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Properties of BK_{Ca} Channels Formed by Bicistronic Expression of *hSlo*α and β1–4 Subunits in HEK293 Cells

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Abstract. Large-conductance Ca^{2+}-activated K^+ (BK_{Ca}) channels are sensitive to both voltage and internal [Ca^{2+}] and are found in many tissues. Their physiological roles range from causing relaxation of smooth muscle to regulating the frequency of action potential firing. There is considerable variation between different tissues in their Ca^{2+}- and voltage-dependence. Much of this variation results from the association of the pore-forming α subunit (hSloα) with different β subunits leading to altered channel properties. Since hSloα alone produces functional BK_{Ca} channels, we have used a bicistronic expression method to ensure that both α and β subunits are expressed, with the β subunit being in excess. Using this method we have investigated the effect of four β subunits (β1 to β4) on cloned BK_{Ca} channels. The four β subunits were individually cloned into a vector that had *hSlo*α cDNA inserted downstream of an internal ribosome entry site. The constructs were transiently transfected into HEK293 cells together with a construct that expresses green fluorescent protein, as a marker for transfection. Fluorescent cells expressed BK_{Ca} channels whose currents were recorded from inside-out or outside-out patches. The currents we measured using this expression system were similar to those expressed in *Xenopus* oocytes by Brenner et al. (Brenner, R., Jegla, T.J., Wickenden, A., Liu, Y., Aldrich, R.W. 2000. Cloning and functional expression of novel large-conductance calcium-activated potassium channel β subunits, hKCNMB3 and hKCNMB4. *J. Biol. Chem.* **275:**6453-6461.)

Key words: Bicistronic expression — Calcium activated potassium channels — hSlo — Activation — Inactivation — Iberiotoxin — Beta-subunits

Introduction

Large-conductance Ca^{2+}-activated K^+ (BK_{Ca}, maxi-K) channels occur in most cells. They are activated by depolarization and the voltage at which they reach half-maximal activation ($V_{1/2}$) is shifted to values that are more negative by an increase in internal [Ca^{2+}]. These channels provide a means of negative feedback by opening in response to both membrane depolarization and rises in intracellular Ca^{2+}. The opening of BK_{Ca} channels can lead to a variety of effects ranging from relaxation of smooth muscle to regulating action potential repolarization. These different roles rely on properties such as the amount and rates of activation, inactivation, and deactivation at various membrane potentials at a particular [Ca^{2+}]. Functional BK_{Ca} channels that are both voltage- and Ca^{2+}-dependent are formed from a tetrameric assembly (Shen et al., 1994) of pore-forming α subunits (*Slo*α) that share close homology with voltage-gated K^+ (Kv) channel α subunits. They differ by having additional hydrophobic segments (S0 to S10 in total) resulting in an extracellular N-terminal and a long cytosolic C-terminal where at least one of the regulatory Ca^{2+}-binding domains resides (Schreiber & Salkoff, 1997).

Although alternative splicing of *Slowpoke*-related genes results in channels with altered activation and phosphorylation properties (Shipston, 2001), tissue-specific expression of accessory β subunits is responsible for many of the fundamental differences between cell types. The first β subunit (now termed β1)

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was identified as a co-immunoprecipitant with the pore forming α subunit from bovine smooth muscle. Co-expression of recombinant α + β1 subunits results in channels that are active at significantly less positive potentials and have slower kinetics compared with the α-subunit expressed alone. Expression of β1 subunits in smooth muscle cells therefore produces BK_{Ca} channels that are active at tonic voltages and Ca^{2+} concentrations.

Recently three additional genes encoding other β-subunits (β2, β3, and β4) have been found, and diversity is further enhanced by alternative splicing of β3 mRNA (Wallner, Meera & Toro 1999; Brenner et al., 2000; Uebele et al., 2000). Channels comprised of α + β2 inactivate rapidly, with a mechanism similar to the N-type inactivation conferred by Kvβ subunits on Kvα channels. Removal of part of the N-terminal of the β2 subunit removes this fast inactivation, leading to channels with biophysical and pharmacological properties similar to those of α + β1 channels. Either β2 subunits or inactivating N-terminal splice variants of β3 are likely to be components of inactivating BK_{Ca} currents observed in hippocampal pyramidal neurones, adrenal chromaffin cells and rat insulinoma cells. In *Xenopus* oocytes non-inactivating β3 subunits fail to upregulate the activity of the α subunit in the manner of β1 or β2 (Brenner et al., 2000).

The β4 subunit gives mixed results when expressed in *Xenopus* ooctyes or mammalian cell lines. The activation and deactivation kinetics are slow, compared with the α subunit alone, but there are discrepancies between studies on how the voltage sensitivity is shifted. Brenner et al. (2000) report both upregulation and downregulation of channels formed from co-injection of mRNA into ooctyes at lower and higher Ca^{2+} concentrations, respectively. In contrast, when the two corresponding DNA plasmids were cotransfected into mammalian cells, the β4 subunit shifted the voltage sensitivity to more positive potentials at all Ca^{2+} concentrations tested (Weiger et al., 2000). A consistent feature of channels comprised of α + β4 subunits is that under normal experimental conditions, they are not blocked by iberiotoxin or charybdotoxin (Meera et al., 2000). It therefore appears that the biophysical properties conferred by the recently described β subunits on recombinant BK_{Ca} channels may depend on the choice of expression system.

In common with other K^+ channels, hSloα alone forms functional BK_{Ca} channels. For this reason it is especially important to ensure that both α and β subunits are expressed when assessing the functional effects of the β subunit. Therefore, the aim of the present study was to develop an expression system that reliably coexpressed hSloα + β subunits in mammalian cells so that the β-subunit protein was likely to be in excess, thus minimizing the probability of channels comprising α subunits alone. To achieve this, a mammalian expression vector containing an internal ribosome entry site (IRES) sequence was used so that both α and β subunits were translated from the same mRNA, thus ensuring the expression of both subunits. It was envisaged that cap-dependent translation of β subunits that are one-fifth the size of the α subunit primary sequence would occur at greater frequency.

Materials and Methods

Molecular Biology

The cDNA encoding the pore-forming BK_{Ca} α subunit, *hSlo*α (KCNMA1), was amplified by the polymerase chain reaction from a construct that had previously been used to generate a stable line (Lippiat, Standen & Davies, 2000). The primers introduced 5′-*Xba*I and Kozak CACCATG sequence, and 3′-*Eag*I restriction sites for cloning into the *Xba*I and *Not*I restriction sites of the pIRES vector (Clontech), downstream of the IRES sequence. A clone that had no PCR errors was selected for further use, and was designated *p*IRES-*hSlo*α.

The four β subunit cDNA sequences (sequences as described by Brenner et al., 2000) were individually amplified from human cDNA samples and libraries (Clontech or Invitrogen) using primers that introduced a 5′-*Nhe*I and Kozak sequence, and 3′-*Xho*I for cloning into the equivalent sites in the *p*IRES-*hSlo*α construct, upstream of the IRES sequence. The β1 sequence (KCNMB1; GenBank U25138) was amplified from bladder, β2 (KCNMB2; AF099137) and β3 (KCNMB3; AF214561) from foetal brain, and β4 (KCNMB4; AF207992) from aorta. Inserts were verified by DNA sequencing and constructs designated pIRES-*hSlo*α + β_x. Fig. 1 shows the concept of the expression system used.

Cell Culture

Human embryonic kidney cells (HEK 293) were cultured in MEM supplemented with 10% v/v FBS, 1% non-essential amino acids, 1% L-glutamine (Gibco BRL), and 1% sodium pyruvate (Sigma). Culture flasks were incubated at 37°C in a humidified atmosphere containing 5% CO_2, and subcultured approximately every 5 days. Cells were transiently transfected with the bicistronic constructs along with a vector containing a green fluorescent protein expression cassette (pEGFP-N1, Clontech) as a marker of likely sites of exogenous DNA expression. Lipofectamine reagent was used (Life Technologies) at a ratio of 6 μl reagent with 1 μg total plasmid DNA per 35 mm well. Cells were seeded into 35 mm petri dishes that had been coated with poly-L-lysine (Sigma) for electrophysiological study.

Electrophysiology

BK_{Ca} channels comprising different subunit composition were recorded from inside-out and outside-out membrane patches excised from the transfected HEK 293 cells. Cells that fluoresced green when illuminated with a UV light source were selected for study.

Glass micropipettes were pulled from thick-walled borosilicate capillaries (Harvard), coated with Sylgard (Dow Corning) and polished to give resistances between 5 and 10 MΩ when filled with experimental solution. Membrane patches were placed in the outflow of a microperfusion system that allowed changing between 6

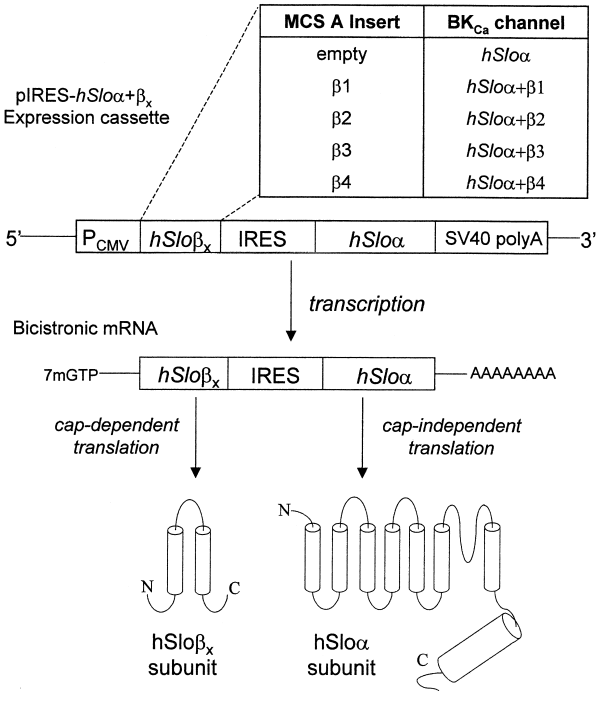

Fig. 1. Outline of the bicistronic expression system used (pIRES). The multiple cloning site A (MCS A) insert between the promoter (P_{CMV}) and the internal ribosome entry site (IRES) dictates the subunits expressed as indicated.

different solutions bathing the patch. Inside-out patches were excised for the biophysical study of voltage- and Ca^{2+}-dependence, whilst pharmacological agents were applied to outside-out patches. When necessary, vesicles associated with inside-out patches were ruptured by taking the patch out of the bath solution transiently. Currents were measured with an Axopatch 200B amplifier, filtered at 5 kHz and digitized at 10 kHz with a DigiData 1200 interface (Axon Instruments). Voltage protocols and data acquisition were controlled by a computer program written using AxoBASIC routines. A P/6 sequence was used to subtract leak and capacitive currents when appropriate. Experiments were done at room temperature (20 to 22°C).

Solutions

The extracellular solution contained (in mM) 50 K_2SO_4, 40 KOH (140 total K^+), 1 $CaCl_2$, 1 $MgCl_2$, and 10 HEPES. The intracellular solutions also contained (in mM) 50 K_2SO_4, 40 KOH and 10 HEPES. To obtain intracellular Ca^{2+} concentrations of 10 μM, 3 μM, and 1 μM, HEDTA (10 mM) was used to buffer Ca^{2+}; for lower Ca^{2+} concentrations, EDTA was used. The total Ca^{2+} to be added to give the desired free concentration was calculated using the program Maxchelator (http://www.stanford.edu/%7Ecpatton/maxc.html). For solutions with [Ca^{2+}] of 30 μM, the Ca^{2+} chelator was replaced by 10 mM sorbitol, and total Ca^{2+} calculated taking into account the solubility product of $CaSO_4$. The pH of each solution was adjusted to 7.2 with HCl or KOH. Sulphate solutions were used to minimize contamination of the solutions by Ba^{2+}, which blocks open BK_{Ca} channels.

Trypsin (type XI bovine pancreatic) was obtained from Sigma and was dissolved directly into intracellular solution to give a final concentration of 1 mg/ml. Stock solutions of iberiotoxin (IbTX, a gift from Pfizer, Sandwich, UK) were made up in water and subsequently diluted by at least 1000-fold in extracellular solution to give the concentrations used in this study. An extracellular solution containing 10 mM tetraethylammonium (TEA^+) chloride was prepared as a blocker of any BK_{Ca} current remaining after IbTX exposure.

Analysis

Activation was measured as conductance since symmetrical K^+ gives a consistent unitary conductance across the voltage range. The data points were fitted with a Boltzmann distribution, which yielded values for $V_{1/2}$, k, and G_{max}

$$G = \frac{G_{max}}{1 + \exp((V - V_{1/2})/k)} \quad (1)$$

Data were normalized by dividing by the fitted maximum G_{max} for each patch and pooled to give mean ± SEM.

Toxin block was studied by expressing the remaining current after each drug exposure as a fraction of the current magnitude of the patch prior to the first drug exposure (f_c). The plots of fractional current, I_f, remaining against concentration [*IbTX*] were fit with the Hill equation (where n is the Hill coefficient):

$$I_f = 1 - \frac{[IbTX]^n}{[IbTX]^n + + IC_{50}^n} \quad (2)$$

Results

Bicistronic Expression of BK_{Ca} α and β subunits

To study subunit combinations of functional BK_{Ca} channels a system was sought which would enable expression of either α subunit alone or the α subunit in combination with an excess of a β subunit to maximize the formation of BK_{Ca}α + βx channels. We tested the suitability of the pIRES expression system by transfecting either the pIRES-*hSlo*α construct or the pIRES-*hSlo*α + β2 construct and examining the resulting currents in inside-out macropatches. Co-transfection of pEGFP-N1 (to produce green fluorescent protein) with these other constructs was used as a marker for expression. Over 90% of cells that fluoresced green when illuminated with UV light on the microscope yielded patches with BK_{Ca} currents. When the pIRES-*hSlo*α construct, which had the *hSlo*α sequence downstream of the internal ribosome entry sequence and no β-subunit sequence, was transfected into the HEK cells, the BK_{Ca} currents recorded were similar to those expressed in an HEK + hSloα stable line that we have generated previously (Lippiat et al., 2000). Example currents are shown in Fig. 2, demonstrating that *hSlo*α cDNA could be translated in HEK cells independently of the mRNA 5′ cap, and mediated by the encephalomycarditis virus IRES sequence.

To determine if an open reading frame upstream of the IRES sequence could be reliably cotranslated, the pIRES-*hSlo*α + β2 construct was transiently transfected. The β2 subunit confers N-type inactivation by means of an intracellular N-terminal domain

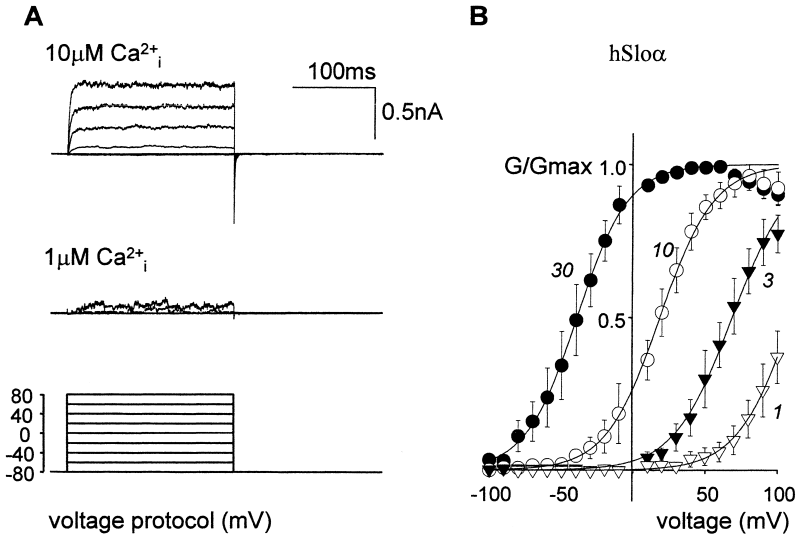

Fig. 2. (*A*) The voltage- and Ca^{2+}-dependent properties of hSloα channels recorded in an inside-out patch exposed to 1 and 10 μM Ca^{2+}. Both pipette and bath had 140 mM K^+ and the voltage protocol used for this and all subsequent recordings is shown below. (*B*) Normalized conductance-voltage relation for the activation of hSloα channels at different $[Ca^{2+}]_i$ indicated in italics (μM) next to the corresponding curve. The symbols show the mean data (± SEM) from 4 patches. The lines show the best fits of the data to Eq. 1 giving values of 19.0, 20.0, 18.6, and 17.7 mV for k, and 110.1, 68.7, 18.6, and −39.3 mV for $V_{1/2}$ in 1, 3, 10, and 30 mM Ca^{2+}, respectively.

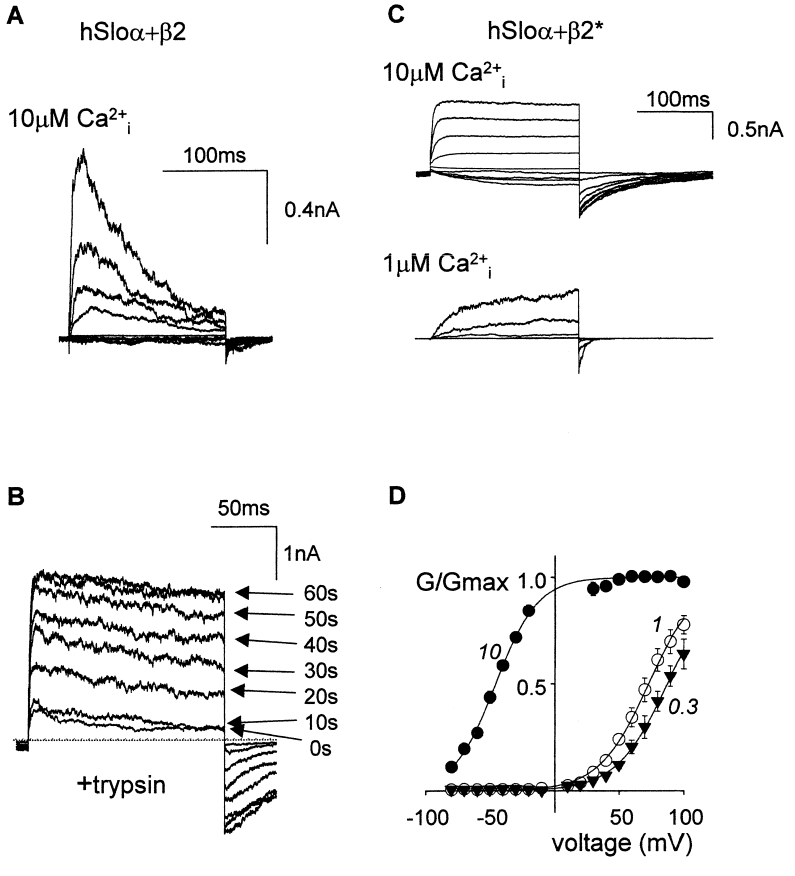

Fig. 3. (*A*) Currents recorded from an inside-out patch excised from HEK293 cells expressing hSloα + β2. The patch was exposed to 10 μM Ca^{2+} and voltage steps from −80 to +80 mV were applied (as shown in Fig. 1). The currents inactivated markedly during the 150 ms pulses. (*B*) The inactivation was readily removed by exposing the patch to 1 mg/mL trypsin. After 60 s in trypsin, the inactivation seen with pulses from −80 to 80 mV was virtually removed. (*C*) Currents observed following trypsin treatment of hSloα + β2 channels (hSloα + β2*) in the presence of 1 and 10 μM Ca^{2+} as indicated. (*D*) Normalized conductance-voltage relation for the activation of hSloα + β2* channels at different $[Ca^{2+}]_i$ indicated in italics (μM) next to the corresponding curve. The symbols show the mean data (± SEM) from 4 patches. The lines show the best fits of the data to Eq. 1 giving values of 19.7, 19.2, and 16.3 mV for k and 87.4, 72.9, and −45.7 mV for $V_{1/2}$ in 0.3, 1 and 10 mM Ca^{2+}, respectively.

(Wallner et al., 1999). Currents recorded from patches from these cells inactivated completely (Fig. 3) showing that the pIRES constructs can produce the two types of BK_{Ca} channel subunit from the same population of mRNA molecules that can coassemble to form functional BK_{Ca} channels. Since the pIRES-*hSlo*α + β2 construct formed BK_{Ca} channels that inactivated completely, this indicated that virtually all the channels were heteromultimers comprising α and β2 subunits, thus confirming the suitability of the pIRES expression system for investigating subunit combinations.

Biophysical Properties of *hSlo*α + β1–4 Channels

Non-inactivating currents were recorded when HEK cells were transiently transfected with pIRES-*hSlo*α + β1, pIRES- *hSlo*α + β3, and pIRES- *hSlo*α + β4

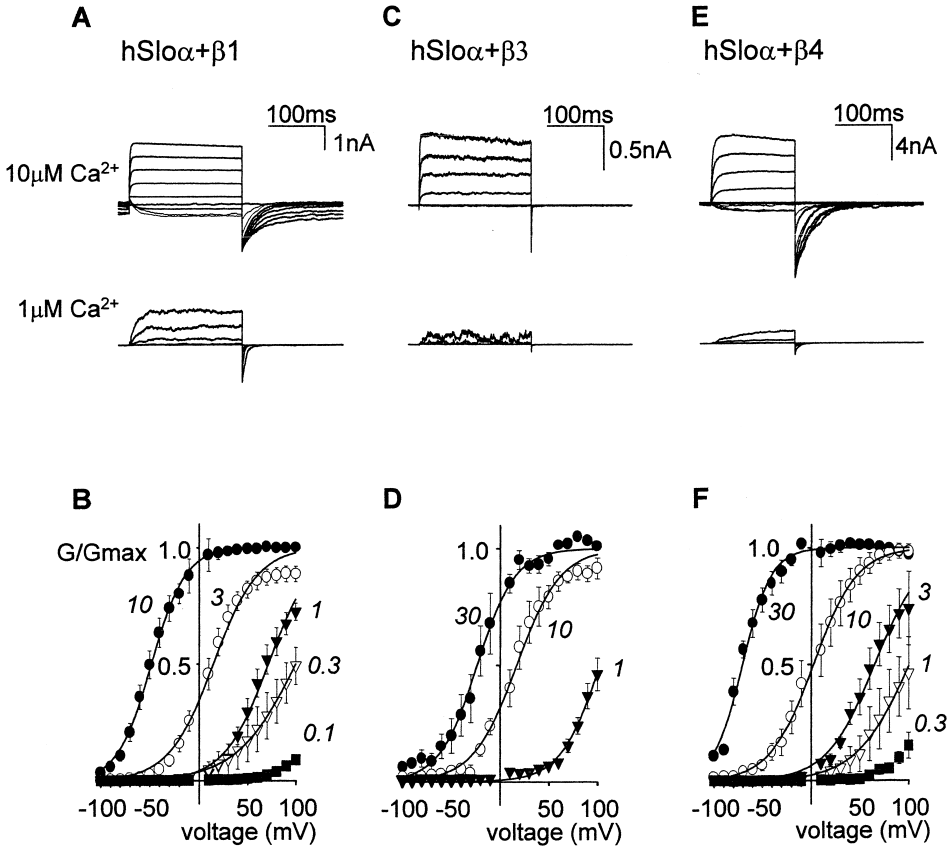

Fig. 4. (*A*, *C*, *E*) Examples of currents recorded from inside-out patches excised from HEK293 cells expressing hSloα + β1, hSloα + β3, and hSloα + β4, respectively. The $[Ca^{2+}]$ was 1 or 10 μM as indicated. The normalized conductance-voltage relations are plotted below in panels *B*, *D*, and *F* at different $[Ca^{2+}]_i$ indicated in italics (μM) next to the corresponding curve. The symbols show the mean data (± SEM) from ≥5 patches. The lines show the best fits of the data to Eq. 1, and the $V_{1/2}$ values returned are plotted in Fig. 5*B*.

(Fig. 4), whereas currents produced by pIRES-*hSloα* + β2 inactivated completely, as described above. As expected from previous reports (e.g., McManus et al., 1995; Dworetzky et al., 1996; Meera et al., 1996) cotranslation of the β1 subunit gave channels that were active at Ca^{2+} concentrations lower than those required to observe currents from the α subunit alone. Also, in comparison with hSloα currents, the rates of deactivation of *hSloα* + β1 channels were slower (Fig. 5*D*).

The inactivation conferred by the β2 subunit could be removed within about 100 s by application of 1 mg/mL trypsin to the intracellular face of the patch (Fig. 3*B*). Following removal of inactivation, the voltage- and Ca^{2+}-dependence of the hSloα + β2 current were similar to those observed with hSloα + β1 described above.

Transfection of the pIRES- *hSloα* + β3 construct produced channels with activation properties similar to those of hSloα channels over the same Ca^{2+} range (Fig. 4*C*). In addition, the currents activated and deactivated rapidly, which is also characteristic of hSloα channels. In contrast, the β4 subunit did alter the properties of the α subunit. Over the range of Ca^{2+} concentrations used, the depolarization required to activate hSloα + β4 channels was more negative than that required to activate hSloα (Fig. 4*E*), although not as negative as that able to activate hSloα + β1. Another feature of hSloα + β4 channels was that they deactivated more slowly than the hSloα channels, though not as slowly as hSloα + β1 channels (Fig. 5).

Fig. 5*A* shows a comparison of the activation curve for each combination of subunits in the presence of 10 μM intracellular Ca^{2+}. The curve representing hSloα + β2 was obtained following a brief application of trypsin to completely remove inactivation. The voltages that induced half-maximal activation ($V_{1/2}$), obtained by fitting Boltzmann distributions to each data set, were (mean ± SEM) 17.0 ± 5.9 mV, −49.1 ± 4.2 mV, −45.6 ± 0.18 mV, 17.7 ± 13.8 mV, and 4.5 ± 8.4 mV for hSloα, and hSloα coexpressed with β1, β2 (after trypsin), β3 and β4, respectively (n = 3 to 6). The calcium sensitivity of $V_{1/2}$ is also shown over a range of concentrations. This shows clearly how β1, β2, and β4 shift the voltage dependence of activation to more negative potentials (Fig. 5*B*). Fig. 5*C* and *D* shows the relative time courses of both activation at +80 mV and deactivation at −80 mV of currents with 10 μM intracellular Ca^{2+}. Although there is little difference in the activation time course under these conditions (although it is notable that the hSloα + β3 current activates the fastest), the β subunits significantly alter the deactivation. Channels assembled with β1, β2, and β4 subunits have slower deactivation under these conditions.

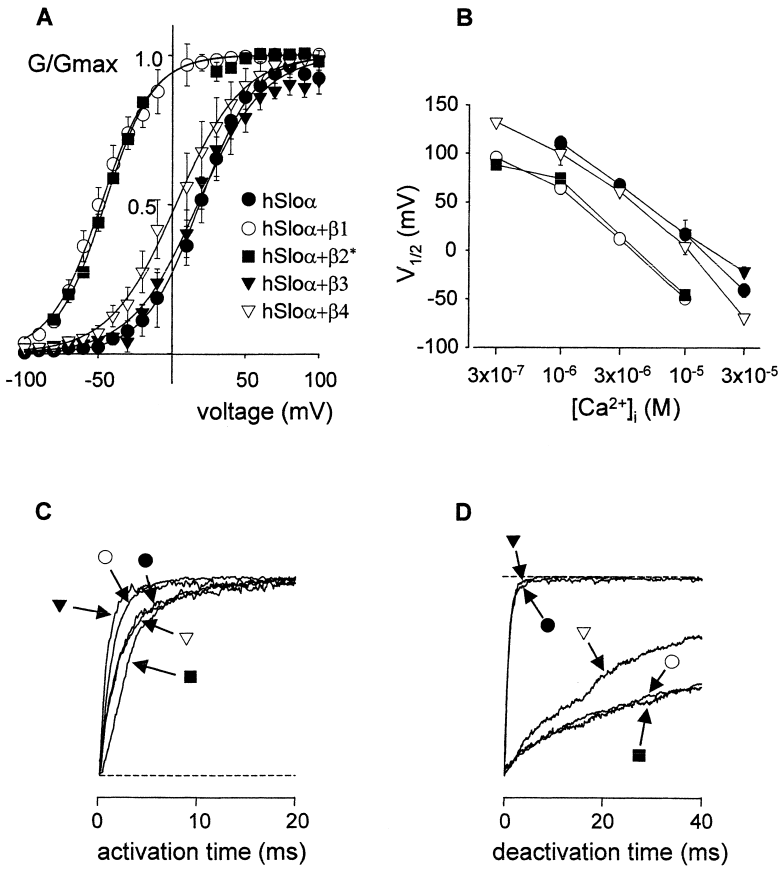

Fig. 5. (*A*) Comparison of the conductance-voltage curves obtained from inside-out patches with 10 μM Ca^{2+} for hSloα, hSloα+β1, hSloα+β2*, hSloα+β3, and hSloα+β4, as indicated. These data are the same as those shown in Figs. 2, 3 and 4. (*B*) Plot of the values of $V_{1/2}$ at different [Ca^{2+}] for hSloα, hSloα+β1, hSloα+β2*, hSloα+β3, and hSloα+β4. The symbols show the mean ± SEM of between 4 and 6 patches. (*C*) Activation at +80 mV and (*D*) deactivation of currents at −80 mV. The symbols representing the constructs expressed are as indicated on graph *A*.

Sensitivity to Iberiotoxin

It has been reported that the β4 subunit in particular decreases the apparent block by the specific BK_{Ca} channel blocker iberiotoxin (IbTX) by slowing down 1000 times its association (Meera et al., 2000). We tested the ability of IbTX to block BK_{Ca} channels comprising hSloα and different β subunits expressed in HEK 293 cells. Increasing concentrations of IbTX were applied to outside-out patches at two-minute intervals, followed by 10 mM TEA^+ to block any residual current (Fig. 6). Channels formed by the expression of the α subunit alone were blocked slowly by IbTX in the 30 to 300 nM range. Tenfold higher concentrations of IbTX were required to block α+β1. Just as coexpressing β3 failed to alter activation kinetics, α+β3 channels seemed to have the same sensitivity as the α subunit to IbTX. At concentrations up to 1 μM, IbTX did not affect currents conducted by α+β4 channels even though block by 10 mM TEA^+ was unaffected. The IC_{50} values fitted to the pooled data were, in decreasing sensitivity, 33 nM, 39 nM, and 371 nM for α, α+β3, and α+β1 channels, respectively (Fig. 6*B*). We were unable to obtain a value for α+β4 channels because the IC_{50} value appeared considerably higher than the concentrations used.

Discussion

The properties of BK_{Ca} channels modulated by different members of the family of hSloβ subunits could be reliably studied by transient transfection of bicistonic expression constructs. Channel proteins formed by cap-independent translation of the α-subunit cDNA (*hSloα*) downstream of the IRES, appeared to be fully functional and qualitatively indistinguishable from the same clone expressed previously with a conventional expression vector (Lippiat et al., 2000). The presence of BK_{Ca} current in excised patches could therefore act as a marker for the expression of an open reading frame upstream of the IRES sequence. When different β subunit cDNA inserts were cloned into the pIRES-*hSloα*, the resulting BK_{Ca} channels differed with respect to activation kinetics, including relaxation time course, and sensitivity to the selective blocker iberiotoxin. These altered characteristics were similar to those observed by coinjection of *hSloα*- and *hSloβ*-subunit mRNA into *Xenopus* oocytes (Brenner et al., 2000).

These alterations of channels expressed in HEK cells in general agree with previous descriptions using the *Xenopus* oocyte expression system, (McManus et al., 1995; Wallner et al., 1999; Brenner et al., 2000; Meera et al., 2000). In particular, the β3 subunit

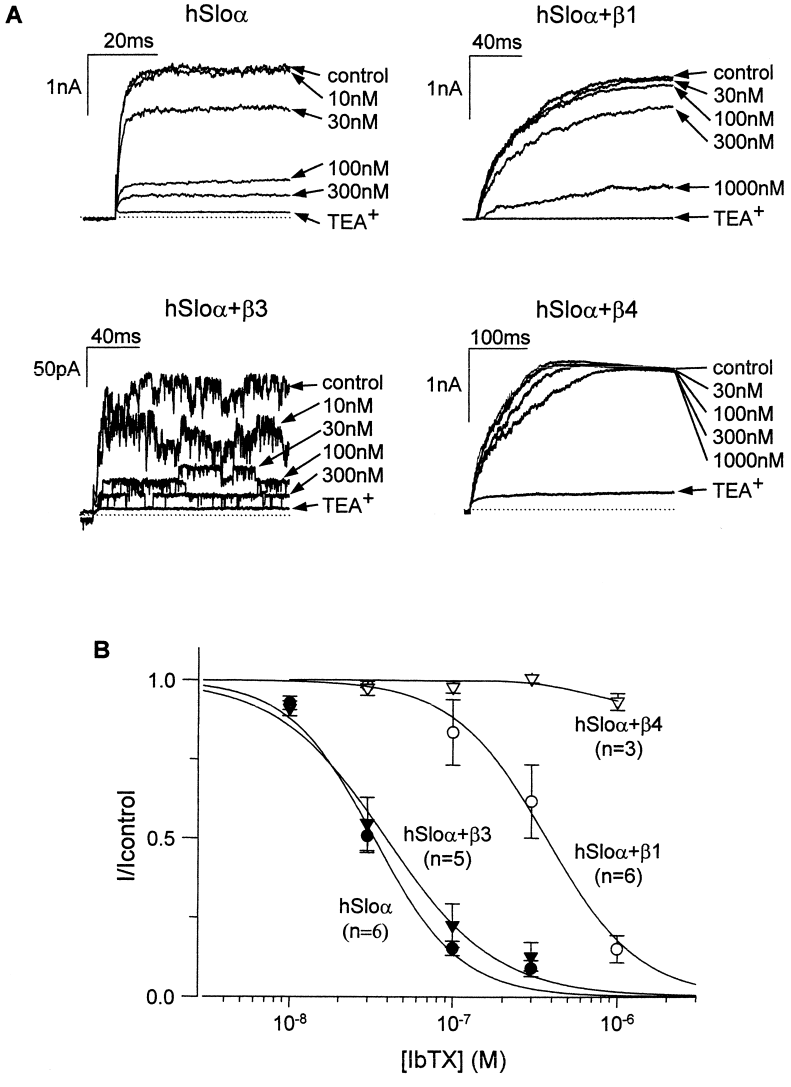

Fig. 6. (*A*) Examples of the block of hSloα, hSloα + β1, hSloα + β3, and hSloα + β4 channels by IbTx at the concentrations indicated and 10 mM TEA^+. The recordings were obtained from outside-out patches at +80 mV and with 10 μM Ca^{2+} in the pipette. (*B*) Plots of the concentration dependence of block by IbTx of hSloα, hSloα + β1, hSloα + β3, and hSloα + β4 as indicated. The curves show the best fits of the data to Eq. 2, giving IC_{50} values of 33, 371, and 39 nM, and Hill coefficients of 1.65, 1.53, and 1.29 for hSloα, hSloα + β1, and hSloα + β3, respectively. An accurate fit for the hSloα + β4 data was not possible. Some patches in the presence of TEA had residual leak current, as no leak subtraction was used in these recordings.

failed to significantly enhance activation kinetics of hSloα expressed in HEK cells, which was also the case in oocytes. Brenner et al. (2000) were able to show that the β3 subunit did indeed assemble with the pore-forming α subunit by demonstrating that transplanting the β2 inactivation domain onto β3 resulted in inactivating BK_{Ca} currents. Furthermore, Uebele et al. (2000) described the existence of variants of β3 that arise from alternative splicing about the 5′ end of the channel gene. These N-terminal splice variants of β3 confer varying degrees of inactivation, so implying association between the subunit types.

The properties of hSloα + β4 channels described in the present study are also consistent with those expressed in oocytes (Brenner et al., 2000) in that the deactivation kinetics are slower than those of hSloα channels and that voltage-dependent activation is shifted to more negative potentials at most calcium concentrations. However, when Weiger et al. (2000) studied hSloα + β4 channels formed by expression in HEK 293 cells, as used in the present study, they found that β4 also slowed kinetics but shifted activation to more positive potentials under all calcium concentrations used. This discrepancy could be due to variation in the hSloα subunit used in the respective studies or to functional differences between channels expressed in mammalian and amphibian cells. Splice variants of the mammalian α subunit exist that vary with respect to activation kinetics and calcium sensitivity (Tseng-Crank et al., 1994), which may also result in different responses to functional association with β subunits. However, comparison of the primary sequences of the *hSloα* clones used for our study (bladder), by Brenner et al. (2000; myometrium), and by Weiger et al. (2000; brain) suggests that the same splice variant seems to have been used.

Alteration of iberiotoxin sensitivity by coexpression with β1 or β4 was also consistent with previous findings (Dworetzky et al., 1996; Meera et al., 2000, respectively). The fact that there was no inhibition of hSloα + β4 channels under all conditions tested is

further evidence that the bicistronic expression system was producing a saturating β:α subunit ratio. Although it could be argued that the block by iberiotoxin may not have reached steady state, the exposure time to iberiotoxin in our experiments is fairly typical of those used to test for the presence of BK_{Ca} channels in native tissue. An interesting observation is that hSloβ subunits that alter activation kinetics also affect toxin binding. With the N-terminal inactivation domain deleted, the β2 subunit increases the activity of hSloα channels similarly to β1. Furthermore the effects of β2 on toxin sensitivity are also similar to β1 (Wallner et al., 1999). The binding of toxin to the putative high-affinity binding site on the β1 subunit (Hanner et al., 1997) does not appear to result in channel inhibition. Coexpression of β4 alters activation kinetics differently from β1 and β2, and also affects toxin sensitivity differently. We found that coexpressing β3 subunit did not have a significant effect on channel inhibition; likewise there was no effect on channel activation.

Since the properties of BK_{Ca} channels comprising different combinations of α + β subunits expressed using this system correspond to those described previously using other means, it can be concluded that bicistronic expression of subunits is an efficient and robust system for functional study of ion channels and their accessory subunits. This eliminates the uncertainty of relative expression levels associated with cotransfection of subunits cloned into independent vectors, and the possibility of altered function when expressed in an amphibian oocyte.

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