An Activator of Calcium-Dependent Potassium Channels Isolated from a Medicinal Herb

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Received April 7, 1993

ABSTRACT: Large-conductance calcium-dependent potassium (maxi-K) channels play an important role in regulating the tone of airway smooth muscle and the release of bronchoconstrictive substances from nerves in the lung. Crude extracts of *Desmodium adscendens*, a medicinal herb used in Ghana as a treatment for asthma, inhibit binding of monoiodotyrosine charybdotoxin (^{125}I-ChTX) to receptor sites in bovine tracheal smooth muscle membranes that have been shown to be associated with maxi-K channels. Using this assay, three active components have been purified and identified by NMR and MS. Comparison with authentic samples revealed the three active components as the known triterpenoid glycosides dehydrosoyasaponin I (DHS-I), soyasaponin I, and soyasaponin III. The most potent of these compounds, DHS-I, is a partial inhibitor of ^{125}I-ChTX binding (K_i = 120 nM, 62% maximum inhibition). Inhibition of ^{125}I-ChTX binding is primarily due to a decrease in the observed maximum number of binding sites, with a smaller decrease in affinity. DHS-I increases the rate of toxin dissociation from its receptor, suggesting that modulation of ChTX binding occurs through an allosteric mechanism. DHS-I reversibly increases the open probability of maxi-K channels from bovine tracheal smooth muscle incorporated into planar lipid bilayers when applied to the intracellular, but not the extracellular, side of the membrane at concentrations as low as 10 nM. In contrast, DHS-I had no effect on several other types of potassium channels or membrane transporters. This natural product is the first example of a high-affinity activator of calcium-dependent potassium channels and is the most potent known potassium channel opener.

Large-conductance, calcium-activated potassium (maxi-K)[1] channels catalyze the movement of potassium ions across cell membranes in response to increases in internal calcium concentration and membrane potential (Marty, 1981; Pallotta et al., 1981; Latorre et al., 1982). Recordings of maxi-K channels in patch clamp and lipid bilayer experiments have provided a wealth of information concerning the biophysical and pharmacological properties of these channels and suggest that there may be a family of related maxi-K channel proteins (Blatz & Magleby, 1987; Latorre et al., 1989; McManus, 1991; Toro & Stefani, 1991). In contrast, knowledge of the molecular components of these channels and of the role of these channels in cellular function is just emerging. A cDNA corresponding to the *Drosophila slowpoke* locus (Atkinson et al., 1991) was shown to encode calcium-activated potassium channels with properties related to mammalian maxi-K channels (Adelman et al., 1992). This important discovery may provide an entry into the molecular realm of mammalian maxi-K channels. Discovery of peptide toxins derived from scorpion venom that are potent blockers of maxi-K channels provides tools to investigate the physiological roles of these channels (Garcia et al., 1991). With this approach, recent experiments have shown that opening of calcium-dependent potassium channels by an indirect mechanism contributes to the relaxation of airway smooth muscle induced by β_2-agonists (Kume et al., 1989, 1992; Jones et al., 1990) and inhibits release of bronchoconstrictors, including tachykinins and acetylcholine, from neurons in the airway (Stretton et al., 1992; Miura et al., 1992). An activator of these channels is therefore expected to relax airway smooth muscle and to attenuate neurogenic inflammation in the lung resulting from local tachykinin release.

Desmodium adscendens (Sw.) DC. var. *adscendens* (Papillonaceae) is a medicinal herb used in Ghana as a treatment for asthma and other diseases associated with smooth muscle contraction (Ampofo, 1977). Extracts of the plant material can inhibit contractions of guinea pig ileum caused by electrical field stimulation (Addy, 1989) and contractions of sensitized guinea pig airway smooth muscle induced by antigen, arachidonic acid, or leukotriene D_4 (Addy & Burka, 1988, 1989). The plant contains several different components that contribute to this relaxation of smooth muscle, some of which have been shown to inhibit the NADPH-dependent monooxygenase pathway of arachidonic acid metabolism (Addy & Schwartzman, 1992).

In this paper we report the purification and identification of three components of *D. adscendens* that increase the open probability of maxi-K channels by a direct mechanism. These compounds were found because they inhibit the binding of monoiodotyrosine charybdotoxin (^{125}I-ChTX) to maxi-K

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[1] Abbreviations: ChTX, charybdotoxin; DHS-I, dehydrosoyasaponin I; EGTA, ethylene glycol bis(β-aminoethyl ether)-*N*,*N*,*N*′,*N*′-tetraacetic acid; Hepes, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; ^{125}I-ChTX, monoiodotyrosine ChTX; maxi-K channel, large-conductance, calcium-activated potassium channel; POPC, 1-palmitoyl-2-oleoylphosphatidylcholine; POPE, 1-palmitoyl-2-oleoylphosphatidylethanolamine.

0006-2960/93/0432-6128$04.00/0

channels in smooth muscle membranes. They are the first potent agonists of maxi-K channels and are the most potent potassium channel agonists known. The discovery of these compounds provides new probes to examine the mechanisms that control the gating of maxi-K channels. A preliminary report of these findings has appeared in abstract form (McManus et al., 1993).

MATERIALS AND METHODS

Materials. ^{125}I-ChTX (2200 Ci/mmol) was obtained from New England Nuclear, and unlabeled ChTX was bought from Peptides International. Glass fiber filters (GF/C) were purchased from Whatman. POPE and POPC were purchased from Avanti Lipids Inc., Birmingham, AL. Highly purified sarcolemmal membrane vesicles were prepared from bovine tracheal smooth muscle (Slaughter et al., 1987) and bovine aortic smooth muscle (Slaughter et al., 1989b) by methods previously described. The membrane fraction at the 8–30% interface of a sucrose density gradient was resuspended in 160 mM NaCl and 20 mM Tris-HCl, pH 7.4, rapidly frozen in liquid N_2, and stored at −70 °C. Binding activities were stable for at least 1 year. Authentic soyasaponin I and soyasapogenol B were prepared from soybeans as described previously (Kitagawa et al., 1976). Soyasapogenol E was prepared by oxidation of soyaspogenol B (Steffens et al., 1986).

Isolation and Structure Determination. Dried and ground leaves of *D. adscendens* (175 g) were extracted for 48 h with H_2O in a Soxhlet apparatus, and the aqueous extract was concentrated approximately 3-fold *in vacuo*. The concentrate was extracted three times with an equal volume of water-saturated nBuOH, and the nBuOH extracts were pooled and concentrated *in vacuo* to a brownish syrup (7.16 g). A portion of this syrup (5.6 g) was chromatographed on a 5 cm × 100 cm column of Sephadex LH-20 eluted with methanol at 12.5 mL/min (18-mL fractions). Fractions 21–30 (1.7 g) were pooled and contained compounds **1–3**. Column chromatography of a portion (1.19 g) of the above LH-20 fraction pool on Baker C_{18} silica gel (350 g) eluted at 15 mL/min (15-mL fractions) with 40% CH_3CN/60% H_2O yielded a mixture of **1–3** in fraction pool 30–50 (168 mg). Compounds **1–3** were separated by high-speed countercurrent, chromatography (Ito Multilayer Countercurrent Chromatograph, P.C., Inc., Potomac, MD) on a no. 14 coil (volume = 300 mL) using a solvent system consisting of $CHCl_3$/MeOH/H_2O, 7:13:8, lower phase mobile, 800 rpm forward rotation, and head-to-tail elution at 2.5 mL/min collecting 10-mL fractions. Fractions 40–50 contained pure component **2** (11.6 mg), fractions 23–28 (3.3 mg) contained pure component **3**, and fractions 20–21 contained impure **1**. Further purification of fraction pool 20–21 by HPLC on Whatman Partisil 5 ODS 3 with a mobile phase of 40% CH_3CN/H_2O at 3.0 mL/min yielded 3.9 mg of pure **1**.

NMR spectra were recorded on a Varian XL-300 spectrometer. Mass spectra were recorded on a Finnigan-MAT Model MAT212. The structures of **1–3** were established by comparison of the ^{1}H and ^{13}C NMR and mass spectra with authentic soyasaponin I and soyasapogenols B and E and with literature values for **1** (Kitagawa et al., 1982, 1988; Konoshima et al., 1991). The ^{1}H NMR spectrum of the permethyl derivative of **2**, prepared in DMSO using a solution of dimsyl carbanion, was identical with that reported for the permethyl derivative of soyasaponin I (Kitagawa et al., 1976).

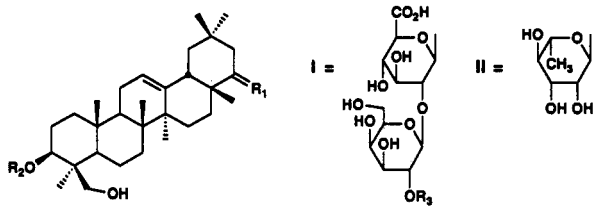
Binding Experiments. Binding of ^{125}I-ChTX to bovine tracheal and aortic smooth muscle sarcolemmal membranes was performed as previously described (Vazquez et al., 1989). Briefly, membranes were incubated with ^{125}I-ChTX, in the presence or absence of test compounds, in a medium consisting of 20 mM NaCl, 20 mM Tris-HCl, pH 7.4, and 0.1% bovine serum albumin. Reactions were carried out at room temperature until equilibrium was achieved. At the end of the incubation period, samples were diluted with 4 mL of ice-cold 100 mM NaCl and 20 mM Hepes/Tris, pH 7.4, filtered through GF/C filters that have been presoaked in 0.5% poly(ethylenimine), and washed twice with ice-cold medium. Nonspecific binding was assessed in the presence of 10 nM ChTX. Quadruplicate assays were routinely performed under each experimental condition, and the data were averaged. The standard error of the mean of these results was typically less than 3%. Data from saturation experiments were subjected to a Scatchard analysis, and linear regression was performed to yield the equilibrium dissociation constant (K_d) and maximum receptor concentration (B_{max}). Data from competition experiments were fit with an inhibition function of the form

$$^{125}\text{I-ChTX bound (\% of control)} = 100 - M/(1 + K_i/[L])$$

where M is the maximum observed inhibition of ^{125}I-ChTX binding, [L] is the concentration of test compound, and K_i is the concentration that causes half-maximal inhibition. The dissociation rate constant for ChTX (k_{-1}) was determined from the inverse of the time constant of an exponential function fit to a plot of ligand dissociation *versus* time. Membrane protein concentration was determined by the Bradford method (Bradford, 1976) using bovine serum albumin as a standard.

Electrophysiological Experiments. Planar lipid bilayers of ~250 pF were cast from a solution of POPE and POPC in a 7/3 molar ratio in decane at 50 μg/mL painted across a 250 μM hole in a partition separating two aqueous compartments. Currents flowing across the bilayer were measured with standard voltage clamp methods. Plasma membrane vesicles from bovine tracheal smooth muscle or bovine aortic smooth muscle were fused with the bilayer until a single maxi-K channel was observed. The orientation of maxi-K channels incorporated into the bilayer was determined from the voltage and calcium sensitivity of the channels. Currents were stored on an FM tape recorder (Racal 4DS, Racal Recorders, Vienna, VA) or digitally recorded on a video cassette tape with a VR-10 digital data recorder (Instrutech Corp., Elmont, NY). Measurements of channel open probability and of event durations were done as previously described (McManus & Magleby, 1988) after playing the data into a DEC 11/73 computer. For Figure 4, toxin-blocked events were defined as closed intervals longer than 500 ms, which was 10 times longer than the longest time constant in the distribution of control closed times. Toxin-unblocked times included all open and closed intervals separated by toxin-blocked events.

The solutions used in the experiments shown in Figures 3 and 4 contained 150 mM KCl, 10 mM Hepes, and 10 μM $CaCl_2$, pH 7.20. For Figure 3, the $CaCl_2$ concentration was raised to 20 μM in the internal solution, and for Figure 4, 1 mM EGTA and 30 μg/mL BSA were added to the external solution. Patch clamp recordings of currents flowing through maxi-K channels in membrane patches excised from cultured bovine aortic smooth muscle were made using conventional techniques (Hamill et al., 1981). Experiments were done at room temperature (22–24 °C).



Compound	R_1	R_2	R_3	K_i ChTX (μM)
Soyasaponin I	H, OH	I	II	6
Dehydrosoyasaponin I	O	I	II	0.1
Soyasaponin III	H, OH	I	H	1
Soyasapogenol B	H, OH	H	-	>100

FIGURE 1: Structures of triterpenoid glycosides isolated from *D. adscendens*. K_i(ChTX) gives the concentration of the listed compound that gives half-maximal inhibition of ^{125}I-ChTX binding (as in Figure 2) to calcium-dependent potassium channels in bovine aortic sarcolemmal membrane vesicles.

RESULTS

Isolation and Structure Determination. ^{125}I-ChTX binds to a single class of binding sites in both bovine aortic (Vazquez et al., 1989) and tracheal (Slaughter et al., 1989a) smooth muscle sarcolemma that is associated with maxi-K channels. Crude extracts of *D. adscendens* inhibited binding of ^{125}I-ChTX to plasma membrane vesicles from smooth muscle, suggesting that a component(s) of the extract interacted with maxi-K channels. The ^{125}I-ChTX binding assay and electrophysiological assays guided fractionation of the crude aqueous *D. adscendens* extract through nBuOH extraction, C_{18} reverse-phase column chromatography, and countercurrent chromatography, yielding three active components. These were identified by NMR, MS, and comparison with authentic samples as the known triterpenoid glycosides dehydrosoyasaponin I (**1**, DHS-I), soyasaponin I (**2**), and soyasaponin III (**3**) (Figure 1). These three soyasaponins have also been reported as constituents of *Desmodium styracifolium* (Kubo et al., 1989).

Binding Studies. When bovine tracheal sarcolemmal membranes were incubated with ^{125}I-ChTX in the presence of increasing concentrations of DHS-I, we observed a concentration-dependent inhibition of toxin binding that reached a maximum level of 62% (Figure 2A). This inhibition of toxin binding was well described by a single-site inhibition curve with a K_i value of 120 nm. Similar results were obtained in experiments using bovine aortic smooth muscle membranes (Figure 1). Saturation experiments done with increasing concentrations of ^{125}I-ChTX in the absence or presence of 1 μM DHS-I indicate that inhibition of toxin binding is primarily due to a decrease in the observed number of binding sites for ^{125}I-ChTX from 0.34 to 0.19 pmol/mg of protein and a smaller increase in the K_d for toxin binding from 0.13 to 0.15 nM (Figure 2B). DHS-I (5 μM) caused a 1.6-fold increase in the rate of ^{125}I-ChTX dissociation from its receptor (Figure 2C). These data suggest that DHS-I binds to maxi-K channels in tracheal smooth muscle and allosterically destabilizes ^{125}I-ChTX bound to the channel.

Electrophysiological Experiments. In Figure 3, DHS-I increased the open probability of a single maxi-K channel from bovine tracheal smooth muscle incorporated into a planar lipid bilayer. Channel open probability was not increased after addition of 500 nM DHS-I to the outside face of the channel. Subsequent addition of 100 nM to the inside caused an 80-fold increase in channel open probability from 0.0058 to 0.48. This increase in channel open probability was reversed after washout of DHS-I from the inside. In similar experiments, increases in the open probability of maxi-K channels were observed after exposure to DHS-I concentrations as low as 10 nM.

DHS-I also increased the open probability of maxi-K channels from bovine aortic smooth muscle examined in patch clamp experiments using excised inside-out membrane patches and after channel incorporation into lipid bilayers (data not shown). Similar agonist effects of DHS-I were seen when the internal calcium concentration was buffered in the micromolar range with 5 mM EGTA, arguing that channel activation was not due to calcium contamination.

The pattern of channel openings observed after exposure to DHS-I differed from that observed when channel open probability was increased by raising internal calcium or membrane potential. The triterpene glycoside induced periods of very high channel open probability lasting several seconds interspersed with periods of lower activity similar to control channel gating. In contrast, raising channel open probability by increasing calcium or membrane potential led to a more homogeneous pattern of channel gating. A possible explanation of this behavior is that the periods of high open

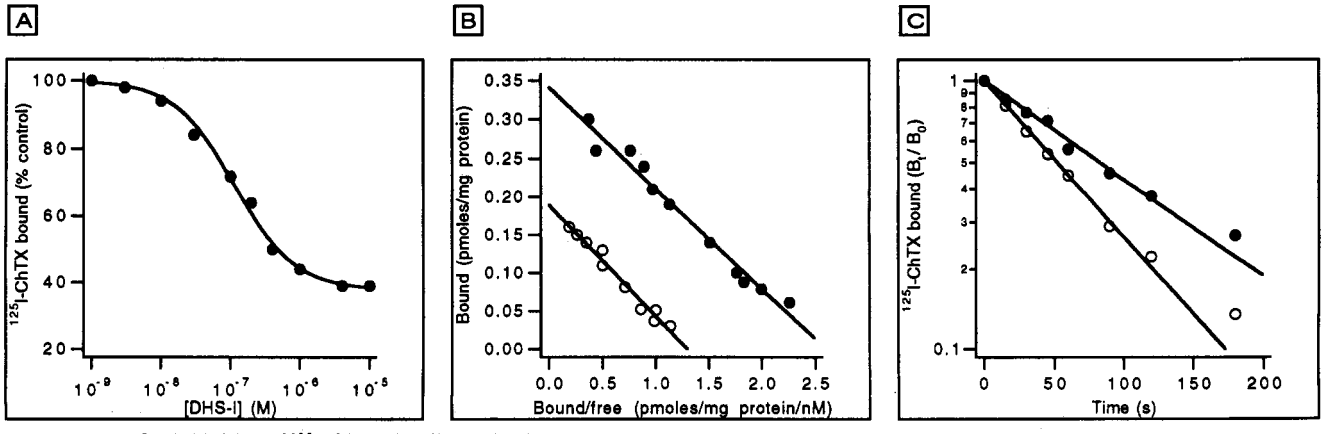


FIGURE 2: DHS-I inhibition of ^{125}I-ChTX binding to bovine tracheal smooth muscle membranes. (A) Specific binding observed after incubation with 27 pM ^{125}I-ChTX and varying concentrations of DHS-I is plotted relative to an untreated control. (B) Scatchard plot of effects of DHS-I on ^{125}I-ChTX binding. Membranes were incubated with increasing concentrations of ^{125}I-ChTX in the absence (●) and presence (○) of 1 μM DHS-I until equilibrium was achieved. (C) Effects of DHS-I on the rate of ^{125}I-ChTX dissociation. Membranes were incubated with 27 pM ^{125}I-ChTX until equilibrium was achieved. At zero time, 10 nM ChTX was added in the absence (●) or presence (○) of 5 μM DHS-I, and ^{125}I-ChTX remaining bound was determined as a function of time.

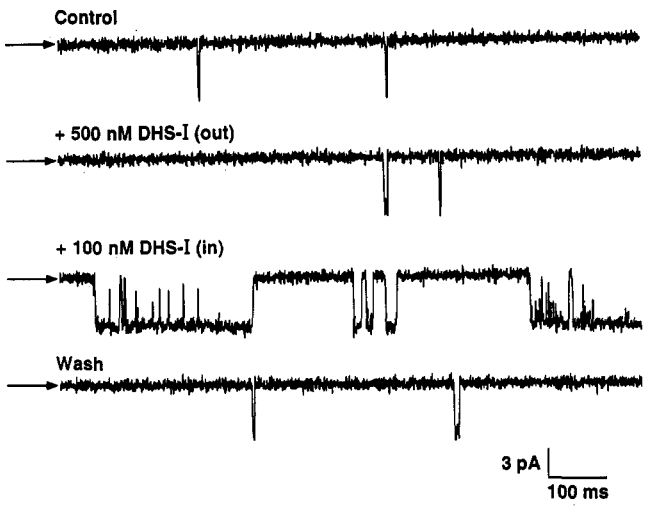


FIGURE 3: Effects of DHS-I on gating of the large-conductance calcium-dependent potassium channel. Currents are recorded from a single maxi-K channel from tracheal smooth muscle incorporated into a lipid bilayer and held at −20 mV. The closed channel current level is indicated by the arrows to the left.

probability observed in the presence of DHS-I represent intervals when the drug is bound to the channel.

High concentrations of DHS-I (1 μM) did not activate maxi-K channels in the virtual absence of internal calcium (1 mM EGTA, 0 added calcium; not shown), suggesting that DHS-I cannot substitute for calcium to activate the channel. Thus, DHS-I does not act independently as an agonist for maxi-K channels but rather increases channel opening in the presence of the natural ligand, calcium.

DHS-I was identified on the basis of its ability to inhibit ^{125}I-ChTX binding to a site on the external face of maxi-K channels. Evaluation of DHS-I in electrophysiological experiments revealed it to be a reversible activator of this channel only when applied to the internal side of the membrane. In order to investigate the interaction between DHS-I binding to a site on the internal face of the channel and ChTX binding to a site on the external face, we measured the effects of DHS-I on the kinetics of ChTX block of maxi-K channels from tracheal smooth muscle incorporated into lipid bilayers (Figure 4). After addition of 10 nM ChTX to the external solution, normal channel gating was interrupted by long silent periods lasting about 1 min that were not seen in the absence of toxin (Figure 4A). The long silent periods represent intervals when ChTX is bound to the channel. Subsequent addition of 300 nM DHS-I to the inside caused two effects: the open probability of the channel during the unblocked periods increased to nearly 1, and the durations of the toxin-blocked intervals decreased. ChTX blocks maxi-K channels by a simple bimolecular reaction (Anderson et al., 1988), allowing the rates of toxin dissociation and association to be calculated from the time constants of the distributions of toxin-blocked (Figure 4B) and -unblocked (Figure 4C) times, respectively. The rate of toxin association decreased slightly from 7.4×10^6 to 5.6×10^6 M^{-1} s^{-1}, while the rate of toxin dissociation increased by 2.3-fold from 0.017 to 0.039 s^{-1}. These data further support the idea that DHS-I binds to a site on maxi-K channels and destabilizes ^{125}I-ChTX bound to a different site near the external entrance to the pore.

Specificity of DHS-I. We have evaluated the effects of DHS-I on other ion channels and membrane transport systems. DHS-I (200–300 nM inside) had no effect on the open probability of ATP-sensitive potassium channels in membrane patches excised from mouse pancreatic β cells or small conductance, calcium-dependent potassium channels in patch-

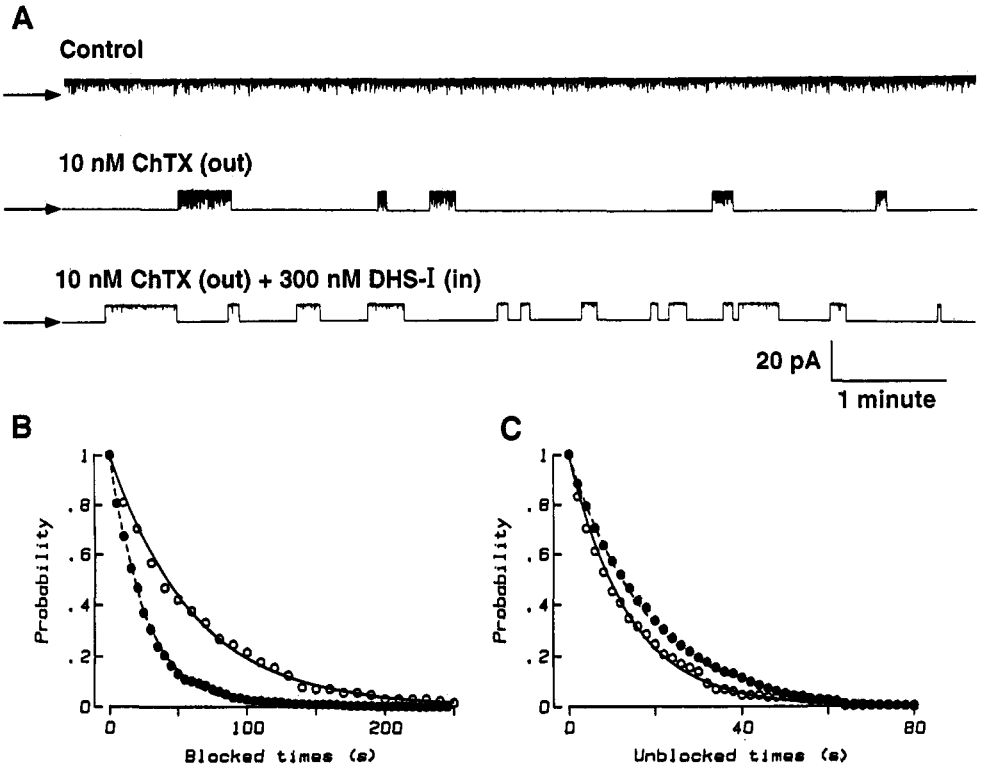


FIGURE 4: Influence of DHS-I on ChTX block of a single maxi-K channel in a bilayer. (A) shows a recording of a single maxi-K channel in control, after addition of 10 nM ChTX to the outside chamber, and after further addition of 300 nM DHS-I to the inside. The durations of toxin-blocked (B) and -unblocked (C) intervals are plotted as cumulative dwell-time distributions. The dwell times from control are plotted as (○), and the dwell times measured in the presence of 300 nM DHS-I are plotted as (●). The solid lines are single-exponential fits to the control data with time constants of 60.2 s for the blocked times and 13.5 s for the unblocked times. The dashed lines are single-exponential fits to the data collected in the presence of DHS-I with time constants of 25.8 s for the blocked times and 17.7 s for the unblocked times.

es excised from human T-lymphocytes. Voltage-dependent potassium currents in human thymocytes were also unaffected by similar external concentrations of DHS-I. Calcium fluxes through L-type calcium channels in GH_3 cells and sodium–calcium exchange in purified porcine cardiac sarcolemmal membranes were also unaffected by high concentrations (up to 10 μM) of DHS-I. ^{125}I-ChTX binding to voltage-dependent potassium channels in rat brain synaptic plasma membranes (Vazquez et al., 1990) and [^{3}H]ouabain binding to porcine cardiac sarcolemmal membranes were unaffected by high concentrations (10–100 μM) of DHS-I.

Structure–Activity Relationship. We examined the effects of structural variants of DHS-I on maxi-K channels from bovine aortic smooth muscle (Figure 1). Replacing the carbonyl group in ring E with an alcohol increased the K_i for inhibition of ^{125}I-ChTX binding by 60-fold and caused a similar loss of potency in activating maxi-K channels in excised membrane patches. When compounds with an alcohol in ring E were compared, the disaccharide was 6-fold more potent than the trisaccharide, while the aglycon form was more than 100-fold weaker than the disaccharide. This defined structure–activity relationship suggests that both the triterpene and sugar moieties are important determinants for the activity of these compounds.

DISCUSSION

The data presented in this paper demonstrate the purification and identification of three components from the African medicinal herb, *D. adscendens*, that increase the open probability of maxi-K channels by a direct mechanism. These triterpenoid glycosides are the first nanomolar activators of calcium-dependent potassium channels and are the most potent potassium channel agonists found to date.

These three compounds were identified because they inhibit ^{125}I-ChTX binding to maxi-K channels in smooth muscle membranes. The most potent of these compounds, DHS-I, was shown to bind to a site located on the internal face of the channel, causing an increase in channel open probability and also allosterically destabilizing ChTX bound to a different site near the external entrance to the pore. Evidence for an allosteric interaction between ChTX and DHS-I comes from binding experiments, where DHS-I was a partial inhibitor of ^{125}I-ChTX binding, was a noncompetitive inhibitor in saturation experiments, and increased the rate of ^{125}I-ChTX dissociation. Further evidence of a negative allosteric interaction was observed in electrophysiological recordings with maxi-K channels incorporated into lipid bilayers. DHS-I applied to the intracellular side caused a 2.3-fold increase in the rate of ChTX dissociation from a site on the external side of the channel. This negative allosteric interaction between ChTX and DHS-I differs from the positive allosteric interaction observed between the physiological activator of this channel which is calcium, and ChTX. In electrophysiological experiments using maxi-K channels from rat skeletal muscle incorporated into lipid bilayers, internal calcium causes 5–7-fold increase in the rate of association of ChTX with the channel and has little effect on toxin dissociation (Anderson et al., 1988). This suggests that the conformation of the channel after activation by DHS-I may differ from the active conformation that results from calcium binding.

The binding site(s) for the triterpene glycosides is (are) located on the intracellular side of the membrane, as are the binding sites for calcium. However, these channel activators cannot substitute for calcium in causing channel opening. Activation of the channel by DHS-I only occurred in the presence of calcium. This implies that the binding site(s) for DHS-I on the channel is (are) likely to differ from the binding sites for calcium. The binding site(s) on the channel for DHS-I may be as large as the entire DHS-I molecule as chemical modifications on either end of the structure cause large changes in activity.

Cromakalim and pinacidil are prototypes of two well-known structural classes of potassium channel agonists (Edwards & Weston, 1990; Longman & Hamilton, 1992). These compounds open ATP-sensitive potassium channels in ventricular myocytes (Sanguinetti et al., 1988; Arena & Kass, 1989; Escande et al., 1989) and smooth muscle (Standen et al., 1989; Clapp & Gurney, 1992; Noack et al., 1992; Silberberg & Van Breemen, 1992) that are blocked by glyburide, suggesting that these channels differ from maxi-K channels. In most tissues, 1–100 μM amounts of these compounds are required to cause channel opening, making these compounds much less potent as potassium channel agonists than is DHS-I. Cromakalim (1–10 μM) has been reported to cause a modest increase in the open probability of maxi-K channels in aortic smooth muscle (Gelband et al., 1989), although we have observed no effect of cromakalim (10–30 μM) on maxi-K channels from bovine aortic or tracheal smooth muscle in lipid bilayer or patch clamp experiments (unpublished observations). Nevertheless, any possible effects of chromakalim on maxi-K channels are unlikely to be related to the smooth muscle relaxation caused by cromakalim because these pharmacological effects are blocked by glyburide but not by ChTX (Edwards & Weston, 1990; Longman & Hamilton, 1992; Winquist et al., 1989; Jones et al., 1990). Therefore, DHS-I and the related triterpenoid glycosides identified in this study are the most potent direct activators of maxi-K channels described to date.

DHS-I is a component of a medicinal herb used therapeutically in the treatment of asthma and dysmenorrhea (Ampofo, 1977). Its ability to open calcium-dependent potassium channels could explain, at least in part, the antispasmogenic and spasmolytic effects observed with this plant extract *in vitro* and may contribute to the known *in vivo* therapeutic effects of the plant which relate to smooth muscles of the lung and uterus, tissues known to express maxi-K channels (McCann & Welsh, 1986; Perez et al., 1993). Opening of maxi-K channels would be expected to cause membrane hyperpolarization, suppression of electrical activity, and relaxation of smooth muscle. The intracellular site of action and the chemical properties of the triterpene glycosides suggest that the *in vivo* effects of DHS-I would have a slow onset and be weaker than our observed *in vitro* effects, which may correlate with the fact that the medicinal herb must be given chronically to elicit therapeutic effects in patients.

ACKNOWLEDGMENT

We thank Dr. A. Ford-Hutcheson for his suggestion to examine extracts of *D. adscendens* in the ^{125}I-ChTX binding assay, Dr. Y.-K. Lam for useful discussions, and Ms. D. Zink for mass spectra.

REFERENCES

Addy, M. E. (1989) *Int. J. Crude Drug Res. 27*, 81–91.
Addy, M. E., & Burka, J. F. (1988) *Can. J. Physiol. Pharmacol. 66*, 820–825.
Addy, M. E., & Burka, J. F. (1989) *Phytother. Res. 3*, 85–90.
Addy, M. E., & Schwartzman, M. L. (1992) *Phytother. Res. 6*, 245–250.
Adelman, J. P., Shen, K.-Z., Kavanaugh, M. P., Warren, R. A., Wu, Y.-N., Lagrutta, A., Bond, C. T., & North, R. A. (1992) *Neuron 9*, 209–216.

Ampofo, O. (1977) *World Health 26*, 28–33.
Anderson, C. S., MacKinnon, R., Smith, C., & Miller, C. (1988) *J. Gen. Physiol. 91*, 317–333.
Arena, J. P., & Kass, R. S. (1989) *Am. J. Physiol. 257*, H2092–H2096.
Atkinson, N. S., Robertson, G. A., & Ganetzky, B. (1991) *Science 253*, 551–553.
Blatz, A. L., & Magleby, K. L. (1987) *Trends Neurosci. 10*, 463–467.
Bradford, M. M. (1976) *Anal. Biochem. 72*, 248–254.
Clapp. L. H., & Gurney, A. M. (1992) *Am. J. Physiol. 262*, H916–H920.
Edwards, G., & Weston, A. H. (1990) *Pharmacol. Ther. 48*, 237–258.
Escande, D., Thuringer, D., Guern, S. L., Courteix, J., Laville, M., & Cavero, I. (1989) *Pfluegers Arch. 414*, 669–675.
Garcia, M. L., Galvez, A., Garcia-Calvo, M., King, V. F., Vazquez, J., & Kaczorowski, G. J. (1991) *J. Bioenerg. Biomembr. 23*, 615–646.
Gelband, C. H., Lodge, N. J., & Van Breeman, C. (1989) *Eur. J. Pharmacol. 167*, 201–210.
Hamill, O. P., Marty, A., Neher, E., Sakmann, B., & Sigworth, F. J. (1981) *Pfluegers Arch. 391*, 85–100.
Jones, T. R., Charette, L., Garcia, M. L., & Kaczorowski, G. J. (1990) *J. Pharmacol. Exp. Ther. 255*, 697–705.
Kitagawa, I., Yoshikawa, M., & Yoshioka, I. (1976) *Chem. Pharm. Bull. 24*, 121–129.
Kitagawa, I., Yoshikawa, M., Wang, H. K., Saito, M., Tosirisuk, V., Fujiwara, T., & Tomita, K. (1982) *Chem. Pharm. Bull. 30*, 2294–2297.
Kitagawa, I., Taniyama, T., Murakami, T., & Yoshikawa, M. (1988) *Yakugaku Zasshi 108*, 547.
Konoshima, T., Kozuka, M., Haruna, M., & Ito, K. (1991) *J. Nat. Prod. 54*, 830–836.
Kubo, T., Hamada, S., Nohara, T., Wang, Z., Hirayama, H., Ikegami, K., Yasukawa, K., & Takido, M. (1989) *Chem. Pharm. Bull. 37*, 2229–2231.
Kume, H., Tokuno, H., & Tomita, T. (1989) *Nature 341*, 152–154.
Kume, H., Graziano, M. P., & Kotlikoff, M. I. (1992) *Proc. Natl. Acad. Sci. U.S.A. 89*, 11051–11055.
Latorre, R., Vergara, C., & Hidalgo, C. (1982) *Proc. Natl. Acad. Sci. U.S.A. 79*, 805–809.
Latorre, R., Oberhauser, A., Labarca, P., & Alvarez, O. (1989) *Annu. Rev. Physiol. 51*, 385–399.
Longman, S. D., & Hamilton, T. C. (1992) *Med. Res. Rev. 12*, 73–148.
Marty, A. (1981) *Nature 291*, 497–500.
McCann, J. D., & Welsh, M. J. (1986) *J. Physiol. 372*, 113–127.
McManus, O. B. (1991) *J. Bioenerg. Biomembr. 23*, 537–560.
McManus, O. B., & Magleby, K. L. (1988) *J. Physiol. 402*, 79–120.
McManus, O. B., Giangiacomo, K. L., Harris, G. H., Addy, M. E., Reuben, J. P., Kaczorowski, G. J., & Garcia, M. L. (1993) *Biophys. J. 64*, 3a.
Miura, M., Belvesi, M. G., Stretton, C. D., Yaciub, M. H., & Barnes, P. J. (1992) *J. Physiol. 455*, 1–15.
Noack, T., Edwards, G., Deitmer, P., & Weston, A. H. (1992) *Br. J. Pharmacol. 107*, 945–955.
Pallotta, B. S., Magleby, K. L., & Barrett, J. N. (1981) *Nature 293*, 471–474.
Perez, G. J., Toro, L., Erulkar, S. D., & Stefani, E. (1993) *Am. J. Obstet. Gynecol. 168*, 652–660.
Sanguinetti, M. C., Scott, A. L., Zingaro, G. J., & Siegl, P. K. (1988) *Proc. Natl. Acad. Sci. U.S.A. 85*, 8360–8364.
Silberberg, S. D., & Van Breemen, C. (1992) *Pfluegers Arch. 420*, 118–120.
Slaughter, R. S., Welton, A. F., & Morgan, D. W. (1987) *Biochim. Biophys. Acta 904*, 92–104.
Slaughter, R. S., Kaczorowski, G. J., & Garcia, M. L. (1989a) *J. Cell. Biol. 107*, 143a.
Slaughter, R. S., Shevell, J. L., Felix, J. P., Garcia, M. L., & Kaczorowski, G. J. (1989b) *Biochemistry 28*, 3995–4002.
Standen, N. B., Quayle, J. M., Davies, N. W., Brayden, J. E., Huang, Y., & Nelson, M. T. (1989) *Science 245*, 177–180.
Steffens, J. C., Lynn, D. G., & Rjopel, J. L. (1986) *Phytochemistry 25*, 2291–2298.
Stretton, D., Motohiko, M., Belvesi, M. G., & Barnes, P. J. (1992) *Proc. Natl. Acad. Sci. U.S.A. 89*, 1325–1329.
Toro, L., & Stefani, E. (1991) *J. Bioenerg. Biomembr. 23*, 561–576.
Vazquez, J., Feigenbaum, P., Katz, G., King, V. F., Reuben, J. P., Roy-Contancin, L., Slaughter, R. S., Kaczorowski, G. J., & Garcia, M. L. (1989) *J. Biol. Chem. 264*, 20902–20909.
Vazquez, J., Feigenbaum, P., King, V. F., Kaczorowski, G. J., & Garcia, M. L. (1990) *J. Biol. Chem. 265*, 15564–15571.
Winquist, R. J., Heany, L. A., Wallace, A. A., Baskin, E. P., Stein, R. B., Garcia, M. L., & Kaczorowski, G. (1989) *J. Pharmacol. Exp. Ther. 248*, 149–156.