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Pharmacology of a cloned potassium channel from mouse brain (MK-1) expressed in CHO cells: effects of blockers and an 'inactivation peptide'

[1]Brian Robertson & David G. Owen

Electrophysiology Laboratory, Wyeth Research, Taplow, Maidenhead SL6 0PH

1 Chinese hamster ovary cells (CHO), maintained in cell culture, were stably transfected with DNA for the MK-1 voltage-activated potassium channel, previously cloned from a mouse brain library.

2 Voltage-activated currents were recorded by the whole-cell patch clamp method. In CHO cells transfected with the vector only, there were no significant outward voltage activated currents. However, large outward voltage-activated potassium currents were always observed in those cells which had been transfected with the vector containing the DNA encoding for MK-1.

3 These potassium currents activated from -40 mV, and reversed at the potassium equilibrium potential. The half-maximal conductance of MK-1 was at -10 mV and had a slope factor of 11 mV when fitted with a Boltzmann function. There was only very slight ($<10\%$) inactivation of MK-1 even at very large positive voltages.

4 MK-1 was reversibly blocked by: 4-aminopyridine (4-AP, 0.1–4 mM), Toxin I (10–100 nM), mast cell degranulating peptide (1 μM), tetraethylammonium (TEA, 4–10 mM), tedisamil (100 μM), quinine (100 μM) and ciclazindol (100 μM); all applied to the outside of the cell from a 'U tube' rapid perfusion system. 4-AP may block closed as well as open MK-1 potassium channels.

5 A synthetic 20 amino acid peptide derived from the N-terminus sequence of the *Shaker* B potassium channel (the 'inactivation peptide') produced dramatic inactivation of MK-1 when applied to the inside, but not the outside of the cell. Reducing peptide concentration or 'degrading' the peptide produced less inactivation.

6 The block of MK-1 by the synthetic inactivation peptide was quite different in time dependence from block by *internal* TEA (0.4–4 mM), which probably blocks much more quickly but less potently than the peptide.

Keywords: MK-1; cloned potassium channel; patch clamp; CHO cells; mouse brain; neurones

Introduction

Voltage-activated potassium channels are important modulators of neuronal excitability in the mammalian CNS. In conjunction with electrophysiological techniques, pharmacological agents which block K^+ channels have been vital in isolating major sub-types of K^+ channels in the CNS, thus permitting their characterization. However, in many cases blockers are not necessarily specific for sub-types of K^+ channels identified electrophysiologically. Naturally-occurring toxins isolated from snakes and scorpions, in particular, have proved amongst the most specific and certainly the most potent blockers (Dreyer, 1990; Strong, 1990), although even here we find apparently selective toxins such as the *B*K blocker, charybdotoxin, blocking voltage-activated K^+ currents (Strong, 1990) and the *s*K blocker, quinine, inhibiting delayed rectifier-like K^+ channels (McFadzean & England, 1992). The explosion of molecular cloning of K^+ channels appears only to confirm the inadequacies of existing pharmacological blockers, since there appear to be more cloned K^+ channels than those distinguished pharmacologically. Fortunately however, the cloning and expression of K^+ channels offers electrophysiologists for the first time the opportunity to study unequivocally the properties of single populations of sub-types of ion channels (see Stühmer, 1991 for review).

We have taken advantage of cloning and expression techniques and obtained a mouse brain K^+ channel, MK1 (Kv1.1, Chandy, 1991), which is stably-expressed in transformed Chinese hamster ovary (CHO) cells. This channel is widely expressed in mouse brain, most notably in the hippocampus (Adams *et al.*, 1991; 1993), and may be an important target for K channel toxins, such as Toxin I, which is known to block homologous channels, RBK-1 and RCK-1 (Stühmer *et al.*, 1988; Wang *et al.*, 1992). The present study describes the actions of a number of important K^+ channel blockers commonly used as pharmacological tools, on this single identified K^+ channel. We also describe modification of MK-1 by the so-called 'inactivation ball' of the *Shaker* B K^+ channel which has been shown to be responsible for N-type inactivation in this archetypal K^+ channel (Hoshi *et al.*, 1991), suggesting that the receptor for this peptide is well conserved. Importantly, these experiments have been performed, for the first time, in a mammalian cell system. A preliminary account of some of his work has been previously reported (Robertson & Owen, 1993).

Methods

Transfection and cell culture procedures

CHO-K1 cell lines were kindly supplied by Dr Bruce Tempel (University of Washington, Seattle, U.S.A.). Two separate lines of CHO cells were used, one containing the control host plasmid (pZMK228R) only, whilst the other cell line contained the plasmid with the cDNA insert for the mouse brain potassium channel gene MK-1 (pZMK6.2SS, Tempel *et al.*, 1988). Cells were grown in tissue culture in 75 ml flasks in a medium containing: 92% RPMI (Sigma); 5% foetal bovine serum (ICN Flow, High Wycombe); 1% L-glutamine (ICN Flow); 1% antibiotic solution (Sigma) and 1% geneticin solution (0.3 mg ml^{-1}, geneticin sulphate in 10% HEPES

[1] Author for correspondence.

buffered distilled water solution). Flasks of cells were maintained in an incubator with an atmosphere containing 5% CO_2 at 37°C. Cells were subsequently plated onto 35 mm plastic petri dishes (Gibco, Paisley, Strathclyde) at least 2 h before electrophysiological recording.

Voltage clamp and electrophysiological recording solutions

CHO cells were voltage-clamped by use of an Axopatch 1C amplifier (Axon Instruments, U.S.A.) and standard whole-cell patch clamp methods. Patch electrodes were made from aluminosilicate glass (SM150F, Clark Electromedical Instruments, Reading), with a 'bullet' shaped tip to minimize access resistance. Electrodes had a resistance of 1–3 MΩ after heat polishing on a Narashige microforge.

Electrodes were back-filled with an 'intracellular solution' which comprised (in mM): K aspartate 120, KCl 20, $MgCl_2$ 1, MgATP 2, EGTA (ethylene glycol-*bis*(β-aminoethyl ether) *N*,*N*,*N*′,*N*′-tetraacetic acid) 10, HEPES (N-[2-hydroxyethyl]-piperazine-*N*′-[2-ethanesulphonic acid]) 10, adjusted to pH 7.4 with NaOH. The intracellular solution was stored frozen in 1 ml aliquots and defrosted just before use.

Cells were continuously perfused at 3 ml min^{-1} with an 'extracellular solution' which comprised (in mM): NaCl 135, $MgCl_2$ 4, EGTA 1, KCl 5, glucose 25 and HEPES 10; adjusted to pH 7.4 with NaOH. Drugs and other compounds were dissolved in the extracellular solution and applied directly to cells via a 'U-tube' rapid perfusion system. All experiments reported here were conducted at room temperature (21–26°C).

The potassium channel blockers 4-aminopyridine (4-AP), tetraethylammonium chloride (TEA) and quinine HCl were obtained from Sigma Chemical Company. Ciclazindol was from Wyeth-Ayerst; tedisamil was a generous gift from Kali-Chemie Pharma (Hannover, Germany); Toxin I was purified by John Stow of Physical Chemistry at Wyeth Research from crude *Dendroaspis polylepis polylepis* snake venom (obtained from Sigma); mast cell degranulating peptide trifluoroacetate (MCDP) was purchased from Research Biochemicals Incorporated (Natick, MA, U.S.A.); and the *Shaker* B 'inactivation peptide' (of sequence MAAVAGLYGLGEDRQHRK KQ) was synthesized by Peptide Products Ltd (Salisbury, Wiltshire), and was confirmed by n.m.r. as >95% pure. All peptides were kept frozen and added to solutions just before experiments. Voltage-gated currents were elicited and recorded with pClamp software (version 5.5.1, Axon Instruments) running on a Dell 220 PC with an Axolab 1100 interface. Currents were routinely sampled at a rate of 0.6 kHz or faster, and written directly to hard disk for later analysis with either Clampfit software on the PC, or Axograph software (both from Axon Instruments) on a Macintosh IIci. Cells were usually held at − 100 mV, and cell leak and capacity artefacts were removed from current traces on-line with a p-on-4 subtraction procedure. Graphical analysis and current-voltage plots etc. were prepared using Kaleidagraph software. Curves (e.g. exponentials and Boltzmanns) were fitted using algorithms designed to minimize sum of squared errors.

Results

The results will be presented in three parts. The first section will focus on the physiological properties of MK-1, the second will describe some of the pharmacology of MK-1 and the final section will be concerned with the pharmacology and physiology of MK-1 channels modified with the *Shaker* B 'inactivation' peptide.

Electrophysiology of MK-1

Currents in untransfected CHO cells In CHO cells that had been transfected with the vector only, and not the cDNA insert for MK-1, no significant outward currents were observed (see e.g. Figure 1a, $n = 6$ cells). This indicates that unmodified CHO cells have little or no active potassium conductances of their own. However, on a faster time scale, small (<250 pA) voltage-activated inward currents could sometimes be observed (see Figure 1b). These currents were presumably carried by sodium ions through either sodium, or possibly under these recording conditions, calcium channels. No further analysis of these inward currents was undertaken, and it was not necessary to block these since they were not resolved in all CHO cells and because of their small amplitude and duration did not compromise the recording of MK-1 currents.

Currents in transfected CHO cells In cells that had been transfected with MK-1 a large 'delayed rectifier' type of

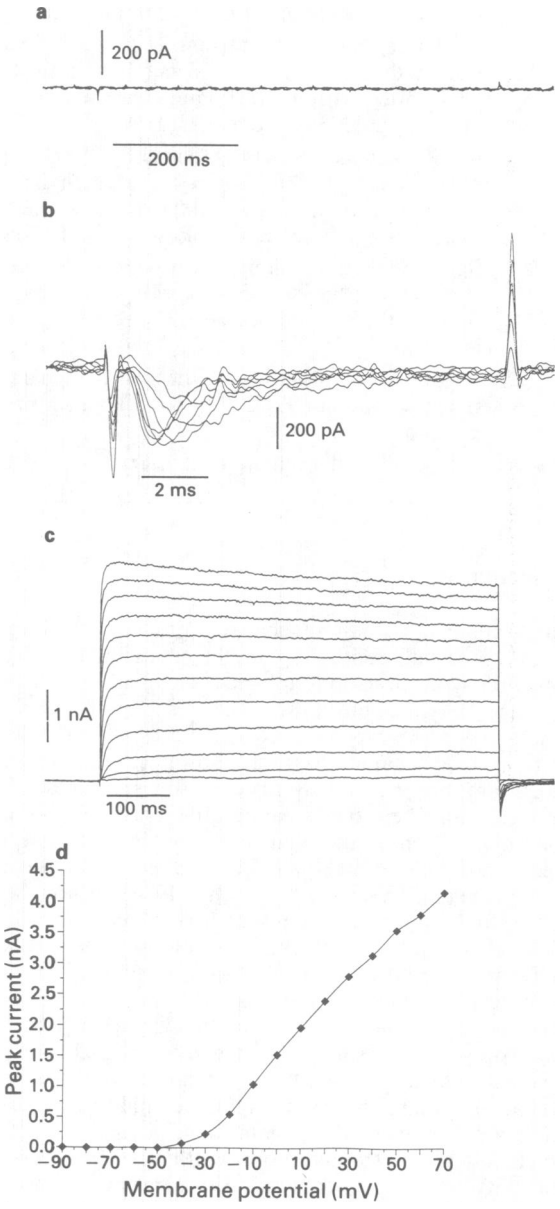

Figure 1 (a) No outward current in a CHO cell transfected with control vector only. Traces are current resulting from voltage steps from − 30 to + 90 mV from a holding potential of − 100 mV. (b) Some untransfected CHO cells have small voltage-activated inward sodium currents, elicited here on voltage steps from − 5 to + 25 mV from a holding potential of − 90 mV. (c) Activation of potassium current in CHO cells transfected with the cDNA for MK-1. Currents activated on voltage steps from − 40 to + 70 mV. Holding potential − 100 mV. (d) Peak current-voltage relationship for one cell transfected with MK-1.

outward current was always seen. (This current has now been observed in recordings from over 260 transfected CHO cells). A typical example of the MK-1 potassium current in response to incremental voltage steps from − 40 to + 70 mV is seen in Figure 1c. A peak current-voltage relationship for MK-1 is shown in Figure 1d, and reveals a gradual increase in current from a threshold of ~ − 40 mV, which may increase to as much as 10 nA at + 70 mV in some cells.

On a faster time scale (see Figure 2a) we observed that MK-1 rose fairly slowly, over several ms, and followed sigmoid kinetics. For the more negative steps there is a short but obvious delay before activation. Figure 2b shows a plot of rise time (measured as 10–90% of peak) versus step potential; each point is the mean of between 9–18 measurements, and the graph shows approximately an e-fold change for 43 mV change in membrane potential. An activation

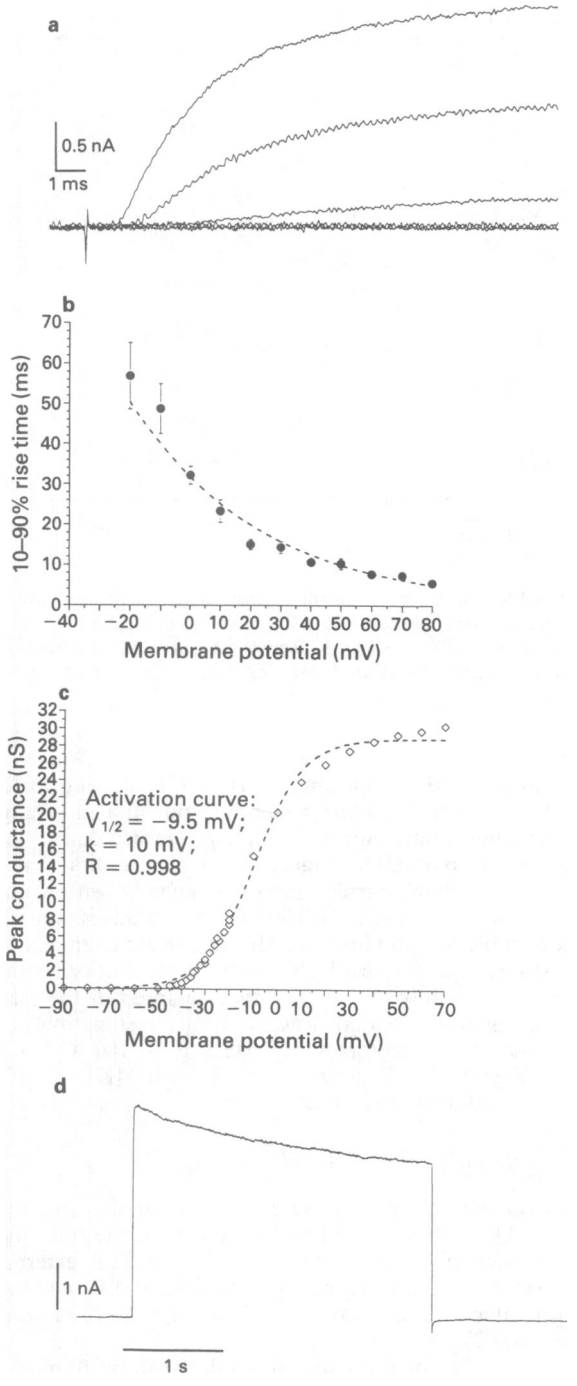

Figure 2 (a) Activation of MK-1 at a higher resolution. Steps between − 80 and + 20 mV in 20 mV increments. Voltage step begins at the point of the residual capacity artefact. V_h = − 100 mV. (b) 10–90% rise time to peak current versus membrane potential. Data from 9–18 cells. Broken curve represents an exponential fit to this data, showing ~e-fold shift for 43 mV change in V_m. R = 0.985. (c) Activation curve for MK-1 from a single cell. Potassium null potential assumed to be − 85 mV, and conductance calculated from peak current. Superimposed is a Boltzmann fit to the data, with R = 0.998. (d) Slow 'inactivation' of MK-1 on a step to + 80 mV from a holding potential of − 100 mV. A single exponential fitted to the decay of this current gives a time constant of 1.8 s.

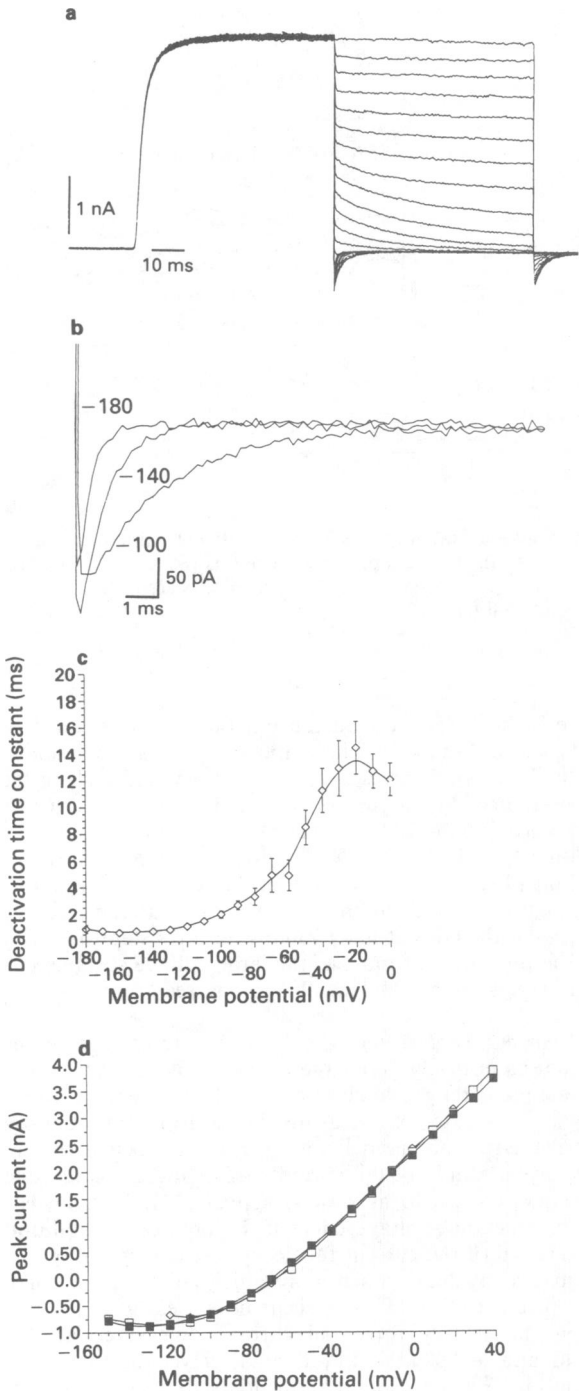

Figure 3 (a) Tail currents following a conditioning step to + 60 mV. Tail steps from − 180 to + 60 mV in 10 mV increments. V_h = − 100 mV. (b) Three tail currents to − 180, − 140 and − 100 mV following a conditioning step to + 60 mV (not shown). (c) Deactivation time constants derived from single exponential fits to tail currents versus membrane voltage. Mean data ± s.e.mean from 5 cells, smooth curve fit by computer. (d) peak amplitudes of tail current versus step potential. Three separate measurements from a single cell. Same conditions as other cells illustrated.

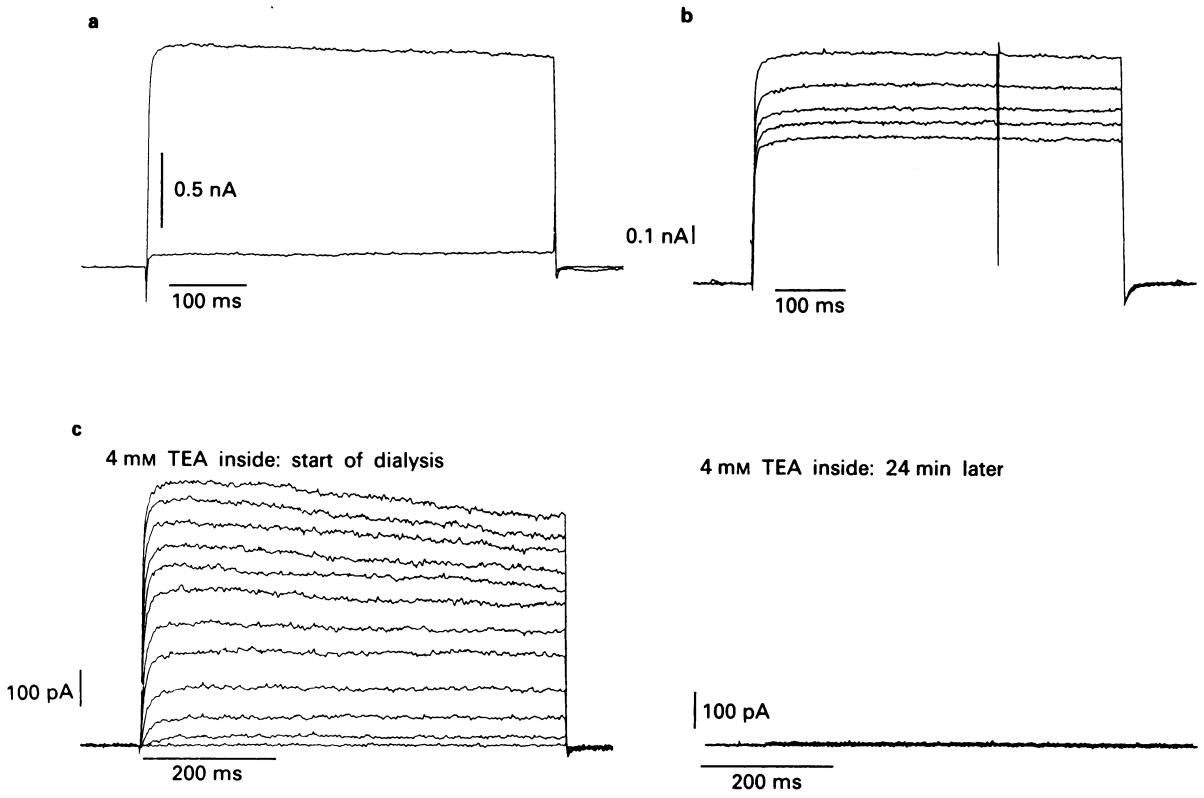

Figure 4 (a) Block of MK-1 by 10 mM tetraethylammonium (TEA) externally; top trace is control, bottom is in the presence of TEA. In this example peak current is reduced to 5% of control. Voltage steps from − 90 to + 70 mV. (b) First minutes' block by 4 mM TEA inside the cell. Each trace is separated by 10 s intervals. Steps from − 100 to + 60 mV. Top trace has a 'zap' artefact. (c) Full current-voltage responses for MK-1 at the start and after several minutes exposure to 4 mM TEA internally. Steps range from − 40 to + 70 mV. V_h = − 100 mV.

curve for the MK-1 conductance is plotted from data from a single cell in Figure 2c. The graph also shows a Boltzmann fit to the data, which gives a $V_{\frac{1}{2}}$ of − 9.5 mV and a slope factor (k) of 10 mV. Mean data on Boltzmann fits from four other cells gave $V_{\frac{1}{2}}$ = − 10.9 mV and k = 11.1 mV.

Some 'inactivation' of MK-1 does occur, albeit very slowly. This phenomenon is only seen on large positive steps (see e.g. Figure 1c), and Figure 2d shows one extreme case, where ~25% of the current inactivated over a 3 s step to + 80 mV. On the time scale of most of the large voltage steps used here (< 1 s), less than 10% inactivation is seen.

Tail current analysis of MK-1 Tail current measurements can tell us not only about the reversal potential of a conductance and thereby give clues as to its ionic identity, but they may also be used to measure deactivation time constants, that is the rate of channel closing from the open state. Figure 3a shows a family of tail currents following a voltage step to + 60 mV, chosen to maximally activate MK-1 channels (see e.g. conductance-voltage curve in Figure 2c). In addition to the reversal of the current the decay rate changes: the tails on negative steps decay much more quickly than those obtained with positive steps. This is seen more clearly in Figure 3b, which shows only three tail currents (measured at − 180, − 140 and − 100 mV) but here displayed at much higher resolution. Fitting single exponentials to these traces yields time constants (τ_ds) of 0.5, 0.9 and 2.9 ms respectively. Plotting such time constants derived from tail current measurements on 5 cells shows a bell shaped dependence of deactivation time constant on membrane potential (Figure 3c). In a few cells, a second, much slower exponential component was observed on tail current measurements, however, this was exceptional and is excluded from the present analysis.

Measuring the peak amplitudes of the tail currents gives a direct measure of the instantaneous current flowing through the MK-1 channels as the membrane potential is changed. The resulting ohmic current from three measurements on a single cell is plotted in Figure 3d, which shows that the outward potassium current increases linearly with voltage, but for inward current considerable rectification is seen. This is presumably due to Goldman-Hodgkin-Katz rectification as a consequence of the relatively low K concentration extracellularly, but possibly also to the extreme speed of the relaxations at negative potentials leading to underestimation of the true peak current (compare e.g. steps to − 180 and − 140 mV in Figure 3b). Experiments with higher $[K]_o$ would be needed to address this problem.

MK-1 and potassium channel blockers

MK-1 potassium currents were blocked rapidly and reversibly by TEA when applied to the outside of the cell. Figure 4a illustrates inhibition of MK-1 by 10 mM TEA externally; the mean reduction in current was to 8.3 ± 1.4% (n = 10) of control values. TEA 4 mM reduced MK-1 to 20% of control values (n = 3).

There was no obvious time-dependence in TEA blockade (see below for examples of kinetic changes with other K^+ channel blockers): the current was simply scaled downwards throughout the voltage step at all potentials. We were also interested in examining TEA block from the inside of MK-1 channels, especially for comparison to the block induced by the *Shaker* peptide discussed below. TEA was included in the intracellular solution in the recording pipette at either 0.4, 2 or 4 mM. In 4 cells with 0.4 mM TEA added inside, 'normal' MK-1 responses were obtained (data not shown); however currents evoked with large depolarizing steps were much noisier than those observed in control solution. It is assumed that this noisiness is due to a kind of flickery block due to

TEA transiently occluding MK-1 channels (see Spruce *et al.*, 1987).

When 4 mM TEA was applied internally to the CHO cells a fairly rapid and substantial block of MK-1 was obtained. Figure 4b shows five consecutive traces evoked by a voltage step from − 100 to + 60 mV. Each trace is separated by an interval of 10 s, and shows TEA gradually blocking MK-1. Once again, note that there is no overall change in the *kinetics* of MK-1 and the current is simply reduced. Another example is shown in Figure 4c, illustrating that 4 mM TEA eventually blocks MK-1 completely, leaving no outward current at all even on the largest depolarizing steps. Identical results were obtained in all 5 cells tested.

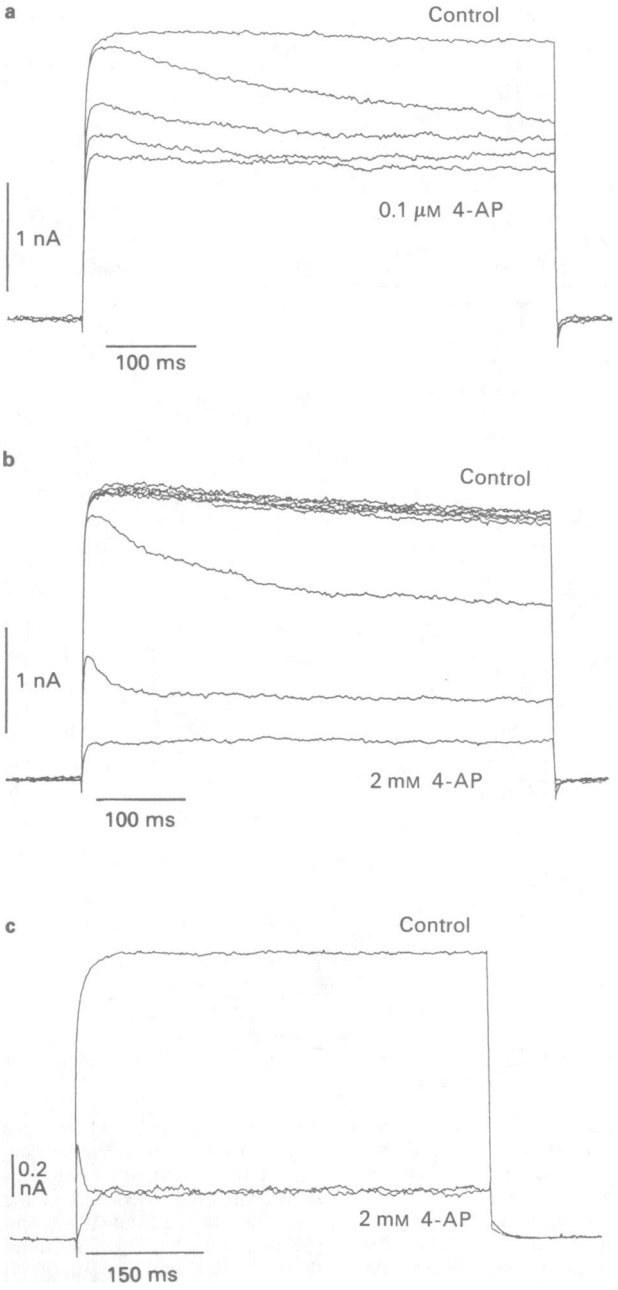

Figure 5 (a) Inhibition of MK-1 evoked by a voltage step to + 60 mV by 0.1 mM 4-aminopyridine (4-AP) externally. Traces separated by 10 s. Final steady-state inhibition is to 56% of control in this cell. (b) Progressive block by 2 mM 4-AP. Largest traces are several control steps, followed by three identical steps after exposure to 4-AP. Steady-state block is 12% of control in this example. (c) 4-AP inhibits MK-1 in the absence of voltage steps. Top trace is control, middle trace is after 60 s exposure to 2 mM 4-AP (note small decay phase at start of current), followed by equilibrium block 60 s later. Final block is 16% of control.

Although the concentrations of TEA employed here were very limited, it is of interest that 4 mM TEA is slightly more effective in blocking MK-1 from the inside than the outside (i.e. 100% block with 4 mM_i, versus ~90% block with 10 mM_o).

External 4-aminopyridine (4-AP) blocks MK-1 potassium channels: 0.1 mM reduced peak current at + 60 mV to 63.8 ± 2.4% ($n = 4$) of control values, whilst a higher concentration, 2 mM, reduced peak current to 9.1 ± 2.3% ($n = 5$). Block by 4-AP came on quickly and was fully and rapidly reversible. However some interesting effects were also seen during 4-AP block. For instance in Figure 5a we can see that in addition to the progressive block of MK-1 (step from − 100 to + 60 mV) that during the first few steps in the drug there is more block at the end of the voltage step than at the beginning, until finally a steady-state block is achieved (bottom trace). These changes are much more obvious with 2 mM 4-AP (Figure 5b), where there is a clear decay phase in MK-1 current before the drug block reaches equilibrium (bottom trace). As might be expected the decay induced in MK-1 is faster for the higher concentration of 4-AP. Similar kinetic changes were seen in every cell tested with 4-AP, and these transformations in current decay suggest that 4-AP is blocking *open channels during the voltage step*. We believe that 4-AP may also block MK-1 channels which are in the *closed* state. This is suggested from experiments like those illustrated in Figure 5c where three identical steps to + 60 mV are given at 60 s intervals. These long intervals are spent at a holding potential of − 100 mV, where it is assumed that all or certainly most of the MK-1 potassium channels remain closed. Immediately after the first step, 4-AP was applied, no further steps were made for 60 s, and then the middle trace was recorded. This showed that much, but not all of the current was already blocked. The brief exponential decay of some of

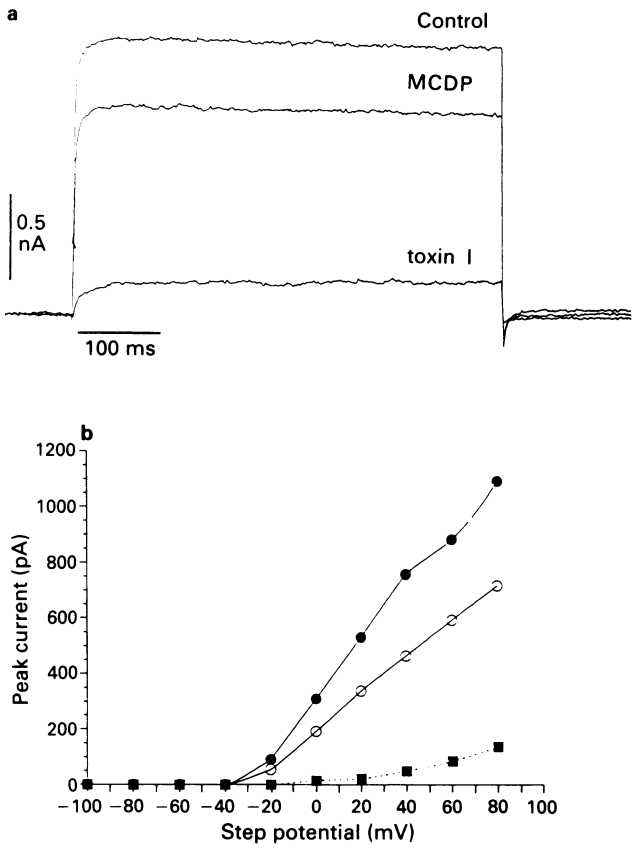

Figure 6 MK-1 is inhibited by mast cell degranulating protein (MCDP, 1 μM) and toxin I (100 nM). (a) Shows control (top), MCDP (middle) and toxin I (bottom) currents recorded on a voltage step from − 100 to + 60 mV. (b) Current-voltage relation for this same cell: (●) control; (○) 1 μM MDCP; ■ toxin I.

this current is due to 4-AP block of open channels. The final trace (bottom) 60 s later shows the final block achieved with this concentration of 4-AP. Together, these experiments suggest that 4-AP may block MK-1 channels in both closed and open states.

MK-1 is also blocked by two animal-derived toxins, Mast Cell Degranulating Peptide (MCDP), which is a 23 amino acid peptide from bee (*Apis mellifera*) venom; and by the 60 amino acid toxin I isolated from *Dendroaspis polylepis polylepis* snake venom.

MCDP was tested at 1 μM, at which concentration MK-1 is reduced to 77 ± 2.6% ($n = 7$) of control values at + 60 mV. Toxin I is much more potent however, 100 nM reducing MK-1 to 10.6 ± 1.7% ($n = 6$) of control under similar conditions. Toxin I (50 nM) reduced peak current to 63% of control in 2 experiments. Some of these results are illustrated in Figure 6, of which part (a) shows control, MCDP and toxin I traces obtained on voltage jumps from − 100 to + 60 mV in a single cell. No kinetic changes are obvious. Figure 6b illustrates peak current-voltage plots for this same cell in the presence and absence of the toxins. There is no voltage-dependence of block. It is worth noting that although block by these toxins was reversible on washing out, it usually took longer for the current to recover fully than with the other non-peptide inhibitors used. Also, the rising phases of MK-1 currents became much slower after removal of toxin I from the perfusate: we can think of no simple explanation for this. Perhaps the toxins induce extra inactivated or closed states in the K^+ channel, resulting in slower opening kinetics. Stühmer *et al.* (1988) have observed a similar phenomenon with MCDP washout on RCK-1 channels.

Tedisamil is a Class III antiarrhythmic which has recently been shown to block certain voltage- and calcium-activated potassium currents in a variety of peripheral and central tissues (see e.g. Dukes *et al.*, 1990). It has been suggested that this drug is a simple 'open channel blocker', and we were interested to see if these K^+ channels cloned from mouse brain were similarly affected. At a concentration of 100 μM, tedisamil markedly altered both the peak amplitude and the decay of MK-1; example traces may be seen in Figure 7. The best way of characterizing the effects of such compounds is not through simply measuring inhibition of peak as we have

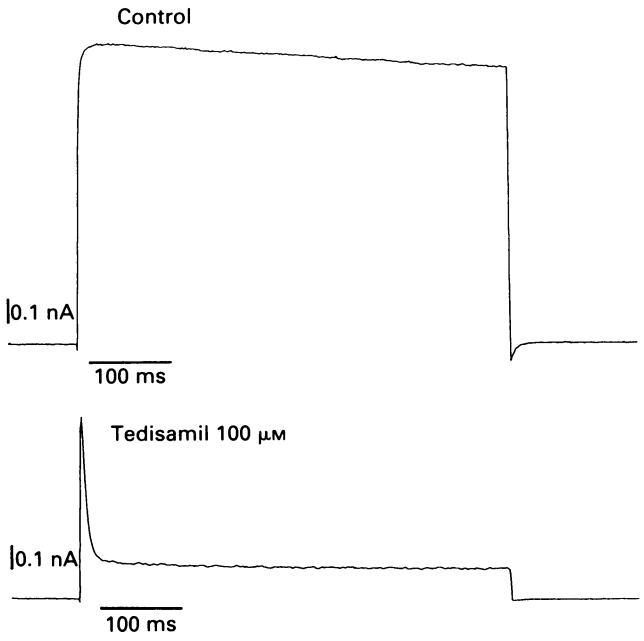

Figure 7 Block of MK-1 by 100 μM tedisamil. Averaged traces (between 4 and 8 separate records) show control and drug responses. Peak current and charge transfer are reduced to 60% and 11% respectively in this example. τ_d in control is 5.5 s, decreasing to 5.1 ms and 137 ms in tedisamil. Step to + 60 mV from − 100 mV.

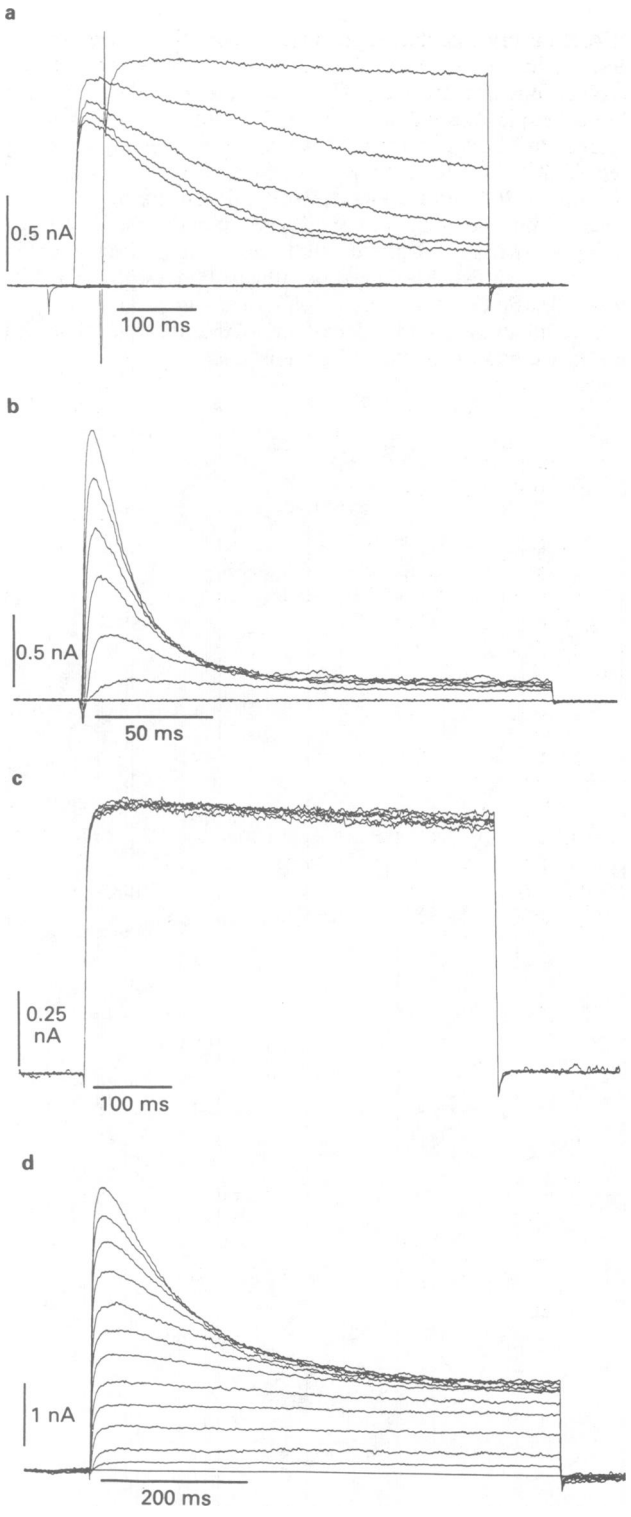

Figure 8 (a) *Shaker* B peptide rapidly modifies MK-1. The bottom, flat traces were recorded in cell attached mode, before bursting into the cell with a 'zap'. The top trace is the initial current recorded just after (see large artefact) establishing whole-cell recording. Other traces are sequential steps 10 s apart showing the gradual acceleration of current. (Steps from − 100 to + 60 mV). (b) Current responses to voltage steps from − 80 to + 80mV increasing in 20 mV increments after peptide effect has equilibrated. Time constants of decay fitted to these traces are as follows: − 20 mV: 63.8 ms; 0 mV: 37.2 ms; + 20 mV: 27.9 ms; + 40 mV: 22.5 ms; + 60 mV: 18.7 ms; + 80 mV: 16.6 ms. $V_h = -100$ mV. (c) MK-1 is not modified when the *Shaker* B peptide ball is applied *outside* the cell. Traces recorded during a voltage step from − 100 to + 60 mV with 400 μM peptide externally. (d) Current in response to incremental voltage steps from − 40 to + 70 mV, with 'degraded' peptide inside. Time constants of decay are: + 10 mV: 370 ms; + 20 mV: 294 ms; + 30 mV: 218 ms; + 40 mV: 170 ms; + 50 mV: 145 ms; + 60 mV: 125 ms; + 70 mV: 111 ms.

before, but by calculating the more illustrative *charge transfer* during the voltage step (obtained by integrating the total current over the duration of the step). The peak current was reduced to 52.7 ± 6.3% of control ($n = 5$) whilst the overall charge transferred during the voltage step was diminished to only 14.8 ± 2.7% of control ($n = 5$). The average decay phase of MK-1 in the presence of 100 μM tedisamil was 8.9 ± 1.5 ms ($n = 5$). A slower phase was sometimes also measured, being ~100 ms. Moreover tedisamil inhibition was only slowly reversible; this is consistent with the postulated intracellular site of action.

The antimalarial drug, quinine HCl has been reported to possess K^+ channel blocking activity (see e.g. McFadzean & England, 1992). At 100 μM quinine reduced MK-1 peak outward current to 25.1 ± 4.6% of control values ($n = 6$), whilst charge transfer was reduced to 16.7 ± 4.1% ($n = 6$). A little time-dependence of quinine block was observed.

The anorectic agent, ciclazindol, has recently been shown to have inhibitory actions at both ATP-dependent and voltage-gated potassium channels. Here we show that at least at the relatively high concentrations used here, ciclazindol (100 μM) reduced peak MK-1 current to 49.2 ± 4.7% of control ($n = 6$), and charge transfer was similarly reduced to 49.1 ± 6.3% of control values ($n = 6$).

Modification of MK-1 currents by the Shaker *B peptide*

The best studied K^+ channel at present is the *Shaker* B channel cloned from *Drosophila* muscle. Unlike MK-1 K^+ channels these *Shaker* channels close quickly after opening. In a recent series of elegant experiments it has been shown that this inactivation mechanism is due to part of the N-terminal sequence of the channel, some 20 amino acids, acting as a molecular 'ball' entering and occluding the inside mouth of the channel (Zagotta *et al.*, 1990). We were interested to determine if a similar mechanism could be induced in the essentially non-inactivating MK-1 channel. To this end we included a synthetically manufactured copy of the *Shaker* B 20-mer peptide in the patch pipette solution at a concentration of 400 μM. (A total of 53 CHO cells were recorded with the *Shaker* peptide inside – all were modified). Figure 8a shows the typical response obtained during the first minutes' dialysis with the peptide. The bottom (flat) traces are in cell-attached mode before bursting into the CHO cell, then we have burst the patch with a 'zap' during the voltage step, and straightaway get our normal, sustained MK-1 current. However in the following few seconds as the ball dialyses into the cell (there are 10 s intervals between traces) the current was transformed into an inactivating potassium current. The next part of the Figure (8b) shows a current-voltage family of modified MK-1 once the effect has stabilized (steps from − 40 to + 80 mV, compared this with e.g. Figure 1c, noting difference in timescales).

The peptide proved to be ineffective when applied at 400 μM outside the cell (Figure 8c, $n = 5$) since no inactivation was induced. This result suggests that the blocking mechanism may be selective for the intracellular face of the channel. The peptide was also tested at 200 μM intracellularly, but very little inactivation was evident ($n = 6$). Attempts were also made to degrade the peptide to determine if the blocking action was dependent on some native structure in the molecule. Initially the peptide was 'aged' for 24 h in the intracellular solution at room temperature, but MK-1 was still quickly transformed into a rapidly inactivating K^+ current ($n = 3$). A further sample of this 'aged peptide' solution was then heated at 60°C for 2 h and vigorously sonicated before testing, and a typical result ($n = 3$) is shown in Figure 8d. It is clear that the current treated with this

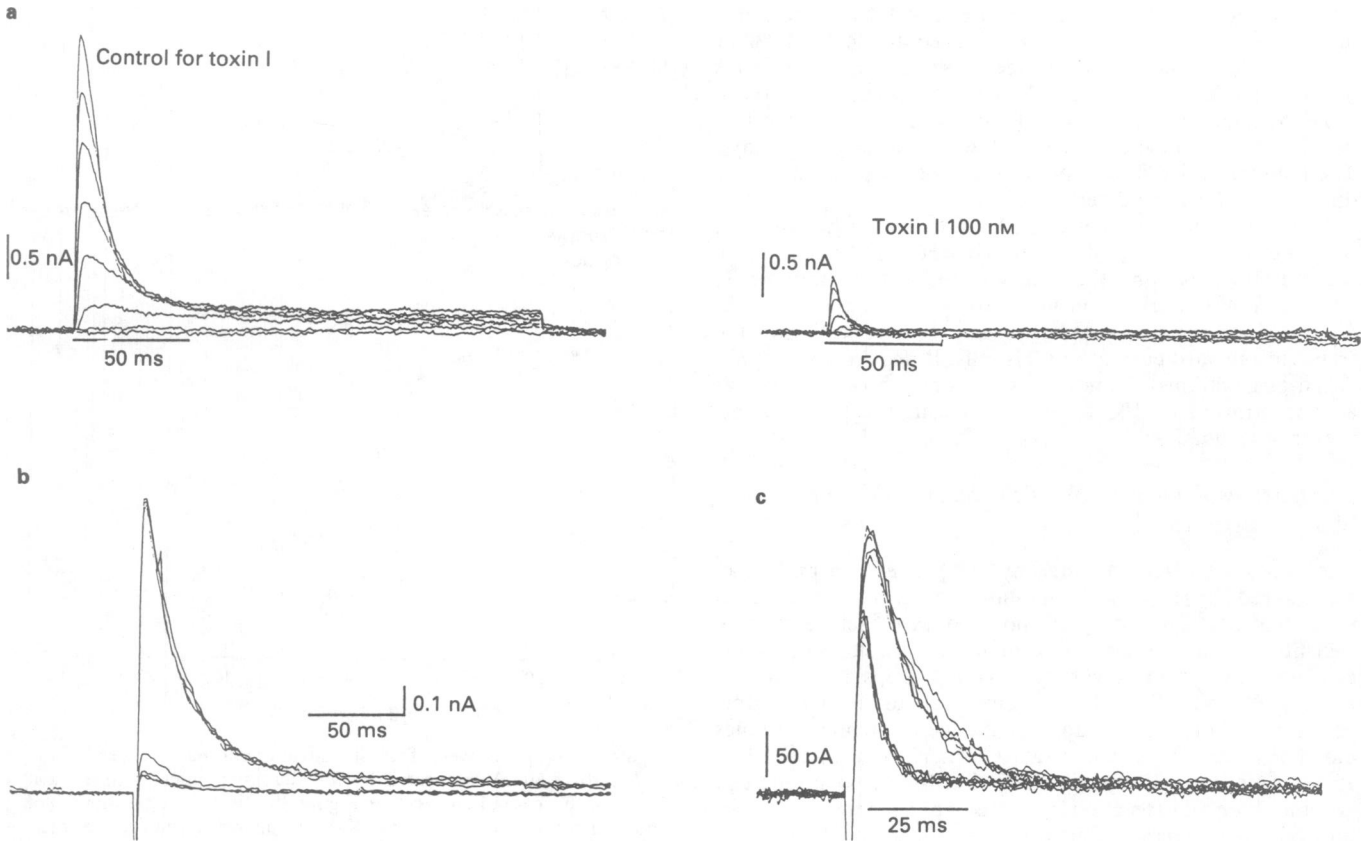

Figure 9 (a) Block of peptide-modified MK-1 by 100 nM toxin I. Peak current at + 60 mV was reduced to 23% of control in this example. (b) Tetraethylammonium 10 mM also blocks modified MK-1. In this example peak outward current was reduced to 5% of control. (c) Inhibition of peptide-treated MK-1 by 100 μM tedisamil. Top traces control, lower traces in drug. In this cell peak current was reduced to 63% of control; charge transfer to 57% of control, and τ_d changed from 12.2 in control to 4.6 ms in tedisamil (mean values from 6 separate records).

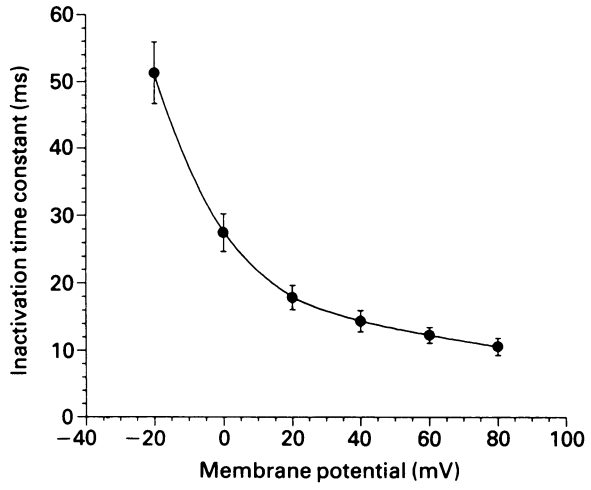

Figure 10 Time constants of inactivation of peptide-modified MK-1 change with membrane voltage. Data points are mean τ_ds obtained from single exponential fits on currents from 10 cells (± s.e.mean). Smooth curve fitted by computer.

'degraded' peptide inactivates much more slowly; indeed the fitted time constants are of the order of hundreds rather than the tens of milliseconds seen with the normal peptide e.g. τ_d for the step to + 60 mV is 125 ms in this example, compared to a mean value of 12 ms (see below) for control peptide.

As may be expected, the basic pharmacology of the 'transformed' MK-1 was unaltered. Sensitivity to Toxin I (100 nM, $n = 7$, e.g. Figure 9a) and external TEA (10 mM, $n = 7$, Figure 9b) were unchanged, and the current decay was also accelerated by tedisamil (100 μM, $n = 4$, Figure 9c). Tedisamil (100 μM) suppressed peak current to 64 ± 4.4%; charge transfer during the voltage step was reduced to 44.3 ± 14% of control values; and decay rate decreased from 55.4 ± 28 ms in control solution to 6.8 ± 1.9 ms (all measurements from 4 separate cells, note that control value is larger than normal because one of these cells had a slower than average decay). The final value for decay rate in tedisamil is not substantially different between modified and unmodified MK-1 current.

In three separate cells where MK-1 had been transformed with the *Shaker* B peptide we tested whether the decay rate could be increased by the oxidising reagent, chloramine T, which removes inactivation in a variety of K^+ channels. (At 500 μM chloramine T completely removes inactivation of the transient outward current in GH_3 cells: B. Robertson, unpublished observations). Somewhat surprisingly however, even at a concentration of 500 μM, no change in decay rate whatsoever was obtained.

Electrophysiological assay of the block induced by the Shaker *B peptide*

The 'block' of MK-1 channels by the *Shaker* B peptide may be assayed by a variety of standard electrophysiological measurements. The decay of modified MK-1 currents were well fitted with a single exponential, and the time constants of inactivation (τ_d) at a variety of voltage steps are plotted in Figure 10. Over the voltage range − 20 to + 80 mV, time constants become more rapid as the step potential becomes more positive. The mean τ_d at + 60 mV from 12 cells was 12 ± 1.2 ms. It is thought unlikely that the inactivation step, i.e. the blocking reaction itself is voltage-sensitive, since previous single channel studies on *Shaker* channels show no voltage sensitivity for this step (Hoshi *et al.*, 1990). However, the voltage-dependence of the decay rate closely follows the activation curve for unmodified MK-1 (see Figure 2c). The steepest change in τ_d occurs over the range − 20 to + 20 mV, which corresponds to the steepest part of the conductance/activation curve. From + 20 to + 80 mV both the inactiva-

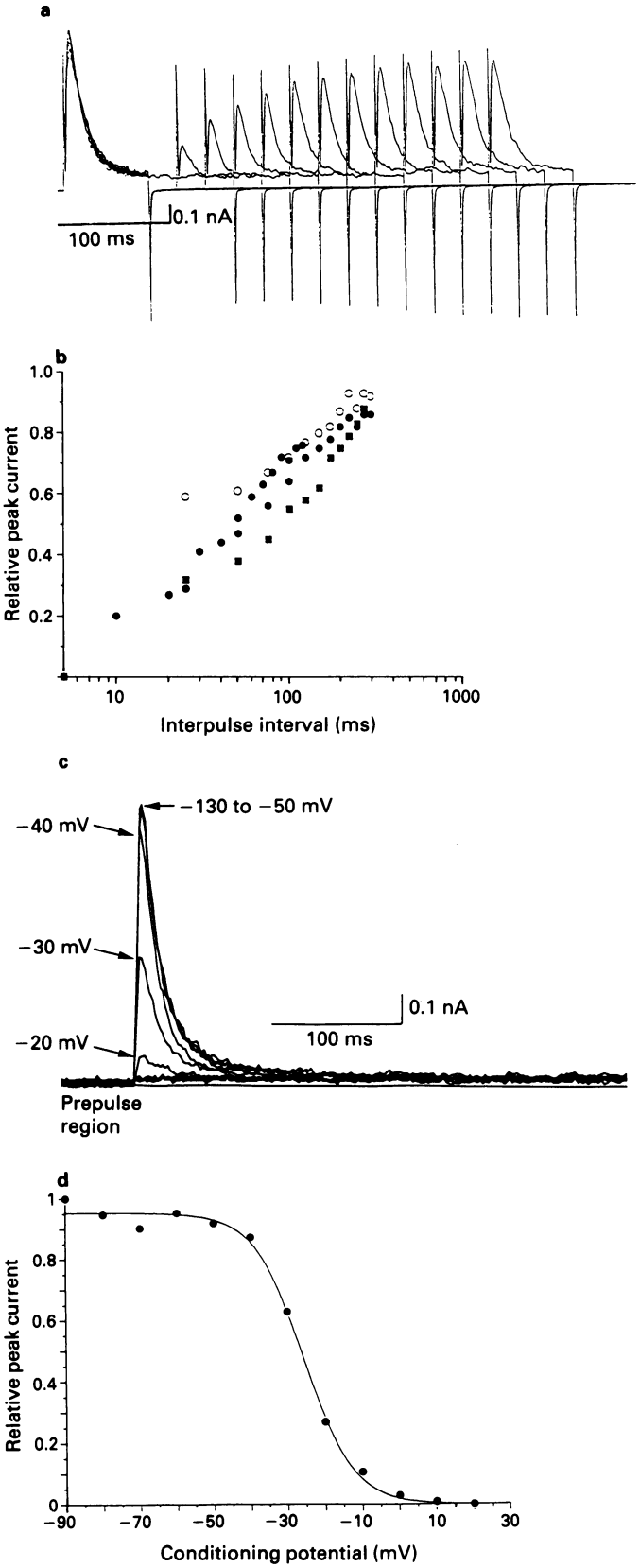

Figure 11 (a) Recovery from inactivation: a pair of steps from − 100 to + 60 mV separated by an increasing interval (increment 25 ms in this case). [Same cell as Figure 8b]. (b) Semilogarithmic plot showing recovery of modified MK-1 with increasing interpulse interval. Data from three separate cells, denoted by different symbols. (c) Example data used for construction of h-infinity curve. Traces are current obtained on a step top + 60 mV after various conditioning steps from − 130 to − 20 mV, as labelled. Preceding 1 s conditioning step only partly shown. (d) Steady-state inactivation curve for four cells, normalised to peak current value. Boltzmann fit to this data gives a $V_{\frac{1}{2}} = -25$ mV, and a slope factor $k = -7.6$ mV; $R = 0.998$.

tion time constant and the activation curves show a much shallower dependence on voltage. The simplest explanation for the voltage dependence of τ_d is that inactivation is coupled to activation, and that as the probability and speed of channel opening increase at more positive voltages, then the blocking particle will enter the channel sooner, quickening the decay of the ensemble current.

Two pulse experiments provide a valuable tool for probing the gating kinetics of voltage-activated channels. Experiments of the sort illustrated in Figure 11a can be used to measure the rate of recovery from inactivation: a pair of identical pulses to + 60 mV are separated by a variable time (t) at the holding potential (− 100 mV). The first step is long and large enough to inactivate the modified MK-1 channels completely. The cell is then repolarized to − 100 mV to initiate recovery from inactivation and then the second pulse provides a measure of the number of channels back in a conducting state. The traces illustrated show the current recovering gradually as the interpulse interval is lengthened. Results from 3 other cells are plotted semilogarithmically in Figure 11b. Recovery from inactivation is ~90% complete in 300 ms and is full in 1 s. (An interval of 1–10 s between voltage pulses was used for all the standard protocols employed here).

The steady-state voltage-dependence of inactivation for peptide-modified MK-1 was investigated with 1 s prepulses ranging from − 130 to + 30 mV, followed by a test pulse to + 60 mV. The test pulse was used to determine the fraction of channels not inactivated by the prepulse, and some traces are shown in Figure 11c. It is clear from these traces that the prepulses from − 130 to − 50 mV have no effect on the test pulse current; however, the pulses from − 40 to − 20 mV have a profound effect on the amplitude of this current. These and other results are plotted graphically in Figure 11d, and the data from four cells is well fit with a Boltzmann distribution with $V_{\frac{1}{2}}$ = − 25 mV and a slope factor k of − 7.8 mV. As expected, the greatest degree of inactivation occurs over the range where MK-1 activates most steeply.

Discussion and conclusions

The combination of the techniques of molecular biology and electrophysiology provide unprecedented access to single, identified ion channels from the mammalian brain in a convenient and robust preparation. Here we have studied the properties of the MK-1 potassium channel cloned from mouse brain, which is now known to be located in several significant neuronal structures (Adams *et al.*, 1991; 1993). In addition, because the channel is expressed here in a mammalian cell line, we get closer to the true native environment of MK-1 compared to oocyte expression systems.

The expression of the MK-1 message in CHO cells results in a slowly activating, essentially non-inactivating potassium current much like the classical 'delayed rectifier' first observed by Hodgkin and Huxley in squid axon. Full activation of the MK-1 current takes several milliseconds even at very positive potentials, and is preceded by a short delay (see also Houamed *et al.*, 1989), presumably due to the presence of multiple closed states before channel opening. The activation parameters are similar to those reported for homologous channels RBK-1 and RCK-1 from the rat brain (Stühmer *et al.*, 1988; Christie *et al.*, 1989) but the half maximal conductance value is almost 20 mV more positive. This may be due to the fact that MK-1 is here expressed in a mammalian, as opposed to an amphibian expression system. The slope of the conductance-voltage curve obtained in the present study (11 mV) is about mid-way between values previously reported for homologous channels, i.e. 6–14 mV (Stühmer *et al.*, 1988; Christie *et al.*, 1989; Klumpp *et al.*, 1991). In every case so far reported, MK-1 and its rat homologues have inactivation rates measured in seconds as opposed to the rapid, millisecond inactivation seen for some other members of the *Shaker* potassium channel family. The tail currents measured here predominantly had a single time constant, with a bell-shaped dependence on membrane potential. A single time constant for deactivation, of approximately the same duration, has been observed for RCK-1 channels by Koren *et al.* (1990). The mean values obtained here at very negative potentials appear to saturate at ~0.7 ms, implying an approximate value for the MK-1 channel closing rate of ~1400 s^{-1}. However, the true value for channel closing is likely to be faster because of the limited bandwidth available in the present study.

TEA was effective in blocking MK-1 channel from both the inside and the outside, as has been shown previously for RBK-1 and RCK-1. For these two rat channels, the K_i s for internal and external block are roughly similar, being around 0.3 mM (Christie *et al.*, 1989; Taglialatela *et al.*, 1991; Newland *et al.*, 1992). The binding sites for block of several potassium channels by internal and external TEA have been localized to a sequence of only 19 amino acids that lie between the S5 and S6 regions of the channel (the H5 region). Recent work on the *Shaker* K channel has shown that the pore-blocking site for external TEA may be constructed from only four aromatic tyrosine residues, one from each channel subunit, which interact simultaneously with the blocker (Kavanaugh *et al.*, 1991; Heginbotham & MacKinnon, 1992). TEA did not induce any kinetic changes in MK-1; the gradual equivalent shift of peak and final current and the flickery block seen here when TEA was included inside the cell support the conclusion that TEA is a rapid channel blocker (Spruce *et al.*, 1987).

4-AP proved more effective than TEA in blocking MK-1. Published K_i values for RBK-1 and RCK-1 vary between 0.16 and 1 mM (Stuhmer *et al.*, 1988; Christie *et al.*, 1989; Wang *et al.*, 1992), which are in accordance with the inhibition by 4-AP seen here. Klumpp *et al.* (1991) have reported a K_i value of 1.1 mM for 4-AP inhibition of mouse retinal MK-1. 4-AP proved a much more interesting blocker than TEA, since our results suggest that 4-AP is able to block open MK-1 channels during the voltage step, as well as closed channels between voltage steps. Wagoner & Oxford (1990) have also suggested that 4-AP blocks closed and open transient potassium channels in GH_3 cells. Unfortunately, there are no details yet from site-directed mutagenesis studies of where 4-AP may bind to cause inhibition of MK-1 or related K^+ channels.

The toxins MCDP and toxin I proved to be potent inhibitors of MK-1. Perhaps this channel is one of the CNS targets of these toxins, thereby causing the powerful convulsive effects of these compounds when they are injected intracerebroventricularly (see Harvey & Anderson, 1991 for review). (However, RCK2- and RCK5-subtype potassium channels are also sensitive to these toxins). Recent studies indicate that both toxins induce a long lasting increase in excitability in rat isolated hippocampal slices, reminiscent of long term potentiation (Southan & Owen, 1992). MCDP was much less effective than toxin I in blocking MK-1; previous reports comparing these two toxins on similar, slowly inactivating potassium channels bear this out, with K_i values for the snake toxin being 4 to 18 times greater than the apian inhibitor (e.g. Stansfeld *et al.*, 1987; Stühmer *et al.*, 1988; Wang *et al.*, 1992).

Tedisamil, which is a novel Class III antiarrythmic (see e.g. Beatch *et al.*, 1991), was a dramatic blocker of MK-1, producing a marked inactivation of the current in both peptide-modified and unmodified states. This action is consistent with tedisamil being an open-channel blocker, as previously suggested (Dukes *et al.*, 1990). It was originally believed that tedisamil was selective for rapidly inactivating voltage-gated potassium currents, but has since been shown to be an effective blocker of both non-inactivating and calcium-activated K^+ currents in central nervous and peripheral tissues (e.g. McLarnon & Wang, 1991; Pfrunder & Kreye, 1991). The slow washout of the responses observed here are

concordant with an intracellular site of action, but this mechanism appears *independent* of the intracellular block by the inactivation peptide. Since tedisamil also blocks central potassium channels including MK-1, this drug may be useful for the modulation of CNS transmission.

Quinine has been used principally to block large-conductance calcium activated K^+ channels, but McFadzean & England (1992) have recently shown that this compound is an extremely effective blocker of the slowly inactivating K^+ current in anococcygeal smooth muscle cells. Similar results were reported by Fatherazi & Cook (1991), who found that quinine was more effective against ATP-sensitive K^+ channels and a delayed rectifier K^+ current than against Ca^{2+}-activated K^+ currents in pancreatic islet cells. However, Christie *et al.* (1989), who used quinine to exclude the possibility that they were recording Ca^{2+}-activated K^+ currents, found no inhibition of RBK-1 by 100 μM quinine: this is surprising in view of the close similarity between MK-1 and RBK-1 (only 3 amino acids are different out of 497). The antagonism of slow, voltage-activated K^+ channels and Ca^{2+}-activated K^+ channels by both quinine and tedisamil suggest that many of these different sub-types of potassium channel share some common structural features.

We found that relatively high concentrations of ciclazindol (100 μM) block MK-1, and Noak *et al.* (1992) report that this compound antagonizes both voltage- and ATP-dependent K^+ channels. Whether these effects are relevant to the (presumably) centrally-mediated anorectic actions of ciclazindol is not clear.

We have demonstrated that the 'inactivation peptide', from the last 20 amino acids of the N-terminal sequence of *Shaker* B potassium channels, is capable of blocking MK-1 channels from mouse brain. This block is characterized by the transformation of the current from a 'delayed rectifier' type to a rapidly inactivating, 'A-type' outward current. The peptide was ineffective however when added to the outside surface of MK-1 channels, suggesting that the receptor site for the ball is only present on the cytoplasmic surface. Isacoff *et al.* (1991) have recently studied the properties of this putative receptor, and find that mutations of certain highly conserved residues between S4 and S5 segments markedly influence the normal inactivation in *Shaker* B potassium channels and that induced in DRK-1 channels. This region is also conserved in MK-1 channels; it would be of interest to perform similar site-directed mutations to prove that this region also forms part of the ball peptide receptor site on this channel. These studies indicate that the binding site has been conserved through evolution of the *Shaker* potassium channel family.

A fairly high concentration of peptide was required in this study to produce appreciable inactivation, certainly when compared to the original studies on *Shaker* B channels. The K_d for the ball peptide on its 'own' channels is 8 μM, which is identical to the K_d obtained by Foster *et al.* (1992) for the peptide on calcium-activated potassium channels from rat brain and skeletal muscle. However, there are significant differences in K_d even between the 'same' channels, as Toro *et al.* (1992) report a K_d of 95 μM for ball inhibition of K_{Ca} channels from smooth muscle. One reason for this may be that even though the 'receptor site' may be identical in sequence between channels, the electrostatic environment or diffusional approach may be made quite different by changes in remote regions. It is also possible that a greater concentration of peptide was required here because it was applied to the channels via whole-cell dialysis, and we do not know the actual concentration of the molecule at the critical region on the inside membrane surface. All of the other studies of ball-induced inhibition of K channels have been conducted with either inside-out patches or in bilayers, where the concentration of the peptide is known exactly.

Structural work has suggested that the ball peptide may adopt an α-helical conformation (Lee *et al.*, 1992), but it is thought unlikely that there is much tertiary structure associated with the molecule. In contrast, the equivalent inactivation particle from RCK-4 channels (which display rapid inactivation) is possibly more complex, due to the presence of cysteine in the molecule. Removal of these cysteines alters inactivation properties (Ruppersberg *et al.*, 1991). These authors have indeed suggested that the cysteine groups may be an important route for coupling cellular metabolism to potassium channel inactivation.

Several lines of evidence indicate that it is unlikely that the peptide induces inactivation through some 'non-selective' action, since single point modifications to the peptide markedly alter its effectiveness (Hoshi *et al.*, 1990). Firm evidence that the ball peptide actually physically blocks the ion conduction pathway in K^+ channels has come from experiments demonstrating competition between internal TEA and the inactivation process (Choi *et al.*, 1991). Additionally, Demo & Yellen (1991) demonstrated that recovery from inactivation may be speeded up by increasing K^+ externally (as if K ions entering the pore knock the ball out of its blocking site) and show in single channel experiments that the inactivation ball must leave the channel before it can close.

Further new developments made possible by molecular biology have been in the exploration of transmitter-channel coupling (Hoger *et al.*, 1991), characterization of the action of clinically useful drugs (Snyders *et al.*, 1992) and in the precise mapping of ion channel subtypes on individual neurones (Sheng *et al.*, 1992). In conclusion, it is clear that the marriage of the techniques of molecular biology and electrophysiology now permits unparalled access to the study of ion channels present in the mammalian brain.

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References

ADAMS, L.A., HOUAMED, K.M., DORSA, D.M. & TEMPEL, B.L. (1993). Glial- and neuronal-specific K^+ channel gene expression in the CNS of the mouse. *Proc. Natl. Acad. Sci. U.S.A.*, (in press).

ADAMS, L.A., HOUAMED, K.M. & TEMPEL, B.L. (1991). Glial- and neuronal-specific K^+ channel gene expression in the CNS of the mouse. *Soc. Neurosci. Abst.*, **17**, 1279.

BEATCH, G.N., ABRAHAM, S., MACLEOD, B.A., YOSHIDA, N.R. & WALKER, M.J.A. (1991). Antiarrythmic properties of tedisamil (KC8857), a putative transient outward K^+ current blocker. *Br. J. Pharmacol.*, **102**, 13–18.

CHANDY, K.G.C. (1991). Simplified gene nomenclature. *Nature*, **352**, 26.

CHOI, K.L., ALDRICH, R.W. & YELLEN, G. (1991). Tetraethylammonium blockade distinguishes two inactivation mechanisms in voltage-activated K^+ channels. *Proc. Natl. Acad. Sci. USA.*, **88**, 5092–5095.

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.

DEMO, S.D. & YELLEN, G. (1991). The inactivation gate of the *Shaker* K^+ channel behaves like an open channel blocker. *Neuron*, **7**, 743–753.

DREYER, F. (1990). Peptide toxins and potassium channels. *Rev. Physiol. Biochem. Pharmacol.*, **115**, 93–136.

DUKES, I.D., CLEEMAN, L. & MORAD, M. (1990). Tedisamil blocks the transient and delayed rectifier K^+ currents in mammalian cardiac and glial cells. *J. Pharmacol. Exp. Ther.*, **254,** 560–569.

FATHERAZI, S. & COOK, D.L. (1991). Specificity of Tetraethylammonium and Quinine for three K channels in insulin-secreting cells. *J. Membr. Biol.*, **120,** 105–114.

FOSTER, C.D., CHUNG, S., ZAGOTTA, W.N., ALDRICH, R.W. & LEVITAN, I.B. (1992). A peptide derived from the *Shaker* B K^+ channel produces short and long blocks of reconstituted Ca^{2+}-dependent K^+ channels. *Neuron*, **9,** 229–236.

HARVEY, A.L. & ANDERSON, A.J. (1991). Dendrotoxins: snake toxins that block potassium channels and facilitate transmitter release. In *Snake Toxins*. ed. Harvey, A.L. pp. 131–164. New York, Pergamon Press Inc.

HEGINBOTHAM, L. & MACKINNON, R. (1992). The aromatic binding site for tetraethylammonium ion on potassium channels. *Neuron*, **8,** 483–491.

HOGER, J.H., WALTER, A.E., VANCE, D., YU, L., LESTER, H.A. & DAVIDSON, N. (1991). Modulation of a cloned mouse brain potassium channel. *Neuron*, **6,** 227–236.

HOSHI, T., ZAGOTTA, W.N. & ALDRICH, R.W. (1990). Biophysical and molecular mechanisms of *Shaker* potassium channel inactivation. *Science*, **250,** 533–538.

HOSHI, T., ZAGOTTA, W.N. & ALDRICH, R.W. (1991). Two types of inactivation in *Shaker* K^+ channels: effects of alterations in the carboxy-terminal region. *Neuron*, **7,** 547–556.

HOUAMED, K., TEMPEL, B. & ALMERS, W. (1989). Mouse brain gene MBK-1 codes for a voltage-sensitive channel of the delayed rectifier type. *Soc. Neurosci. Abstr.*, **15,** 538.

ISACOFF, E.Y., JAN, Y.N. & JAN, L.Y. (1991). Putative receptor for the cytoplasmic inactivation gate in the *Shaker* K^+ channel. *Nature*, **353,** 86–90.

KAVANAUGH, M.P., VARNUM, M.D., OSBORNE, P.B., CHRISTIE, M.J., BUSCH, A.E., ADELMAN, J.P. & NORTH, R.A. (1991). Interaction between tetraethylammonium and amino acid residues in the pore of cloned voltage-dependent potassium channels. *J. Biol. Chem.*, **266,** 7583–7587.

KLUMPP, D.J., FARBER, D.B., BOWES, C., SONG, E.-J. & PINTO, L.H. (1991). The potassium channel MBK1 (Kv1.1) is expressed in the mouse retina. *Cell. Mol. Neurobiol.*, **11,** 611–622.

KOREN, G., LIMAN, E.R., LOGOTHETIS, D.E., NADAL-GINARD, B. & HESS, P. (1990). Gating mechanism of a cloned potassium channel expressed in frog oocytes and mammalian cells. *Neuron*, **2,** 39–51.

LEE, C.W.B., ALDRICH, R.W. & GLERASCH, L.M. (1992). Conformational studies of the N-terminal domain of the *Shaker* B K^+ ion channel by CD and NMR. *Biophys. J.*, **61,** A379.

MCFADZEAN, I. & ENGLAND, S. (1992). Properties of the inactivating outward current in single smooth muscle cells isolated from the rat anococcygeus. *Pflügers Arch.*, **421,** 117–124.

MCLARNON, J.G. & WANG, X.-P. (1991). Actions of cardiac drugs on a calcium-dependent potassium channel in hippocampal neurons. *Mol. Pharmacol.*, **39,** 540–546.

NEWLAND, C.F., ADELMAN, J.P., TEMPEL, B.L. & ALMERS, W. (1992). Repulsion between tetraethylammonium ions in cloned voltage-gated potassium channels. *Neuron*, **8,** 975–982.

NOAK, Th., EDWARDS, G., DIETMER, P., GREENGRASS, P., MORITA, T., ANDERSSON, P.-O., CRIDDLE, D., WYLLIE, M.G. & WESTON, A.H. (1992). The involvement of potassium channels in the action of ciclazindol in rat portal vein. *Br. J. Pharmacol.*, **106,** 17–24.

PFRUNDER, D. & KREYE, V.A.W. (1991). Tedisamil blocks single large-conductance Ca^{2+}-activated K^+ channels in membrane patches from smooth muscle cells of the guinea-pig portal vein. *Pflügers Arch.*, **418,** 308–312.

ROBERTSON, B. & OWEN, D. (1993). Actions of some blockers and the *Shaker* B 'inactivation' peptide on a mouse brain cloned potassium channel (MK-1) expressed in Chinese Hamster ovary (CHO) cells. *J. Physiol.*, **459,** 92P.

RUPPERSBERG, J.P., STOCKER, M., PONGS, O., HEINEMANN, S.H., FRANK, R. & KOENEN, M. (1991). Regulation of fast inactivation of cloned mammalian $I_K(A)$ channels by cysteine oxidation. *Nature*, **352,** 711–714.

SHENG, M., TSAUR, M.-L., JAN, Y.N. & JAN, L.Y. (1992). Subcellular segregation of two A-type K^+ channel proteins in rat central neurons. *Neuron*, **9,** 271–284.

SNYDERS, D.J., KNOTH, K.M., ROBERDS, S.L. & TAMKUN, M.M. (1992). Time-, voltage-, and state-dependent block by quinidine of a cloned human cardiac potassium channel. *Mol. Pharmacol.*, **41,** 322–330.

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

SPRUCE, A.E., STANDEN, N.B. & STANFIELD, P.R. (1987). The action of external tetraethylammonium ions on unitary delayed rectifier potassium channels of frog skeletal muscle. *J. Physiol.*, **393,** 467–478.

STANSFELD, C., MARSH, S., PARCY, D., DOLLY, J.O. & BROWN, D. (1987). Mast cell degranulating peptide and dendrotoxin selectively inhibit a fast activating K^+ current and bind to common neuronal proteins. *Neurosci.*, **23,** 893–902.

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

STUHMER, W. (1991). Structure-function studies of voltage-gated ion channels. *Annu. Rev. Biophys. Biochem.*, **20,** 65–78.

STUHMER, W., STOCKER, M., SAKMANN, B., SEEBURG, P., BAUMANN, A., GRUPE, A. & PONGS, O. (1988). Potassium channels expressed from rat brain cDNA have delayed rectifier properties. *FEBS Lett.*, **242,** 199–206.

TAGLIATELA, M., VANDONGEN, A.M.J., DREWE, J.A., JOHO, R.H., BROWN, A.M. & KIRSCH, G.E. (1991). Patterns of internal and external tetraethylammonium block in four homologous K^+ channels. *Mol. Pharmacol.*, **40,** 299–307.

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

TORO, L., STEFANI, E. & LATORRE, R. (1992). Internal blockade of a Ca^{2+}-activated K^+ channel by *Shaker* B inactivating 'ball' peptide. *Neuron*, **9,** 237–245.

WANG, S.-Y., CASTLE, N.A. & WANG, G.K. (1992). Identification of RBK-1 potassium channels in C6 astrocytoma cells. *Glia*, **5,** 146–153.

WAGONER, P.K. & OXFORD, G.S. (1990). Aminopyridines block an inactivating potassium current having slow recovery kinetics. *Biophys. J.*, **58,** 1481–1489.

ZAGOTTA, W.N., HOSHI, T. & ALDRICH, R.W. (1990). Restoration of inactivation in mutants of *Shaker* potassium channels by a peptide derived from ShB. *Science*, **250,** 568–571.

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