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The β subunit of the high-conductance calcium-activated potassium channel contributes to the high-affinity receptor for charybdotoxin

(transient expression/toxin binding/Western blots/crosslinking)

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ABSTRACT **Transient expression of either α or α+β subunits of the high-conductance Ca^{2+}-activated K^+ (maxi-K) channel has been achieved in COS-1 cells. Expression has been studied using charybdotoxin (ChTX), a peptidyl inhibitor that binds in the pore on the α subunit. Although some properties of monoiodotyrosine-ChTX (^{125}I-ChTX) binding to membranes derived from each type of transfected cells appear to be identical, other parameters of the binding reaction are markedly different. Under low ionic strength conditions, the affinity constant for ^{125}I-ChTX measured under equilibrium binding conditions is increased *ca.* 50-fold in the presence of the β subunit. The rate constant for ^{125}I-ChTX association is enhanced *ca.* 5-fold, whereas the dissociation rate constant is decreased more than 7-fold when the β subunit is present. These data indicate that functional coassembly of maxi-K channel subunits can be obtained in a transient expression system, and that the β subunit has profound effects on ^{125}I-ChTX binding. We postulate that certain negatively charged residues in the large extracellular loop of β attract the positively charged ^{125}I-ChTX to its binding site on α through electrostatic interactions, and account for effects observed on ligand association kinetics. Moreover, another residue(s) in the loop of β must contribute to stabilization of the toxin-bound state, either by a direct interaction with toxin, or through an allosteric effect on the α subunit. Certain regions in the extracellular loop of the β subunit may be in close proximity to the pore of the channel, and could play an important role in maxi-K channel function.**

High-conductance Ca^{2+}-activated K^+ (maxi-K) channels display characteristic biophysical and pharmacological properties that distinguish them from other ion channels. Maxi-K channels are activated by both membrane depolarization and intracellular Ca^{2+}, and exhibit a large potassium conductance (200–300 pS), as well as high selectivity for this cation. These channels are sensitive to inhibition by external application of a number of peptidyl toxins, such as charybdotoxin (ChTX) and iberiotoxin (1). Maxi-K channels are also modulated by a series of small molecule natural products; indole diterpene alkaloids such as paxilline represent the most potent and selective nonpeptidyl blockers of this channel discovered to date (2); glycosylated triterpene channel agonists, like dehydrosoyasaponin I, bind to the cytoplasmic face of the protein and enhance its activity (3). The maxi-K channel has been purified to homogeneity from tracheal (4) and aortic smooth muscle (5) and shown to be composed of two structurally distinct subunits, α and β. Although expression of the α subunit of the maxi-K channel in *Xenopus* oocytes yields channels that are activated by Ca^{2+} and voltage (6), coexpression of β with the α subunit has profound effects on the biophysical and pharmacological properties of the channel. In the presence of the β subunit, there is a shift in the midpoint of channel activation of 80–100 mV in the hyperpolarized direction (7–9). This shift is equivalent to that produced by a 10-fold increase in Ca^{2+} concentration. Thus, the β subunit may change the overall conformation of the channel in such a way that the transitions to the open state are more favored. In addition, the agonist activity of the glycosylated triterpenes requires the presence of the β subunit (8).

Heterologous expression of the α subunit yields channels that are sensitive to ChTX. This is expected since ChTX binds in the pore formed by four α subunits to block the ion conduction pathway. However, possible contributions of the β subunit to the ChTX receptor have not been explored. In this study, the characteristics of the interaction of monoiodotyrosine-ChTX (^{125}I-ChTX) with membranes derived from COS-1 cells transiently transfected with either α or α and β subunits of the maxi-K channel have been determined. The results of these studies indicate that the β subunit causes a significant enhancement in toxin affinity of *ca.* 50-fold through an effect on both association and dissociation kinetics. Thus, it appears that certain regions in the large extracellular loop of β are in close proximity to the toxin binding site and may even participate in it and in other aspects of channel function.

MATERIALS AND METHODS

Materials. Restriction enzymes and pCI-neo vector were from Promega. COS-1 cells (culture CRL1650) were obtained from American Type Culture Collection. All tissue culture media and the LipofectAMINE reagent were from GIBCO. Fetal bovine serum was purchased from HyClone. Recombinant *N*-glycanase was from Genzyme. Digitonin special grade (water soluble) was obtained from Biosynth Technologies (Skokie, IL). GF/C glass fiber filters were purchased from Whatman. Affinity-purified alkaline phosphatase-conjugated goat anti-rabbit IgG, nitroblue tetrazolium (NBT), 5-bromo-4-chloro-3-indoyl phosphate *p*-toluidine salt (BCIP), Tween 20, Triton X-100, and paxilline were obtained from Sigma. Disuccinimidyl suberate (DSS) was from Pierce. ChTX was obtained from Peninsula Laboratories and radioiodinated as described (10). ^{125}I-Na was obtained from DuPont/NEN. 4′,6-Diamidino-2-phenylindole was from Molecular Probes.

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Abbreviations: ChTX, charybdotoxin; ^{125}I-ChTX, monoiodotyrosine-ChTX; maxi-K channel, high-conductance Ca^{2+}-activated K^+ channel; DSS, disuccinimidyl suberate.

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Prestained molecular mass standards and polyvinylidene difluoride membranes (Immobilon) were obtained from Bio-Rad. All other reagents were obtained from commercial sources and were of the highest purity commercially available. The human α clone huR2 (+) (7), and the bovine β clone (11) were subcloned into pCI-neo vector.

Transfection of COS-1 Cells and Membrane Preparation. COS-1 cells were grown in DMEM supplemented with defined fetal bovine serum, penicillin/streptomycin, and L-glutamine under 10% CO_2, 100% humidity at 37°C. For transfections, cells were seeded into T-225 cm^2 flasks at an initial density of 3×10^6 cells per flask and left to acclimate overnight. Transfections were performed in phenol red-free/serum-free DMEM (pr/sf DMEM) supplemented with L-glutamine. For transfections involving the α subunit, 100 μl pr/sf DMEM was mixed with 100 μl of LipofectAMINE (GIBCO) and 10 μg of α DNA with 100 μl of pr/sf DMEM per T-225 flask being transfected. The two solutions were then combined and incubated for 30 min at room temperature. After incubation, each flask was rinsed with 10 ml pr/sf DMEM and filled with 30 ml of the same medium. The transfection mixture was diluted up to 1 ml with pf/sf DMEM and added to the flask. Cells were allowed to incubate with the transfection medium for 5 hr under 10% CO_2, 100% humidity at 37°C. For transfections involving the α and β subunits, the same protocol was employed with the addition of 3 μg of β DNA per T-225 flask. After incubation, the transfection medium was removed and 60 ml DMEM was added to each flask. The cells were grown for 72 hr. Cells were then scraped from the flasks and collected by centrifugation. Membranes were prepared as described (12). The final membrane pellet was resuspended in 100 mM NaCl/20 mM Hepes·NaOH, pH 7.4. Aliquots were quickly frozen in liquid N_2 and stored at −70°C.

Binding Assays. The interaction of ^{125}I-ChTX with COS-1 membranes was carried out in a medium consisting of 10 mM NaCl/20 mM Tris·HCl, pH 7.4/0.1% BSA. Other experimental conditions are given in the figure legends. Separation of bound from free ligand was done as described (10).

Crosslinking Experiments. Covalent incorporation of ^{125}I-ChTX into membranes in the presence of the bifunctional crosslinking reagent, DSS, was carried out as described (13).

Antibody Production. Polyclonal serum raised against residues 913–926 of the α subunit ($\alpha_{913–926}$), and residues 118–132 of the β subunit ($\beta_{118–132}$) of the maxi-K channel were obtained as described (14, 15). Antibodies were affinity-purified on their respective antigen columns as described (14).

Immunoblot Analysis. After SDS/PAGE, proteins were transferred onto polyvinylidene difluoride membranes. After transfer, the membrane was incubated with 10% skim milk powder/0.5% (wt/vol) Triton X-100/0.1% (wt/vol) Tween 20 in TBS buffer (150 mM NaCl/20 mM Tris·HCl, pH 7.4) for 3 hr at 22°C and then with either antiserum $\alpha_{913–926}$ or $\beta_{118–132}$ diluted in 1% skim milk powder/0.5% Triton X-100/0.1% Tween 20 in TBS for 12 hr at 4°C. After several washes with 0.5% Triton X-100/0.1% Tween 20 in TBS, affinity-purified alkaline phosphatase-conjugated goat anti-rabbit IgG at 1:2,000 dilution was added and blots were incubated for 2 hr at 22°C. Membranes were washed as before and developed in 100 mM Tris·HCl, pH 9.5/100 mM NaCl/5 mM $MgCl_2$/0.033% NBT/0.017% BCIP. The reaction was stopped by addition of 0.5 M EDTA/Tris, pH 7.4, up to a final concentration of 20 mM.

Polyacrylamide Gel Electrophoresis. Samples were resuspended in SDS/PAGE sample buffer containing 1% 2-mercaptoethanol, incubated at 37°C for 120 min, and subjected to SDS/PAGE.

Analysis of Data. Data from saturation experiments were analyzed according to the equation $B = B_{max}/[1 + (kd/L)^{nH}]$, where B_{max} is the maximum number of receptors, K_d is the dissociation constant, nH is the pseudo-Hill coefficient, and L is the free ligand concentration. The dissociation rate constant (k_{-1}) was determined directly from the first order plot of ligand dissociation vs. time. The association rate constant (k_1) was determined by measuring the kinetics of toxin binding at different ligand concentrations and determining k_{obs} at each toxin concentration. k_{obs} is the slope of the pseudo-first-order plot $\ln[LR]_e/([LR]_e\text{-}[LR]_t)$ vs. time, where $[LR]_e$ is the concentration of toxin-receptor at equilibrium. k_1 was determined as the slope of the plot of k_{obs} vs. ChTX concentration. In this plot, the y intercept represents k_{-1}. In some experiments, k_1 was determined from the equation $k_1 = k_{obs}$ ($[LR]_e/[L] \times B_{max}$).

RESULTS

Expression of α and β Subunits of the maxi-K Channel in COS-1 Cells. To assess whether COS-1 cells are an appropriate system for transient expression of the α and β subunits of the maxi-K channel, transfected cells were subjected to immunostaining with affinity-purified site-directed antibodies raised against each subunit. Mock transfected cells yielded very low levels of staining with either antibody. In contrast, after 72 hr of transfection with either α or $\alpha+\beta$ subunits, cells displayed very prominent staining with each corresponding antibody (data not shown). The percentage of cells that could be transfected employing a DEAE-dextran procedure was estimated to be between 10 and 20% after quantitating cell nuclei with 4′,6-diamidino-2-phenylindole.

The expression of α and β subunits of the maxi-K channel at the plasma membrane level was determined from immunoblot experiments. As shown in Fig. 1*A*, membranes derived from cells transfected with either α or $\alpha+\beta$ displayed immunoreactivity with anti-$\alpha_{913–926}$ made against a peptide contained within the α subunit of the maxi-K channel. No immunostaining was observed with membranes derived from either untransfected cells, from cells transfected with the β subunit alone, or if incubation was done in the presence of the antigenic peptide (data not shown). The expression of the β subunit of the maxi-K channel was determined from corresponding immunoblots employing an anti-$\beta_{118–132}$ antibody. Membranes prepared from untransfected cells, or from cells

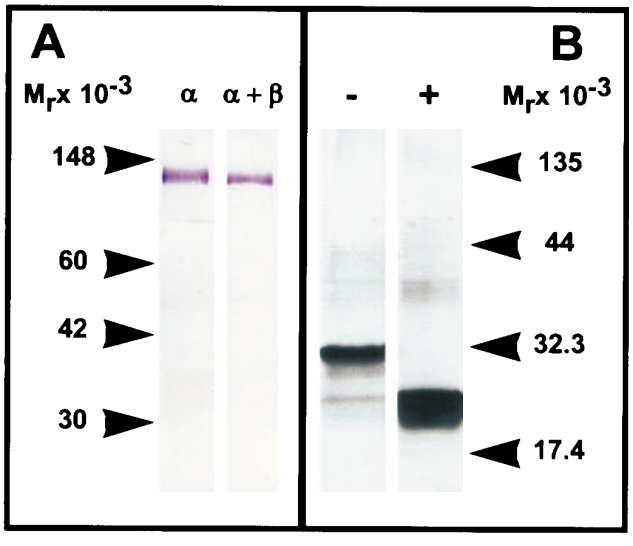

FIG. 1. Immunoblot analysis of the α and β subunits of the maxi-K channel. Membranes prepared from COS-1 cells transiently transfected with either α or $\alpha+\beta$ subunits of the maxi-K channel were separated by SDS/PAGE under reducing conditions and immunoblotted using anti-$\alpha_{913–926}$ (*A*), or anti-$\beta_{118–132}$ (*B*). In *B*, $\alpha+\beta$ membranes were incubated overnight with recombinant *N*-glycanase (+) and compared with membranes treated identically, but in the absence of enzyme (−).

transfected with the α subunit alone, did not display any immunoreactivity with this antibody. In marked contrast, when the transfection employed α and β subunits, there was immunoreactivity for a polypeptide with an M_r of 31,000 (Fig. 1*B*). To confirm that this polypeptide corresponds to the β subunit of the maxi-K channel, membranes were subjected to treatment with *N*-glycanase to remove putative N-linked sugars. After enzymatic treatment, the immunoreactivity was then associated with a polypeptide of M_r 22,000, which corresponds to the core protein of the β subunit. In all cases, immunostaining was prevented in the presence of the antigenic peptide. These data indicate that both subunits of the maxi-K channel can be expressed in COS-1 cells, and that modification of the β subunit by covalent addition of sugars occurs to the same extent as found in native tissue (4).

Functional Expression of maxi-K Channel Subunits. Binding of radiolabeled peptides such as ^{125}I-ChTX can be used as a measure of the expression of the maxi-K channel complex. It is well documented with native maxi-K channels that when such an iodinated peptide is bound to its receptor site in the pore of the channel, covalent incorporation of radioactivity takes place exclusively in the β subunit in the presence of a bifunctional crosslinking reagent (4, 13, 15). To determine whether functional association of α and β subunits occurs in COS-1 cells, membranes derived from cells transfected with either α or $\alpha+\beta$ subunits were incubated with ^{125}I-ChTX and then exposed to the crosslinking reagent, DSS. Experimental conditions were chosen so that the amount of ^{125}I-ChTX bound was identical in each membrane preparation. After the addition of DSS and separation by SDS/PAGE, covalent incorporation of radioactivity could be observed into the β subunit of the channel (Fig. 2), suggesting, in fact, that α and β subunits are associated in the complex in a similar way as occurs in native tissue. No incorporation of toxin occurred in membranes containing the α subunit alone.

A number of pharmacological agents have been identified that modify maxi-K channel function (16). Members of the family of indole diterpene alkaloids are potent inhibitors of channel activity. However, their effect on ChTX binding to its receptor differs. Compounds such as paxilline and verruculogen enhance toxin binding, whereas aflatrem, paspalitrem A and C, and penitrem A inhibit the toxin's interaction through an allosteric mechanism. We monitored binding of ^{125}I-ChTX to membranes containing either α or $\alpha+\beta$ subunits of the maxi-K channel in the absence or presence of two representative members of the indole diterpene family: paxilline and aflatrem. Regardless of whether or not the β subunit is present, paxilline causes a concentration-dependent stimulation of toxin binding, whereas inhibition is observed in the presence of aflatrem (Fig. 3). These data indicate that the site of action of indole diterpenes is most likely restricted to the α subunit of the maxi-K channel and that the presence of β does not modify the coupling between the receptor sites for these compounds and ChTX.

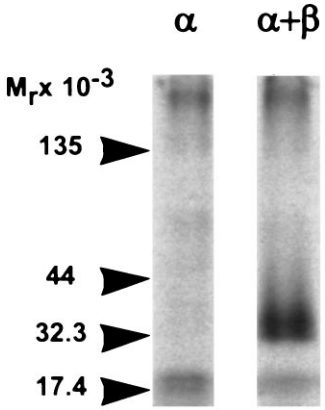

FIG. 2. Crosslinking of ^{125}I-ChTX to the β subunit of the maxi-K channel. Membranes prepared from COS-1 cells transiently transfected with either α or $\alpha+\beta$ subunits were incubated with ^{125}I-ChTX in the presence of 5 μM paxilline, and then reacted with DSS. Samples were subjected to SDS/PAGE. The migration of molecular weight standards is shown.

Binding Properties of ^{125}I-ChTX Are Altered in the Presence of the β Subunit. It is well established that in the presence of the β subunit, the midpoint of channel activation is shifted 80–100 mV in the hyperpolarized direction (7–9). However, effects of the β subunit on other parameters, such as toxin binding, have not been explored. For this purpose, membranes containing α or $\alpha+\beta$ subunits were incubated with increasing concentrations of ^{125}I-ChTX under equilibrium binding conditions. Fig. 4 shows saturation isotherms of specific ^{125}I-ChTX binding to either type of membrane. In each case, ^{125}I-ChTX binds to a single class of sites, as reflected by the fact that the Hill coefficient of both slopes is equal to one. Although the data are presented normalized to the B_{max} of each preparation, this parameter is not significantly different between either set of membranes, suggesting that the β subunit does not affect expression levels of α. In marked contrast, toxin affinity is dramatically altered. Thus, in the presence of β subunit, the K_d for ^{125}I-ChTX is *ca.* 1 pM, whereas in its absence, the affinity decreases *ca.* 50-fold. These data are representative of several different transfection experiments, and average values are presented in Table 1.

The large change in toxin affinity caused by the β subunit could be due to an effect on either the kinetics of ligand association or dissociation, or on both rate constants. To determine which parameter(s) of the binding reaction is responsible for the shift in toxin affinity, on and off rates for ^{125}I-ChTX were measured to each set of membranes. Fig. 5 shows the results of the dissociation experiments. In each case, ligand dissociation follows a monoexponential function with time, indicative of a first-order reaction. The semilogarithmic transformation of these data gives a straight line that goes through zero, confirming the presence of a single class of receptor sites. It is evident from the data that in the presence of the β subunit, the half-time of toxin dissociation has been significantly decreased by *ca.* 7-fold. Thus, β causes a stabilization of the toxin-bound state. The results of multiple dissociation experiments employing several different membrane preparations are included in Table 1; the same trend is noted throughout.

To determine the rate constant for ligand association, ^{125}I-ChTX binding was monitored as a function of time at different ligand concentrations, always employing pseudo-first-order binding conditions (i.e., $<10\%$ ligand depletion). The data were linearized as indicated in *Materials and Methods* to yield a k_{obs} value at each ligand concentration. A plot of k_{obs} vs. ligand concentration gives a straight line in which the y intercept represents k_{-1} and the slope of the curve is k_1. Results of these experiments are also presented in Table 1 and indicate that the β subunit causes an increase in the rate constant for association of *ca.* 5-fold. Thus, the combined effects of β on association and dissociation kinetics is to produce a very large increase in toxin affinity.

DISCUSSION

The results presented in this study demonstrate that the two structural components of the maxi-K channel, the α and β subunits, can be transiently expressed in functional form in COS-1 cells. Moreover, and most important, coexpression of α and β subunits causes a dramatic effect on the properties of ^{125}I-ChTX interaction when compared with those found with the α subunit alone.

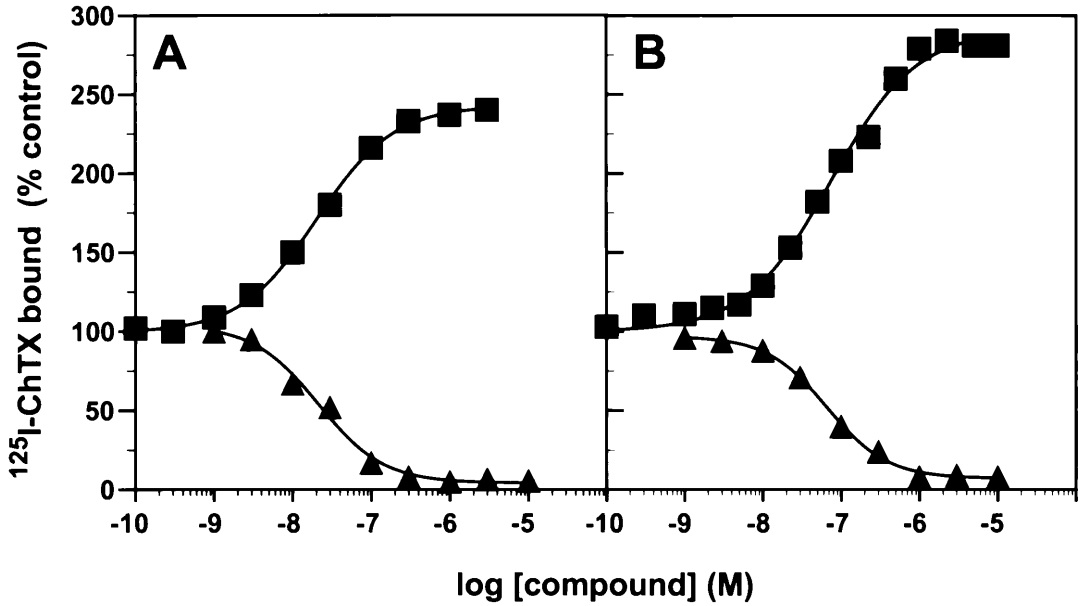

FIG. 3. Modulation of ^{125}I-ChTX binding by paxilline and aflatrem. Membranes prepared from COS-1 cells transiently transfected with either α (*A*) or $\alpha+\beta$ (*B*) subunits of the maxi-K channel were incubated with ^{125}I-ChTX in the absence or presence of increasing concentrations of either paxilline (■) or aflatrem (▲) until equilibrium was achieved. Specific binding was assessed relative to an untreated control.

It has previously been shown that the β subunit produces a marked change in certain biophysical and pharmacological properties of α (7–9). Thus, the midpoint of channel activation is shifted 80–100 mV in the hyperpolarized direction, and this change is equivalent to that produced by a 10-fold increase in Ca^{2+} concentration. It is reasonable to hypothesize that the functional role of maxi-K channels in different tissues could be regulated by the presence or absence of a β subunit. Likewise, modulation of maxi-K channel activity by dehydrosoyasaponin I (a channel agonist) requires the presence of a β subunit (8).

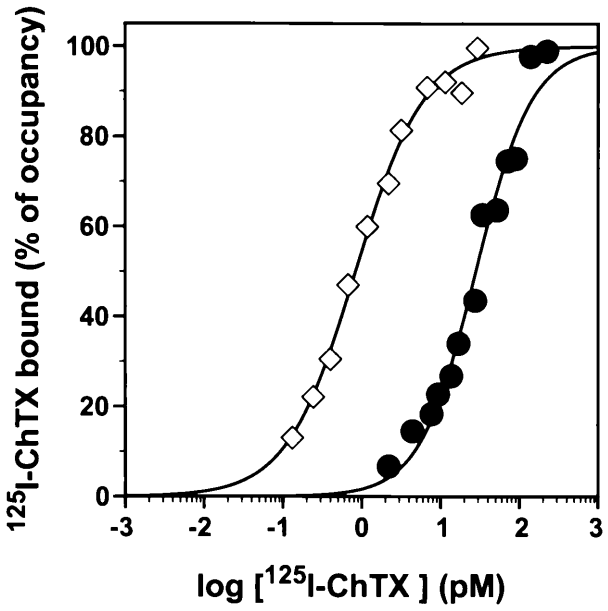

FIG. 4. Binding of ^{125}I-ChTX to either α or $\alpha+\beta$ subunits of the maxi-K channel. Membranes prepared from COS-1 cells transiently transfected with either α (●) or $\alpha+\beta$ subunits (◇) of the maxi-K channel were incubated with increasing concentrations of ^{125}I-ChTX in a medium consisting of 10 mM NaCl, 20 mM Tris·HCl (pH 7.4), 0.1% BSA, 0.1% digitonin, and 5 μM paxilline, until equilibrium was achieved. Nonspecific binding was determined in the presence of 10 nM ChTX. Specific binding data are presented relative to the maximum receptor occupancy.

This is a remarkable observation in view of the fact that dehydrosoyasaponin I binds to a site accessible only from the cytoplasmic side of the channel, and that the postulated cytoplasmic domains of the β subunit do not appear large enough to accommodate such an interaction surface. It is conceivable that the β subunit changes the overall conformation of α in such a way that the transitions to the open state are more favored and that the binding site for dehydrosoyasaponin I becomes available. In contrast, the site of action of indole diterpene blockers appears to be exclusively on the α subunit.

There is no doubt that expression of the α subunit alone produces maxi-K channels that are voltage-dependent, Ca^{2+}-activated, and ChTX-sensitive (6–9). It has therefore been postulated that the receptor site for ChTX is formed from residues contributed solely by the pore-forming subunit. However, the results presented in this study clearly indicate that the β subunit also contributes to the formation of the toxin receptor. The large difference in toxin affinity when β is coexpressed with α is very significant. Moreover, the effects of β can be demonstrated to be due to changes in both the kinetics of toxin association and dissociation. How can these results be interpreted? Since the binding experiments were carried out under low ionic strength conditions, it is possible that the effects of β on association kinetics are due to the presence of negatively charged residues in the extracellular loop of β that attract the positively charged peptide to its binding site through an electrostatic interaction. If this is true, then the effect of β on toxin association should disappear as the ionic strength of the medium is increased. Unfortunately, it is quite difficult to perform binding experiments with ^{125}I-ChTX under the conditions required to test this hypothesis, because of the large

Table 1. Binding of ^{125}I-ChTX to α and $\alpha+\beta$ membranes

Membrane	K_d, pM	k_{-1}, s^{-1}	k_1, $M^{-1}\cdot s^{-1}$
α	45.14 ± 5.92 (n = 4)	0.0029 (n = 3)	5.5×10^7 (n = 3)
$\alpha+\beta$	0.84 ± 0.11 (n = 5)	0.0004 (n = 5)	2.6×10^8 (n = 3)
$\alpha/\alpha+\beta$	53.7	7.3	0.21

The equilibrium dissociation constant (K_d) and the rate constants of association (k_1) and dissociation (k_{-1}) for ^{125}I-ChTX binding to membranes derived from COS-1 cells transiently transfected with either α or $\alpha+\beta$ subunits of the maxi-K channel are presented. These values are the average of several different experiments.

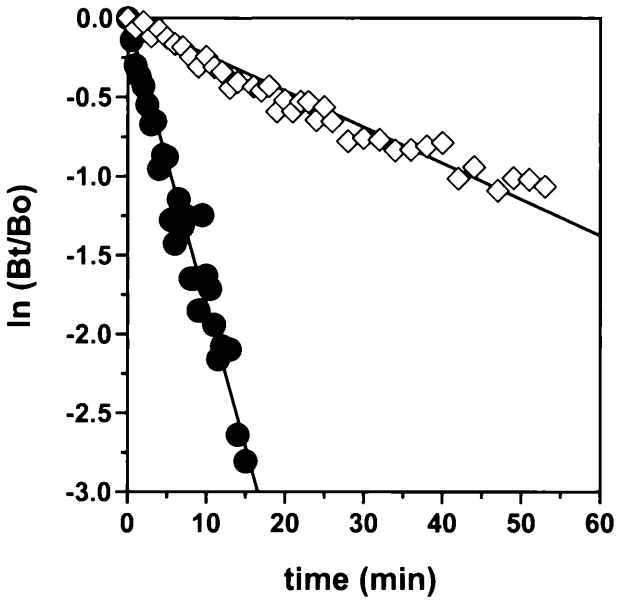

FIG. 5. Dissociation kinetics of ^{125}I-ChTX. Membranes prepared from COS-1 cells transiently transfected with either α (●) or $\alpha+\beta$ subunits (◇) of the maxi-K channel were incubated with ^{125}I-ChTX at room temperature in a medium consisting of 10 mM NaCl, 20 mM Tris·HCl (pH 7.4), 0.1% BSA, 0.1% digitonin, and 5 μM paxilline. Dissociation kinetics were initiated by adding 10 nM ChTX and incubating at room temperature for different periods of time. A semilogarithmic transformation of the first-order dissociation rates is shown.

drop in toxin affinity that occurs as the ionic strength of the medium is elevated. However, it may be possible to identify those residues in the β subunit that are responsible for these effects by site-directed mutagenesis. In this context, it is worth mentioning that the large extracellular loop of β contains 16 negatively charged residues. Some of these are located in close proximity to Lys-69, the residue involved in the crosslinking reaction with ^{125}I-ChTX (15). These data imply that Lys-69 is at a maximum distance of 11 Å from Lys-32 in ChTX (17). Therefore, residues close to Lys-69 could be positioned in close proximity to the ChTX receptor. As an alternative explanation, we can consider the possibility that in the presence of the β subunit, the open probability of the channel may be enhanced. This phenomenon could explain the effects of β on toxin binding if ^{125}I-ChTX would bind with different affinities to open vs. closed conformations of the channel. It has previously been demonstrated for the rat skeletal muscle maxi-K channel that association rates of ChTX increase about 7-fold in going from the closed to the open channel conformation, whereas dissociation rates are independent of the channel's open probability (18). Thus, a change in the channel's open probability would only be reflected in association kinetics of ^{125}I-ChTX. However, the open probability of both α and $\alpha+\beta$ channels in this study must be identical and close to zero since 5 μM paxilline (a channel blocker) was present in these experiments. It is, therefore, very unlikely that the effect of the β subunit on association rates of ^{125}I-ChTX could be due to differences in the open probability of the channels.

The results of the dissociation experiments indicate that the presence of β causes a stabilization of the toxin-bound state. This effect could be due to either a direct participation of β in the formation of the ChTX receptor, or an allosteric effect in which β changes the conformation of the α subunit. In the former case, it should be feasible to identify which residue(s) of the β subunit participates in making contact with specific residues of ChTX. Obviously, further investigation is needed to obtain a more detailed picture of those crucial residues of the β subunit that are responsible for the large increase in ^{125}I-ChTX binding affinity.

Are the β subunit-dependent changes in toxin affinity as measured in the present study related to functional inhibition of maxi-K channels by ChTX? To answer this question, it would be necessary to determine the effect of ChTX on single channel recordings from either α or $\alpha+\beta$ channels under conditions in which the open probability of the channels is identical. If predictions are correct, only the toxin's mean blocked time would be affected in physiological ionic strength-recording solutions. Although these experiments have not been carried out systematically, it is possible to compare available data from the literature regarding the effects of ChTX on maxi-K channels from either bovine aortic smooth muscle (19) or rat skeletal muscle (20) incorporated into planar lipid bilayers. The experimental conditions from these two studies are very similar, and therefore the kinetic parameters of toxin binding to the channel can be compared. Whereas there are no significant differences in the on-rates of ChTX binding to either smooth or skeletal muscle maxi-K channels (5 vs. 4.5×10^6 $M^{-1}\cdot s^{-1}$, respectively), there is a large difference in the toxin dissociation rate: from 0.015 s^{-1} in smooth muscle to 0.067 s^{-1} in skeletal muscle. Thus, the toxin-bound state is *ca.* 4.5-fold longer lived in smooth muscle maxi-K channels. One possibility that would account for these results is that maxi-K channels from rat skeletal muscle do not have a β subunit, or that the putative β subunit is different from the one present in smooth muscle. Consistent with this idea is the finding that Northern blots give a very weak signal for β in skeletal muscle (21), and that it is not possible to observe the protein in Western blots of skeletal muscle employing antibodies raised against the smooth muscle β subunit (data not shown). The data presented in this study suggest a close proximity of some regions of the extracellular loop of β to the outer vestibule of the pore of α, which leads to very significant changes in ^{125}I-ChTX binding. Whether or not the extracellular loop of β plays additional roles in maxi-K channel function remains to be determined.

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