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Modulation of inhibitory post-synaptic currents (IPSCs) in mouse cerebellar Purkinje and basket cells by snake and scorpion toxin K^+ channel blockers

[1,2]Andrew P. Southan & [1]Brian Robertson

[1]Electrophysiology Group, Department of Biochemistry, Imperial College of Science, Technology and Medicine, Exhibition Road, London SW7 2AY

1 Using an *in vitro* mouse cerebellar slice preparation and whole-cell electrophysiological recording techniques we have characterized Purkinje and basket cell inhibitory post-synaptic currents (IPSCs), and examined the effects of a number of selective peptidergic K^+ channel blockers.

2 Spontaneous IPSC amplitude ranged from ~10 pA up to ~3 nA for both cell types [mean values: Purkinje cells -122.8 ± 20.0 pA ($n=24$ cells); basket cells -154.8 ± 15.9 pA ($n=26$ cells)]. Frequency varied from ~3 up to ~40 Hz, [mean values: basket cells 14.9 ± 1.7 Hz ($n=26$ cells); Purkinje cells 17.9 ± 2.2 Hz ($n=24$ cells)]. 5 μM bicuculline eliminated virtually all spontaneous currents.

3 IPSC rise times were fast (~0.6 ms) and the decay phase was best fit with the sum of two exponential functions (τ_1 and τ_2: ~4 ms and ~20 ms, $n=40$; for both cell types).

4 The snake toxins alpha-dendrotoxin (α-DTX) and toxin K greatly enhanced IPSC frequency and amplitude in both cell types; the closely related homologues toxin I and gamma-dendrotoxin (γ-DTX) produced only marginal enhancements (all at 200 nM).

5 Two scorpion toxins, margatoxin (MgTX) and agitoxin-2 (AgTX-2) had only minor effects on IPSC frequency or amplitude (both at 10 nM).

6 Low concentrations of tetraethylammonium (TEA; 200 μM) had no overall effect on cerebellar IPSCs, whilst higher concentrations (10 mM) increased both the frequency and amplitude.

7 The results suggest that native K^+ channels, containing Kv1.1 and Kv1.2 channel subunits, play an influential role in controlling GABAergic inhibitory transmission from cerebellar basket cells.

Keywords: Potassium channels; mouse cerebellum; dendrotoxins; scorpion toxins; inhibitory synaptic transmission

Introduction

Neurotransmitter release is dependent on calcium entry during the presynaptic action potential (Katz, 1969). For invertebrate preparations such as the squid stellate ganglion (Augustine, 1990) and *Aplysia* sensory neurones (Hochner *et al*., 1986), a delayed-rectifier type K^+ conductance regulates the release of excitatory neurotransmitter by limiting the entry of calcium during presynaptic action potentials. In the vertebrate CNS relatively little is known about the contribution of such presynaptic K^+ currents in controlling neurotransmitter release. We are interested in determining whether neurotransmitter release in mammalian central synapses is limited by presynaptic K^+ currents and to this end have chosen to study the inhibitory synaptic connections between cerebellar basket cells and Purkinje neurones.

Purkinje cells are the sole efferent pathway from the cerebellar cortex and their output is largely controlled by basket cells through unique GABAergic axon collaterals terminating directly on (or close by) the Purkinje cell body and axon hillock, forming the pericellular basket and 'pinceau' regions respectively (Ramón y Cajal, 1911; Palay & Chan-Palay, 1974). Since the Purkinje cell axon initial segment is the region of action potential generation (Andersen *et al*., 1963; Stuart & Häusser, 1994), these synapses are perfectly located to influence the final outcome of cerebellar integration. Immunoreactivity to DTX-sensitive potassium channel α-subunit antibodies has been shown to be selectively localized to basket cell terminal processes (e.g. McNamara *et al*., 1993) and we have recently shown, through targeted patch-clamp recordings, that α-DTX blocks a proportion of the voltage-gated potassium current in basket cell nerve terminals, whilst somatic currents remained unaffected (Southan & Robertson, 1998). These α-DTX sensitive K^+ channels clustered in nerve terminal regions could play a substantial role in regulating the release of GABA at this synapse.

The dendrotoxins are a family of homologous proteins isolated from mamba snake venom, consisting of 59–61 amino acids in a single chain, cross linked by three disulphide bridges. They are potent and selective blockers of particular types of neuronal voltage-gated K^+ channels (Dolly *et al*., 1994; Harvey, 1997), with their molecular targets being the cloned channel subunits Kv1.1, 1.2 and 1.6 (Stühmer *et al*., 1989). Toxin K and δ-DTX (which are almost identical) are even more selective than the other DTX homologues and block only Kv1.1 subunits (Hopkins *et al*., 1996; Robertson *et al*., 1996). Furthermore, two toxins recently isolated from scorpion venom, margatoxin (MgTX) and agitoxin-2 (AgTX-2), are also high affinity blockers of certain Kv subunits expressed in oocytes (Garcia *et al*., 1994; Hopkins *et al*., 1996; Koch *et al*., 1997) and may be useful adjuncts to the DTXs when examining native CNS K^+ channels.

This paper describes some properties of IPSCs originating from basket cell terminals making synaptic contact with both Purkinje cells and other basket cells, and investigates how their amplitude and frequency are altered by a variety of DTX homologues (α-DTX, δ-DTX, toxin I and toxin K), two

[2] Author for correspondence.

scorpion toxins (MgTX and AgTX-2) and tetraethylammonium (TEA).

Methods

Preparation of tissue and solutions

Experiments were performed on mouse cerebellar slices using established methodology (Southan & Robertson, 1998). In brief, male TO mice (3–5 weeks old; Charles River) were decapitated following cervical dislocation and the brain immediately dissected out and placed in a chilled (~4°C) oxygenated, sucrose-based artificial cerebro-spinal fluid (ACSF) solution. This sucrose ACSF was identical to the standard ACSF (see below) with the exception of isosmotic substitution of sodium chloride with sucrose. Saggital cerebellar slices (250 μm thick) were then cut in cold sucrose ACSF solution using a Vibroslice (Campden Instruments, Loughborough, U.K.). Slices were then transferred to a submerged-type incubation chamber containing the standard ACSF solution saturated with 95% O_2/5% CO_2 at room temperature (20–23°C). The standard ACSF contained (in mM):- NaCl 124, KCl 3, $NaHCO_3$ 26, NaH_2PO_4 2.5, $MgSO_4$ 2, $CaCl_2$ 2, D-glucose 10 and was maintained at pH 7.35–7.45 with 95% O_2/5% CO_2.

Patch clamp recording

Slices were placed in a glass bottomed recording chamber (volume ~1 ml) and perfused at 3–5 ml min^{-1} with ACSF. Neurones were visualized with a Zeiss fluid immersion lens (numerical aperture 0.9) at 630× magnification using an Axioskop FS microscope (Carl Zeiss, Oberkochen, Germany) equipped with differential interference contrast (DIC) and fluorescence optics (excitation wavelength 395–440 nm, Carl Zeiss). Basket cells were selected according to their characteristic size and location in the lower third of the molecular layer (Palay & Chan-Palay, 1974); their identity was confirmed by fluorescence microscopy following electrophysiological recording (see Southan & Robertson, 1998). Purkinje cells were identified by their large size and distinctive arrangement in the cerebellar folia. Somatic whole-cell patch-clamp recordings were made using an EPC-9 amplifier (HEKA Electronik, Lambrecht, Germany), controlled by Pulse software (v8.05; HEKA) running on a Macintosh computer (Power PC, 7500/100). Patch electrodes were fabricated from filamented borosilicate glass (GC150-F10, Clark Electromedical Instruments, Reading, U.K.) using a PP83 microelectrode puller (Narishige, Tokyo, Japan). Electrode resistance was typically between 3 and 6 MΩ when filled with an intracellular solution consisting of (in mM): KCl 140; $MgCl_2$ 1; $CaCl_2$ 1; EGTA 10; HEPES 10; pH 7.3. For recording from basket cells the intracellular solution was supplemented with 1–4 mg ml^{-1} lucifer yellow (Sigma, Poole, U.K.) to facilitate subsequent unambiguous identification. For both cell types spontaneous postsynaptic currents were recorded at a holding potential of −90 mV, responses were filtered at 2 or 3 kHz and sampled between 6 and 9 kHz. 70–90% series resistance compensation was used throughout. Data analysis was carried out using Axograph software (v3.5; Axon Instruments, California, U.S.A.), with all detected events included in the analysis being confirmed by eye as spontaneous synaptic currents during the analysis procedure. Amplitude and frequency data are expressed as mean value ± s.e.mean, where n = number of cells. Statistical significance was determined using a Students t- or Wilcoxon signed rank test (Statview II, Abacus Concepts, California, U.S.A.).

Drugs and peptidergic toxins

Toxin I and toxin K were purified from black mamba *Dendroaspis polylepis* venom using previously described methods (Robertson *et al*., 1996), and were a kind gift from John Stow. The other dendrotoxins (α-DTX and γ-DTX) were obtained from Alomone Laboratories (Jerusalem, Israel) as were Agitoxin-2 (AgTX-2) and Margatoxin (MgTX). Tetraethylammonium (TEA), bicuculline methiodide and tetrodotoxin (TTX) were obtained from Sigma (Poole, U.K.). All drugs were bath applied and salts were ANALAR or equivalent grade and obtained from BDH Laboratory Supplies or Sigma (Lutterworth, U.K.).

Results

Spontaneous synaptic currents in basket and Purkinje neurones

In control ACSF, at a whole-cell recording potential of −90 mV, spontaneous inward currents with a rapid rising phase and slower exponential decay phase were always observed in both Purkinje neurones (Figure 1) and basket cells. For both cell types the currents had variable amplitudes, ranging from less than 10 pA up to around 3 nA [mean values: Purkinje neurones −122.8 ± 20.0 pA (n = 24 cells); basket cells −154.8 ± 15.9 pA (n = 26 cells)]. The frequency of spontaneous currents exhibited wide cell to cell variability for both types of neurone (Purkinje cell range 3.8 to 40.2 Hz, mean 17.9 ± 2.2 Hz, n = 24 cells; basket cell range 2.3–33 Hz, mean 14.9 ± 1.7 Hz, n = 26 cells). Rise time (10–90%) and decay time constants were determined using a sample of 40 currents of similar amplitude (~150 pA) from 10 Purkinje cells and 10 basket cells chosen at random. Rise times were ~0.5–0.6 ms for both Purkinje neurones and basket cells. The majority (~90%) of currents were best fit with a double exponential decay time course; τ_1 and τ_2 being 4.3 ± 0.2 and 23.1 ± 1.5 ms respectively in Purkinje neurones (n = 40, Figure 1a) and 4.9 ± 0.3 ms and 20.9 ± 1.6 ms for basket cells (n = 40). Application of 5 μM bicuculline methiodide (Figure 1b) blocked virtually all spontaneous currents in both cell types, therefore these events were characterized as spontaneous IPSCs mediated by GABA (Llano & Gerschenfeld, 1993). Bicuculline-resistant events were comparatively infrequent, having maximum amplitudes less than 100 pA [mean amplitude and cumulative frequency: −25.3 ± 1.1 pA/1.9 Hz for Purkinje neurones (n = 3) and −23.1 ± 3.9 pA/1.2 Hz for basket cells (n = 3)]. Such events had significantly faster decay kinetics than bicuculline-sensitive events. Their contribution to the total spontaneous activity was only a minor fraction of the overall spontaneous synaptic activity.

TTX and spontaneous synaptic currents

TTX (1 μM) dramatically reduced the frequency and amplitude of spontaneous IPSCs in both Purkinje neurones (Figure 1c) and basket cells. Effects reached steady-state within 3 min, from which point all measurements were taken. TTX reduced Purkinje cell IPSC mean amplitude and frequency to −42.4 ± 1.1 pA and 5.2 ± 0.6 Hz from control values of −104.4 ± 1.1 pA and 20.4 ± 2.4 Hz (n = 3 cells, P < 0.05). Basket cell values were similar, namely −60.7 ± 4.2 pA and 3.5 ± 0.3 Hz in TTX from control values of −142.7 ± 3.8 pA and 24.3 ± 5.1 Hz (n = 3 cells, P < 0.05). In both cell types infrequent large amplitude events (up to ~1 nA) persisted in TTX solution. Rise time and decay time

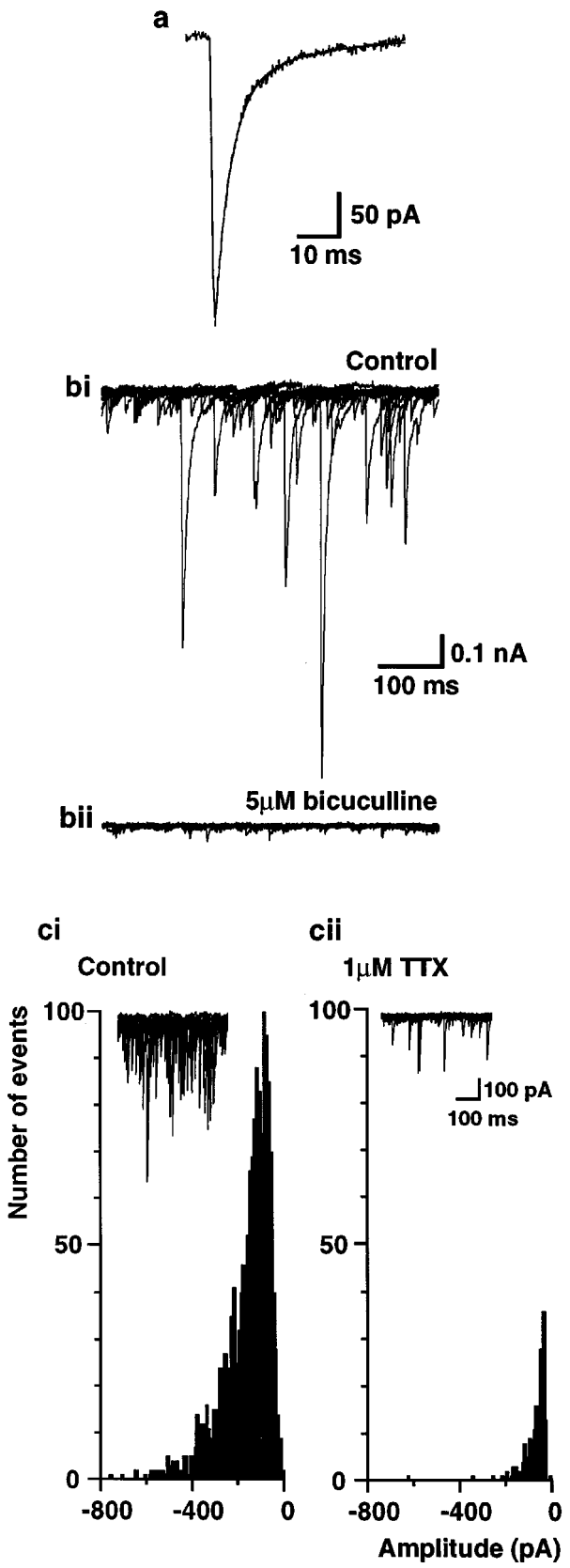

Figure 1 Properties of Purkinje cell IPSCs. (a) In the majority of cases Purkinje cell IPSC decay was best fit using double exponential functions, shown superimposed on the current trace. In this example τ_1 and τ_2 are 3.9 and 21.2 ms. (bi and bii) Virtually all spontaneous activity is blocked by 5 μM bicuculline. (ci and cii) TTX reduced both the frequency and amplitude of IPSCs in Purkinje cells. Raw data traces are 10 consecutive sweeps superimposed from control and after 3 min in TTX solution. Amplitude histograms is from the same cell as (a). 45 s samples, 10 pA bin width. Mean values are: (ci) -145.0 ± 2.7 pA/33.8 Hz (cii) -68.5 ± 4.8 pA/3.8 Hz.

constants (τ_1 and τ_2) in the presence of TTX were 0.6 ms, 3.3 ± 0.2 ms and 16.5 ± 2.8 ms respectively for Purkinje neurones ($n=3$ cells, 40 events) and 0.5 ms, 3.6 ± 0.3 ms and 14.9 ± 1.8 ms for basket cells ($n=3$ cells, 40 events) indicating a modest, but statistically significant ($P<0.05$), reduction in both IPSC decay time constants in TTX solutions.

Effects of dendrotoxins

We have recently demonstrated a dramatic increase in both the amplitude and frequency of IPSCs in Purkinje cells following exposure to 200 nM α-DTX (Southan & Robertson, 1998). 200 nM α-DTX also induces a similar augmentation of basket cell IPSCs (Table 1). We have now examined the effects of a further range of dendrotoxins in both Purkinje neurones and basket cells. Toxin effects were apparent within 1 min of application, and achieved steady-state after 3 min; all measurements were taken after this time. Since toxin effects were only partially reversible, the slice was discarded after one application. After several minutes in toxin, activity was decreased, perhaps due to synaptic fatigue (Vincent & Marty, 1996). All measurements reported here were obtained before such fatigue occurred. For all the DTX-homologue experiments, a high concentration of toxin, relative to potency in cloned channels, was chosen; high concentrations of toxins are required for enhancement of synaptic activity in hippocampal slices (Southan & Owen, 1997).

Figure 2 shows example IPSC traces and amplitude histograms obtained from Purkinje cells before and after application of the two most potent dendrotoxins (α-DTX and toxin K) in our IPSC assay. Table 1 presents mean amplitude and frequency data obtained from a number of Purkinje and basket cells using α-DTX, toxin K, toxin I and γ-DTX at a concentration of 200 nM. Changes in spontaneous IPSC mean amplitude and frequency are presented for 3–5 separate cells for each toxin, and toxin effectiveness presented as a toxin/control ratio. For instance, α-DTX increases IPSC mean amplitude ~1.6 times, and mean frequency ~2.7 fold in Purkinje cells. Overall effectiveness of these toxins is quantified as ι, the 'Activity Index', which represents the overall change in synaptic activity in the presence of each toxin (amplitude ratio × frequency ratio). In both Purkinje cells and basket cells there exists a striking contrast between the marked enhancement seen with α-DTX and toxin K, and the minor enhancements (or even reduction in activity), observed with toxin I and γ-DTX (Table 1).

Dendrotoxins in the presence of TTX

We also examined the effects of 200 nM α-DTX and 200 nM toxin K in Purkinje neurones and basket cells perfused with ACSF containing 1 μM TTX (Table 2). In these experiments the increase in IPSC mean amplitude seen with α-dendrotoxin was modest (~10 pA increase in Purkinje cells; ~26 pA increase in basket cells), but statistically significant ($P<0.05$). In Purkinje neurones, toxin K had no significant effect on IPSC mean amplitude in the presence of TTX, but in basket cells an ~8 pA increase in mean amplitude was observed ($P<0.05$). No statistically significant effects on mean frequency were observed. These actions are minimal compared to the dramatic effects seen in control ACSF.

Effects of scorpion toxins

Agitoxin-2 (AgTX-2) and Margatoxin (MgTX) are potent blockers (IC_{50}'s ranging from 9 pM to <1 nM) of the cloned

Table 1 Summary of the effects of dendrotoxin homologues on Purkinje cell and basket cell spontaneous IPSC amplitude and frequency

DTX (all at 200 nM)	*Control IPSC amplitude* (pA)	*Toxin IPSC amplitude* (pA)	*Toxin/control ratio*	*Control IPSC frequency* (Hz)	*Toxin IPSC frequency* (Hz)	*Toxin/control ratio*	*Activity index* (ι)
Purkinje cells							
α-DTX ($n=4$)	−108.7±1.8	−176.6±1.4*	1.62	14.3±3.4	38.2±5.6*	2.67	4.33
Toxin K ($n=5$)	−120.4±1.7	−170.4±1.5*	1.42	18.3±4.2	32.8±8.1*	1.79	2.54
Toxin I ($n=3$)	−55.8±1.2	−69.8±1.3*	1.25	12.3±1.8	15.9±2.5*	1.29	1.61
γ-DTX ($n=4$)	−128.4±2.0	−153.8±2.5*	1.20	21.6±6.2	20.9±5.7	0.97	1.16
Basket cells							
α-DTX ($n=3$)	−242.2±4.0	−440.5±3.4*	1.82	20.7±7.4	73.8±25.7*	3.57	6.50
Toxin K ($n=4$)	−76.3±4.1	−139.0±4.0*	1.82	7.0±0.8	19.1±3.9*	2.73	4.97
Toxin I ($n=4$)	−268.1±5.7	−304.3±7.2*	1.14	22.2±5.7	20.1±3.1	0.91	1.04
γ-DTX ($n=5$)	−141.0±3.0	−148.1±3.7	1.05	9.4±2.1	7.8±2.2	0.83	0.87

*Denotes $P<0.05$. The activity index (ι) is the product of the toxin/control amplitude ratio and the toxin/control frequency ratio.

Table 2 TTX drastically reduces the potentiation of Purkinje and basket cell spontaneous IPSC amplitude and frequency by α-DTX and toxin K

DTX (all at 200 nM)	*Control IPSC amplitude* (pA)	*Toxin IPSC amplitude* (pA)	*Toxin/control ratio*	*Control IPSC frequency* (Hz)	*Toxin IPSC frequency* (Hz)	*Toxin/control ratio*	*Activity index* (ι)
Basket cells							
α-DTX ($n=4$)	−167.3±4.9	−193.4±5.1*	1.16	5.0±1.4	6.0±2.3	1.20	1.39
Toxin K ($n=3$)	−54.0±1.3	−62.6±1.5*	1.16	11.7±2.2	12.5±5.3	1.07	1.24
Purkinje cells							
α-DTX ($n=3$)	−63.9±1.1	−73.5±0.9*	1.15	7.6±1.3	9.8±1.9	1.29	1.48
Toxin K ($n=4$)	−48.1±1.4	−45.4±1.2	0.94	4.7±1.0	4.4±0.6	0.94	0.88

*Denotes $P<0.05$.

Table 3 Synopsis of the actions of the scorpion toxins MgTX and AgTX-2 on Purkinje cell and basket cell spontaneous IPSC amplitude and frequency

Scorpion toxin (all at 10 nM)	*Control IPSC amplitude* (pA)	*Toxin IPSC amplitude* (pA)	*Toxin/control ratio*	*Control IPSC frequency* (Hz)	*Toxin IPSC frequency* (Hz)	*Toxin/control ratio*	*Activity index* (ι)
Basket cells							
MgTX ($n=3$)	−134.4±2.7	−198.2±5.2*	1.47	16.5±3.3	18.3±2.8	1.11	1.63
AgTX-2 ($n=3$)	−181.9±4.1	−153.8±4.7*	0.85	12.1±1.8	10.4±1.6	0.86	0.73
Purkinje cells							
MgTX ($n=3$)	−76.2±1.8	−96.7±2.7*	1.27	16.5±3.8	12.0±3.2	0.73	0.93
AgTX-2 ($n=3$)	−165.6±6.9	−135.6±3.6*	0.82	17.1±7.9	16.8±11.5	0.98	0.80

*Denotes $P<0.05$.

homomultimeric potassium channels Kv1.1, Kv1.2 and Kv1.3 expressed in *Xenopus* oocytes (Hopkins *et al*., 1996). In the present experiments in the cerebellum, using a high concentration (10 nM) of these blockers, MgTX was found to increase, whilst AgTX-2 decreased, IPSC mean amplitude for both cell types ($P<0.05$). Mean amplitude and frequency data for the scorpion toxins are presented in Table 3.

Tetraethylammonium (TEA)

Two concentrations of the broad-spectrum K^+ channel blocker TEA (200 μM and 10 mM) were examined for effects on spontaneous IPSCs in Purkinje neurones and basket cells. Both 200 μM and 10 mM TEA induced a small increase in the mean amplitude of spontaneous IPSCs. Full results are presented in Table 4.

Discussion

We have examined the actions of K^+ channel selective toxins at identified inhibitory synapses in the mammalian cerebellum. Major points to note are that α-DTX and toxin K caused profound facilitation of IPSC amplitude and frequency whilst γ-DTX and toxin I produced no, or only slight, increases. Surprisingly, two scorpion toxins (AgTX-2 and MgTX) had little facilitatory effect on spontaneous IPSCs.

Basic properties of IPSCs in mouse cerebellum

In the mouse, basket cell synaptic development is almost complete after day 20 (Larramendi, 1969). We therefore chose to study mice between 21 and 35 days old to ensure that cerebellar connectivity was 'mature'. The vast majority of

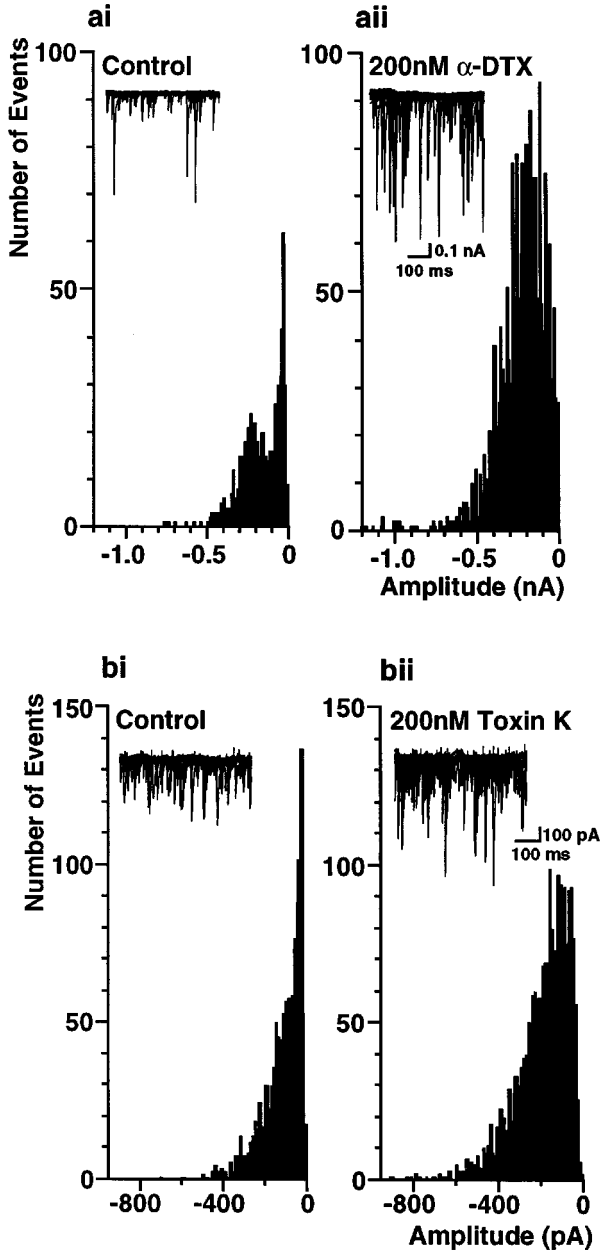

Figure 2 Purkinje cell spontaneous IPSC amplitude and frequency are dramatically enhanced by α-DTX and toxin K. Inset figures show example IPSCs from Purkinje individual neurones. Amplitude histogram data is mean data derived from 4 neurones for each toxin, 45 s samples, 10 pA bin width. Mean amplitude and frequency values are (ai) Control: −155.9±4.8 pA/15.2 Hz (aii) α-DTX: −231.7±3.2 pA/49.4 Hz; (bi) Control: −121.9±2.7 pA/29.0 Hz (bii) Toxin K: −183.6±2.7 pA/49.1 Hz.

spontaneous synaptic events recorded from basket and Purkinje cells were blocked by bicuculline, indicating they were IPSCs mediated by $GABA_A$ receptor activation (Vincent *et al*., 1992). The results also show that mouse Purkinje cell and basket cell IPSCs exhibit similar characteristics, having comparable rise times, decay time constants, frequency and amplitude values. The similar IPSC kinetics suggest that the $GABA_A$ receptor subunit composition is similar for both neurone types.

Effects of dendrotoxin homologues on IPSCs

For both cell types marked increases in ι, our index of overall IPSC activity, were found with α-DTX and toxin K. Toxin I was less potent, and γ-DTX had marginal effects. Dendrotoxins facilitate release of a number of neurotransmitter substances, including acetylcholine at the neuromuscular junction (Barrett & Harvey, 1979) and glutamate in the hippocampus (Southan & Owen, 1997). These effects are most likely to be due to selective blockade of presynaptic voltage-gated K^+ channels (see Harvey, 1997 for review). We have recently shown that α-DTX blocks a proportion (~40%) of voltage-gated K^+ current in basket cell nerve terminals, but, interestingly, not in basket cell somata (Southan & Robertson, 1998). Furthermore, we have never observed block of somatic voltage-gated K^+ currents by any of the above DTX homologues, in either basket cells (Southan & Robertson, 1998); Purkinje neurones (manuscript in preparation) or hippocampal CA1 pyramidal neurones (Southan & Owen, 1997). These electrophysiological results lead us to propose that DTX-sensitive K^+ channels are concentrated in the distal projections of basket cells, including the nerve terminal, and play a key role in regulating GABA release.

Antibody labelling experiments reveal high concentrations of Kv1.1 and Kv1.2 α-subunits selectively localized in basket cell nerve terminal structures (e.g. McNamara *et al*., 1993; Wang *et al*., 1993; Sheng *et al*., 1994; Rhodes *et al*., 1995, 1996). Low nM concentrations of α-DTX block homomultimeric channels comprised of these Kv1.1 and Kv1.2 α-subunits in expression systems (Stühmer *et al*., 1989; Grissmer *et al*., 1994). Similarly, toxin I blocks both Kv1.1 and 1.2 α-subunits (Robertson *et al*., 1996). Toxin K (and δ-DTX) selectively blocks Kv1.1, but not Kv1.2 α-subunits in oocytes (Hopkins *et al*., 1996; Robertson *et al*., 1996). However, toxin K does block heteromultimeric Kv1.1/1.2 subunits (Bell & Robertson, unpublished observations). γ-DTX has not yet been fully characterized or sequenced (Harvey, 1997), although it is reported that this toxin is a potent blocker of Kv1.1 channels in Chinese hamster ovary cells (Owen *et al*., 1997).

Immunohistological evidence (Wang *et al*., 1993; Rhodes *et al*., 1995) suggests that it is likely that Kv1.1 and 1.2 exist

Table 4 Comparison of two concentrations of TEA (200 μM and 10 mM) on spontaneous basket and Purkinje cell IPSCs

Blocker	*Control IPSC amplitude* (pA)	*TEA IPSC amplitude* (pA)	*TEA/control ratio*	*Control IPSC frequency* (Hz)	*TEA IPSC frequency* (Hz)	*TEA/control ratio*	*Activity index* (ι)
Basket cells							
TEA (200 μM, $n=4$)	−142.5±4.4	−205.6±7.8*	1.44	9.3±1.9	7.0±1.1	0.75	1.08
TEA (10 mM, $n=4$)	−51.1±1.3	−67.1±1.7*	1.31	18.4±5.0	22.9±4.6	1.24	1.62
Purkinje cells							
TEA (200 μM, $n=4$)	−102.4±1.5	−120.6±2.2*	1.18	23.2±6.4	20.9±6.8	0.90	1.06
TEA (10 mM, $n=4$)	−148.5±2.9	−240.1±3.9*	1.62	5.9±1.4	8.2±1.6	1.39	2.25

*Denotes $P<0.05$.

together in heteromultimeric K^+ channel complexes in basket cell synaptic terminals, but proof of this requires direct recording from terminals, or the synaptic output of such terminals, in conjunction with selective pharmacological tools. In our experiments, using ι as the activity index, the rank order of potency for both basket and Purkinje cells was: α-DTX > toxin K > toxin I > γ-DTX (γ-DTX having only marginal effects). Since basket cells innervate and inhibit each other, this may suggest that the complement of K^+ channels is similar in all basket cell terminals, independent of target neurone. Unfortunately, these ι values do not help us to distinguish the contributions of Kv1.1 or Kv1.2 subunits to terminal K^+ currents at an absolute level, since potency figures for toxin block of cloned channel subunits vary considerably between expression systems and experimental groups (see Chandy & Gutman, 1995; Robertson, 1997). However, the potency order does suggest that homomultimeric Kv1.2 channels alone do not contribute significantly to regulating GABA release, and the dendrotoxins act by blocking heteromultimeric assemblies of Kv1.1 and Kv1.2 channels. The lack of effect of γ-DTX on IPSC amplitude and frequency was surprising since Owen *et al.* (1997) and Hopkins *et al.* (1996) report that γ-DTX is an effective blocker of Kv1.1 channels and our own oocyte studies reveal that γ-DTX is a relatively weak blocker of Kv1.2 channels, with slow onset (unpublished observations). However, this toxin has no effect on hippocampal CA1 neurones' glutamatergic potentials (250 nM), whilst other DTX-homologues (α-DTX, toxins K and I) produce significant enhancements (Southan & McIntosh, unpublished observations), with α-DTX again being the most potent. A study of the potency of this toxin, and the other dendrotoxins, on Kv1.1/Kv1.2 heteromultimers is clearly warranted and may help to explain the potency order in our IPSC experiments.

TTX blocked most of the actions of α-DTX and toxin K indicating that the effects of dendrotoxins are mainly dependent on Na^+-dependent action potential propagation. However, some modest increases in amplitude were still apparent in TTX solution. It is possible that a small toxin-induced depolarization of the nerve terminal region coupled with propagation of TTX-insensitive action potentials (e.g. Ca^{2+} spikes) could account for this effect.

Lack of effect of AgTX-2 and MgTX on IPSCs

We had hoped that the scorpion toxins AgTX-2 and MgTX would give further clues regarding the identity of K^+ channels regulating inhibitory transmission. However, neither of these toxins appeared to stimulate IPSC amplitude or frequency (indeed, AgTX-2 reduced ι). The reasons for this are unclear, since both toxins block Kv1.1 and Kv1.2 currents expressed in oocytes (Garcia *et al.*, 1994; Hopkins *et al.*, 1996). MgTX also binds with high affinity (~ 80 fM) to cerebellar membranes, and immunoreactive labelling shows dense staining in the basket cell terminal layer (Koch *et al.*, 1997). Our chosen concentration of the scorpion toxins was orders of magnitude in excess of the IC_{50} obtained on cloned channels, and effectiveness of these batches of toxins were confirmed in our own oocyte experiments (unpublished observations), where significant block of voltage-activated currents was obtained for Kv1.1 and Kv1.2 homomultimers and Kv1.1/Kv1.2 heteromultimers. We can offer no simple explanation for the discrepancy between the very high affinity binding obtained by Koch *et al.* (1997) and lack of effect in our present physiological assay.

Actions of TEA

Low concentrations (200 μM) of TEA did not dramatically affect IPSC frequency or amplitude, however 10 μM TEA moderately increased ι. Again, we can explain these results in terms of the relative selectivity of TEA for Kv1 channels, as homomultimeric Kv1.1 channels have an IC_{50} ~300 mM for TEA, whilst homomultimeric Kv1.2 channels are effectively resistant to concentrations of TEA even up to 100 mM (Christie *et al.*, 1990; Chandy & Gutman, 1995). Heteromultimeric channels comprised of Kv1.1 and Kv1.2 channels have an IC_{50} for TEA around 6 mM (Christie *et al.*, 1990). The actions of TEA on cerebellar IPSCs are more consistent with block of heteromultimeric Kv1.1 / Kv1.2 containing channels than either subunit alone.

We suggest that some of the native K^+ channels involved in GABA release are heteromultimers containing Kv1.1 and Kv1.2 subunits. Basket interneurones fire spontaneously (Eccles *et al.*, 1967), even in the absence of excitatory input (Häusser & Clark, 1997), suggesting that they play a major role in controlling tonic activity of Purkinje cells and interneurones in the cerebellar cortex. This tonic firing presumably depends upon the basket cells' complement of voltage-activated conductances, with a key role played by K^+ currents. DTX block of Kv1.1/Kv1.2-containing channels could lead to increased IPSC frequency by increasing somatic firing, through block of an I_d-like current (Brew & Forsythe, 1995). However, we have found no DTX or 4AP-sensitive component in whole-cell recordings from basket cell somata (Southan & Robertson, 1998). We propose that DTX-sensitive K^+ currents are localized to axonal and terminal compartments (corroborating antibody labelling), and blocking these K^+ channels increases the likelihood of action potential transmission through axonal and terminal branch points. Llano *et al.* (1997) and Vincent & Marty (1996) have shown that block of basket neurone K^+ currents by internal Cs^+ leads to increases in IPSC amplitude and intracellular Ca^{2+} signals in axons and terminals, effects also consistent with increased transmission to nerve terminals and GABA release sites. IPSC amplitude could also be increased by enhanced Ca^{2+} entry through broadening of terminal action potentials (Augustine, 1990).

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