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Comparison of the effects of drugs on hyperexcitability induced in hippocampal slices by withdrawal from chronic ethanol consumption

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1 The effects of drugs, previously demonstrated to have a range of effects on the behavioural signs of ethanol withdrawal hyperexcitability, were examined in area CA1 in isolated hippocampal slices prepared after withdrawal from chronic ethanol *in vivo*.

2 The decreases seen after the ethanol treatment in the thresholds for production of single and multiple population spikes were prevented when the dihydropyridine calcium channel antagonist, isradipine, was included in the perfusion medium at 4 μM.

3 Another dihydropyridine, felodipine, which had no activity against withdrawal signs *in vivo*, did not affect the changes in field potentials, at concentrations up to 10 μM.

4 Diltiazem, which increased withdrawal hyperexcitability *in vivo*, had no effect on the withdrawal changes in field potentials at 30 μM; higher concentrations affected the control slices.

5 The novel anticonvulsant, gabapentin, at 1 μM but not at 100 nM, significantly decreased the signs of withdrawal hyperexcitability in the hippocampal slices. When the CCK_B antagonist, CI988, was added to the bathing medium, at 1 μM, there were small, but significant decreases in the withdrawal hyperexcitability.

6 The results showed that the actions of these drugs on the changes in the field potentials in isolated hippocampal slices were very similar to their previously demonstrated effects on the convulsive signs of ethanol withdrawal *in vivo*, but differences were seen in the corresponding comparison with anxiolytic actions *in vivo*.

Keywords: Ethanol; hippocampus; withdrawal; calcium channel antagonists; field potentials

Introduction

Cessation of prolonged ethanol consumption causes a withdrawal or abstinence syndrome. In man this consists of tremor, agitation, delirium and confusion, and full convulsions can occur. The syndrome can be modelled in rodents, in which tremors, convulsions and anxiety-related behaviour are seen during withdrawal from prolonged ethanol intake (Goldstein & Pal, 1971). The expression of the syndrome can be decreased by some anticonvulsant drugs, such as benzodiazepines or chlormethiazole, but the effects of these drugs are not selective as they decrease many types of convulsive behaviour, of different origins. We have demonstrated neuronal changes that may be responsible for the signs of the withdrawal syndrome. Increases have been found in dihydropyridine-sensitive calcium currents (Whittington *et al*., 1995) and in both NMDA receptor mediated and AMPA/kainate receptor mediated excitatory postsynaptic potentials (e.p.s.ps) (Whittington *et al*., 1995; Molleman & Little 1995). No decreases were seen in $GABA_A$-mediated inhibitory postsynaptic potentials (i.p.s.ps) in hippocampal neurones (Whittington *et al*., 1992), although such changes have often been suggested to underlie the withdrawal syndrome, and no changes were found in $GABA_B$-mediated i.p.s.ps (Molleman & Little, 1995).

We have previously shown that dihydropyridine calcium channel antagonists prevented the tremor and convulsive components of the ethanol withdrawal syndrome in animal models. This has been demonstrated with nitrendipine, nimodipine and isradipine (Little *et al*., 1986; Littleton *et al*, 1990). This effect was selective, as few other behavioural effects were seen at the doses used. The effects of the stereoisomers of isradipine showed the same pattern as their effects on calcium currents *in vitro* (Littleton *et al*., 1990). However, another dihydropyridine calcium channel antagonist, felodipine, did not show any effects on the behavioural signs of ethanol withdrawal, even at high doses (Watson *et al*., 1994). Diltiazem is a calcium channel antagonist that binds at a different site on the channel complex from that at which the dihydropyridines bind. This compound increased the severity of the behavioural signs of withdrawal in mice (Watson & Little, 1994a). The anticonvulsant, gabapentin, binds to a novel site in the CNS (Suman-Chauhan *et al*., 1993), but its mechanism of action is not fully understood. This compound significantly decreased all the behavioural signs of the ethanol withdrawal syndrome in mice (Watson *et al*., 1995; Watson & Little, 1997a). The CCK_B antagonist, CI988, slightly decreased the convulsive aspects of the ethanol withdrawal syndrome (Wilson & Little, 1995) and prevented several of the signs of anxiety-related behaviour (Wilson *et al*., 1998).

We have previously demonstrated neuronal hyperexcitability in field potentials in isolated hippocampal slices after chronic ethanol treatment *in vivo* (Whittington & Little, 1990; Morton *et al*., 1992). The changes included decreases in the stimulation thresholds for elicitation of both single and multiple population spikes. The present study compares the actions of the drugs described in the preceding paragraph on the changes in field potentials in isolated hippocampal slices

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prepared after chronic ethanol administration. The aim was to determine how far the effects of the drugs in the *in vitro* model mirrored their actions on the behavioural signs of withdrawal. The concentrations of the drugs were therefore chosen, where possible, on the basis of the CNS concentrations measured after administration of the doses used in our previous behavioural studies. Where this information was not available, values for concentrations were obtained from the literature.

Methods

Chronic ethanol treatment

Mice of the C57 strain were given ethanol via the drinking fluid; this is the method used in our previous electrophysiological studies. Male mice, C57 strain, 25–30 g, were given ethanol, 24% v/v as sole fluid for 12–18 weeks. Control animals drank water only. Ethanol intake was measured every four days; the ethanol intake values throughout the treatment were between 10 and 14 g kg^{-1} day^{-1}. The mice were weighed at regular intervals during the treatment and there were no significant differences between the weights of the chronic ethanol group and the controls. The light cycle was 9h 00min to 21h 00min and the animals were housed 10 per cage.

Slice preparation

The mice were killed by cervical dislocation followed by decapitation between 9h 00min and 10h 00min, with no prior removal from ethanol treatment. The brain was removed and the dissection was kept wet at all times with oxygenated (95% O_2/5% CO_2) artificial cerebrospinal fluid (ACSF) at 23°C. Transverse hippocampal slices, 400 μm thick, were taken with a McIlwain tissue chopper. The time of dissection was taken as 0 h after ethanol withdrawal.

One hippocampal slice was used from each mouse. Data shown are for sample sizes of 4 to 6 mice for each treatment.

Bathing fluid composition

The composition of the ACSF bathing medium was as follows (mM): NaCl 124, KCl 3.25, KH_2PO_4 1.25, $NaHCO_3$ 20.0, $MgSO_4$ 2.0, $CaCl_2$ 2.0 and D-glucose 10.0.

Concentrations of drugs used

The concentrations of the drugs used were chosen where possible on the basis of previous data obtained from behavioural and binding studies. Isradipine was chosen as a 'reference' compound, to provide a positive control group, as we have already demonstrated the effects of this substance on ethanol withdrawal hyperexcitability in isolated hippocampal slices.

Measurements made of the brain concentrations of isradipine, following an effective acute dose, in mice withdrawing from ethanol, gave concentrations of between 1 and 2 μM, 2 h to 6 h after intraperitoneal injection (Ripley & Little, 1995). In addition, previous work in isolated hippocampal slices, demonstrated that the active (+)-stereoisomer of this compound decreased the ethanol withdrawal hyperexcitability at a concentration of 2 μM.

In earlier work the *ex vivo* binding of felodipine was compared directly with that of nitrendipine (Watson *et al*., 1994) and showed that felodipine produced a similar degree of displacement of labelled nitrendipine when given *in vivo* (intraperitoneal administration) at a dose one fifth lower than that of nitrendipine. We have data showing that the brain concentrations following an acute dose of nitrendipine required for protective action in mice withdrawing from ethanol were between 2 and 6 μM (Whittington & Little, 1991a). Felodipine was therefore first used at 1 μM, but when this was found to produce no changes, the concentration was raised to 10 μM, to determine whether or not any effects could be seen.

Data were not available from behavioural work to suggest a suitable concentration of diltiazem. Preliminary experiments were therefore carried out to determine what concentration of diltiazem might affect the field potentials in hippocampal preparations from control animals. These demonstrated that concentrations over 30 μM significantly inhibited the amplitude of control field potentials, particularly paired-pulse potentiation. Hence 30 μM was used to determine whether or not there were any selective effects on the field potentials in slices prepared after chronic ethanol treatment *in vivo*.

Although we have demonstrated the protective action of gabapentin on withdrawal signs *in vivo*, brain concentrations after the effective doses are not yet available. The concentrations of gabapentin, 100 nM and 1 μM, were therefore chosen from the literature. Binding data for gabapentin (Suman-Chauhan *et al*., 1993) and data from cell culture experiments (Wamil & McLean, 1994), indicated a suitable concentration of 100 nM. However, data showing the brain plasma levels following an acute anticonvulsant dose of gabapentin (Welty *et al*., 1993), suggested that a suitable concentration would be 1 μM. The effects of both these concentrations were therefore examined in the present study.

The situation with CI988 was similar to that of gabapentin, in that brain concentrations after the doses used in our own behavioural studies were not available. Data from experiments on the actions of CI988 on isolated ventromedial hypothalamic neurones (Hughes *et al*., 1990) indicated that 100 nM and 1 μM would be suitable concentrations of CI988 to use in the present studies.

Addition of drugs to the bathing fluid

Gabapentin, (4-[[2-[[3-(1H-indol-3-yl)-2-methyl-1-oxo-2-[[tricyclo[3.3.1.7.]dec-2-yloxyl)carbonyl]amino]propyl]amino]-1-phenetyl]amino-4]oxo[R[9R,R]]-N-methyl-D-glucamine CI988) and diltiazem were dissolved in water and added to the aCSF at 1000 fold dilution. Isradipine and felodipine were dissolved in dimethylsulphoxide (DMSO) at 5000 times the final concentration, then added to the aCSF as required. The final concentration of DMSO in the bathing solution was not more than 0.02%. Previous results (Whittington & Little, 1991b) have shown this concentration to have no effect on field potentials in hippocampal slice preparations.

The drugs used were present in the bathing fluid from the start of the experiment (i.e. before the time of dissection). This experimental design was used because previous results (Whittington & Little, 1990) demonstrated time-dependent changes over 7 h in field potentials in hippocampal slices prepared from mice on withdrawal from chronic ethanol treatment.

Electrophysiological recordings

The hippocampal slices were maintained at the junction between the perfusion medium (aCSF at 30°C, flow rate 1.5 ml min^{-1}). Slices from ethanol-treated and control animals were alternated between the two perfusion chambers. Extracellular recordings were taken from stratum pyramidale

in area CA1. Recording eletrodes were made from 1.2 mm diameter glass filled with 2 M KCl, with resistances between 5 and 15 MΩ.

Orthodromic stimulation of Schaffer collateral/commissural fibres was given with square-wave pulses at constant current, 50 μs duration, either singly or in pairs 70 ms apart. The stimulating electrodes were 2 insulated nickel/chromium wires, 0.025 mm in diameter. The stimulating electrodes were placed on the surface of the slice such that the slice surface was not broken. The recording electrode was lowered approximately 100 μm into the slice each time.

At the completion of each day's experiment, the recording chambers and tubing were washed through with 0.5 M NaOH for at least 15 min, followed by 0.1 M citric acid for at least 15 min, followed by distilled water for at least 15 min, to ensure removal of any residual drug.

Slice integrity and consistency of electrode placement between slices

Slice integrity was validated on the basis of three parameters: threshold for production of single population spikes less than 100 mA, maximum spike height over 4 mV and no multiple spikes at stimulus strengths up to 1.25 × threshold. Certain precautions were taken to ensure that the comparisons between hippocampal slices from different animals were valid. In order to limit the variability of electrode placement between experiments, a number of anatomical markers was used (Whittington & Little, 1990). The stimulating electrodes were placed at the interior of the cell body layer of the CA3 region at the apex of the cell-body layer is curvature towards the dentate gyrus. The recording electrodes were placed at the exterior of the cell-body layer of the CA1 region, on an imaginary line through the ends of the arc of granular cells in the dentate gyrus.

Stimulus/recording protocol

The thresholds for production of single population spike, multiple population spike from a single pulse and multiple population spike from the second pulse of a paired pulse were determined, with stimuli at 0.1 Hz. A cut-off stimulus current was set at 350 μA to avoid damage to the slice. The threshold for multiple population spike from the second pulse of a paired pulse was found by applying two pulses, 70 ms apart, at 0.1 Hz. The appearance of a second population of spike immediately after the first was taken as the threshold for multiple firing.

Measurements were taken every 15 min, from 105 min after dissection (approximately 90 min after insertion into the recording chamber), until 7 h after start of dissection, i.e. 7 h after start of ethanol withdrawal. Our previous work has demonstrated that this is the time period over which signs of withdrawal hyperexcitability are seen in hippocampal slices. Between each recording session, pulses at 1.25 × single population spike threshold were applied at 0.033 Hz.

The incidence of 'reverberative firing' was measured in each slice. This pattern of firing was described in our earlier studies on ethanol withdrawal (Whittington & Little, 1993) and differs from the multiple firing in that there is a second set of population spikes after the first multiple firing pattern. In the reverberative firing there is a consistent interval between the first set of population spikes elicited by an e.p.s.p. and the appearance of a second burst of spikes, superimposed on the same population e.p.s.p. The patterns of single, multiple and reverberative firing are illustrated in Figure 1.

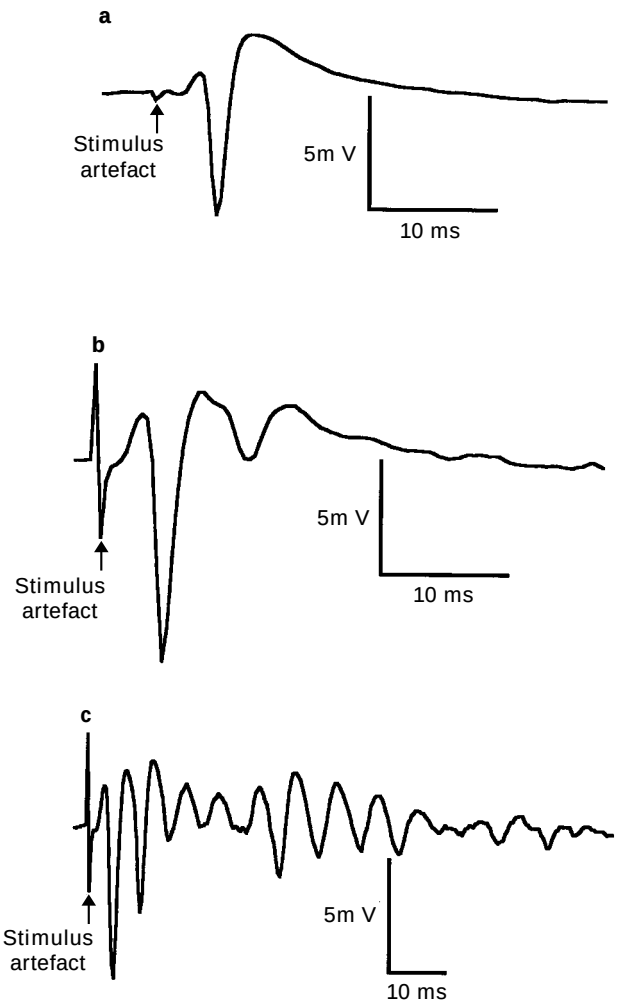

Figure 1 Examples of traces obtained during recordings from hippocampal slices. (a) A single population spike superimposed on the population e.p.s.p. (b) The appearance of a second population spike after the first was taken as the threshold for multiple population spiking. (c) Reverberative firing pattern. Many population spikes are superimposed on the population e.p.s.p.; the increase in size of these population spikes beginning at the sixth spike indicates the reverberative firing. Arrows indicate stimulus artifacts.

Statistical analysis

Single population spike data were analysed by Student's *t* tests at each time-point. Owing to the cut-off point imposed on the data, a non-parametric test was required for the multiple population spike data; the Mann-Whitney U-test was used. The incidence of reverberative spiking data was analysed by Fisher's exact probability test.

Results

The effects of ethanol withdrawal on the thresholds for production of population spikes are illustrated in Figures 2 to 6. The single and multiple spike thresholds were decreased in slices prepared after the ethanol treatment. Single-spike thresholds, between 2 h and 7 h ($P<0.05$), multiple spiking on the first response, between 5 h and 7 h ($P<0.05$), and multiple spiking on the second response (not previously studied) between 2 h 45 min and 7 h ($P<0.01$). The multiple spike thresholds from a single stimulus, in the presence or absence of

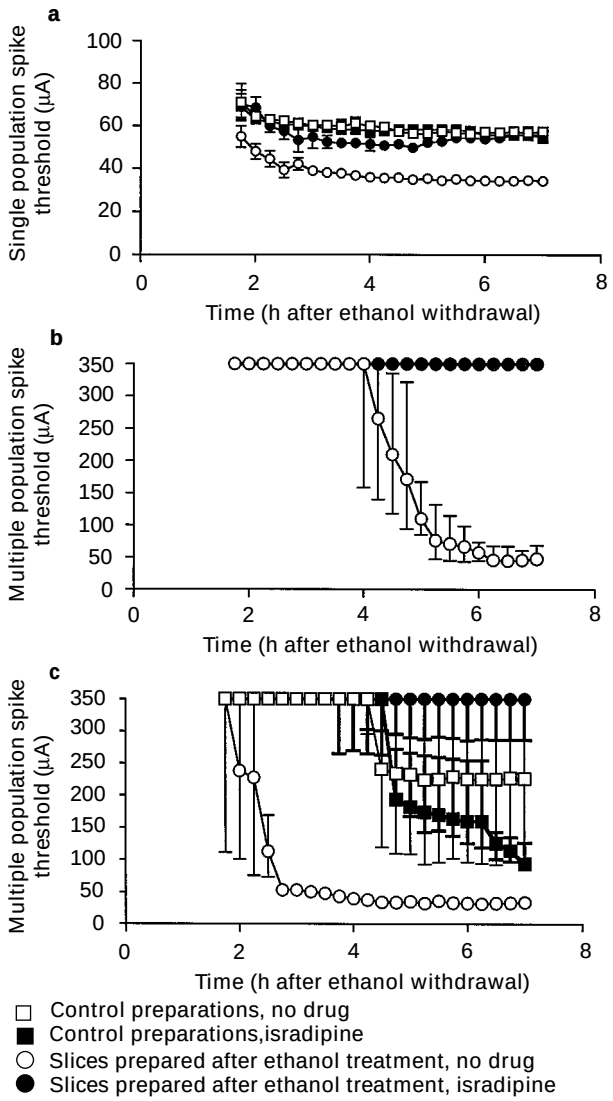

Figure 2 The effects of isradipine, 4 μM, on stimulation thresholds for the production of single (a) population spikes, the multiple population spikes on the first response (b) and multiple population spikes on the second response (c). Values are mean for single spike thresholds and medians and interquartile ranges for multiple spike thresholds; a cut-off was made at 350 μA stimulation to avoid tissue damage. Vertical lines show s.e.mean.

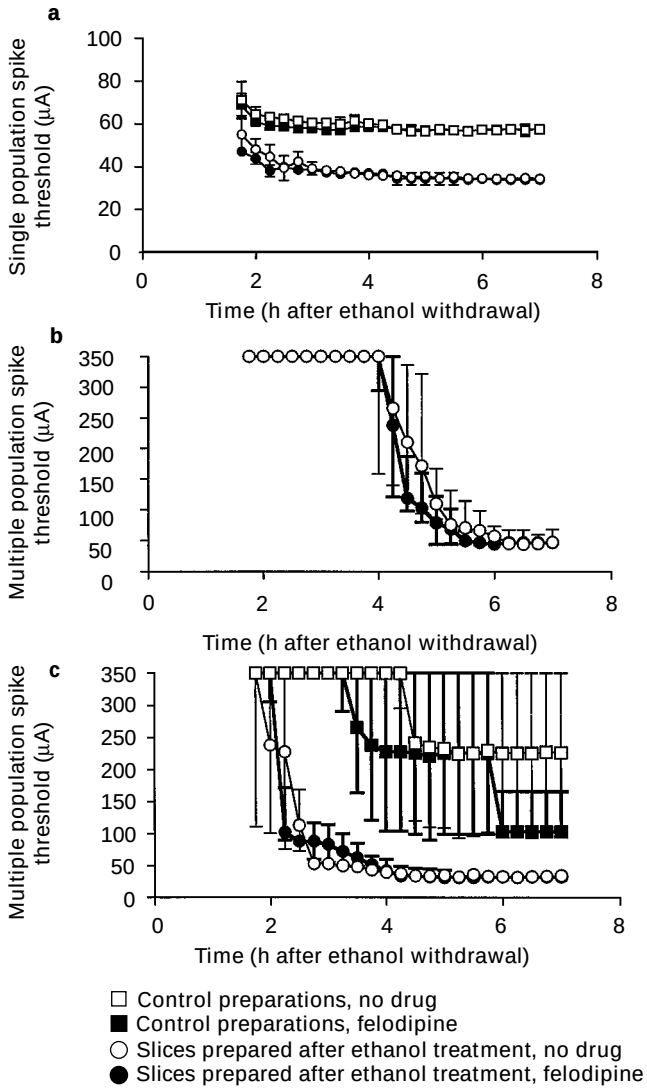

Figure 3 The effects of felodipine, 10 μM, on stimulation thresholds for the production of single (a) population spikes, the multiple population spikes on the first response (b) and multiple population spikes on the second response (c). Values are mean for single spike thresholds and medians and interquartile ranges for multiple spike thresholds; a cut-off was made at 350 μA stimulation to avoid tissue damage. Vertical lines show s.e.mean.

drugs, in control preparations did not fall below the cut-off value of 350 μA. These data are omitted from the figures.

In the presence of isradipine, 4 μM, all the changes in threshold seen in slices prepared after ethanol treatment were almost completely prevented (Figure 2). The effect was significant (compared with the thresholds in slices from ethanol-treated animals in the absence of drug) when comparisons were made between 2 h and 7 h from ethanol withdrawal for the single spike thresholds ($P<0.01$), between 5 h and 7 h from withdrawal for the multiple spiking on the first response ($P<0.05$) and between 4 h and 7 h for the multiple spiking on the second response ($P<0.01$). There were no effects of isradipine on any of the thresholds in preparations from control animals.

Felodipine, added to the bathing medium at 1 μM, had no effect on any of the changes seen during withdrawal (data not shown). In view of this lack of effect, the experiments were repeated with the concentration of this compound raised to 10 μM. These results are illustrated in Figure 3, and it can be seen that, even at this higher concentration, this compound had no effect on the signs of ethanol withdrawal hyperexcitability in the field potentials. No effects of either concentration of felodipine were seen in control preparations.

Diltiazem, 30 μM had no significant effect on single spike thresholds, or multiple spike thresholds on the first response or on the second response during withdrawal (Figure 4). The concentration of this compound was not raised, as earlier studies indicated a significant effect on field potential amplitudes of control preparations at concentrations higher than 30 μM.

At 100 nM, gabapentin did not alter the thresholds in slices from either control or ethanol-treated animals (data not shown). However, at 1 μM this compound significantly attenuated the decreases in all three types of threshold (Figure 5). The effects were significant (compared with the thresholds in slices from ethanol-treated animals in the absence of drug) between 2 h and 7 h from withdrawal for the single spike thresholds ($P<0.05$), between 5 h and 7 h for the multiple

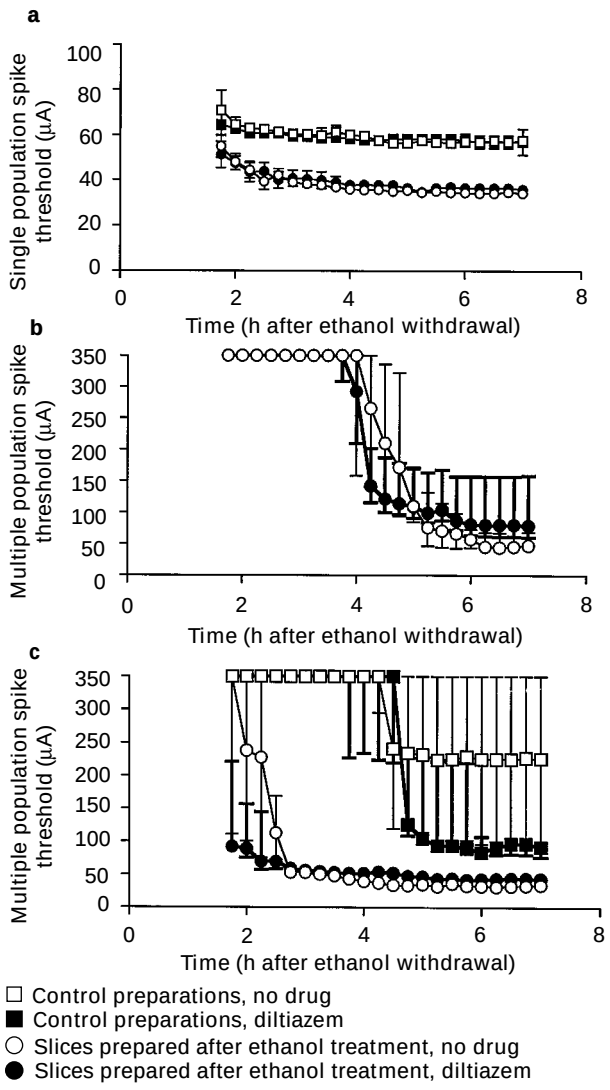

Figure 4 The effects of diltiazem 30 μM, on stimulation thresholds for the production of single (a) population spikes, the multiple population spikes on the first response (b) and multiple population spikes on the second response (c). Values are mean for single spike thresholds and medians and interquartile ranges for multiple spike thresholds; a cut-off was made at 350 μA stimulation to avoid tissue damage. Vertical lines show s.e.mean.

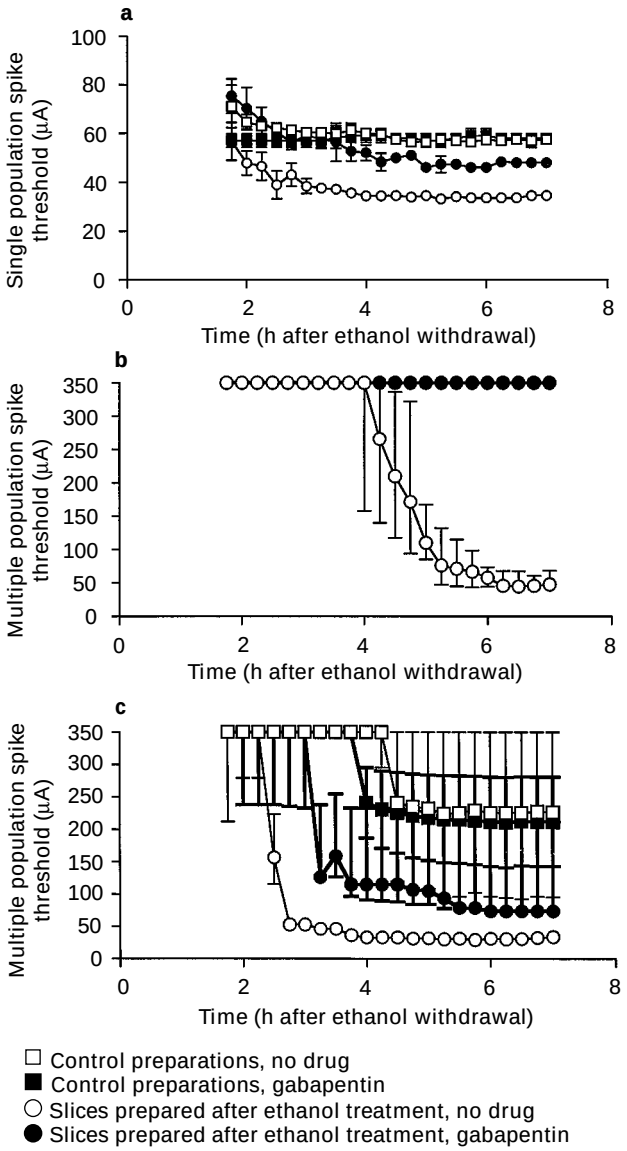

Figure 5 The effects of gabapentin 1 μM, on stimulation thresholds for the production of single (a) population spikes, the multiple population spikes on the first response (b) and multiple population spikes on the second response (c). Values are mean for single spike thresholds and medians and interquartile ranges for multiple spike thresholds; a cut-off was made at 350 μA stimulation to avoid tissue damage. Vertical lines show s.e.mean.

spike threshold for the first response ($P<0.05$) and between 3 h and 7 h for the multiple spike threshold for the second response ($P<0.05$). No effects were seen on the thresholds in control preparations at either concentrations of gabapentin.

The CCK_B antagonist, CI988, had a small, but significant effect on the changes in thresholds caused by ethanol withdrawal when added to the bathing medium at 1 μM (Figure 6), but not at 100 nM (data not shown). The effects of the 1 μM concentration were significant (compared with the thresholds in slices from ethanol-treated animals in the absence of drug) between 4 h 30 min and 6 h 15 min for the single spike thresholds ($P<0.05$), between 5 h and 6 h 15 min for the multiple spike thresholds for the first response ($P<0.05$) and between 3 h and 6 h 15 min for the multiple spike thresholds for the second response ($P<0.05$). The actions of this compound appeared to decrease during the last 1 h of the recording period, even though it was still present in the bathing medium at this time; there were no significant differences in any of the thresholds after 6 h 15 min.

Reverberative spiking patterns were seen in all slices prepared after chronic ethanol treatment, when no drugs were added to the bathing medium, and were similar to those previously reported in hippocampal slices after ethanol withdrawal (Whittington & Little, 1993). Table 1 shows a comparison of the incidence of this type of spiking after the various drug treatments. Isradipine and gabapentin, at the higher concentrations, significantly decreased the appearance of this spiking pattern. No signs of such spiking activity were seen in control preparations in the absence or presence of any of the drugs.

Discussion

The effects of the compounds on withdrawal hyperexcitability in the hippocampal slices were extremely similar to their actions in our behavioural studies. Isradipine and gabapentin

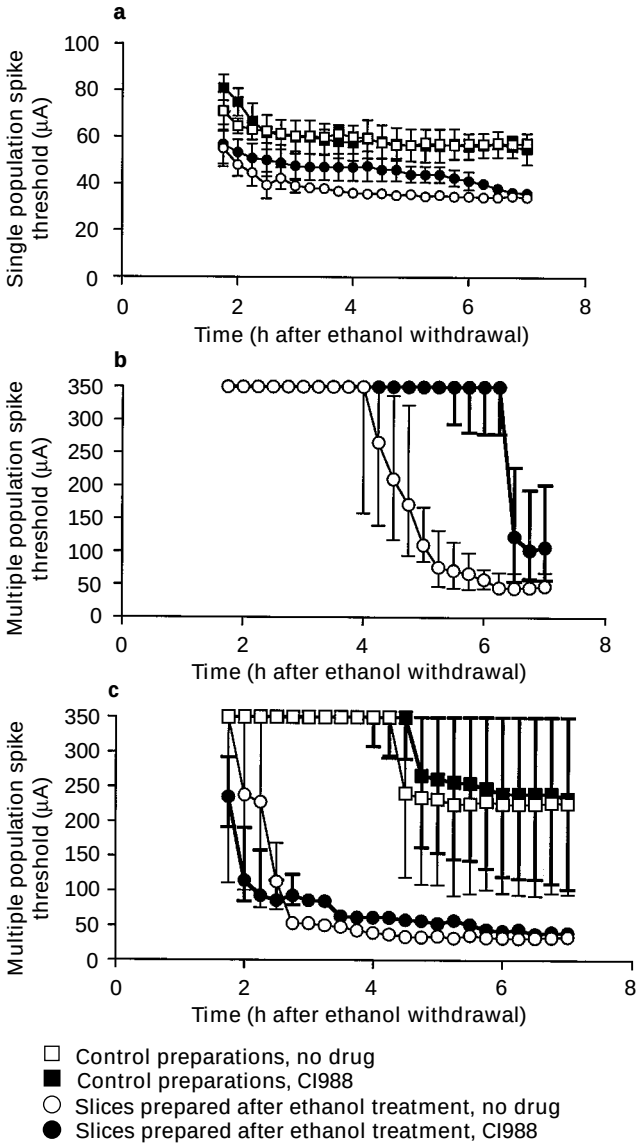

Figure 6 The effects of CI988, 1 μM, on stimulation thresholds for the production of single (a) population spikes, the multiple population spikes on the first response (b) and multiple population spikes on the second response (c). Values are mean for single spike thresholds and medians and interquartile ranges for multiple spike thresholds; a cut-off was made at 350 μA stimulation to avoid tissue damage. Vertical lines show s.e.mean.

Table 1 The incidence of reverberative spiking patterns in hippocampal slices prepared after chronic ethanol treatment *in vivo*

Drug	*Percentage of slices showing reverberative spiking*	P *Value for comparison with spiking incidence in the absence of drug*
None	100	
Isradipine 1 μM	100	$P>0.1$
Isradipine 4 μM	0	$P<0.01$
Felodipine 1 μM	100	$P>0.1$
Felodipine 10 μM	100	$P>0.1$
Diltiazem 30 μM	50	$P>0.1$
Gabapentin 100 nM	100	$P>0.1$
Gabapentin 1 μM	0	$P<0.01$
CI988 100 nM	100	$P>0.1$
CI988 1 μM	50	$P>0.1$

were very effective in decreasing the signs of hyperexcitability in the hippocampal slices, CI988 showed some protective effects, while felodipine had no action. These results are all consistent with the *in vivo* effects of the drugs on the convulsive aspects of the ethanol withdrawal syndrome (Ripley & Little, 1995; Watson *et al*., 1994; Wilson & Little, 1995; Watson & Little, 1997a). An important aspect of this similarity was that the effects of the drugs on the ethanol withdrawal hyperexcitability were selective, as no effects were seen on control preparations.

The lack of effect of felodipine, even when the concentration was raised as high as 10 μM, was entirely consistent with the results previously obtained *in vivo*. In our previous paper on the effects of felodipine on the behavioural signs of ethanol withdrawal (Watson *et al*., 1994), the possible reasons for the lack of effect of this compound was discussed.

The finding that diltiazem had no protective action against the withdrawal hyperexcitability was also in agreement with the behavioural data (Watson & Little, 1994a), but there was a difference from the behavioural effects of this drug in that no increase was seen in the neuronal excitability in the slices. The severity of the convulsive aspects of the withdrawal syndrome *in vivo* were increased by this drug (Watson & Little, 1994a). It is possible that increases in some measurements of excitability might have been apparent at higher concentrations of diltiazem, but previous studies (unpublished results) showed that, above 30 μM, diltiazem decreased the amplitude of population spikes in control preparations. The present results suggest that the increase in withdrawal hyperexcitability *in vivo* may have been due to actions on brain areas other than the hippocampus, and through sites other than neuronal calcium channels. Increases in the *in vivo* convulsive actions of the compounds bicuculline and pentylenetetrazol were also seen after administration of diltiazem (Watson & Little, 1994).

The effects of calcium channel antagonists on the hippocampal slice have previously been shown to be selective for the hyperexcitability due to ethanol withdrawal, as the effects of the $GABA_A$ antagonist, bicuculline were unaffected (Whittington & Little, 1991b). We have also previously shown that an antagonist at N-methyl-D-aspartate (NMDA)-receptors prevents the signs of ethanol withdrawal hyperexcitability in isolated hippocampal slices (Ripley & Little, 1995). Although the calcium channel antagonist, nitrendipine, has been demonstrated to have actions at the NMDA receptor complex (Skeen *et al*., 1993), it is unlikely that the effects this type of compound produces on withdrawal hyperexcitability are mediated by this site, since other protective compounds, isradipine and nimodipine, were found not to interact with the NMDA complex.

We have demonstrated that the new anticonvulsant, gabapentin, is effective in decreasing both the convulsive and anxiety-related aspects of the ethanol withdrawal syndrome *in vivo* (Watson *et al*., 1995; Watson & Little, 1997a). The site of action of gabapentin is uncertain, but a discrete binding site has been demonstrated in the CNS (Hill *et al*., 1993), which does not correspond to any other established binding site. A recent study (Gee *et al*., 1996) has suggested that this site is identical with the $\alpha 2\gamma$ subunit of the calcium channel. Rock *et al*. (1993) showed that, at therapeutic concentrations, gabapentin had no effects on inhibitory (GABA and glycine) or excitatory (NMDA- and non-NMDA receptor mediated) responses, or voltage-dependent calcium currents, in rodent cultured neurones. However, it is possible that effects of gabapentin are produced when the neurones are perturbed in some way, for example by withdrawal from chronic ethanol treatment, resulting in effects not seen in the normal situation.

We have previously shown that the density of binding sites in the central nervous system labelled by dihydropyridine calcium channel antagonists was increased after chronic ethanol treatment (Dolin *et al*., 1987) and that neuronal calcium channel activity was also increased (Whittington *et al*., 1995; Molleman & Little, 1997).

In vivo, CI988 had only a small action on the convulsive signs of withdrawal during the first 12 h of the withdrawal period (Wilson & Little, 1995), but greater effects were seen when the effects of this compound were tested later in the withdrawal period, at 16 h, when it prevented the responses to handling, the decreases in the convulsive thresholds for NMDA and the anxiety-related behaviour measured by the plus-maze test (Wilson *et al*., 1998). The latter effects were produced at doses as low as 0.1 mg kg^{-1}, although a claim has been made that CI988 does not penetrate the blood/brain barrier well and it did not displace labelled ligand from CCK_B receptors after intravenous administration until the dose was raised to 30 mg kg^{-1} (Patel *et al*., 1994). The time course of the results obtained *in vivo* during ethanol withdrawal cannot be compared directly with those on hippocampal slices *in vitro*, owing to the metabolism of alcohol *in vivo*, but it does appear that the pattern of appearance of effects of CI988 are different in these two types of experiment.

A dissociation has previously been demonstrated between the effects of drugs in preventing the convulsive behaviour during the withdrawal phase and their effects on anxiety-related behaviour seen during this time. The calcium channel antagonists prevented the increases in the convulsive responses to handling and the changes in thresholds of convulsive drugs (Littleton *et al*., 1990; Watson & Little, 1997b), but did not alter the changes in behaviour measured on the elevated plus maze (File *et al*., 1989). Gabapentin prevented both the convulsive and anxiety-related aspects of the ethanol withdrawal syndrome (Watson *et al*., 1995; Watson & Little, 1997a), while CI988 significantly decreased the anxiety-related behaviour measured in the elevated plus maze after withdrawal from chronic ethanol intake (Wilson & Little, 1996), but had only minor effects against the convulsive aspects of withdrawal (Wilson & Little, 1998). Comparisons can therefore be made between these *in vivo* effects of the compounds and their actions on the various aspects of ethanol withdrawal hyperexcitability in the hippocampal slices. Isradipine and gabapentin decreased all the changes seen *in vitro*, while CI988 had some effects, although these appeared to decrease towards the end of the recording period. It appears therefore that the actions of the drugs on ethanol withdrawal hyperexcitability in hippocampal slices parallel their protective effects on the convulsive aspects of withdrawal *in vivo*, but do not reflect their actions on anxiety-related behaviour.

In conclusion, the effects of the compounds on the signs of ethanol withdrawal hyperexcitability in the isolated hippocampal slice were found to parallel their anticonvulsive effects on such withdrawal hyperexcitability *in vivo*. However, differences were seen between the pattern of actions of the different compounds on hippocampal hyperexcitability and on anxiety-related behaviour *in vivo*.

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