3 subunits shift the midpoint of the activation curve toward more negative voltages and slow the deactivation process. In addition, the Kvβ1.3 subunit converts hKv1.5 from a delayed rectifier with a modest degree of slow inactivation to a channel with both fast and slow components of inactivation. In the present study, we have analyzed the effects of bupivacaine and a permanently charged analog [*R*(+)-*N*-methyl-bupivacaine (RB^+1C)] on Kvα1.5 and Kvα1.5+Kvβ1.3 channels expressed in human embryonic kidney 293 cells using the whole-cell configuration of the patch-clamp technique. Block induced by RB^+1C binding to its external receptor site was not modified by the presence of this β subunit. However, hKvα1.5+Kvβ1.3 channels were ~4-fold less sensitive to bupivacaine than hKv1.5 channels in the absence of β subunits (IC_{50} = 47.5 ± 5.1 versus 13.1 ± 0.8 μM, respectively, $p < 0.01$). Quinidine was also less potent to block Kvα1.5+Kvβ1.3 channels than Kvα1.5 channels (IC_{50} = 49.6 μM versus 6.2 μM, respectively). These results suggest that the Kvβ1.3 subunit does not modify the affinity of the charged bupivacaine for its external receptor site but markedly reduces the affinity of bupivacaine and quinidine for their internal receptor site in hKv1.5 channels.

Voltage-gated K^+ channels (Kv) are multimeric membrane proteins composed of four α subunits that are often associated with accessory β subunits. Not only are α subunit mutations responsible for human disease but also β subunits (minK and MiRP1) are involved in severe pathologies, such as the congenital long QT syndrome (Splawski et al., 1997; Abbott et al., 1999). The hKv1.5 channel encodes the ultrarapid delayed rectifier K^+ current present in the human atrium but not in the ventricle (Fedida et al., 1993; Snyders et al., 1993; Wang et al., 1993; Feng et al., 1997). It is also widely expressed in the vascular system (Coppock and Tamkun, 2001). Two β subunits that show overlapping expression patterns with Kv1.5, Kvβ1.3, and Kvβ2.1 shift the midpoint of the activation curve toward more negative voltages and slow deactivation (England et al., 1995; Uebele et al., 1996). The Kvβ2.1 subunit increases the degree of slow inactivation of the current, whereas Kvβ1.3 converts hKv1.5 from a delayed rectifier with a modest degree of slow inactivation to a channel with both fast and slow components of inactivation (Uebele et al., 1996; Uebele et al., 1998). The regional distribution of Kvβ1.3 subunits in the myocardium is not homogeneous, with higher expression in the ventricle than in atria (Wang et al., 1996). If β subunits alter the pharmacology of the Kv α subunits, the molecular targets of local anesthetics and/or antiarrhythmic drugs will vary greatly depending on β subunit expression. Therefore, a further knowledge of the effects of regulatory β subunits present in the human myocardium is required. Quinidine-induced block of Kvα1.5 is not modified by Kvβ2.1 subunit (Yeola et al., 1996). However, the possible effects of Kvβ1.3 on drug-hKv1.5 channel interactions have not been yet analyzed.

This study was undertaken to determine the pharmacological consequences of the interaction between Kvα1.5 and Kvβ1.3 subunits. Bupivacaine, its quaternary ammonium derivative [*R*(+)-*N*-methyl-bupivacaine (RB^+1C)], and quinidine sensitivity were examined. Bupivacaine is a very cardiotoxic local anesthetic that can prolong the QT interval and

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ABBREVIATIONS: RB^+1C, *R*(+)-*N*-methyl-bupivacaine; IV, current-voltage; HEK, human embryonic kidney.

induce *torsades de pointes* (Kasten and Martin, 1985; Kasten, 1986; Covino, 1987). At the same concentrations needed to block Na^+ channels, bupivacaine binds to two different receptors on hKv1.5 channels, one external and one internal (Valenzuela et al., 1995; Longobardo et al., 2000, 2001). Quinidine is a classic antiarrhythmic agent that has been studied extensively (Snyders et al., 1992; Yeola et al., 1996). The internal bupivacaine binding site is the same as that described for quinidine (Yeola et al., 1996; Franqueza et al., 1997). In the present study, we have analyzed the effects of bupivacaine, RB^+1C, and quinidine on hKv1.5 channels expressed alone or with Kvβ1.3 in β subunit-free HEK293 cells (Uebele et al., 1996) and those produced by quinidine on Kvα1.5+Kvβ1.3. These results are in contrast to our previous studies with these three drugs using hKv1.5 channels expressed in *Ltk*$^-$ cells that endogenously express the Kvβ2.1 subunit (Uebele et al., 1996; Longobardo et al., 2000; González et al., 2001). A preliminary report of the present study has been published in abstract form (Navarro-Polanco et al., 2001).

Materials and Methods

Expression Systems and Transfection. Human Kv1.5 (−22–1894 nt) and Kvβ1.3 (−53–1500 nt) were inserted in tandem into the same pBK vector with the Kv1.5 subunit placed 3′ to the Kvβ1.3 subunit and behind an internal ribosome entry sequence, thus generating a dual cistronic mRNA as described previously (Kwak et al., 1999). The pBK construct used for Kv1.5 alone has been described previously (Uebele et al., 1998). HEK293 cells were cultured in minimal essential medium supplemented with 10% bovine fetal serum, penicillin-streptomycin (Sigma, St. Louis, MO), and nonessential amino acids 1%. Transfection of hKvα1.5 or Kvα1.5+Kvβ1.3 channel (0.3 μg) and reporter plasmids CD8 (1.6 μg) or green fluorescent protein pCI was performed by use of LipofectAMINE (10 μl). Before experimental use, cells were incubated with polystyrene microbeads precoated with anti-CD8 antibody (Dynabeads M450; Dynal Biotech, Oslo, Norway), as described previously (González et al., 2001) or viewed under fluorescence optics to identify the transfected cells.

Electrophysiological Technique and Data Acquisition. The intracellular pipette filling solution contained 80 mM K-aspartate, 50 mM KCl, 3 mM phosphocreatine, 10 mM KH_2PO_4, 3 mM MgATP, 10 mM HEPES-K, and 5 mM EGTA, and was adjusted to pH 7.25 with KOH. The bath solution contained 130 mM NaCl, 4 mM KCl, 1.8 mM $CaCl_2$, 1 mM $MgCl_2$, 10 mM HEPES-Na, and 10 mM glucose, and was adjusted to pH 7.40 with NaOH. Permanently charged *R*(+)-bupivacaine [RB^+1C ((*R*)-(+)-1-butyl-1-methyl-2′,6′-pipecoloxylidide)] (Astra, Södertälje, Sweden) was dissolved in ethanol (50%). Bupivacaine and quinidine (Sigma) were dissolved in distilled deionized water to yield stock solutions of 10 mM.

Recordings were made with an Axopatch 1C patch-clamp amplifier (Axon Instruments, Union City, CA) using the whole-cell configuration of the patch-clamp technique (Hamill et al., 1981). Currents were recorded at room temperature (21–23°C) at a stimulation frequency of 0.1 Hz and sampled at 4 kHz after antialias filtering at 2 kHz. Data acquisition and command potentials were controlled with the use of pClamp 6.0.1 (Axon Instruments). The average pipette resistance was 2.1 ± 0.5 MΩ (n = 25). GigaOhm seal formation was achieved by suction (10 ± 1 GΩ, n = 15). Capacitive surface area and access resistance were 9.1 ± 0.4 pF (n = 15) and 3.3 ± 0.4 MgΩ (n = 15), respectively. Usually, 80% compensation of the effective access resistance was obtained, which leads to a mean uncompensated access resistance of 2.0 ± 0.4 MΩ. Because maximum hKv1.5 current amplitudes at +60 mV averaged 4.8 ± 0.8 nA, no significant voltage errors (<5 mV) were expected with the electrodes used. Origin 6.1 software (OriginLab Corp., Northampton, MA) and custom-made programs were used to perform least-squares fitting and for data presentation.

Drug-induced block was measured at the end of 200-ms depolarizing pulses from −80 to +60 mV. The degree of inhibition obtained for each drug concentration was used to calculate the IC_{50} and n_H values from the fitting of these values to a Hill equation of the form: $1/[1 + (IC_{50}/[D])^{nH}]$ (Valenzuela et al., 1995).

Statistical Analysis. Data are presented as mean values ± S.E.M. Comparisons between mean values in control conditions and mean values in the presence of drug for a single variable were performed by use of paired Student's t test. One-way analysis of variance was used to compare more than two groups. Statistical significance was set at $p < 0.05$.

Results

Figure 1 shows original records for hKvα1.5 channels expressed alone (Fig. 1A) and with Kvβ1.3 (Fig. 1B) obtained after applying depolarizing pulses from a holding potential of −80 to +60 mV in 10-mV steps. Tail currents are shown after repolarization to −40 mV. In the absence of β subunits, the hKv1.5 channels inactivate by 11.6 ± 0.7% (n = 40) at the end of 200-ms depolarizing pulses to +60 mV. In the presence of Kvβ1.3, this current exhibited a fast initial but incomplete inactivation that averaged 77.6 ± 4.0% (n = 12) with a time constant of 3.7 ± 0.2 ms (n = 12). In addition, the Kvβ1.3 subunit slows the time course of the deactivation process that became monoexponential (Fig. 1, bottom).

Effects of RB^+1C on hKvα1.5 Subunits Alone and in the Presence of Kvβ1.3. RB^+1C, externally applied, blocks hKv1.5 channels expressed in *Ltk*$^-$ cells that endogenously express Kvβ2.1 subunits (Uebele et al., 1996) in a time- and voltage-independent manner (Longobardo et al., 2000). However, when it is applied internally, this drug mimics bupivacaine effects (Valenzuela et al., 1995; Franqueza et al., 1997; Longobardo et al., 2000). To analyze whether Kvβ1.3 and/or Kvβ2.1 modify the interaction between RB^+1C and hKvα1.5 subunits, we studied its effects on hKvα1.5 subunits expressed alone or coexpressed with Kvβ1.3 in HEK293 cells. Figure 2A shows current traces through hKvα1.5 channels in the absence and in the presence of RB^+1C (50 μM). RB^+1C inhibited hKv1.5 current by 22 ± 9% (n = 4) in a time- and voltage-independent manner. Figure 2B shows the effects of RB^+1C on hKvα1.5 coexpressed with Kvβ1.3 subunits. Under these conditions, RB^+1C blocked K^+ current by 16 ± 2% (n = 4). Thus, neither the Kvβ2.1 nor the Kvβ1.3 subunits altered RB^+1C block of Kv1.5 via its external receptor site ($p > 0.05$).

Effects of Bupivacaine on hKvα1.5 Subunits. Fig. 3A shows the effects of bupivacaine (20 μM) on hKvα1.5 channels expressed without a β subunit in HEK293 cells. At the end of 200-ms depolarizing pulses to +60 mV, bupivacaine inhibited this current by 61 ± 3% (n = 8). Bupivacaine decreased the current at all membrane potentials tested (Fig. 3B), although block increased more at positive than negative membrane potentials, consistent with an open channel block mechanism. All these data are similar to those found when blocking effects of bupivacaine on hKv1.5 channels were studied in *Ltk*$^-$ cells (González et al., 2001), indicating that the Kvβ2.1 subunit does not modify bupivacaine affinity of hKv1.5 channels.

Figure 3, C and D, shows superimposed current traces

obtained in the absence and in the presence of bupivacaine. Figure 3C shows the effects of bupivacaine (20 and 50 μM) after applying depolarizing pulses from a holding potential of −80 to +60 mV. Bupivacaine (20 μM) induced a fast initial decay of the current with a time constant (τ_{Block}) of 8.9 ± 1.8 ms (n = 7) that decreased as the drug concentration increased. From the τ_{Block} values obtained at different bupivacaine concentrations (from 10 to 50 μM), the association (k) and the dissociation (l) rate constants were derived ($\tau_{Block} = k \times [D] + l$) (Fig. 3C). The k value was faster than that reported previously (González et al., 2001) in the presence of Kvβ2.1 subunits (3.5 ± 0.2 $\mu M^{-1}s^{-1}$, n = 22, versus 2.2 ± 0.3 $\mu M^{-1}s^{-1}$, n = 16, $p < 0.05$). Similarly, the l value was also faster in the absence of Kvβ2.1 (46.0 ± 3.3 s^{-1}, n = 22, versus 17.6 ± 2.5 s^{-1}, n = 16, $p < 0.05$). These similar changes in the association and dissociation rate constants explain the similar IC_{50} values obtained in the absence and in the presence of Kvβ2.1 subunits (see below). Time dependence of bupivacaine-induced block was also observed in the deactivation process (Fig. 3D). Under control conditions, the deactivation of hKvα1.5 was fitted to a biexponential function, the fast (τ_f) and the slow (τ_s) time constants averaging 12.5 ± 2.1 ms and 47.9 ± 7.0 ms (n = 5), respectively. Bupivacaine (20 μM) slowed this process, increasing the τ_f and τ_s values to 29.2 ± 6.0 ms (n = 5, $p < 0.05$) and 124.2 ± 30.7 ms (n = 5, $p < 0.05$), respectively. Moreover, the contribution of the fast time constant to the total process of deactivation decreased in the presence of bupivacaine from 0.72 ± 0.09 ms to 0.37 ± 0.09 ms ($p < 0.05$). In all these experiments, superimposed tail currents recorded in the absence and in the presence of bupivacaine exhibited a "crossover" phenomenon suggestive of an open channel block mechanism and similar to that observed in the presence of Kvβ2.1 subunits (González et al., 2001).

Effects of Bupivacaine on hKvα1.5 Subunits in the Presence of Kvβ1.3. Fig. 4A shows current through hKvα1.5+Kvβ1.3 in the absence and in the presence of bupivacaine (100 μM). Bupivacaine decreased this current at +60 mV by 62 ± 4% (n = 4); i.e., its potency was ~4-fold lower than in hKvα1.5 channels expressed alone or in the presence of Kvβ2.1 subunits. Figure 4B shows the IV relationship of hKvα1.5 when expressed with Kvβ1.3. Block in-

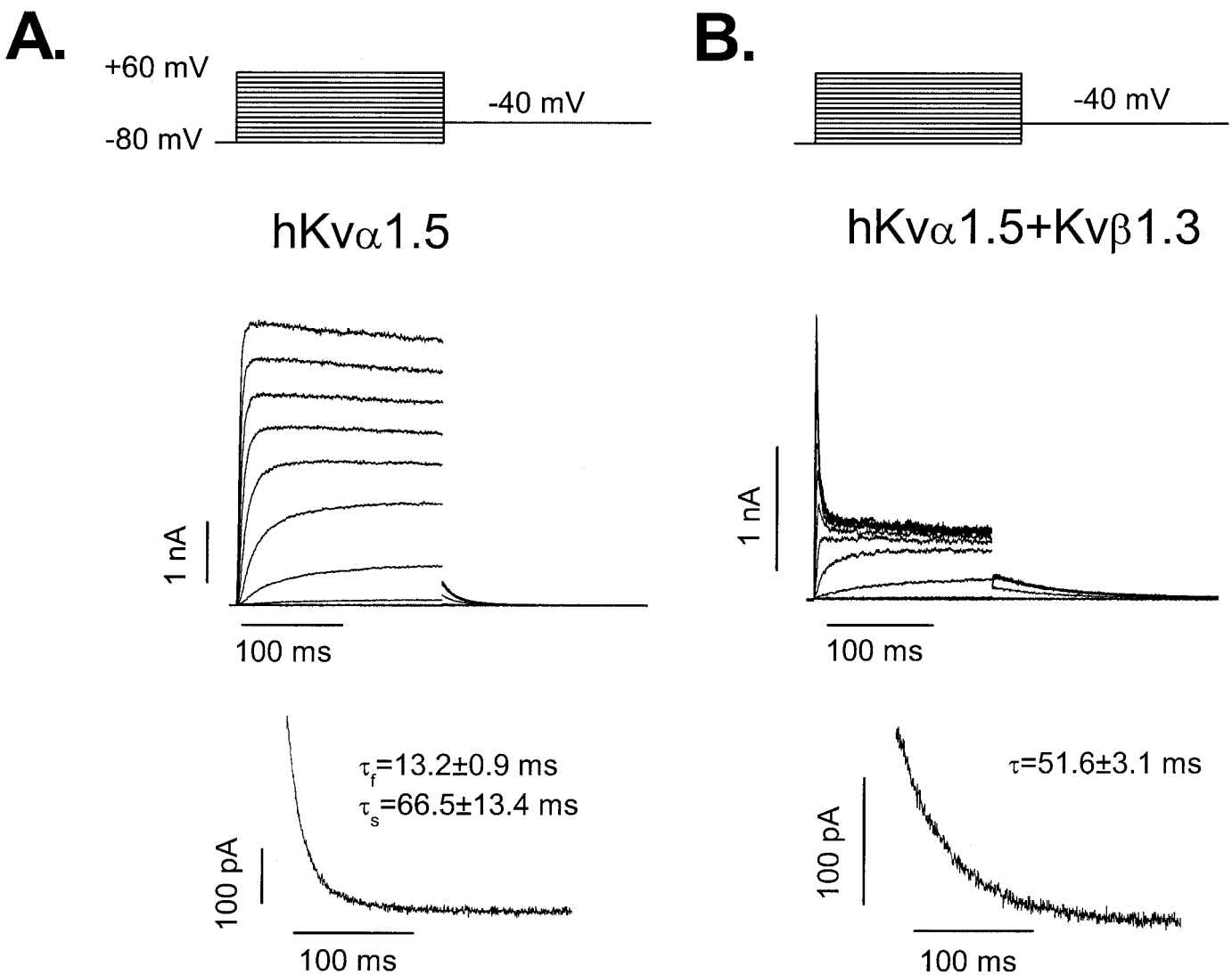

Fig. 1. Original records obtained upon depolarization from a holding potential of −80 mV to +60 mV in 10 mV steps and upon repolarization to −40 mV. A, current records obtained from the activation of Kvα1.5 subunits. B, current records of hKvα1.5 channels in the presence of the Kvβ1.3 subunit. Note that the deactivation process (bottom) is slower in the presence of Kvβ1.3.

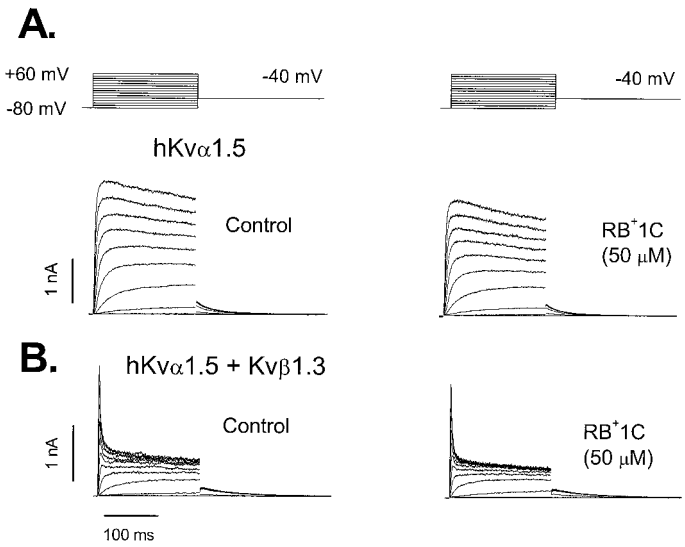

Fig. 2. Effects of RB^+1C (50 μM) on hK$v\alpha$1.5 alone (A) or in the presence of the K$v\beta$1.3 subunit (B). RB^+1C induced the same degree of block under both experimental situations.

duced by bupivacaine was voltage dependent, achieving a maximum value at 0 mV and decreasing at more positive membrane potentials (74 ± 2% versus 62 ± 4%, measured at 0 and +60 mV, respectively, $n = 4$, $p < 0.05$). Figure 5 shows the concentration dependence of bupivacaine block of hK$v\alpha$1.5 and hK$v\alpha$1.5+K$v\beta$1.3 channels when using as index of block the suppression of the current at the end of 200-ms depolarizing pulses to +60 mV. In addition, Fig. 5 shows the concentration dependence of bupivacaine block of hK$v\alpha$1.5+K$v\beta$2.1 channels taken from a previous study (dashed line) (González et al., 2001). IC_{50} values for blocking hK$v\alpha$1.5 and hK$v\alpha$1.5+K$v\beta$2.1 channels were similar (13.1 ± 0.8 μM, $n = 25$, versus 8.9 ± 1.4 μM, $n = 22$, respectively, $p > 0.05$), whereas the IC_{50} value for the blockade of hK$v\alpha$1.5+K$v\beta$1.3 channels was ~4-fold higher (IC_{50} = 47.5 ± 5.1 μM, $p < 0.01$). Under both experimental conditions, the n_H values were close to unity, averaging 0.96 ± 0.05 and 0.74 ± 0.06 for K$v\alpha$1.5 and K$v\alpha$1.5+K$v\beta$1.3 channels, respectively. When the IC_{50} values were calculated fixing the n_H value at 1, the obtained IC_{50} values were 12.7 ± 1.6 μM and 47.6 ± 7.8 μM for K$v\alpha$1.5 and K$v\alpha$1.5+K$v\beta$1.3

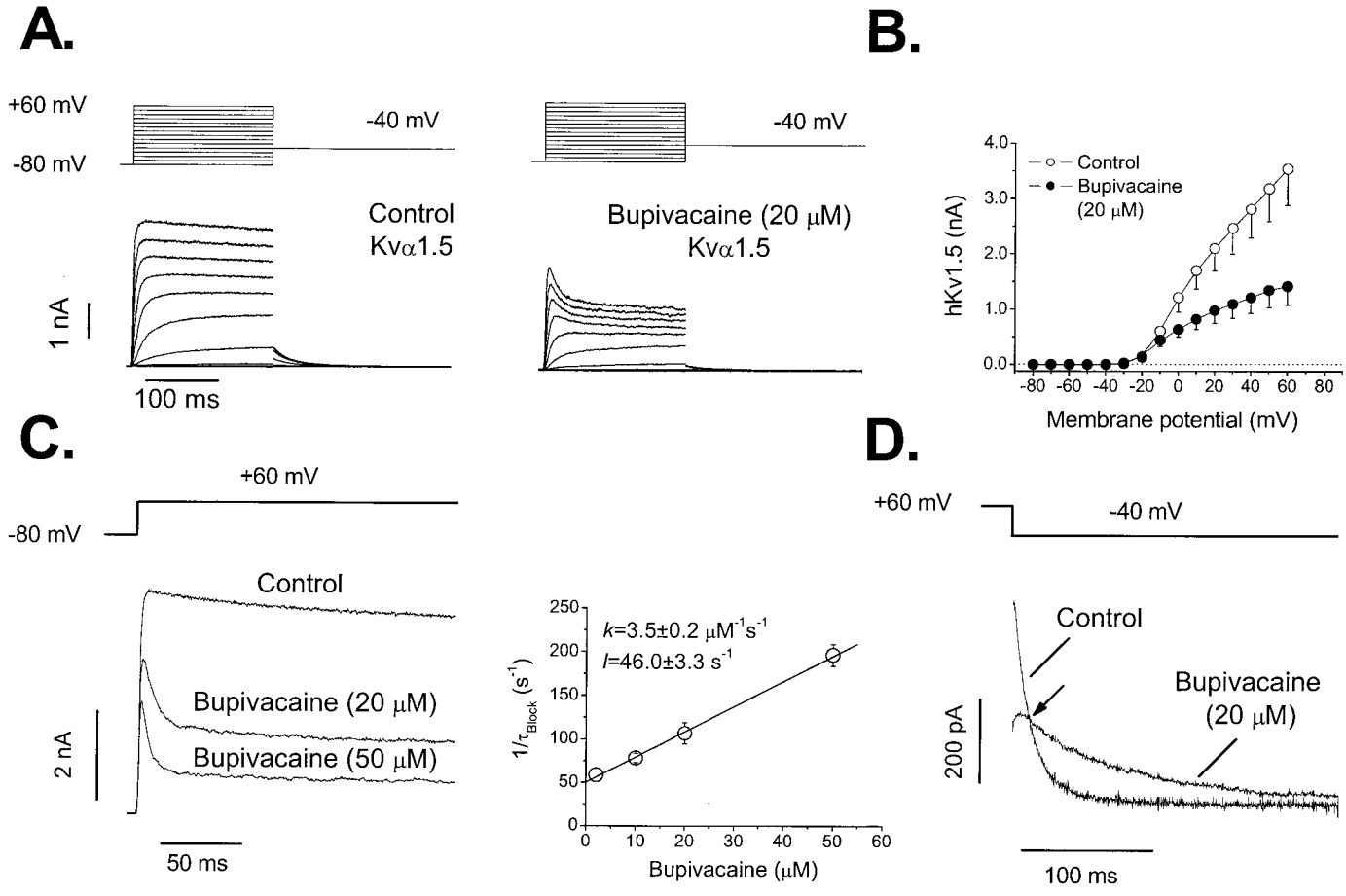

Fig. 3. Effects of bupivacaine (20 μM) on hK$v\alpha$1.5 channels. A, current records obtained in the absence and in the presence of bupivacaine. B, IV relationships obtained in the absence (○) and in the presence of bupivacaine (●). Block induced by bupivacaine measured at +60 mV averaged 61 ± 3%. Each point represents the mean ± S.E.M. of seven experiments. C, original records obtained after depolarization from −80 to +60 mV in the absence and in the presence of bupivacaine (20 and 50 μM). Note the fast initial decay of the current induced by the drug. Relationship between $1/\tau_{Block}$ and bupivacaine concentration. The fast time constant of the biexponential fit of the current traces in the presence of different bupivacaine concentrations were considered a good approximation of τ_{Block} values (see text). For a first-order blocking scheme, a linear relation is expected: $1/\tau_{Block} = k \times [\text{Bupivacaine}] + l$. The solid line represents the linear fit, from which the apparent binding and unbinding rate constants were obtained. D, tail currents obtained upon repolarization from +60 to −40 mV in the absence and in the presence of bupivacaine. Arrow, "crossover" characteristic of an open-channel block mechanism.

channels, respectively; suggesting that binding of one drug molecule was necessary to block K^+ channel efflux.

Block induced by bupivacaine of hKvα1.5 expressed with Kvβ1.3 subunits was also time dependent (Fig. 6). The time constant of the fast inactivation in the absence and in the presence of bupivacaine (100 μM) averaged 3.40 ± 0.02 ms and 2.34 ± 0.09 ms (n = 4, $p < 0.05$), respectively. This acceleration of the fast inactivation was concentration-dependent. To quantify the kinetics of block of bupivacaine on Kvα1.5+Kvβ1.3 channels, we plotted the ratio between the drug-sensitive current and the current in control conditions [$(I_{Control} - I_{Drug})/I_{Control}$] during the first 12 ms in the presence of 10, 30, and 100 μM bupivacaine (Fig. 6B, inset). Block exponentially increased during depolarization and the time constant of this process was faster at higher bupivacaine concentrations. Thus, the time constant of this process was considered a good index of development of block (τ_{Block}). From the τ_{Block} values obtained at different bupivacaine concentrations, the k and l values were derived, averaging 5.9 ± 0.5 $\mu M^{-1}s^{-1}$ (n = 11) and 252.3 ± 33.0 s^{-1} (n = 11), respectively. The IC_{50} value obtained from these values (42.8 μM = l/k) was very similar to that obtained from the concentration-response curve (47.6 μM). Time dependence of block was again observed in the deactivating process, which was slower in the presence than in the absence of drug. Indeed, bupivacaine (100 μM) increased the time constant of deactivation from 43.5 ± 1.6 ms (n = 5) to 112.7 ± 31.1 ms (n = 5, $p <$ 0.05). As in the absence of Kvβ1.3 subunit, superimposed tail currents recorded in the absence and in the presence of bupivacaine exhibited a crossover phenomenon (Fig. 6C).

Effects of Quinidine on hKvα1.5+Kvβ1.3 Channels. Quinidine and bupivacaine share a common receptor site at hKv1.5 channels that is located at the S6 segment and that involves a polar (T505) and a hydrophobic amino acid (V512) (Yeola et al., 1996; Franqueza et al., 1997). To determine whether quinidine block of Kv1.5 channels is modified also, we studied the effects of this drug on Kvα1.5+Kvβ1.3 channels transiently transfected in HEK293 cells. Figure 7A shows current traces through hKvα1.5+Kvβ1.3 in the absence and in the presence of quinidine (100 μM). Quinidine

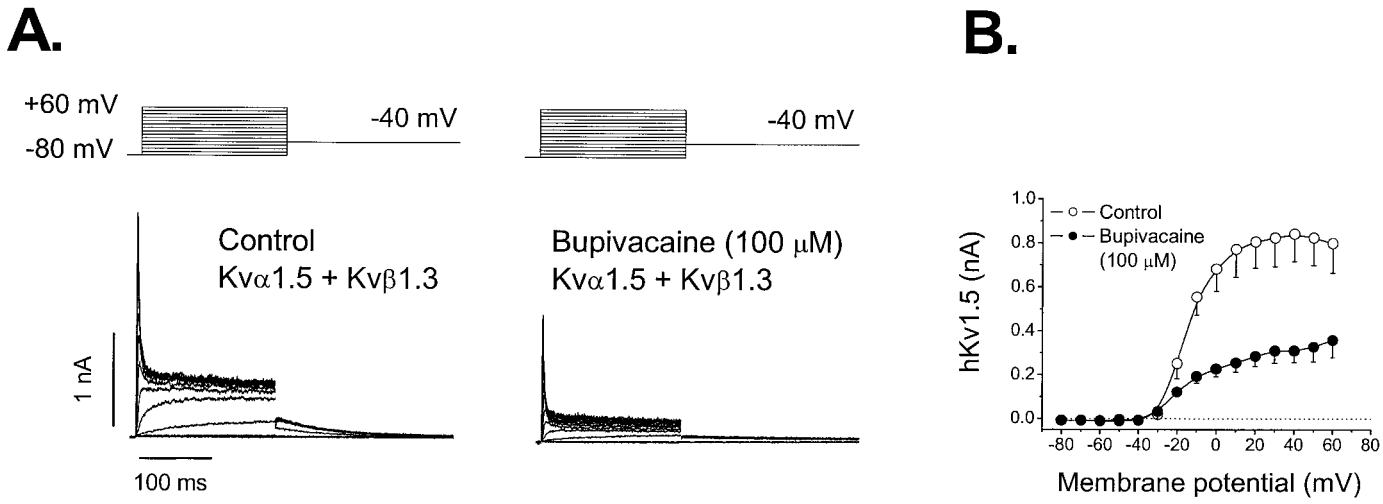

Fig. 4. Effects of bupivacaine (100 μM) on hKv1.5 channels in the presence of the Kvβ1.3 subunit. A, original records obtained in the absence and in the presence of bupivacaine. B, IV relationship obtained in the absence (○) and in the presence of bupivacaine (●). Block induced by bupivacaine measured at +60 mV averaged 62 ± 4%. Each point represents the mean ± S.E.M. of four experiments.

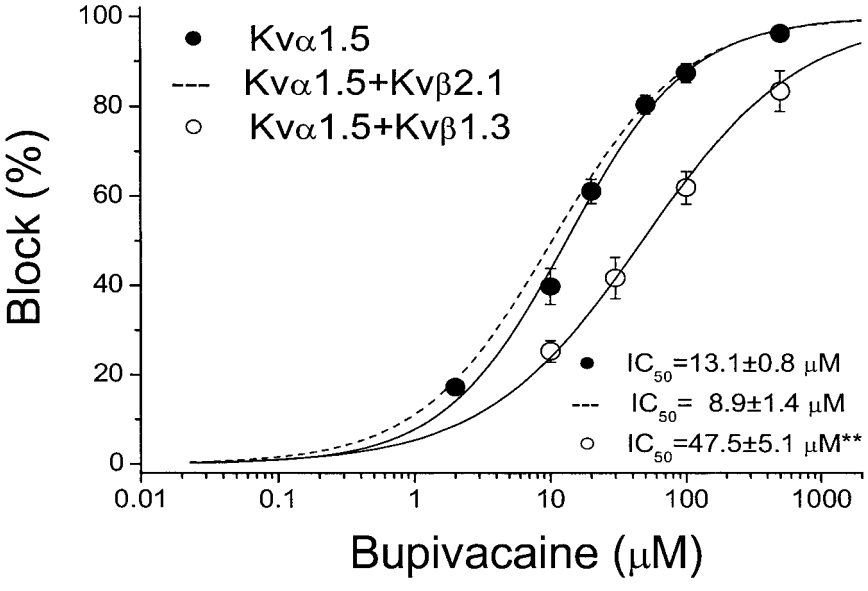

Fig. 5. Concentration dependence of bupivacaine-induced block of hKvα1.5 channels in the absence and in the presence of Kvβ1.3 subunits. Dashed line represents the dose-response curve obtained for bupivacaine block of hKvα1.5 channels expressed in *Ltk*$^-$ cells that endogenously express Kvβ2.1 subunits (taken from González et al., 2001). Reduction of current (relative to control) at the end of depolarizing steps from −80 to +60 mV was used as index of block. Each point represents the mean ± S.E.M. of three to seven experiments. The continuous line represents the fit of the experimental data to a Hill equation. **, $p < 0.01$.

decreased this current at +60 mV by 62 ± 9% (n = 5); i.e., its potency was ~8-fold lower than in hKvα1.5 channels expressed alone or in the presence of Kvβ2.1 subunits (Snyders et al., 1992; Yeola et al., 1996). Figure 7B shows the concentration-response curve for the blocking effects of quinidine on Kvα1.5+Kvβ1.3 measured at the end of 200-ms depolarizing pulses to +60 mV. The dashed line represents the concentration dependence of quinidine block of Kvα1.5+Kvβ2.1 as reported previously (Snyders et al., 1992), which is similar to that observed in Kvα1.5 (Yeola et al., 1996). IC_{50} values in the absence and in the presence of Kvβ1.3 averaged 6.2 μM and 49.6 ± 4.2 μM (n = 12, $p < 0.05$), respectively.

Discussion

Whereas Kvβ1.3 and Kvβ2.1 do not modify the effects of RB^+1C on the external binding site of bupivacaine on hKvα1.5 channels, Kvβ1.3, but not Kvβ2.1, reduces bupivacaine and quinidine affinity for its internal receptor site on the hKvα1.5 subunit. Quinidine and bupivacaine block hKv1.5 channels after binding to an external and to an internal receptor site (Yeola et al., 1996; Franqueza et al., 1997; Longobardo et al., 2000, 2001). Although the molecular determinants of the external binding site are unknown, quinidine, and bupivacaine share a common internal receptor site located at the S6 segment that involves a polar interaction with T505 and a hydrophobic interaction with V512 (Yeola et al., 1996; Franqueza et al., 1997). The membrane impermeant form of bupivacaine, RB^+1C, produced a similar degree of block of hKvα1.5 channels expressed in HEK293 cells in the absence or in the presence of Kvβ1.3. This block was also similar to that previously characterized in hKv1.5 channels expressed in *Ltk*$^-$ cells, which endogenously express Kvβ2.1 subunit (Uebele et al., 1996; Longobardo et al., 2000). Moreover, block was time- and voltage-independent, as that found in Kvα1.5 assembled with Kvβ2.1, indicating that the assembly of hKvα1.5 subunits with Kvβ1.3 or Kvβ2.1 subunits does not modify the binding of the charged form of bupivacaine to its external receptor site in hKv1.5 channels.

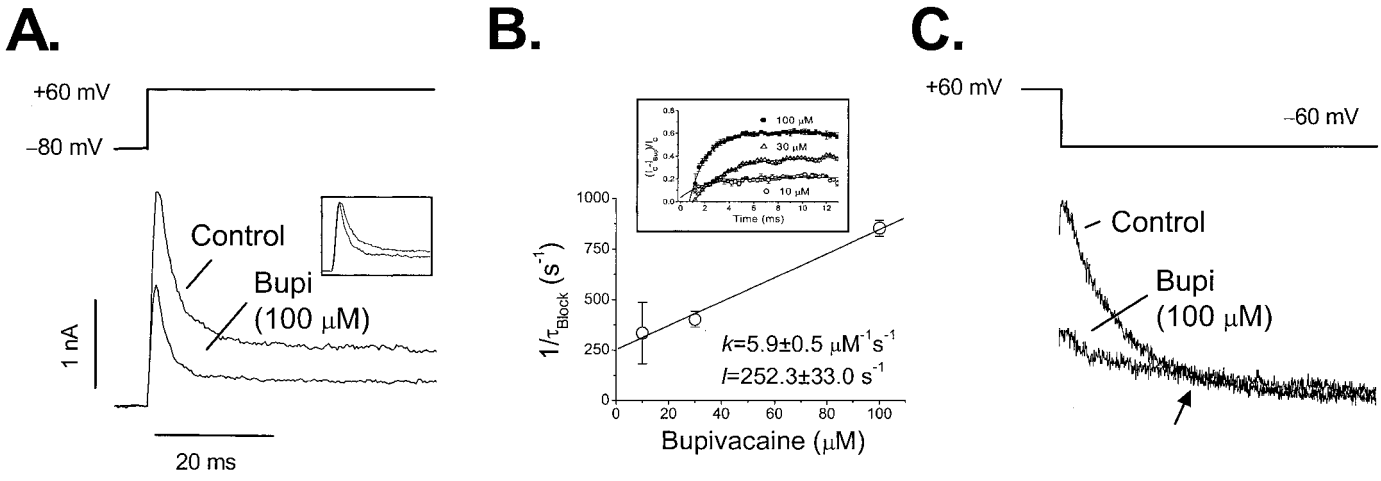

Fig. 6. Time-dependent effects of bupivacaine block of Kvα1.5+Kvβ1.3 channels. A, original records obtained upon depolarization from −80 to +60 mV in the absence and in the presence of bupivacaine. Time constant of fast inactivation induced by Kvβ1.3 was faster in the presence of bupivacaine. Inset, records obtained in the absence of drug normalized to the control value. B, relationship between $1/\tau_{Block}$ and bupivacaine concentration. τ_{Block} values were obtained from the fit of the sensitive current $[(I_{Control} - I_{Drug})/I_{Control}]$ during the first 12 ms (inset) at different bupivacaine concentrations (from 10 to 100 μM). Each point represents the mean ± S.E.M. of three to four experiments. For a first-order blocking scheme, a linear relation is expected: $1/\tau_{Block} = k \times [\text{Bupivacaine}] + l$. The solid line represents the linear fit, from which the apparent binding and unbinding rate constants were obtained. C, tail currents recorded upon repolarization from +60 to −40 mV in the absence and in the presence of drug exhibited a crossover (arrow).

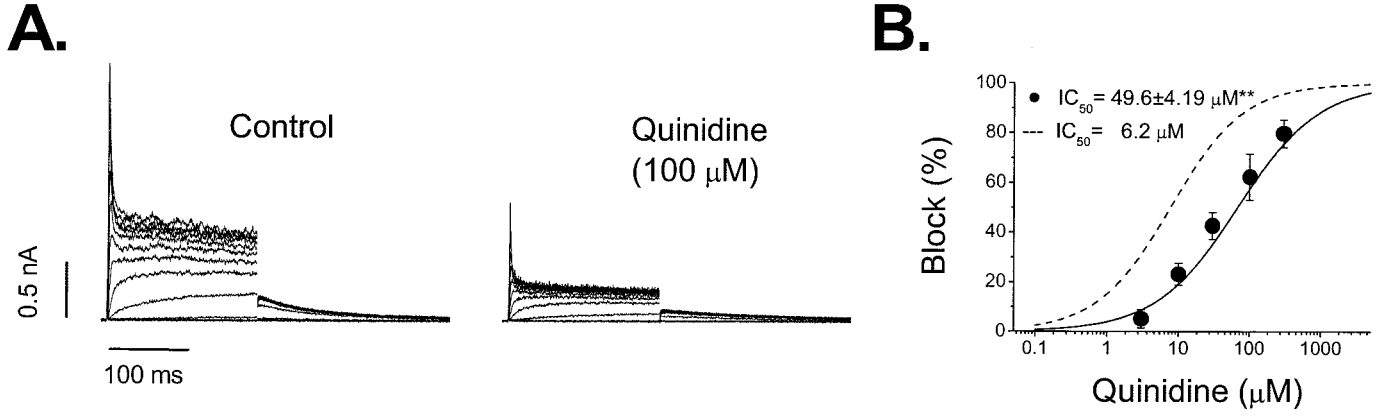

Fig. 7. Effects of quinidine (100 μM) on hKvα1.5+Kvβ1.3 channels. A, original records obtained in the absence and in the presence of bupivacaine. B, concentration dependence of quinidine-induced block of hKvα1.5+Kvβ1.3 channels. Reduction of Kvα1.5+Kvβ1.3 current (relative to control) at the end of depolarizing steps from −80 to +60 mV was used as index of block. Each point represents the mean ± S.E.M. of three to five experiments. The continuous line represents the fit of the experimental data to a Hill equation. The dashed line represents the dose-response curve obtained for quinidine block of hKvα1.5 channels expressed in *Ltk*$^-$ cells that endogenously express Kvβ2.1 subunits (taken from Snyders et al., 1992). **, $p < 0.01$.

Bupivacaine binding to its internal site inhibited current through hKvα1.5 (Fig. 3) or hKvα1.5+Kvβ2.1 (González et al., 2001) channels to a similar extent (IC_{50} values of 13 μM and 9 μM, respectively), and in a similar fashion. Under both experimental conditions, bupivacaine induced an initial decay of the current and block increased at positive potentials over which channel activation occurred. Moreover, the superposition of the tail currents recorded under control conditions and in the presence of bupivacaine shows a 'crossover' between them, indicating fast recovery from block during deactivation. All these findings are consistent with an open channel block mechanism (Armstrong, 1971). Kinetics of block differed mostly in the dissociation rate constant that was faster in the absence of Kvβ2.1, suggesting that block obtained with this subunit somehow stabilizes the bupivacaine-hKvα1.5 interaction. Bupivacaine blocked hKvα1.5+Kvβ1.3 channels to a lesser extent than hKvα1.5 or hKvα1.5+Kvβ2.1 channels (IC_{50} = 48 μM). This lower potency to block Kvα1.5+Kvβ1.3 was accompanied by a dramatic increase in the dissociation rate constant (46 s^{-1} versus 252 s^{-1}), thus indicating a less stable drug-channel interaction when Kvβ1.3 is present. As with bupivacaine, quinidine sensitivity of Kvα1.5+Kvβ1.3 channels increased ~8-fold in the presence of the Kvβ1.3 subunit (IC_{50} = 50 μM), suggesting a common mechanism of action for local anesthetics and antiarrhythmic drugs, probably at their common internal receptor site at the S6 segment.

The most striking difference between current through hKvα1.5 or hKvα1.5+Kvβ2.1 and hKvα1.5+Kvβ1.3 channels is the incomplete fast inactivation induced by Kvβ1.3. This fast inactivation involves an open channel block of the hKvα1.5 subunit produced by the N terminus of the Kvβ1.3 subunit (inactivation "ball") (Uebele et al., 1998). These results suggest that the inactivation ball of the Kvβ1.3 subunit may compete with the open channel blocking drugs at the internal receptor site. Thus, this drug receptor site might be the "natural" receptor site for the inactivation ball of the Kvβ1.3 subunit (Yeola et al., 1996; Franqueza et al., 1997). However, internal pore mutations involved in stereoselective bupivacaine block of hKv1.5 channels, such as V512A or T505I, do not affect Kvβ1.3-mediated inactivation (Uebele et al., 1998). Moreover, an external pore mutation (R485Y) that decreases the slow inactivation of hKvα1.5 channels and confers sensitivity to external tetraethylammonium dramatically increased the extent of Kvβ1.3-induced fast inactivation, suggesting that inactivation induced by Kvβ1.3 subunits involves open channel block that is allosterically linked to the external pore (Uebele et al., 1998). Therefore, one explanation for the present results would be that binding of the Kvβ1.3 inactivation particle allosterically modifies bupivacaine binding to the channel. Supporting this idea is the finding that Kvβ1.3 reduced bupivacaine affinity by increasing the dissociation rate constant, which could be indicative of an allosteric change in the drug binding site. Although recent studies suggest that the hydrophobic central cavity of the *Shaker* channel inner pore forms the receptor site for both the inactivation gate and quaternary ammonium compounds (Zhou et al., 2001), there may be significant differences between this work involving *Shaker* and the Kvα1.5/Kvβ1.3 studies reported here. For example, pore mutations that modify internal tetraethylammonium block 10-fold in *Shaker* have minimal effects on quinidine binding to Kv1.5 (Yeola et al., 1996). In addition, the mechanism of action of the Kvβ1.3 N terminus may not be the same as that used by the *Shaker* inactivation ball (Uebele et al., 1998).

Conclusions. The present study demonstrates that the assembly of Kvα1.5 and Kvβ1.3 subunits decreases the block induced by bupivacaine and quinidine on hKv1.5 channels (~4- and ~8-fold, respectively). Therefore, the sensitivity to hKv1.5 channel-blocking drugs will vary depending on the regional distribution of β regulatory subunits. The expression of Kvβ1.3 subunits in the myocardium is not homogeneous, for this subunit is expressed to a higher degree in the ventricle than in atria (Wang et al., 1996). Within various vascular beds, there are marked differences in β subunit expression, whereas Kv1.5 levels change little (Coppock and Tamkun, 2001). Thus, the differential assembly between the Kvα and Kvβ subunits present in the cardiovascular system is another variable to be accounted for in the development of new ion channel modifying agents.

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