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The relative potencies of dendrotoxins as blockers of the cloned voltage-gated K^+ channel, mKv1.1 (MK-1), when stably expressed in Chinese hamster ovary cells

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1 The mKv1.1 voltage-gated K^+ channel has been expressed stably in Chinese hamster ovary cells and whole-cell currents recorded by the patch-clamp method.

2 A range of structurally related peptide toxins (dendrotoxins) from the venom of green mamba (*Dendroaspis angusticeps*) and black mamba (*Dendroaspis polylepis polylepis*) snakes were tested for mKv1.1 channel blocking activity. Their potencies were compared based on EC_{50}s derived from their respective concentration-inhibition relationships.

3 The rank order of potency, thus determined was: Toxin K > γ-dendrotoxin(γ-Dtx) > δ-Dtx > Toxin I = α-Dtx > β-Dtx.

4 Block was independent of voltage and no effects of the toxins on the kinetics of activation were observed. These results are consistent with a mechanism involving the block of closed channels.

5 A wide range of activity was observed even between toxins with an extremely high degree of sequence homology. Toxin K, in particular was an exquisitely potent blocker of the mKv1.1 channel, having an EC_{50} of 30 pM compared with 1.8 nM for δ-Dtx in spite of 95% sequence identity.

Keywords: Dendrotoxins; voltage-gated K^+ channel; clone; Chinese hamster ovary cell

Introduction

The dendrotoxins constitute a family of structurally homologous peptides (57–60 amino acids) found in the venom of the green and black mamba snakes. They have been useful tools with which to characterize voltage-gated K^+ channels in mammalian nerve cells. For example, α-dendrotoxin (α-Dtx) from *Dendroaspis angusticeps* (green mamba) and Toxin I (sometimes known as Dtx-I) from *Dendroaspis polylepis polylepis* (black mamba), have both been shown to block voltage-activated K^+ currents in neurones, enhance synaptic transmission and bind avidly to specific receptors in brain (see Dolly *et al*., 1992; Harvey, 1993). The high affinity binding of dendrotoxins to their receptor has been exploited in the construction of affinity columns used to isolate the receptor protein. This protein fraction consists of at least three structurally and pharmacologically related voltage-gated K^+ channels, Kv1.1, Kv1.2 and Kv1.6 (after Chandy & Gutman, 1993); the predominant species being Kv1.2 (Scott *et al*., 1994). Since the isolation of α-Dtx and Toxin I, a number of homologous peptides have been identified and isolated (Benishin *et al*., 1988) although only a limited number of electrophysiological studies had been carried out with these members of the dendrotoxin family at the start of the present study (Kavanaugh *et al*., 1990; Southan & Owen, 1992; Hall *et al*., 1994; see also Strong, 1990 and Harvey, 1993). Dendrotoxins potentiate synaptic transmission in the hippocampus and can induce a long-lasting potentiation (LTP_K) with varying efficacy (Southan & Owen, 1992). In rat sensory neurones, a number of the dendrotoxins block voltage-gated K^+ channels and in the case of δ-Dtx at least, selective block of one component of the native K^+ current was apparent (Hall *et al*., 1994). Although previous studies have examined the relative potency of various dendrotoxin homologues as blockers of the rat homologue of the Kv1.1 channel, rKv1.1 (also known as RBK1 or RCK1) (Kavanaugh *et al*., 1990), no systematic evaluation of dendrotoxin homologues has been carried-out in the case of the mouse homologue, mKv1.1 (MK1). In the present study, we have compared the activities of a range of dendrotoxin homologues on mKv1.1 which was stably expressed in the mammalian Chinese hamster ovary cell-line (CHO-K1) whereas previous studies (above) have utilized the frog oocyte expression system. A preliminary account of this study has been published previously (Owen *et al*., 1992).

Methods

All experiments were carried out with a CHO-K1 cell-line stably transfected with the mKv1.1 channel in the pZEM-223 vector (Bosma *et al*., 1993). Cells were sub-cultivated according to standard methods and as previously described (Robertson & Owen, 1993). Electrophysiological recordings were made within 1–3 days of plating onto standard 35 mm tissue culture dishes.

Cells were voltage-clamped by use of the whole-cell patch-clamp method with an EPC-9 patch-clamp amplifier (HEKA elektronik) controlled by either an Atari MegaST4 or Macintosh Quadra 800 computer. Cells were routinely held at a potential within the range −60 to −90 mV and mKv1.1 current was evoked with voltage-steps to potentials between +30 and +60 mV. For analysis purposes, a P/4 routine was employed to remove leak currents digitally. Data were analysed with the programmes Review (Instrutech Corp.), PulseFit (HEKA elektronik) and Igor (Wavemetrics Inc.).

Toxins were made up as stock solutions in de-ionized water and stored frozen until diluted for application via a microperfusion system to individual cells. With this system, cells were continuously perfused with a bathing solution consisting of (in mM): NaCl 120, KCl 2.5, $CaCl_2$ 2, $MgCl_2$ 2, glucose 10 and HEPES (N-[2-hydroxyethyl]piperazine-N′[2-ethanesulphonic acid]) 5; pH 7.4, adjusted to 320 mOsm with sucrose, or bathing solution in which Dtx homologues had been diluted. Patch pipettes were filled with a solution consisting of (in mM):

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Kgluconate 140, KCl 5, $MgCl_2$ 2, EGTA (ethylene glycol-bis(β-aminoethyl ether)N,N,N′,N′-tetraacetic acid) 1.1, HEPES 5, magnesium ATP (adenosine 5′-triphosphate) 2, sodium GTP (guanosine 5′-triphosphate) 200 μM; pH 7.2, adjusted to 310mOsm with sucrose. The resistance of recording pipettes was in the range of 4–7 MΩ when filled with the above solution.

α-Dtx, β-Dtx, γ-Dtx and δ-Dtx were obtained from Alomone Labs and in addition were isolated from crude venom of *Dendroaspis angusticeps* according to published methods (Benishin *et al*., 1988; Dolly, 1992). The β-Dtx fraction was found to consist of a mixture of β_1- and β_2-Dtx by capillary electophoresis and subsequently it was the β_2- fraction that was isolated and examined in electrophysiological experiments. Toxin I and Toxin K were purified from the venom of *Dendroaspis polylepis polylepis* by ultra-filtration and cation exchange chromatography, followed by reverse-phase high pressure liquid chromatography (h.p.l.c.). The purity of the toxins was found to be greater than 99.5% by capillary electrophoresis and molecular weights were confirmed by electrospray mass spectrometry as matching published data (e.g. Dufton, 1985). No systematic differences in results were obtained with toxins obtained from Alomone or in-house toxins.

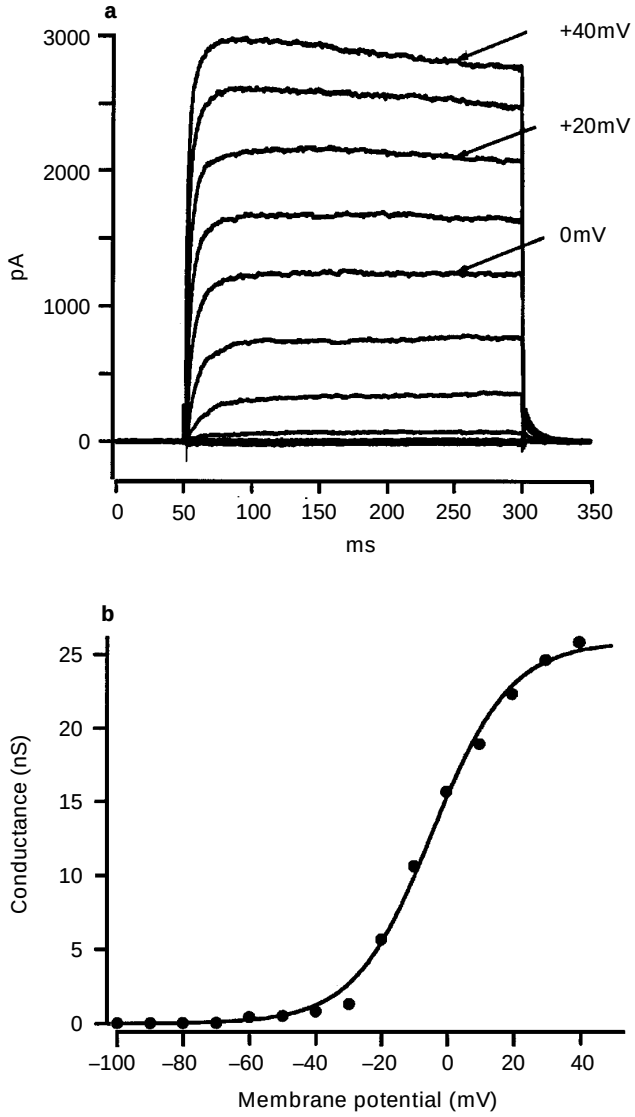

Figure 1 Outward currents expressed in Chinese hamster ovary cells stably transfected with mKv1.1 cDNA. (a) Currents activated by a series of voltage steps (250 ms) from a holding potential of −60 mV to membrane potentials ranging from −100 mV to +40 mV. (b) Conductance was calculated from the peak outward current, assuming a reversal potential of −80 mV, and plotted against membrane potential. The line represents a non-linear least-squares fit of the Boltzmann equation, $G = G_{max}/1 + \exp[(V_h - V)/k]$, to the data. Half-maximal activation voltage (V_h) = −5 mV, slope factor (k) = 11 mV.

Results

Block of mKv1.1 by dendrotoxins

As previously shown (Robertson & Owen, 1993), following a depolarizing voltage step to +60 mV, mKv1.1 currents developed relatively slowly with a rise-time (10–90%) of about 8 ms at +40 mV and decayed only slightly over the course of a 500 ms step (Figure 1a). The steady-state activation curve illustrated in Figure 1a was well fitted by a single Boltzmann function (half-maximal activation voltage −5 mV; slope factor about 11 mV). These values are similar to those obtained previously (Robertson & Owen, 1993).

α-Dtx blocked over half (55.7±5.4%), n=6) of the mKv1.1 current at a concentration of 10 nM (Figure 2a). The block was dose-related (5 nM blocked 30.3±7.6%, n=3) and reversible on washout of the toxin (not shown). Steady-state inhibition was reached within 2 min and recovery was complete after 3 min washing with control bathing solution. The other *Dendroaspis angusticeps* toxins (β-, γ- and δ-Dtx) also inhibited mKv1.1 currents and with varying potencies (Figure 2b–d). The *Dendroaspis polylepis polylepis* homologues, Toxin I and Toxin K, were also active (Figure 3). In particular, Toxin K was remarkably potent, inhibiting 58% (±3.6%, n=4) of the current at a concentration of 100 pM (Figure 3b).

Block by dendrotoxins is not voltage-sensitive

In order to evaluate whether or not the block by dendrotoxins varied with membrane potential, the amount of suppression of the peak current by toxin was measured at various potentials between −100 mV and +60 mV. No obvious voltage-sensitivity was apparent with any of the toxins apart from a slightly reduced block by a high concentration of Toxin I (100 nM) at positive potentials; eg. 83.8±0.6% at +60 mV (n=3) and 94.7±2.3% at 0 mV (n=3). This could reflect a contribution

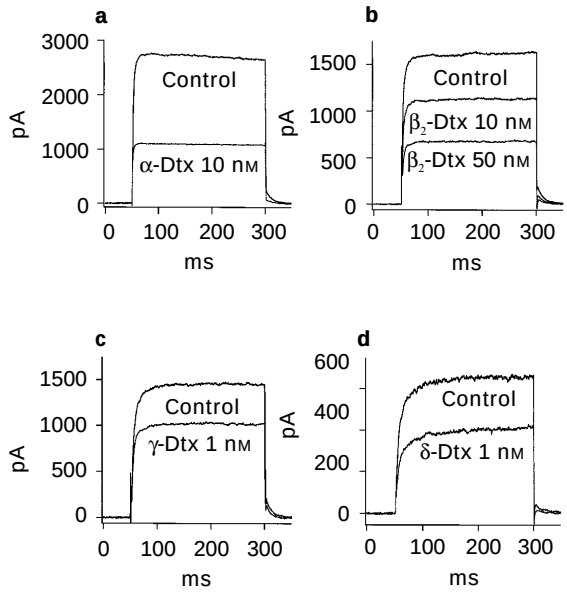

Figure 2 Inhibition of mKv1.1 currents by dendrotoxins from the green mamba. mKv1.1 currents were activated from a holding potential of −60 mV by depolarizing pulses (250 ms duration) to +60 mV. The currents were blocked by external application of all four *Dendroaspis angusticeps* dendrotoxin homologues: (a) 10 nM α-Dtx; (b) 10 nM and 50 nM β_2-Dtx; (c) 1 nM γ-Dtx; (d) 1 nM δ-Dtx.

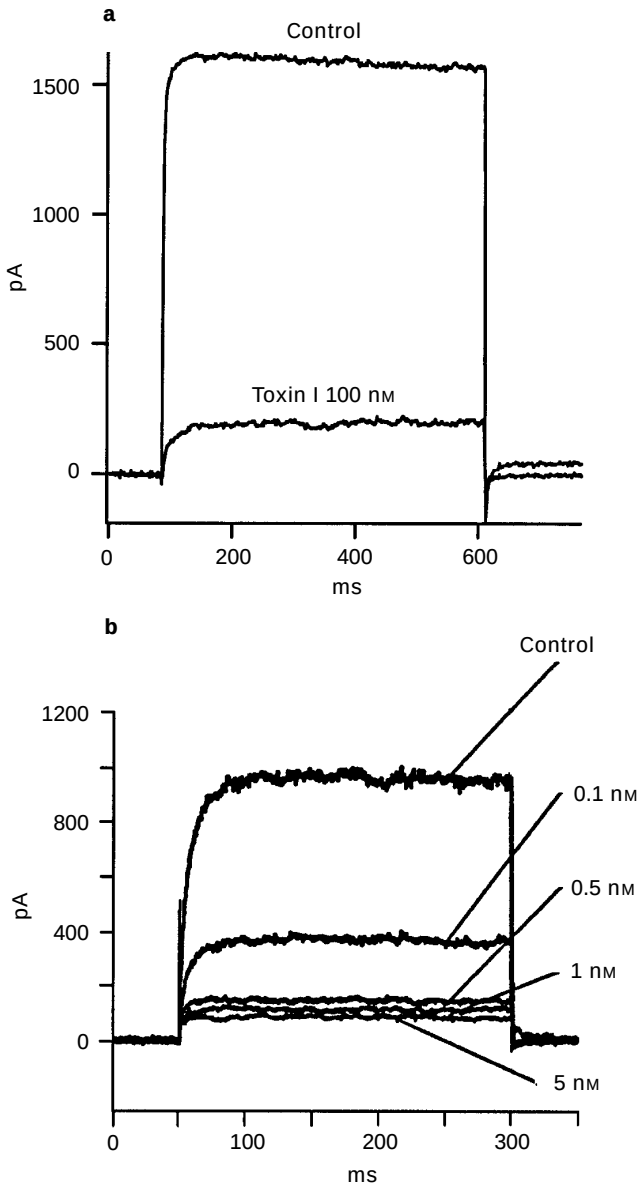

Figure 3 Effects of black mamba toxins, Toxin I and Toxin K. (a) The effect of 100 nM Toxin I on mKv1.1 current activated by a voltage-step (500 ms duration) to +60 mV from a holding potential of −100 mV. About 86% of the current was blocked in this cell (mean from 4 cells was 90.6±1.8%). (b) Block of mKv1.1 by Toxin K. A similar degree of block (85%) to that observed with 100 nM Toxin I (above) was obtained with only 0.5 nM Toxin K and over 50% of the mKv1.1 current was blocked by only 100 pM. Currents illustrated in this panel were activated with a voltage-step (250 ms duration) to +60 mV from a holding potential of −60 mV.

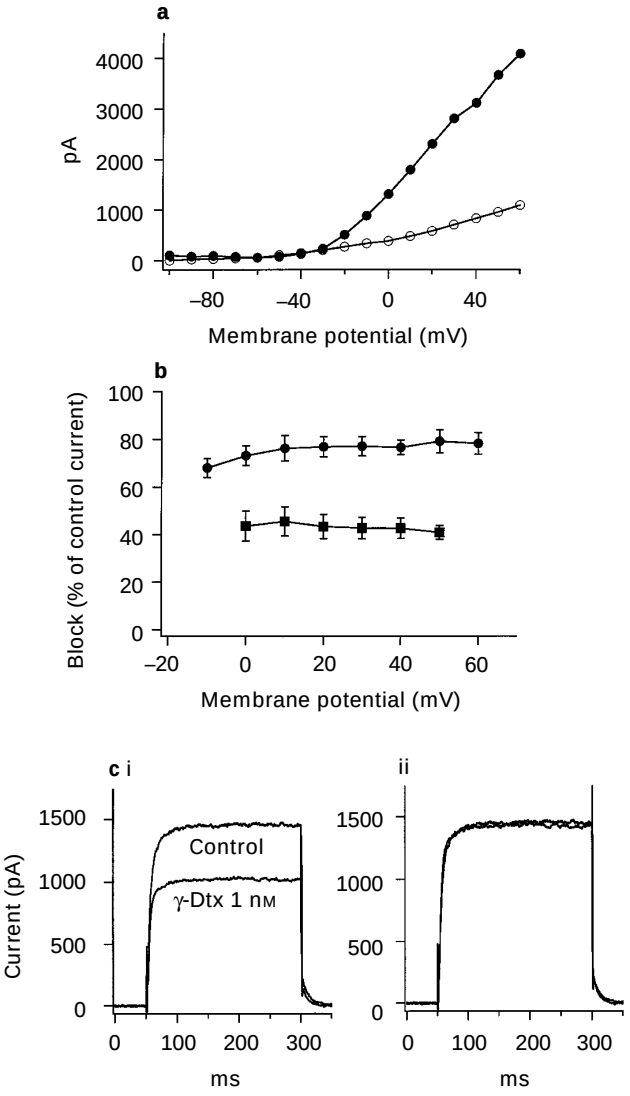

Figure 4 Effects of dendrotoxins at varying membrane potentials. (a) Current-voltage relationship in the presence (○) and absence (●) of Toxin K (1 nM). (b) Lack of voltage-dependence of block of mKv1.1 by 10 nM α-Dtx (■) and 1 nM Toxin K (●). Note: in (a) and (b) the total time in contact with toxin was less than 5 min. (c) No systematic alterations in activation kinetics were seen in the presence of any of the toxins. An example is shown for γ-Dtx where the current recorded in the presence of the toxin (i) has been scaled-up (ii) to exactly match the control current (reproduced from i).

to the whole cell current by a Dtx-resistant 'background' current, although in wild-type CHO-K1 cells and cells that had been transfected with vector alone, such currents were negligible (not shown). α-Dtx (10 nM) and Toxin K (1 nM) in particular were studied over a range of potentials in a number of cells and in these cases no voltage sensitivity was apparent between −10 mV and +60 mV and 0 mV and +50 mV for Toxin K and α-Dtx, respectively (Figure 4b).

No consistent changes were seen, either in the kinetics of activation or inactivation of mKv1.1 currents in the presence of any of the dendrotoxins. By way of illustraiton, Figure 4c shows an example of a current blocked by γ-Dtx. The current spared by γ-Dtx has been scaled and aligned with the control current for comparison. The two traces are identical indicating that the toxin has not altered the rate of activation.

Recovery from block

With the exception of Toxin K and δ-Dtx, dendrotoxins could be readily washed out (within about 3 min). In contrast, recovery from the block induced by Toxin K (Figure 5) or δ-Dtx (not shown) was extremely slow; the time constant of recovery was about 25 min for Toxin K and 18 min for δ-Dtx.

Dose-response relationships

All the toxin homologues blocked current in a concentration-dependent fashion (Figure 6). Dose-response relationships, obtained by pooling data from a number of cells were fitted with a single logistic function in each case and EC_{50} values were estimated (Table 1 and Figure 6). Interestingly, the extrapolated maximal degree of inhibition by β-Dtx and γ-Dtx was only 55–60% in contrast to the other homologues for which the fitted curves predicted a block close to 100% (Figure 6). Toxin K was remarkably potent, having an EC_{50} of only 30 pM (Figure 6).

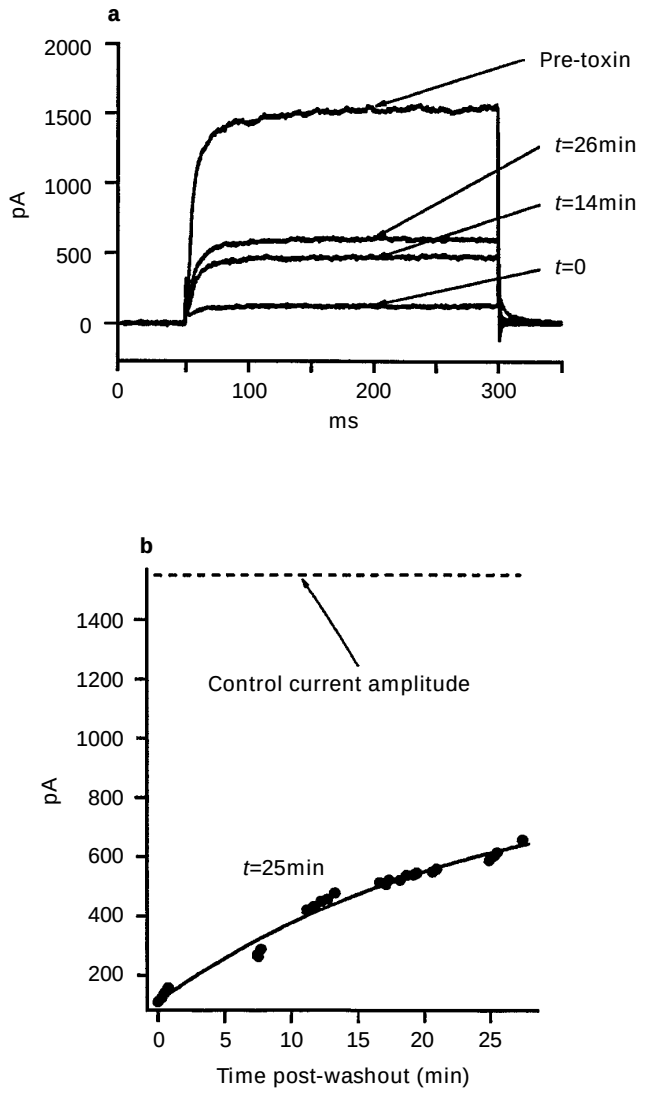

Figure 5 Recovery from block by Toxin K. The mKv1.1 current was blocked with 5 nM Toxin K ($t=0$) and the toxin was then washed-out over a period of 30 min. Following the start of the wash, the amplitude of the current was tested periodically with a 250 ms voltage pulse to +60 mV from the holding potential of −60 mV. (a) Example of raw traces at the times indicated and (b) the current amplitudes plotted *vs* time. (b) The amplitude of the pre-toxin current is indicated by the horizontal dashed line.

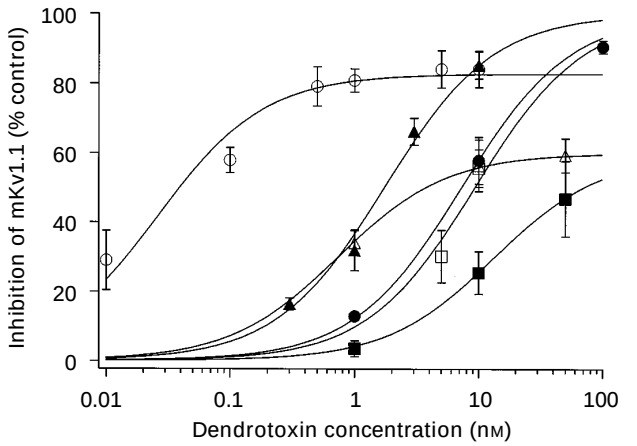

Figure 6 Relative potencies of dendrotoxins as Kv1.1 blockers. Collated data for all the dendrotoxin homologues: (□) α-Dtx; (■) β_2-Dtx; (△) γ-Dtx; (▲) δ-Dtx; (●) Toxin I; (○) Toxin K. Block of the mKv1.1 current is expressed as a percentage of the control current amplitude measured between +40 mV and +60 mV. Logistic curves of the form: $y=y_{max}/(1+(EC_{50}/[toxin])^{n_H})$, were fitted to the data using IGOR (Wavemetrics Inc) and EC_{50} values (±s.e.mean) derived thereby: Toxin K, 30±7 pM; Toxin I, 7.4±0.8 nM; α-Dtx, 9.4±1.7 nM; β_2-Dtx, 13.5±0.7 nM; γ-Dtx, 0.7±0.03 nM; δ-Dtx, 1.8±0.2 nM. Each point on the graph represents the mean of 3–6 cells and the vertical lines represent the s.e.mean. Note that the extrapolated curves predict that at high concentrations α-Dtx, δ-Dtx and Toxin I would block about 100% of the mKv1.1 current while Toxin K appear to plateau at about 80%. The concentration-inhibition curves for β-2-Dtx and γ-Dtx extrapolate to only about 60% of the control current.

Table 1 Summary of the EC_{50} values for inhibition of mKv1.1 currents by dendrotoxins

Toxin	*EC_{50} (nM)*	*s.e.mean*
Toxin K	0.03	0.007
γ-Dtx	0.7	0.03
δ-Dtx	1.8	0.2
Toxin I	7.4	0.8
α-Dtx	9.4	1.7
β_2-Dtx	13.5	0.7

Dtx - dendrotoxin.

Discussion

The effects of dendrotoxins on rKv1.1 channels expressed in *Xenopus* oocytes have been described previously (Kavanaugh *et al*., 1990; Robertson *et al*., 1996). However, the present study is the first that describes a systematic comparison of the effects of members of the dendrotoxin family on voltage-gated K^+ channels (mKv1.1) expressed in a mammalian cell system. The results are of particular interest in two ways: (1) Toxin K is one of the most potent blockers of a voltage-gated K^+ channel discovered to date. (2) There is a large difference between the inhibitory potency of Toxin K on two virtually identical channels, namely rKv1.1 channels (expressed in oocytes) and the mKv1.1 channel described here which is expressed in CHO-K1 cells. Kavanaugh *et al*. (1990) and Robertson *et al*. (1996) found the IC_{50} for Toxin K block of rKv1.1 to be in the nM range, compared with that found for the mouse homologue in the present study which was in the pM range. This is surprising as there is >98% overall homology in the amino acid sequences between the two channels, and identity in the putative pore region (Tempel *et al*., 1988; Christie *et al*., 1989). Three amino acids flanking the pore region in the rKv1.1 channel have been identified as being critical for Dtx activity, these being alanine[352], glutamate[353] and tyrosine[379] (Hurst *et al*., 1991; Tytgat *et al*., 1995). All three residues are conserved across the mouse rat and human species implying either that other residues contribute to the Dtx binding site or that its affinity can be altered by other factors. These might include: differences in the precise geometry of the assembled tetramer or in specific components of the binding site resulting from glycosylation or phosphorylation (for example). Differences in the expression systems might result in variation in post-translational modifications and indeed the degree of phosphorylation may differ between oocyte and mammalian cell-line. While such factors may indeed come into play, the fact that the potency of Toxin K is similar with respect to mouse (30 pM) and human (47 pM) Kv1.1 channels (Robertson *et al*., 1995), in spite of the difference in expression systems, suggests that this is not the only explanation for the difference seen between mouse and rat channels. On the other hand, whereas in the present study, the rank order of potency for mKv1.1 was: Toxin K > γ-Dtx > δ-Dtx > Toxin I = α-Dtx > β-Dtx (see Table 1), in the case of hKv1.1 the order is

different, i.e. δ-Dtx > Toxin K > Toxin I > α-Dtx (Robertson *et al*., 1995). Clearly hKv1.1 does exhibit a different pharmacological profile to mKv1.1, at least in these particular expression systems. The amino acid sequence in the pore and flanking regions is identical for all three Kv1.1 homologues and indeed the handful of differences that do exist (12 out of 495 when rKv1.1 and hKv1.1 are compared) are not in the proximity of the pore. The homology between rat and mouse Kv1.1 channels is extraordinary (>99%) and the only differences between them, that are also found in the human sequence, are two alanine(Ala)/threonine(Thr) switches: Ala480Thr and Thr481Ala. Although this region is close to the C-terminus of the channel and intuitively seems unlikely to participate in Dtx binding, perhaps it can influence the fine structure of the assembled tetramer with respect to the dendrotoxin binding site. Indeed, although a so-called 'assembly domain' has been identified in the N-terminus of Kv channels (Li *et al*., 1992; Shen *et al*., 1993; Yu *et al*., 1996) a number of residues in the C-terminus have also been implicated in the assembly of tetrameric mKv1.1 channels in particular (Hopkins *et al*., 1994). Even subtle changes in the overall geometry of the assembled tetramer could be important in Dtx binding because high-affinity binding requires all four subunits to be Dtx-'receptive' (Tytgat *et al*., 1995).

No evidence of time-dependent block by the dendrotoxins was seen and, with the possible exception of Toxin I at high concentrations, no voltage-dependence was apparent either. The actions of the dendrotoxins are thus consistent with a mechanism involving block of closed-channels and are presumed to bind at a site that does not sense the transmembrane voltage.

Interestingly, two of the toxins (β_2-Dtx and γ-Dtx) appear unable to block all of the mKv1.1 current. This may reflect a high off-rate resulting in rapid binding and unbinding of the toxin. However, this would require single channel analysis to confirm or otherwise. Also, while α-Dtx, β_2-Dtx, γ-Dtx and Toxin I all wash-out quite quickly, δ-Dtx and Toxin K, appear to dissociate extremely slowly from the channel. This could explain the extremely high potency of Toxin K, although δ-Dtx (also very slow to wash-out) is not a strong blocker by comparison with Toxin K and the other homologues.

The striking difference in potency of Toxin K in comparison to the other dendrotoxins may be associated with specific amino-acid residues in the toxin N-terminus since the C-terminus is rather well conserved throughout the dendrotoxins and related peptides such as bovine pancreatic trypsin inhibitor (BPTI) (e.g. see Berndt *et al*., 1993) which does not block K^+ channels. Near the N-terminus of the toxins, on the other hand, there are some obvious differences, between toxins, in the pattern of distribution of positively charged lysine (Lys) and arginine (Arg) residues. For example, δ-Dtx has three consecutive lysine residues, Lys^{15-17} and Toxin K also has a triplet of basic residues, Lys^{15}-Arg^{16}-Lys^{17}. In contrast, α-Dtx and Toxin I each have a single Lys^{19} in the analogous stretch of amino acids. Only two of a triplet of lysines in α-Dtx and Toxin I (Lys^{28-30}), which lie on a β-turn connecting two β-sheets in the tertiary structure (Skarzynski, 1992), are represented in δ-Dtx and Toxin K (Lys^{26} and Lys^{28}). The significance of this latter region of the toxins has been called into question by Danse *et al*. (1994) who showed that a recombinant α-Dtx lacking the Lys^{28-30} triplet could still bind to rat brain receptors and possessed biological activity in the chick biventer preparation. However, other regions are worthy of further consideration. Parallels are apparent between α-Dtx and Toxin I, on the one hand, and δ-Dtx and Toxin K, on the other. For example, whereas α-Dtx has three positively charged residues (Arg^3, Arg^4 and Lys^5) at the N-terminus and Toxin I has two (Arg^4 and Arg^5), δ-Dtx and Toxin K have only one positively charged residue (Lys^5). Similarly, both δ-Dtx and Toxin K have an Arg in position 10, whereas α-Dtx and Toxin I have an asparagine (Asn) in this position (although see Danse *et al*., 1994). The different potencies of Toxin K and δ-Dtx are all the more striking when the sequences are compared. These peptides are almost identical and, apart from one or two conservative substitutions, the only noteworthy difference is a switch at position 11 from an isoleucine in Toxin K to a tyrosin in δ-Dtx.

It is tempting to speculate that those features of the structure of Toxin K which give rise to its extraordinary inhibitory potency on the mKv1.1 channel, may also contribute to its high degree of selectivity for the rKv1.1 channel in comparison to the related rKv1.2 and rKv1.6 channels which was recently obtained (Robertson *et al*., 1996). Thus it may be possible to glean from these naturally occurring ion channel modulators, structural moieties which may be incorporated in the design of novel and specific synthetic channel modulators, although this information would be more forthcoming with more systematic studies of toxin and ion channel homologues in a single expression system.

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References

BENISHIN, C.G., SORENSEN, R.G., BROWN, W.E, KRUEGER, B.K. & BLAUSTEIN, M.P. (1988). Four polypeptide components of green mamba venom selectively block certain potassium channels in rat brain synaptosomes. *Mol. Pharmacol*., **34,** 152–159.

BERNDT, K., GUENTERT, P. & WUETHRICH, K. (1993). Nuclear magnetic resonance solution structure of dendrotoxin K from the venom of *Dendroaspis polylepis polylepis*. *J. Mol. Biol*., **234,** 735–750.

BOSMA, M.M., ALLEN, M.L., MARTIN, T.M. & TEMPEL, B.L. (1993). PKA-dependent regulation of mKv1.1, a mouse *Shaker*-like potassium channel gene, when stably expressed in CHO cells. *J. Neurosci*., **13,** 5242–5250.

CHANDY, K.G. & GUTMAN, G.A. (1993). Nomenclature for mammalian potassium channel genes. *Trends Pharmacol Sci*., **14,** 434.

CHRISTIE, M.J., ADELMAN, J.P., DOUGLASS, J. & NORTH, R.A. (1989). Expression of a cloned rat brain potassium channel in *Xenopus* oocytes. *Science*, **244,** 221–224.

DANSE, J.M., ROWAN, E.G., GASPARINI, S., DUCANCEL, F., VATANPOUR, H., YOUNG, L.C., POORHEIDARI, G., LAJEUNESSE, E., DREVET, P., MÉNEZ, R., PINKASFIELD, S., BOULAIN, J.-C., HARVEY, A.L. & MÉNEZ, A. (1994). On a site by which α-dendrotoxin binds to voltage-dependent potassium channels: site-directed mutagenesis reveals that the lysine triplet 28–30 is not essential for binding. *FEBS Lett*., **356,** 153–158.

DOLLY, J.O. (1992). Peptide toxins that alter neurotransmitter release. In *Handbook of Experimental Pharmacology: Selective Neurotoxicity*, Vol. **102.** ed Herken, H. & Hucho, F. pp. 681–717. Berlin: Springer-Verlag.

DOLLY, J.O., PARCEJ, D.N., SCOTT, V.E., MUNIZ, Z.M., SEWING, S. & PONGS, O. (1992). Molecular architecture of K^+ channels. *Neurochem. Int*., Proc. ESN meeting (Dublin, August, 1992).

DUFTON, M.J. (1985). Proteinase inhibitors and dendrotoxins. *Eur. J. Biochem*., **153,** 647–654.

HALL, A., STOW, J., DOLLY, J.O. & OWEN, D.G. (1994). Blockade by dendrotoxin homologues of voltage-dependent K^+ currents in cultured sensory neurones from neonatal rats. *Br. J. Pharmacol.*, **113,** 959–967.

HARVEY, A.L. (1993) Neuropharmacology of potassium ion channels. *Med. Res. Rev.*, **13,** 81–104.

HOPKINS, W.F., DEMAS, V. & TEMPEL, B.L. (1994). Both N- and C-terminal regions contribute to the assembly and functional expression of homo- and heteromultimeric voltage-gated K^+ channels. *J. Neurosci.*, **14,** 1385–1393.

HURST, R.S., BUSCH, A.E., KAVANAUGH, M.P., OSBOURNE, P.B., NORTH, R.A. & ADELMAN, J.P. (1991). Identification of amino acid residues involved in dendrotoxin block of rat voltage-dependent potassium channels. *Mol. Pharmacol.*, **40,** 572–576.

KAVANAUGH, M.P., OSBORNE, P.B., CHRISTIE, M.J., BUSCH, A.E., HARTSHORNE, R.P. & NORTH, R.A. (1990). Sensitivity to blocking agents of cloned potassium channels expressed in *Xenopus* oocytes. *Soc. Neurosci. Abstr.*, **16,** Abstr. 281.14.

LI, M., JAN, Y.N. & JAN, L.Y. (1992). Specification of subunit assembly by the hydrophilic amino-terminal domain of the Shaker potassium channel. *Science*, **257,** 1225–1230.

OWEN, D.G., ROBERTSON, B., HALL, A. & SCOTT, J.A. (1992). Blockade by peptidic toxins of MK1 potassium currents in CHO cells. *Soc. Neurosci. Abstr.*, **18,** Abstr. 335.1.

ROBERTSON, B. & OWEN, D.G. (1993). Pharmacology of a cloned potassium channel from mouse brain (MK-1) expressed in CHO cells: effects of blockers and an 'inactivation peptide'. *Br. J. Pharmacol.*, **109,** 725–735.

ROBERTSON, B., OWEN, D.G., & NUMANN, R.E. (1995). Actions of some potassium channel-selective toxins on a human cloned potassium channel (hKv1.1) expressed in oocytes from *Xenopus laevis*. *J. Physiol.*, **483,** 72P.

ROBERTSON, B., OWEN, D., STOW, J., BUTLER, C. & NEWLAND, C. (1996). Novel effects of dendrotoxin homologues on subtypes of mammalian Kv1 potassium channels expressed in *Xenopus* oocytes. *FEBS Lett.*, **383,** 26–30.

SCOTT, V.E.S., MUNIZ, Z.M., SEWING, S., LICHTINGHAGEN, R., PARCEJ, D.N., PONGS, O. & DOLLY, J.O. (1994). Antibodies specific for distinct Kv subunits unveil a heterooligomeric basis for sub-types of α-dendrotoxin-sensitive K^+ channels in bovine brain. *Biochemistry*, **33,** 1617–1623.

SHEN, N.V., CHEN, X., BOYER, M.M. & PFAFFINGER, P.J. (1993). Deletion analysis of K^+ assembly. *Neuron*, **11,** 67–76.

SKARZYNSKI, T. (1992). Crystal structure of α-dendrotoxin from the green mamba venom and its comparison with the structure of bovine pancreatic trypsin inhibitor. *J. Mol. Biol.*, **224,** 671–683.

SOUTHAN, A.P. & OWEN, D.G. (1992). Dendrotoxins induce a form of long-lasting synaptic potentiation in hippocampal pyramidal neurones. *Soc. Neurosci. Abstr.*, **18,** Abstr. 628.6.

STRONG, P.N. (1990). Potassium channel toxins. *Pharmacol. Ther.*, **46,** 137–162.

TEMPEL, B.L., JAN, Y.N. & JAN, L.Y. (1988). Cloning of a probable potassium channel gene from mouse brain. *Nature*, **332,** 837–839.

TYTGAT, J., DEBONT, T., CARMELIET, E. & DAENENS, P. (1995). The alpha-dendrotoxin footprint on a mammalian potassium channel. *J. Biol. Chem.*, **270,** 24776–24781.

YU, W., XU, J. & LI, M. (1996). NAB domain is essential for the subunit assembly of both α-α and α-β complexes of *Shaker*-like potassium channels. *Neuron*, **16,** 441–453.

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