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The contrasting effects of dendrotoxins and other potassium channel blockers in the CA1 and dentate gyrus regions of rat hippocampal slices

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1 The effects of potassium channel blocking compounds on synaptic transmission in the CA1 and dentate gyrus regions of the rat hippocampus were examined by means of simultaneous field potential recording techniques in brain slices.

2 4-Aminopyridine (4-AP) enhanced the excitatory postsynaptic potential (e.p.s.p.) and induced multiple population spike responses in both regions. EC_{50} values were 6.7 μM in the CA1 ($n=5$) and 161.7 μM ($n=5$) in the dentate gyrus.

3 Tetraethylammonium (TEA) increased the amplitude and induced broadening of the population spike in both regions. In the dentate gyrus ($n=5$) a single slow spike response was introduced (EC_{50} 12.8 mM) and in the CA1 region ($n=5$) the response was transformed into two wide spikes (EC_{50} 2.6 mM).

4 In the CA1 region all of the dendrotoxins (toxin I, toxin K, α-Dtx and δ-Dtx) induced multiple population spikes and enlarged e.p.s.p. responses. Potentials recorded simultaneously in the dentate gyrus exhibited comparatively minor enhancements. The EC_{50} value for toxin I in the CA1 was calculated to be 237 nM ($n=4$). Estimated EC_{50} values were obtained for α-Dtx (1.1 μM, $n=3$), toxin K (411 nM, $n=4$) and δ-Dtx (176 nM, $n=3$).

5 In the presence of toxin I, DL-2-amino-5-phosphonovaleric acid (APV) induced slight reduction of the late e.p.s.p. phase ($n=3$). 6-Cyano-7-nitroquinoxaline-2,3-dione (CNQX) abolished all population spikes leaving a late slow positive waveform ($n=3$). Co-application of APV and CNQX abolished all postsynaptic responses.

6 Charybdotoxin (CbTx) was significantly less potent than the dendrotoxins and had mixed actions in the CA1 region ($n=3$). Again the dentate gyrus exhibited reduced sensitivity ($n=3$).

7 In the presence of mast cell degranulating peptide (MCDP), enhancement of the CA1 field potential response ($n=5$) was greater than that observed in the dentate gyrus ($n=5$).

8 The results show that some potassium channel modulators can profoundly enhance CA1 region synaptic responses in the absence of notable changes in dentate gyrus excitability. Selective enhancement of defined synaptic pathways by potassium channel modulators may prove to have considerable therapeutic potential.

Keywords: Synaptic transmission; potassium channels; field potential; rat hippocampus; dendrotoxins

Introduction

The excitability of mammalian central neurones is governed to a large extent by the presence of membrane potassium channels (see Halliwell, 1990; Storm, 1990). A variety of voltage-gated and calcium-sensitive potassium conductances have been described in central neurones and they serve to regulate neuronal excitability by controlling factors such as the width of the action potential, interspike interval, firing patterns and neurotransmitter release. This considerable diversity of potassium channel conductances found within mammalian central neurones is reflected by the large number of genes encoding for potassium channel α- (Pongs, 1992) and β-subunits (Rettig *et al*., 1994), which combine together to form functional potassium channels (see Chandy & Gutman, 1995 for a review). By combining molecular biology and immunohistochemical technology, high resolution mapping of some potassium channel α- and β- subunits within brain regions of the mouse and rat has been made possible (Wang *et al*., 1994; Veh *et al*., 1995; McNamara *et al*., 1996; Rhodes *et al*., 1996), and these experiments reveal clustering of certain potassium channel subunits in discrete neuronal structures. This precise placement of certain potassium channel subunits within the cell membrane of central neurones has important implications for broadening our understanding of how central neurones function, and may assist in the development of selective ligands to modulate neuronal excitability in specific brain regions.

The principal aim of the present study was to investigage whether the distribution of potassium channels within the rat hippocampus allows the selective enhancement of synaptic responses within a particular neuronal pathway. We have therefore examined the activity of a panel of potassium channel blocking agents on synaptic responses recorded simultaneously in the CA1 and dentate gyrus regions of the hippocampus. The potassium channel blockers investigaged were 4-aminopyridine (4-AP) and tetraethylammonium (TEA); some dendrotoxin homologues (α-, δ-, toxin I and toxin K); mast cell degranulating peptide (MCDP) and charybdotoxin (CbTx). A preliminary account of this data has been published in abstract form (Southan & Owen, 1995).

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Methods

Preparation of tissue and solutions

Experiments were performed on rat hippocampal slices. Sprague Dawley rats (120–160 g; Charles River) of either sex were decapitated, the brain quickly dissected out, hemisected and placed in chilled oxygenated ($\leqslant 4^{\circ}C$) artificial cerebrospinal fluid (ACSF). Transverse hippocampal slices (350 μm thick) were cut either with a vibroslice (Campden Instruments) or tissue chopper (McIlwain). The slices were then left to incubate at room temperature (20–23°C) for at least one hour submerged in ACSF saturated with 95% O_2/5% CO_2. The ACSF contained (in mM): NaCl 124, KCl 3, $NaHCO_3$ 26, NaH_2PO_4 1.25, $MgSO_4$ 2, $CaCl_2$ 2, D-glucose 10 and was maintained at pH 7.35–7.45 with 95% O_2-5% CO_2.

Field potential recording

Following the recovery period, the CA1 region was isolated by means of a scalpel cut between the CA3 and CA1 regions and the slice then transferred to the recording chamber (submerged type, volume ~1 ml) and continuously perfused with ACSF at 30°C (flow rate 2–3 ml min^{-1}). Orthodromic field potential responses were evoked by bipolar stimulating electrodes (SNE-100; Clark Electromedical Instruments), one positioned in the stratum radiatum and the other stimulating the perforant path, located adjacent to the hippocampal fissure in the outer molecular layer of the upper blade of the dentate gyrus. Glass recording microelectrodes (GC150-F10; Clark Electromedical Instruments) were positioned in the CA1 and dentate gyrus soma regions and field potential responses were recorded with Axoclamp 2A amplifiers (Axon Instruments). Electrodes were filled with ACSF giving typical resistance of $\leqslant 5M\Omega$. Spike responses were digitized at 10 kHz (VR-10 digital data recorder; Instrutech Corp.) and stored on video tape for off line capture (2–5 kHz), and subsequent analysis, with a Macintosh Quadra 950 microcomputer running Axograph (v2.0) software (Axon Instruments). Only slices able to produce single population spike responses with stable amplitude greater than 5 mV in both regions of interest were used in this study. If a slice satisfied this criterion, control population spikes (standardized at ~1 mV amplitude) were evoked (stimulus parameters: 0.1 Hz/0.02 ms/5–30V) for at least 20 min before exposure to a potassium channel blocker. Cumulative dose-response relationships were created by increasing the concentration of potassium channel blocker in the ACSF every twenty minutes. Twenty minutes was found to be a sufficient period of time for drug effects to reach steady state. Four consecutive potentials were averaged for each time point and the area under the population spike(s) measured by use of Acknowledge (v2.0.10, Biopack systems Inc., U.S.A.) or Axograph (v2.0, Axon Instruments, U.S.A.) software packages. The shaded areas shown in Figure 1 illustrate how population spike area was quantified. Measurements from individual experiments were combined and have been expressed as a percentage change from the area of the control response ($\pm$s.e.mean). Statistical significance was determined by means of Student's *t*-test (Statview II, Abacus Concepts Inc.). Dose-response curves were fitted with the logistic function:

$$\text{Response} = \text{maximum response}/1 + (\text{concentration}/EC_{50})$$

Where only minor changes in excitability were observed (i.e. dendrotoxin effects in the dentate gyrus, and the data obtained with MCDP and CbTx in both regions) the data were not fitted with the above function.

Patch clamp recording

CA1 neurone somatic whole cell patch clamp recordings were made by use of Axopatch-1C (Axon Instruments) amplifier, controlled by pCLAMP software (v5.1; Axon Instruments) running on a Dell 325D microcomputer (Dell Computer Corp.). Patch electrodes were fabricated from borosilicate glass (GC150-F10, Clark Electromedical Instruments) by a DMZ Universal Electrode puller (List). Electrode resistance was typically 4–6MΩ when filled with an intracellular medium consisting of (in mM): KCl 140, $MgCl_2$ 1, $CaCl_2$ 1, EGTA 10, Na-ATP 2, HEPES 10; pH 7.3. The ACSF (see above) was supplemented with 1 μM tetrodotoxin to block voltage-activated sodium currents. Potassium currents were leak and capacity subtracted by use of a p/4 protocol and were evoked from a holding potential of −70 mV, filtered at 1 kHz and sampled at 3–5 kHz; 70–90% series resistance compensation was used throughout. Data analysis was carried out by use of pCLAMP (v5.1) and Axograph software (v2.0) (Axon Instruments).

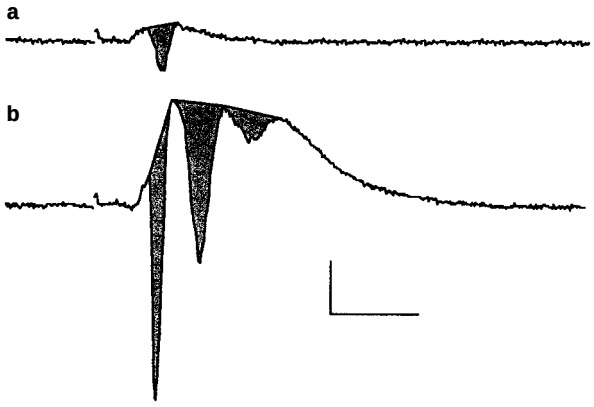

Figure 1 Example potentials illustrating how the field potential area was quantified. The shaded regions correspond to the area measured in control solution (a) and during multiple population spiking in the presence of a potassium channel blocker (b). Calibration bars: vertical 1 mV, horizontal 10 ms.

Drugs

Toxin I and toxin K were purified from black mamba *Dendroaspis polylepis* venom by use of previously described methods (Robertson *et al.*, 1996). The other dendrotoxins (α-Dtx and δ-Dtx) and synthetic charybdotoxin were obtained from Alomone Labs (Jerusalem). Synthetic mast cell degranulating peptide (MCDP) and 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX) were supplied by Research Biochemicals International (U.S.A.). 4-Aminopyridine (4-AP), tetraethylammonium (TEA) and DL-2-amino-5-phosphonovaleric acid (APV) were obtained from Sigma (U.K.). All salts were ANALAR or equivalent grade and were obtained from BDH Laboratory Supplies (U.K.) or Sigma (U.K.).

Results

Standard potassium channel blockers

In both the CA1 region (Figure 2a; $n=5$) and the dentate gyrus (Figure 2b; $n=5$), 4-AP increased the amplitude of the orthodromic population spike response and, at concentrations of 100 μM and above, induced multiple population spike discharges riding upon a greatly enlarged excitatory postsynaptic potential (e.p.s.p.) component. In high concentrations of 4-AP (1–10 mM) the mean increase in population spike area was similar for both regions. For example, in 1 mM 4-AP, enhancements of 493.8±118.8% ($P<0.05$) in the CA1 region and 554.4±133.8% ($P<0.05$) in the dentate gyrus were observed. The CA1 region was found to be more sensitive to low concentrations of 4-AP (1–100 μM) and this is reflected in the EC_{50} values of 6.7 μM in the CA1 and 161.7 μM in the dentate gyrus (Figure 2c).

In the CA1 region, TEA (0.5–30 mM $n=5$) induced a gradual increase in amplitude and a broadening of the population spike. Alongside this, a long slow second spike was introduced (Figure 2d). In 30 mM TEA population spike area had increased by 456.5±175.8% ($P<0.05$) and the EC_{50} for the CA1 region was calculated to be 2.6 mM. In the dentate gyrus, enhancement of spike responses occurred over a similar concentration range to that found for the CA1 region, although the maximum percentage increase in area was greater than that observed in the CA1 region due to the spike response being transformed into a single slow wave (Figure 2e; $n=5$). Mean percentage enhancement was 866.9±88.3% ($P<0.05$) in 30 mM TEA and the EC_{50} value was 12.8 mM (Figure 2f).

Dendrotoxins

In the CA1 region the dendrotoxin homologues were all able to induce a characteristic profile of multiple population spikes riding upon an enlarged e.p.s.p.; although differences between the potency of individual homologues was apparent. Potentials recorded simultaneously in the dentate gyrus exhibited comparatively minor enhancements over the same concentration range and displayed little or no tendency to-

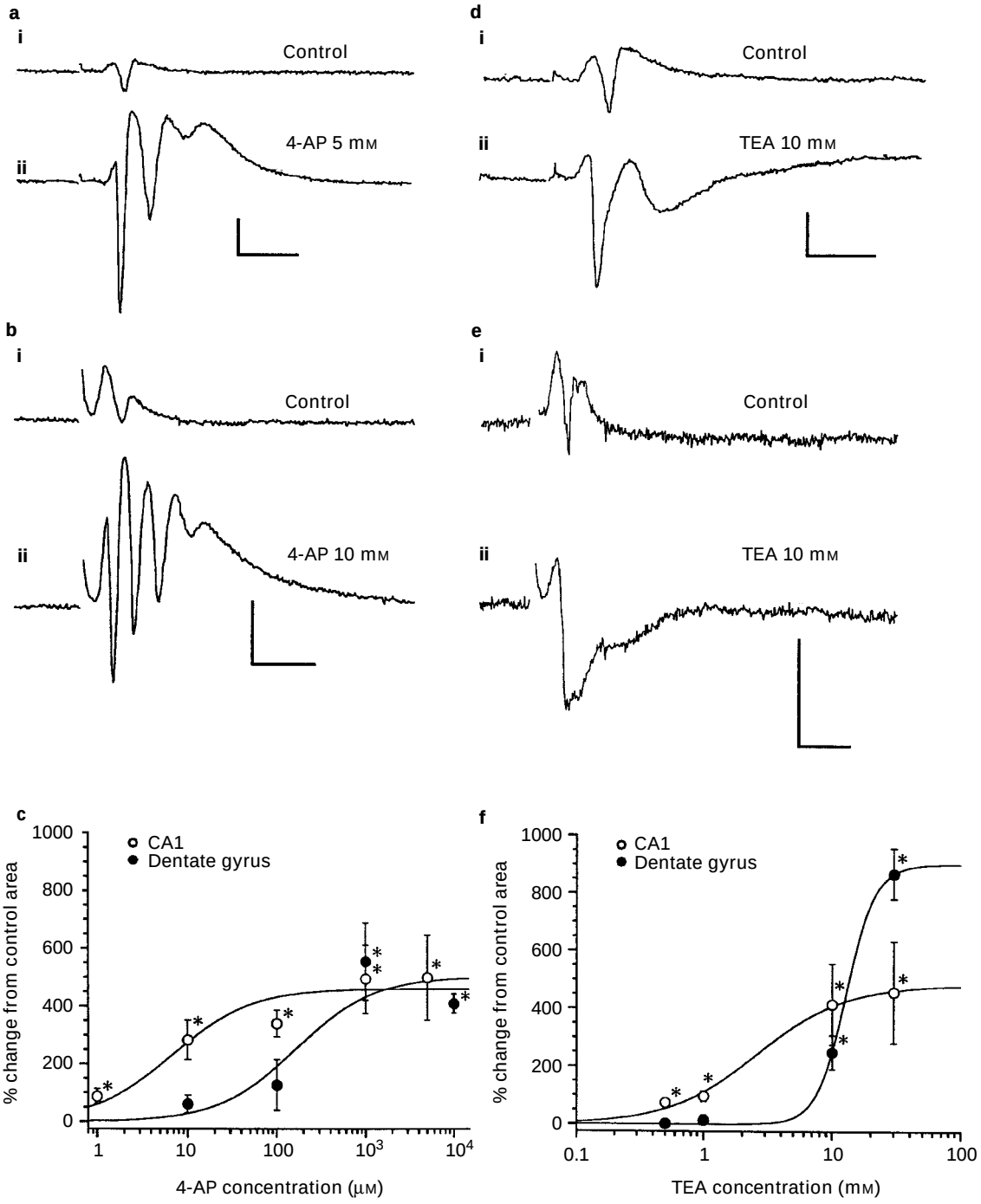

Figure 2 4-AP and TEA modified both CA1 and dentate gyrus field potential responses. (ai) Control CA1 field potential response. (aii) Response from the same slice in the presence of 5 mM 4-AP. (bi) Control dentate gyrus field potential response. (bii) Response from the same slice in the presence of 10 mM 4-AP. (c) Graph illustrating the percentage change in population spike area in both the CA1 region and the dentate gyrus through a range of concentrations of 4-AP. (di) Control CA1 field potential response. (dii) Modification of the response from the same slice by 10 mM TEA. (ei) Control dentate gyrus field potential. (eii) Response from the same slice in 10 mM TEA. (f) Graph illustrating percentage change from control area in both the CA1 and dentate gyrus regions for a range of concentrations of TEA. Calibration bars: vertical 1 mV, horizontal 10 ms. *Significantly different from control value ($P<0.05$).

wards repetitive firing. Due to limited supplies of the dendrotoxins, concentrations were limited to a maximum of 1 μM, with the exception of toxin I, where a full dose-response relationship (1 nM – 10 μM) was obtained. Estimated EC_{50} values are therefore presented below for the CA1 region in the presence of toxin K, α-Dtx and δ-Dtx.

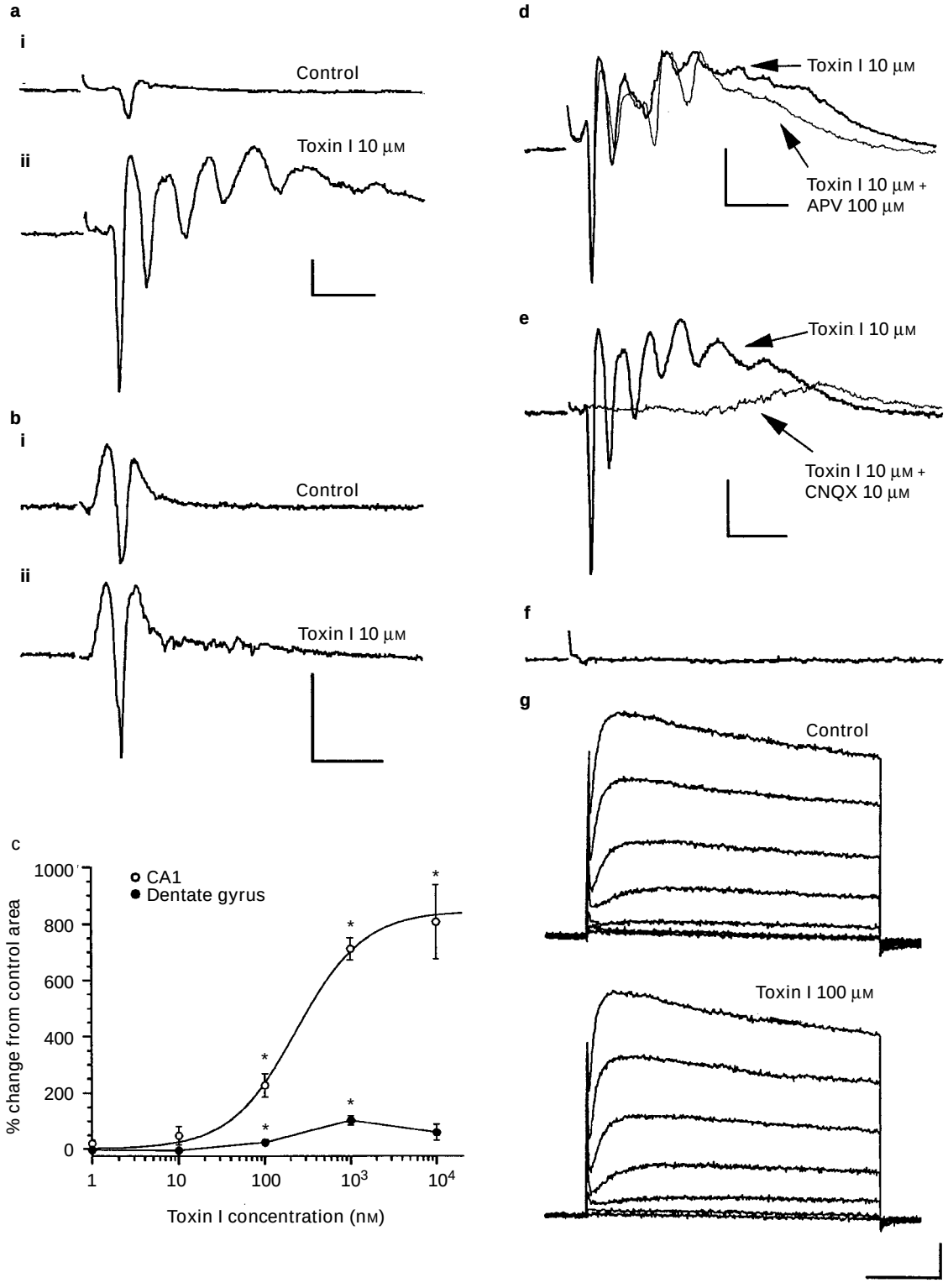

Figure 3 Toxin I had profound effects on CA1 excitability whilst simultaneous recordings in the dentate gyrus region showed little modification. (ai) Control orthodromic CA1 field potential response. (aii) Response from the same slice in the presence of 10 μM toxin I. (bi) Control dentate gyrus field potential response. (bii) Response from the same slice in the presence of 10 μM toxin I. (c) Dose-response relationship showing percentage change from control spike area in the presence of toxin I in the CA1 and dentate gyrus regions. (d) In the presence of 10 μM toxin I, a 20 min application of 100 μM APV slightly reduced the late phase of the enhanced e.p.s.p. (e) In the presence of 10 μM toxin I, a 20 min application of 10 μM CNQX abolished the early e.p.s.p. and spike responses, leaving a late slow positive potential. (f) Co-application of 100 μM APV and 10 μM CNQX abolished postsynaptic responses in the presence of toxin I (10 μM). (g) At a time point where obvious potentiation of orthodromic CA1 field potentials would be evident, 100 nM toxin I had no discernible effect on CA1 neurone somatic potassium currents evoked by 800 ms depolarizing voltage pulses. Calibration bars: field potentials: vertical 1 mV, horizontal 10 ms; Whole cell currents: vertical 1 nA, horizontal 200 ms. *Significantly different from control value ($P<0.05$).

Toxin 1

In the CA1 region, toxin I enhanced orthodromic population spike responses and at concentrations of 100 nM and above considerably enlarged the population e.p.s.p. and induced multiple (up to six additional spikes) population spike discharges (Figure 3a). Potentials recorded simultaneously in the dentate gyrus were only marginally enhanced (Figure 3b). For example, application of 10 μM toxin I increased the mean area under CA1 population spikes by $808.4 \pm 131.4\%$ ($n=4$, $P<0.05$), whilst at the same time point, mean enhancement in the dentate gyrus was by $61.2 \pm 28.7\%$ ($n=4$, $P>0.05$). The EC_{50} value for toxin I-induced enhancement of CA1 region field potential area was 237 nM (Figure 3c).

In further experiments conducted in the presence of 10 μM toxin I, application of the selective NMDA receptor blocker DL-2-amino-5-phosphonovaleric acid (APV; 100 μM), caused a slight reduction of the late e.p.s.p. component (Figure 3d; $n=3$). In three additional experiments in the presence of 10 μM toxin I, application of the selective AMPA receptor blocker (CNQX, 10 μM) abolished the e.p.s.p. and population spikes leaving a late slow positive waveform (Figure 3e). Co-application of CNQX and APV eliminated all postsynaptic responses (Figure 3f).

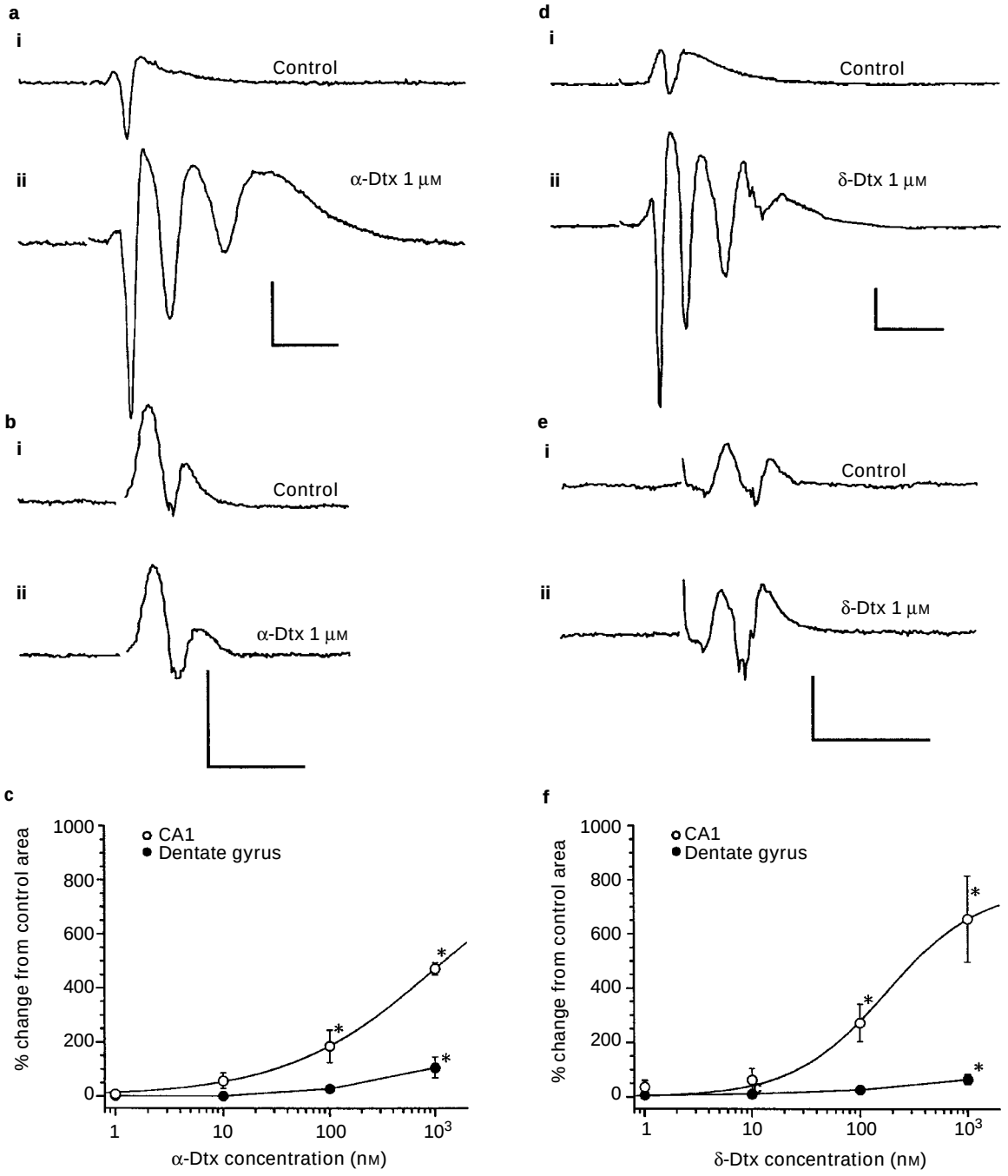

Figure 4 The effects of α-Dtx and δ-Dtx were similar to those of toxin I in both the CA1 and dentate gyrus regions. (ai) Control orthodromic CA1 field potential response. (aii) Response from the same slice in the presence of 1 μM α-Dtx. (bi) Control orthodromic dentate gyrus field potential response. (bii) Dentate gyrus response from the same slice in the presence of 1 μM α-Dtx. (c) Graph showing percentage enhancement of CA1 and dentate gyrus population spike area for a range of concentrations of α-Dtx. (di) Control orthodromic CA1 field potential response. (dii) Response from the same slice in the presence of 1 μM δ-Dtx. (ei) Control orthodromic dentate gyrus field potential response. (eii) Dentate gyrus field potential response from the same slice in the presence of 1 μM δ-Dtx. (f) Graph showing percentage change in CA1 and dentate gyrus population spike area for a range of concentrations of δ-Dtx. Calibration bars: vertical 1 mV, horizontal 10 ms. *Significantly different from control value ($P<0.05$).

α-Dendrotoxin

The effects of α-Dtx (1 nM – 1 μM) were qualitatively similar to those of toxin I in both the CA1 and dentate gyrus regions (Figure 4a and b). In the CA1 region multiple population spikes and an enlarged e.p.s.p. were seen at concentrations of 100 nM and above, with mean enhancement of spike area being by 471.7 ± 22.8% ($P<0.05$) in the presence of 1 μM α-Dtx ($n=3$, Figure 4c). The estimated EC_{50} value in the CA1 region was 1.1 μM. In the dentate gyrus, 1 μM α-Dtx induced a small second population spike (<0.5 mV) in two out of the four potentials examined. The mean maximum enhancement of spike area was 106.6 ± 39.0% ($P<0.05$) at a concentration of 1 μM α-Dtx ($n=4$, Figure 4c).

δ-Dendrotoxin

Again, a marked contrast between the enhancement in the CA1 and the effects in the dentate gyrus was observed during administration of δ-Dtx (1 nM – 1 μM). Significant enlargement of the e.p.s.p. and multiple population spikes were observed in the CA1 region in both 100 nM and 1 μM δ-Dtx (Figure 4d). In 1 μM δ-Dtx the mean enhancement observed was 655.8 ± 64.9% ($P<0.05$, $n=3$) and the estimated EC_{50} value calculated to be 176 nM (Figure 4f). In the dentate gyrus ($n=4$) a second population spike (<0.5 mV) was introduced in one experiment and the maximum mean enhancement of spike area in the presence of 1 μM δ-Dtx was by 64.9 ± 17.8% ($P<0.05$, Figure 4).

Toxin K

In the CA1 region, the effects of toxin K (1 nM – 1 μM) were again qualitatively similar to the other dendrotoxins (Figure 5a), although only minor enhancements were noted at concentrations below 1 μM. With 1 μM toxin K, the mean enhancement in the CA1 region was 689.2 ± 215.2% ($P<0.05$, $n=4$). The curve fit gave an estimated EC_{50} value of 411 nM (Figure 5c). In the dentate gyrus only minor enhancements were noted at concentrations up to and including 1 μM toxin K (maximum mean enhancement being 121.2 ± 41.9%; $P<0.05$, $n=4$; Figure 5b), with one potential exhibiting a small second population spike (<0.5 mV). To demonstrate that the lack of effect of the dendrotoxins was not due to our dentate preparation being 'unhealthy' or inexcitable, we added 50 μM bicuculline to slices previously found to be resistant to enhancement by 1 μM toxin K. Bicuculline was found to enhance markedly dentate gyrus responses in these slices (Figure 5d, $n=2$).

Other polypeptide potassium channel blockers

Charybdotoxin CbTx had mixed effects in the CA1 region over the concentration range tested (1 nM – 1 μM, $n=3$). In two preparations, a single population spike response, having increased width and amplitude, was observed (Figure 6a). The remaining preparation displayed e.p.s.p. enhancement and multiple population spikes at the highest concentration tested. Overall, at a concentration of 1 μM CbTx, there was a mean enhancement of CA1 population spike area of 201 ± 47.3% ($P<0.05$). In the dentate gyrus, spike area increased by 146.4 ± 26.0% ($P<0.05$) with one preparation exhibiting a small second spike (~0.5 mV) in 1 μM CbTx (Figure 6b,c).

Mast cell degranulating peptide In the presence of MCDP (0.64 nM – 6.4 μM) enhancement of CA1 region potentials was again accompanied by relatively small increases in dentate gyrus excitability. Enhancement of CA1 field potentials was not as marked as that seen with the dendrotoxins, with the maximum mean enhancement being 327.8 ± 63.5% (6.4 μM MCDP, $P<0.05$, $n=5$) and only one out of five potentials exhibiting notable repetitive firing (see Figure 6d). In the dentate gyrus, no multiple spiking was observed and the mean

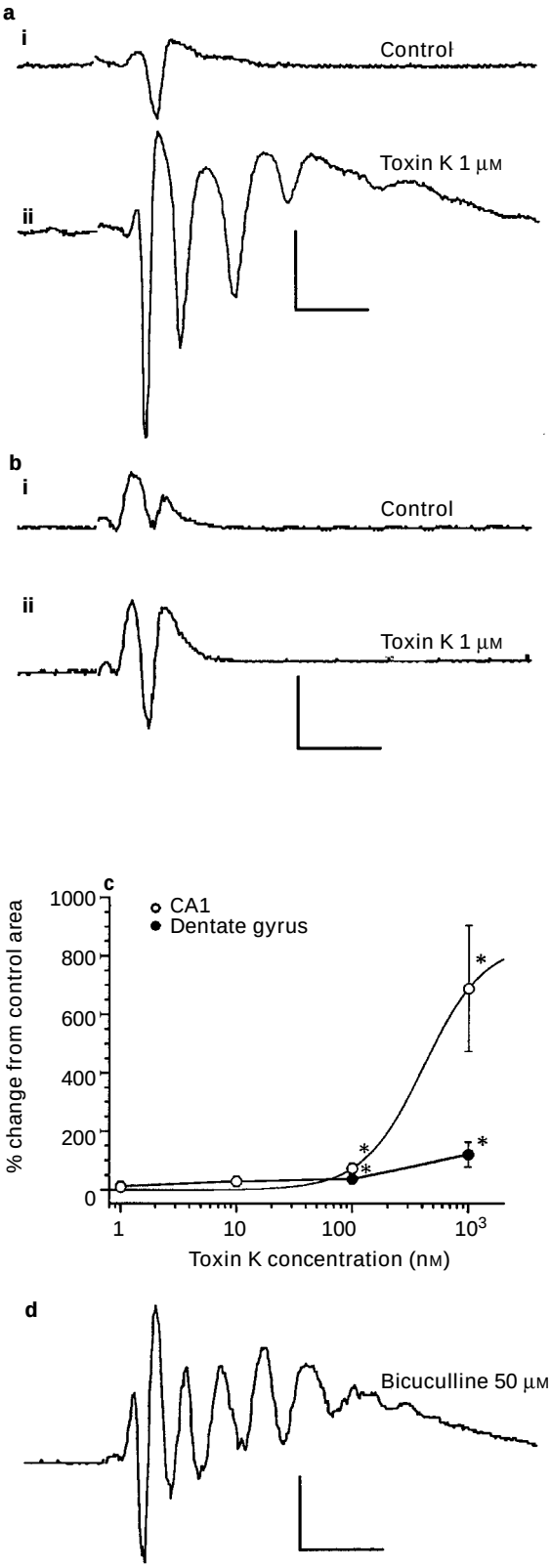

Figure 5 Toxin K had effects comparable to the other dendrotoxins tested, but required higher concentrations to induce significant potentiation of CA1 field potential responses. (ai) Control orthodromic CA1 response. (aii) CA1 response from the same slice following exposure to 1 μM toxin K. (bi) Control orthodromic dentate gyrus field potential response. (bii) Dentate gyrus response from the same slice following exposure to 1 μM toxin K. (c) Dose-response relationship for the change in CA1 and dentate gyrus spike area for a range of toxin K concentrations. (d) Dentate gyrus field potential response following a 10 min exposure to 50 μM bicuculline (same slice as potentials illustrated in b, bicuculline added after 38 min washout of toxin K). Calibration bars: vertical 1 mV, horizontal 10 ms. *Significantly different from control value ($P<0.05$).

enhancement was 35.9±20.3% ($P>0.05$) at 6.4 μM MCDP (Figure 6e, f; $n=5$).

Whole cell patch clamp experiments

In ACSF containing 1 μM tetrodotoxin, CA1 neurone somatic whole cell potassium currents were evoked by a series of depolarizing voltage steps (+10 mV increments) from a holding potential of −70 mV. Toxin I (100 nM) had no significant effect on the whole cell potassium currents ($n=3$; Figure 3g). For example, after ten minutes exposure to toxin I, the mean steady state current amplitude during a step from −70 mV to +10 mV was 108.4±7.3% of control. The amplitude of the fast inactivating 'A type' current was also unaffected during the application of toxin I, mean current amplitude being 96.6±2.7% of control during an identical step. In additional experiments with an identical protocol, 400 nM toxin K ($n=2$) and 100 nM α-Dtx ($n=2$) also had no marked effects on the amplitude and characteristics of CA1 whole cell somatic potassium currents. Mean amplitude was 94.5% and 87.0% of control steady state current and 95.5% and 85.4% of control fast inactivating current, respectively.

Discussion

We have examined the actions of a number of potassium channel blocking agents on orthodromic field potential responses recorded simultaneously in the CA1 and dentate gyrus regions of the rat hippocampal slice preparation. The key findings of this series of experiments were that (i) the dendrotoxins, at the concentrations used, markedly enhanced sy-

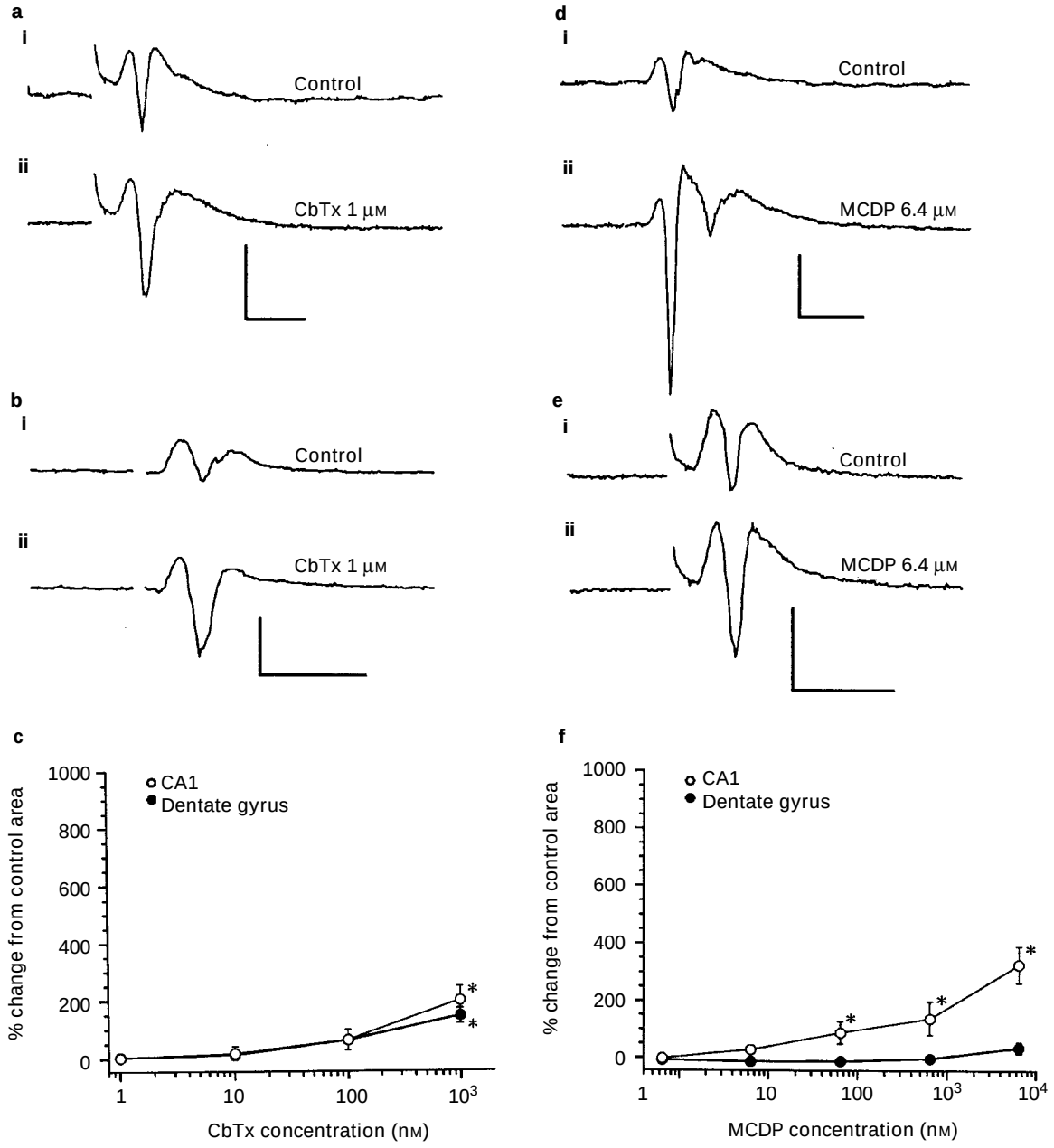

Figure 6 (ai) Control orthodromic CA1 field potential. (aii) Response from the CA1 region of the same slice in the presence of 1 μM charybdotoxin (CbTx). (bi) Control orthodromic dentate gyrus field potential. (bii) Response from the dentate gyrus region of the same slice in the presence of 1 μM CbTx. (c) Graph showing the increase in CA1 and dentate gyrus population spike area for a range of concentrations of CbTx. (di) Control orthodromic CA1 field potential. (dii) Response from the CA1 region of the same slice in the presence of 6.4 μM MCDP. (ei) Control orthodromic dentate gyrus field potential. (eii) Response from the dentate gyrus region of the same slice in the presence of 6.4 μM MCDP. (f) Graph illustrating the increase in CA1 and dentate gyrus population spike area for a range of concentrations of MCDP. Calibration bars: vertical 1 mV, horizontal 10 ms. *Significantly different from control value ($P<0.05$)

naptic responses in the CA1 region and induce comparatively minor changes in dentate gyrus excitability; (ii) the enhancement of CA1 responses by toxin I was dramatically reduced by α-amino-3-hydroxy-5-methyl-4-isoxazolapropionate (AMPA) receptor antagonists; (iii) 4-AP induced multiple population spiking in both regions, although the CA1 region was more sensitive to low concentrations; (iv) the enhancement of CA1 responses by CbTx and MCDP was less than that observed for comparable concentrations of the dendrotoxins, the dentate gyrus again exhibited comparative insensitivity.

The field potential data presented in this study highlights potentially important differences between the sensitivity of two important subfields of the hippocampus to potassium channel blocking agents. Most notably, our experiments demonstrate that the dendrotoxins induce profound enhancement of CA1 region orthodromic potentials which is accompanied by only minor changes in dentate gyrus excitability. We also observed interesting differences between the potency of the individual dendrotoxin homologues in the CA1 region.

Toxin K has been shown to block selectively homomultimeric rat Kv1.1 channels in preference to the other dendrotoxin sensitive channels Kv1.2 and Kv1.6 (Robertson *et al*., 1996 ; Owen *et al*., 1997). Compared to the other dendrotoxins, higher concentrations of toxin K (>100 nM) were required to induce significant potentiation of the CA1 orthodromic field potential. This result may be a reflection of this selectivity of toxin K for Kv1.1 channels. Kv1.1 channel protein immunostaining has been detected in the axons and fine terminals of CA3 neurones of the mouse (Wang *et al*., 1994) and toxin K may enhance synaptic transmission through the CA3-CA1 synapse by blocking Kv1.1 channels in these CA3 terminal processes. Kv1.2 channel protein immunoreactivity has been found in CA1 neurone dendrites and stratum lacunosum moleculare of the rat (Sheng *et al*., 1994) and homomultimeric rat Kv1.2 channels have been shown to be blocked by toxin I, δ-Dtx and α-Dtx (Stühmer *et al*., 1989; Pongs *et al*., personal communication). These toxins may therefore influence CA1 excitability at lower concentrations than toxin K due to blocking activity at both Kv1.1 and Kv1.2 channel subunits. Our preliminary whole cell patch clamp experiments demonstrated that toxin I, toxin K and α-Dtx have no discernible effects on CA1 neurone somatic potassium currents (see also Southan & Owen, 1994) and similar observations have been published for dendrotoxin (Klee *et al*., 1995). This supports the immunohistochemical evidence for the dendrotoxin-sensitive channel subunits being located in nerve terminal compartments in the hippocampus.

The profound effect of CNQX on the repetitive firing induced by toxin I in the CA1 region would indicate that the primary action of this particular dendrotoxin is to enhance the evoked release of glutamate, acting mainly at AMPA receptor coupled channels. The *N*-methyl-D-aspartate (NMDA) component of the response forming a relatively minor, late component of the toxin I enhanced field potential. This result is consistent with the present knowledge of the roles of excitatory amino acid receptors in the CA1 region, NMDA receptors contributing only during prolonged depolarization or repetitive stimulation (Collingridge *et al*., 1988). Similar results have been obtained in the CA1 region with the γ-aminobutyric acid $(GABA)_A$ receptor antagonist bicuculline (Dingledine *et al*., 1986; Williamson & Wheal, 1992) and following kainic acid lesion (Ashwood & Wheal, 1986).

In the dentate gyrus, Kv1.1 and Kv1.6 immunoreactivity has been detected in granule cell dendrites and Kv1.2 immunostaining has been localized in perforant path terminals. These observations suggested that the dendrotoxins would have significant effects in this region. The striking lack of potency of any of the dendrotoxin homologues was therefore particularly surprising. It has previously been established that the dentate gyrus has a higher threshold for seizure type activity than the CA1 and CA3 regions (Schweitzer *et al*., 1992; Bear & Lothman, 1993; Colom & Saggau, 1994). However, the observations from these studies, and our results with 4-AP, illustrate that significant enhancement of the dentate evoked field potential can be achieved given sufficient stimulus. Even in the absence of multiple population spikes, significant changes in the area of the first population spike might have been expected following exposure to the dendrotoxins. However, this was not evident in any of our dendrotoxin experiments and even in the presence of extremely high concentrations of toxin I (10 μM), only very minor potentiation of the evoked population spike was revealed. Some authors have proposed that the resting membrane potential for granule cells could be as high as −85 mV, rendering these neurones less excitable than CA1 neurones (Lambert & Jones, 1990). However, in the same study, the authors noted great difficulty in evoking population spike responses which contrasts with both our experience and other *in vitro* studies (see Lambert & Jones, 1990 for references). The underlying reason for the lack of significant effect of the dendrotoxins in this region is presently unclear. The distribution and/or the subunit composition of the native heteromultimeric channels may provide a tentative explanation for our observations. In contrast to CA3 nerve terminals, Kv1.1 appears to be absent in perforant path nerve terminals and, if dendrotoxin activity at channels with Kv1.1 subunits is the predominant reason for the marked effects on the CA1 field potential, we might expect to see a greatly reduced response in the dentate gyrus.

In addition to its now well-known blocking activity at native BK/maxi-K channels (Miller *et al*., 1985), charybdotoxin has also been shown to block various Kv channels such as Kv1.2, Kv 1.3 (Stühmer *et al*., 1989; Grissmer *et al*., 1994) and Kv1.1 (Stühmer *et al*., 1989) in heterologous expression systems. However, in the present study we used recombinant CbTx which is not a potent blocker of Kv1.1 at least (see Grissmer *et al*., 1994). We observed an increase in width of population spikes which could be due to block of maxi-K Ca^{2+} activated K^+ channels resulting in a slowing of the repolarization phase of individual action potentials (Storm, 1987). The absence of dramatic changes in field potential excitability and the fact that there were no significant differences between the actions of CbTx in the CA1 and dentate gyrus regions, tends to rule-out an involvement of BK and Kv1.3 channels and is consistent with the idea that Kv1.1 subunits may mediate the enhancement of CA1 potentials by dendrotoxin homologues.

MCDP blocks both Kv1.1 and Kv1.2 channels (Stühmer *et al*., 1989; Grissmer *et al*., 1994). The selective nature of this peptide for Kv1.1 and Kv1.2 combined with our observations on the CA1 and dentate gyrus field potentials suggest that MCDP acts in a similar manner to the dendrotoxins, but acts with reduced potency at the native channels.

In heterologous expression systems, 4-AP and TEA display significantly less selectivity for individual potassium channel subtypes and require the use of much higher concentrations compared to the peptide blocking agents used in this study (Grissmer *et al*., 1994). In hippocampal CA1 neurones, the major difference between the actions of the two agents is that 4-AP selectively blocks a small slowly inactivating current I_D at low concentrations (Storm, 1988) and the fast inactivating 'A-type' current at higher concentrations (Segal & Barker, 1984); whilst TEA blocks the essentially non-inactivating, delayed rectifier current (Segal & Barker, 1984). The block of I_D by low micromolar concentrations of 4-AP could be responsible for the selective enhancement of the CA1 field potential in the low concentrations of 4-AP used in this study. The dramatic e.p.s.p. enhancement we observed suggests that 4-AP blocks presynaptic potassium channels in both the dentate gyrus and CA1 regions facilitating glutamate release. The widening of the population spike observed with TEA was presumably due to block of the current underlying the fast after-hyperpolarization and, at the higher concentrations, the introduction of a slow inward Ca^{2+} current, both actions contributing towards slowing postsynaptic spike repolarization (see Storm, 1987). The channel subtypes underlying this activity are at present unclear, although homomultimeric Kv1.2 channels have been shown to be insensitive to TEA (Stühmer *et al*., 1989) and

therefore Kv1.2 subunits are unlikely to play a dominant role in the TEA-induced enhancement of the field potentials.

This series of experiments has demonstrated some interesting and conceivably important actions of potassium channel blocking agents on synaptic responses in the mammalian hippocampus. Knowledge of the distribution and subunit composition of native potassium channels in the CNS may facilitate the design of novel agents to modulate the function of specific channels, thereby altering the excitability of defined neuronal pathways. As our knowledge of the molecular basis of neuronal form and function expands, this approach may prove to have considerable therapeutic potential.

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