Nitrendipine, given during drinking, decreases the electrophysiological changes in the isolated hippocampal slice, seen during ethanol withdrawal

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1 Extracellular recordings were made from mouse isolated hippocampal slices prepared after chronic treatment *in vivo* with either ethanol or ethanol plus the dihydropyridine calcium channel antagonist, nitrendipine.

2 The withdrawal of ethanol caused a variety of changes in the field potentials, as previously reported, including decreases in the thresholds for eliciting single and multiple population spikes, increases in paired pulse potentiation and shifts to the left of the input/output curves.

3 The addition of nitrendipine to the drinking mixture in the chronic ethanol treatment significantly decreased all the changes in the field potentials that were seen after ethanol withdrawal.

4 Addition of nitrendipine to the perfusion medium also decreased the signs of hyperexcitability seen in the hippocampal slices during ethanol withdrawal.

5 The results provide further evidence that neuronal calcium channels may be involved in ethanol dependence and that the adaptive changes caused by chronic ethanol treatment can be modulated by alterations at dihydropyridine-sensitive sites.

Keywords: Ethanol withdrawal; hippocampus; dihydropyridines; Ca^{2+} .

Introduction

The results in the preceding paper (Whittington *et al.*, 1991) showed that a dihydropyridine calcium channel antagonist, nitrendipine, given concurrently with ethanol, during chronic ethanol administration, prevented the ethanol withdrawal syndrome. This was considered to be an adaptive response, as it was seen when the dihydropyridine treatment was stopped 24 h or 48 h before the testing of withdrawal and the CNS concentrations of nitrendipine at the time of testing were too low to produce an acute effect on the withdrawal syndrome. In addition, nitrendipine, given concurrently with ethanol in chronic treatment, has been found to prevent the development of tolerance to ethanol (Little & Dolin, 1987; Dolin & Little, 1989). The increase in the number of dihydropyridine binding sites that occurred after chronic ethanol administration, was also prevented, suggesting a possible causal relationship between the binding site changes and development of ethanol tolerance (Dolin *et al.*, 1988; Dolin & Little, 1989). We have put forward the theory that the effects of concurrently administered calcium channel antagonists on ethanol dependence are due to the prevention of the upregulation of voltage-sensitive calcium channels (Dolin *et al.*, 1988; Dolin & Little, 1989; Littleton & Little, 1989).

Ethanol has been known for some time to affect neuronal calcium mechanisms. It decreased the fast phase of depolarization-induced calcium flux into neurones, an effect to which tolerance developed on chronic treatment (Harris & Hood, 1980; Friedman *et al.*, 1980). Ethanol decreased calcium spikes in cultured neurones (Stokes & Harris, 1982). Carlen *et al.* (1982) reported that ethanol (5–20 mM) increased both inhibitory and excitatory postsynaptic potentials in the isolated rat hippocampus and the hyperpolarization that followed the eliciting of calcium spikes. They concluded that ethanol increased both pre- and postsynaptic calcium-mediated mechanisms.

Only a few electrophysiological studies have been carried out on the effects of chronic ethanol treatment. Acute tolerance was seen to the depression of orthodromically evoked CA1 field potentials occurring at bath concentrations of up to

100 mM ethanol (Durand *et al.*, 1981). Three weeks after withdrawal from chronic ethanol treatment, Durand & Carlen (1984) found decreased after-hyperpolarizations and decreased spike frequency adaptation to depolarizing current in CA1 neurones in rat isolated hippocampal slices. These authors also reported decreased inhibitory postsynaptic potentials (i.p.s.ps) in dentate granule and CA1 neurones. However, when the same measurements were made 24 h after ethanol withdrawal, a different pattern was seen, with increased after-hyperpolarizations (Reynolds *et al.*, 1990). We have described changes in field potentials in the isolated hippocampal slice after chronic ethanol treatment (Whittington & Little, 1990; 1991). These changes included decreased thresholds for eliciting population spikes, following both orthodromic and antidromic stimulation, and increased paired pulse potentiation. They followed different time courses during the withdrawal period, suggesting different origins of the forms of hyperexcitability.

In the present study we have measured the effects of the dihydropyridine calcium channel antagonist, nitrendipine, on these changes in field potentials in the hippocampal slice following chronic ethanol treatment. Nitrendipine was given either during the chronic ethanol treatment *in vivo* or added to the perfusion medium. We have used a treatment schedule for nitrendipine *in vivo* that prevented the behavioural manifestations of ethanol withdrawal (see Whittington *et al.*, 1991). The aim was to see whether or not the nitrendipine treatment affected the changes in neuronal activity, as measured by extracellular field potentials, that occur following ethanol withdrawal. The chronic treatment schedules were chosen to parallel our behavioural and receptor binding measurements (see Whittington *et al.*, 1991).

Some of these results have been presented in abstract form to the British Pharmacological Society.

Methods

Chronic drug treatment

Male mice, C57 strain, 25–30 g, were given ethanol, 24% v/v, as sole fluid, for 12 weeks. The mouse was chosen for these

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studies in order to provide a direct comparison with our earlier behavioural studies. For the chronic nitrendipine treatment the compound was dissolved in the drinking fluid at 1.77 mM. This did not alter the ethanol intake; the mean daily intakes were $15.0 \pm 0.9 \text{ g kg}^{-1}$ daily ($n = 9$) for ethanol alone and $14.4 \pm 0.1 \text{ g kg}^{-1}$ daily ($n = 12$) for ethanol plus nitrendipine. Mice were selected at random from these groups for preparation of the slices. Nitrendipine intake was $56.4 \pm 3.8 \text{ mg kg}^{-1}$ daily. The nitrendipine was removed from the drinking fluid 24 h before the experiments (the ethanol remaining) so that the effects of chronic nitrendipine treatment, rather than any acute action, could be studied. The mice were weighed at regular intervals during the chronic treatment and there were no significant differences between the weights of any of the treatment groups.

Controls drank water only. In separate experiments, nitrendipine was included in the perfusion medium (see below). Owing to the low aqueous solubility of nitrendipine it was not possible to treat animals by this method with nitrendipine in the absence of ethanol. Chronic treatment with nitrendipine alone, by injection, had no effect on the behaviour of mice, as measured by responses to handling (see Whittington *et al.*, 1991).

Hippocampal slices were prepared between 09 h 00 min and 10 h 00 min and perfused with standard Ringer solution. The animals were not withdrawn from the ethanol before preparation of the slices, so ethanol would have been gradually washed out of the slices during the beginning of the recording period.

Electrophysiological recordings

Dissections were made in HEPES medium (see below) and 400 μm slices of hippocampus were cut on a McIlwain chopper. The slices were maintained in recording chambers on nylon mesh and lens tissue, superfused with Krebs solution (see below) at $30^\circ\text{C} \pm 0.5^\circ\text{C}$ and 1.5 ml min^{-1} . Extracellular recordings from stratum pyramidale in area CA1 were started 30 min after the slices were placed in the perfusion bath, that is 45 min from removal of tissues.

Orthodromic stimulation of Schaffer collateral/commissural fibres was given, with paired pulses, constant current, 50 μs duration and 0.1 Hz. The stimulating electrodes consisted of two silver insulated wires, 0.2 mm in diameter, passed through 1 mm diameter silicon tubing. Recording electrodes were made from 1.2 mm diameter electrode glass and filled with 2 M KCl; the resistances were between 5 and 15 M Ω . A 70 ms interval was used for the paired pulses because preliminary experiments showed that it gave the maximum paired pulse potentiation in normal mouse hippocampal slices. A stimulation level of 1.25 times the threshold stimulation for eliciting a single population spike was used in the studies on paired pulse potentiation.

The thresholds for the production of population spikes, and paired pulse potentiation, were measured every 15 min and input/output curves determined at 1, 3, 5 and 7 h from removal of tissues, i.e. from the start of ethanol withdrawal. Measurements were made of the thresholds for elicitation of single population spikes and multiple population spikes (see Figure 1b). The former threshold was the minimum stimulating current required to produce a single population spike superimposed on the excitatory postsynaptic potential (e.p.s.p.) produced by the first of the paired stimuli, as discerned by eye. The threshold for multiple population spikes was established by the minimum stimulating current required to produce a second population spike, seen after the first but superimposed on the same e.p.s.p. (see Figure 2c).

In the studies on the acute actions of nitrendipine, the compound was dissolved in dimethylsulphoxide (DMSO) and then added to the bathing medium at 10 μM . The final DMSO concentration of 0.02%, did not alter the field potentials. (We have not found any action of DMSO on the field potentials at concentrations up to 0.5%). The effects of nitrendipine added

to the bathing medium were studied on control slices and following chronic ethanol administration *in vivo*. In each case the compound was present during the entire recording period, rather than being added after control responses were established. The reason for this was that we wished to study the effects of nitrendipine on the various changes that occur in the field potentials from the beginning of ethanol withdrawal. Comparison was therefore made between rather than within slices.

At the completion of every day's experiments the perfusion chamber was washed through with distilled water for at least 30 min, and washed through every 5 days with 500 mM sodium hydroxide followed by 100 mM citric acid in order to prevent residual drug build up on the sides of the perfusion tubing.

Statistical analysis

The areas of the population spikes were measured by Acorn computer analysis. In every case a minimum of three responses were averaged for each stimulus. The results in the Figures are expressed as mean $\pm$ s.e.mean for each of the different treatments. Five tissues from five different animals were used for each treatment group. Comparisons were made between results from tissues following the different treatments by analysis of variance. The times given in the results section are all from the removal of the tissues. This corresponded with the withdrawal of ethanol.

Input/output relationships were derived from population spike area, and e.p.s.p. slope data. Non-linear regression was used to produce sigmoidal curves, to enable calculation of the stimulus required to produce half-maximal responses. Comparisons of data from these curves were made between different tissues, following the various treatments, by use of Student's nonpaired *t* test.

Certain precautions were taken to ensure that valid comparisons could be made between the field potentials from tissues from different treatment groups (Whittington & Little, 1990). The order of testing and the distribution of the various treatments between the two recording chambers were carefully balanced. The positions of the stimulating and recording electrodes were determined in every tissue by using the ends of the arc of granular cells in the dentate gyrus and the apex of the CA3 region above the fimbria as common reference points between slices.

Composition of solutions

The composition of the perfusion fluid was (mM): NaCl 124, KCl 3.25, NaH_2PO_4 1.25, NaHCO_3 20.0, MgSO_4 2.0, CaCl_2 2.0, and D-glucose 10.0. The pH was 7.2 at 30°C . The composition of the HEPES buffered perfusion medium was (mM): NaCl 120, KCl 2.0, KH_2PO_4 1.25, NaHCO_3 20.0, MgSO_4 2.0, CaCl_2 2.0, D-glucose 10.0 HEPES acid 7.0 and NaHEPES 2.6. The pH was 7.2 at 23°C .

Results

Thresholds for elicitation of population spikes

Chronic nitrendipine treatment The chronic ethanol treatment caused decreases in the threshold for eliciting both single and multiple population spikes, from 3 h onwards, compared with control tissues ($P < 0.005$ and 0.001 respectively). These decreases were smaller after the chronic nitrendipine treatment *in vivo* (Figures 1a and 2a). Examples of some of the traces obtained during measurement of multiple population spike thresholds are shown in Figure 2c. Analysis of variance over the 4 h–7 h period (inclusive) showed significant differences ($P < 0.005$ for eliciting single and $P < 0.001$ for multiple population spikes) when tissues from animals treated with ethanol alone were compared with those from mice treated with ethanol plus nitrendipine. Although the effect of the ethanol treatment did not appear to be completely abolished,

Figure 1 (a) The effect of chronic treatment with nitrendipine on the decrease in thresholds for eliciting single population spikes caused by withdrawal from chronic ethanol treatment. The chronic nitrendipine treatment was stopped 24 h before the ethanol withdrawal. Times given are all from preparation of tissues. This was also the time of ethanol withdrawal (time zero on the horizontal axis). The thresholds in slices from mice treated with ethanol were significantly different from those in slices from mice treated with ethanol and nitrendipine (comparison from 3 h to 7 h inclusive $P < 0.005$). Thresholds are in μA , mean with s.e.mean shown by vertical bars: ($\square$) controls; ($\blacktriangle$) chronic ethanol-treatment; ($\bullet$) chronic ethanol plus nitrendipine treatment. (b) The effect of bath application of nitrendipine on the decrease in thresholds for eliciting single population spikes caused by withdrawal from chronic ethanol treatment. Nitrendipine, $10\mu\text{M}$, was present in the bathing medium from the start of recording. The thresholds measured when nitrendipine was added after ethanol withdrawal were significantly different from those when vehicle was added to slices prepared after ethanol treatment *in vivo* ($P < 0.005$). Thresholds are in μA , mean with s.e.mean shown by vertical bars. Times given are all from preparation of tissues. ($\square$) Controls; ($\blacktriangle$) chronic ethanol treatment; ($\bullet$) chronic ethanol treatment, nitrendipine, $10\mu\text{M}$, in perfusion medium.

comparison of the results for the tissues following chronic treatment with ethanol plus nitrendipine were not significantly different from those from control tissues ($P > 0.1$).

Acute addition of nitrendipine to the bathing medium The decreases in thresholds for eliciting single and multiple population spikes seen after chronic ethanol treatment were reduced when nitrendipine, $10\mu\text{M}$, was added to the bathing medium (Figures 1b and 2b). Analysis of variance over the 4 h to 7 h period (inclusive) showed significant differences between data from slices from ethanol-treated mice recorded with and without nitrendipine in the bathing medium ($P < 0.005$ for both single population spike thresholds and multiple spike thresholds). The extent of protection from the effects of ethanol withdrawal afforded by the acute addition of nitrendipine appeared smaller than that due to chronic nitrendipine intake (compare Figures 1a and 1b and Figures 2a and 2b). The thresholds for eliciting multiple population spikes (Figure 2b) were still significantly different from control values when nitrendipine was perfused in the bathing medium after ethanol withdrawal ($P < 0.001$), but this difference was not significant for the single spike thresholds ($P > 0.1$).

Paired pulse potentiation

Chronic nitrendipine treatment Hippocampal slices from animals that received chronic ethanol treatment showed a transient increase in paired pulse potentiation, seen only between 2 and 4 h from the start of recording, as reported previously (Whittington & Little, 1990; 1991). This effect was also decreased by chronic nitrendipine administration (Figure 3a). The difference between the paired pulse potentiation for controls and ethanol treatment, between 1–4 h from the start of recording, was significant ($P < 0.001$). The difference between ethanol and ethanol plus chronic nitrendipine treatments was also significant ($P < 0.001$), although the paired pulse potentiation after ethanol plus nitrendipine was still higher than the control values ($P < 0.001$).

Acute addition of nitrendipine to the bathing medium The addition of nitrendipine to the medium, at $10\mu\text{M}$, (Figure 3b) also reduced the increase in paired pulse potentiation seen after chronic ethanol treatment ($P < 0.001$ for comparison between ethanol alone and ethanol plus nitrendipine), although there was still a significant difference between ethanol plus nitrendipine and control treatments ($P < 0.001$).

Input/output curves

Chronic nitrendipine treatment The input/output curves showed that the chronic ethanol treatment caused a shift to the left, at the 5 h and 7 h intervals, of the lines for population spike area against stimulus intensity, without changes in the gradient of the line or the maximum responses (Figure 4a). The decrease in the stimulus intensity required to produce half maximal population spikes (S_{50}) was almost completely abolished by the chronic nitrendipine treatment ($P < 0.005$ for comparison of this value for ethanol alone and for ethanol plus nitrendipine, at both 5 h and 7 h after withdrawal), indicating a reduction in the shift to the left of these curves during ethanol withdrawal. After treatment with ethanol plus nitrendipine, the position of the input/output curve and its gradient were not significantly different from the control curve.

Acute addition of nitrendipine to the bathing medium Nitrendipine added to the bathing medium at $10\mu\text{M}$, caused a small, but significant, decrease in the shift to the left of the input/output curve for population spike area caused by the chronic ethanol treatment (Figure 4b). The effect of the ethanol treatment on the stimulation level required to produce half-maximal responses (S_{50}) was significantly decreased by the addition of nitrendipine ($P < 0.01$ at 5 h and 7 h), but the S_{50} remained significantly lower than the control value ($P < 0.01$). The gradient of the line after addition of nitrendipine to the bathing medium was significantly lower than either the line for slices prepared after ethanol treatment or for controls.

Input/output curves for the slopes of the field e.p.s.ps

Chronic nitrendipine treatment The plots of field e.p.s.p. slope against stimulus intensity showed that the ethanol treatment decreased both the slope and the maximum value for this measurement at the 5 h and 7 h time intervals, and shifted the lower part of the curves to the left (Figure 5a). This indicated a greater response of the pyramidal cells for a given stimulus intensity at the lower end of the stimulation range, but smaller responses at higher levels of stimulation.

Figure 5a shows that chronic nitrendipine treatment had a clear effect on the decrease in maximal response caused by the chronic ethanol treatment. The protective effect of nitrendipine on the maximal responses (calculated from the sigmoid curves) was significant at the 7 h time interval ($P < 0.01$, comparison between ethanol alone and ethanol plus nitrendipine).

When the gradients of the sigmoid curves for e.p.s.p. slope against stimulus intensity were compared, the ethanol treatment was found to cause significant decreases in these values

Figure 2 (a) The effect of chronic treatment with nitrendipine on the decrease in thresholds for eliciting multiple population spikes caused by withdrawal from chronic ethanol treatment. Times given are all from preparation of tissues, as described in Figure 1(a). The thresholds in slices from mice treated with ethanol were significantly different from those in slices from mice treated with ethanol plus nitrendipine, from 3 h to 7 h inclusive ($P < 0.001$). Thresholds are in μA , mean with s.e.mean shown by vertical bars. ($\square$) Controls; ($\blacktriangle$) chronic ethanol treatment; ($\blacksquare$) chronic ethanol plus nitrendipine treatment. (b) The effect of bath application of nitrendipine on the decrease in thresholds for eliciting multiple population spikes caused by withdrawal from chronic ethanol treatment. Nitrendipine, $10\text{ }\mu\text{M}$, was present in the bathing medium from the start of recording. The thresholds when nitrendipine was added after ethanol treatment were significantly different from those when vehicle was added ($P < 0.005$). Thresholds are in μA , mean with s.e.mean shown by vertical bars. Times given are all from preparation of tissues. ($\square$) Controls; ($\blacktriangle$) chronic ethanol treatment; ($\bullet$) chronic ethanol treatment, nitrendipine, $10\text{ }\mu\text{M}$, in perfusion medium. (c) Examples of traces recorded from slices during eliciting of multiple population spikes. These recordings were made 7 h after preparation of tissues, that is 7 h from ethanol withdrawal. The stimulation level was $70\text{ }\mu\text{A}$, for all three slices. The scale bar is 2 mV, 20 ms. (i) Control; (ii) chronic treatment with ethanol; (iii) chronic treatment with ethanol plus nitrendipine

at the 3 h and 5 h intervals ($P < 0.01$, comparison between chronic ethanol treatment and controls), whereas the values were not significantly changed after chronic administration of ethanol plus nitrendipine ($P > 0.1$, comparison between controls and ethanol plus nitrendipine). This reflects the prevention by nitrendipine of the decrease, caused by the ethanol treatment, in the maximal responses.

The stimulus intensities required to produce half maximal field e.p.s.p. slopes do not give a good indication of the changes when, as in this instance, the maximum values and gradients of the curves are altered, although when these S_{50} values were calculated, the chronic nitrendipine prevented the decreases due to the chronic ethanol treatment ($P < 0.05$,

comparison between ethanol and ethanol plus nitrendipine-treated groups). The shift to the left of the lower part of the curves appeared to be less affected by the nitrendipine treatment.

Acute addition of nitrendipine to the bathing medium The acute action of nitrendipine on the changes in the field e.p.s.p. slopes showed a qualitatively similar pattern to that of the chronic nitrendipine treatment (Figure 5b). The effect of the ethanol treatment on the maxima of the sigmoid curves for field e.p.s.p. slope against stimulus intensity was completely prevented when nitrendipine was added to the bathing medium, at $10\text{ }\mu\text{M}$ ($P < 0.01$ for comparison between addition

Figure 3 (a) The effect of concurrent chronic treatment with nitrendipine on the increase in paired pulse potentiation caused by withdrawal from chronic ethanol treatment. Times given are all from preparation of tissues, as described in Figure 1(a). The paired pulse potentiation in slices from mice treated with ethanol was significantly different from that in slices from mice treated with ethanol and nitrendipine (1 h to 4 h inclusive, $P < 0.001$). Thresholds are in μA , mean with s.e.mean shown by vertical bars. ($\square$) Controls; ($\blacktriangle$) chronic ethanol treatment; ($\blacksquare$) chronic ethanol plus nitrendipine treatment. (b) The effect of bath application of nitrendipine on the increase in paired pulse potentiation caused by withdrawal from chronic ethanol treatment. Nitrendipine, $10\mu\text{M}$, was present in the bathing medium from the start of recording. The potentiation when nitrendipine was added after ethanol withdrawal was significantly different from that when vehicle was added after ethanol withdrawal ($P < 0.001$). Thresholds are in μA , mean with s.e.mean shown by vertical bars. Times given are all from preparation of tissues. ($\square$) Controls; ($\blacktriangle$) chronic ethanol treatment; ($\bullet$) chronic ethanol treatment, nitrendipine, $10\mu\text{M}$, in perfusion medium.

Figure 4 (a) The effect of concurrent chronic treatment with nitrendipine on the shift to the left in input/output curves derived from population spike measurements caused by withdrawal from chronic ethanol treatment. The diagram illustrates the changes in input/output curves 5 h after ethanol withdrawal. The stimulus intensities required to produce half maximal population spikes were significantly different when recordings from slices from mice treated with ethanol were compared with those from slices from mice treated with ethanol and nitrendipine ($P < 0.005$). Data are given as mean with s.e.mean shown by vertical bars. ($\square$) Controls; ($\blacktriangle$) chronic ethanol treatment; ($\blacksquare$) chronic ethanol plus nitrendipine treatment. (b) The effect of bath application of nitrendipine, $10\mu\text{M}$, on the shift to the left in input/output curves derived from population spike measurements caused by withdrawal from chronic ethanol treatment. The diagram illustrates the changes in input/output curves 5 h after ethanol withdrawal. After the ethanol treatment, the stimulus intensities required to produce half maximal population spikes when nitrendipine was added to the bathing medium were significantly different from the results when vehicle was added ($P < 0.01$). Data shown as mean with s.e.mean shown by vertical bars. ($\square$) Controls; ($\blacktriangle$) chronic ethanol treatment; ($\bullet$) chronic ethanol treatment, nitrendipine, $10\mu\text{M}$, in perfusion medium.

of nitrendipine and addition of vehicle, at 5 h and 7 h into withdrawal).

Nitrendipine, $10\mu\text{M}$, also decreased the changes in the gradient of these curves that were seen 5 h and 7 h after ethanol withdrawal ($P < 0.05$, comparison between slices from mice treated with ethanol, recorded with or without nitrendipine in the bathing medium). However, the effect of the chronic ethanol treatment in causing a shift of the lower part of the curve to the left appeared to be less affected by the nitrendipine.

Lack of effect of nitrendipine in control tissues

The addition of nitrendipine to the perfusion medium at $10\mu\text{M}$ did not alter any aspect of the field potentials recorded from control slices, $P > 0.1$ (Figures 6a and b). There were no changes in either the gradient or the maxima of the input/output curves ($P < 0.01$, not illustrated). Nitrendipine added to the perfusion medium at $10\mu\text{M}$ had no effect on paired pulse potentiation in control tissues (Figure 6c).

Discussion

All the changes in the field potentials in the mouse isolated hippocampal slice preparation caused by ethanol withdrawal were significantly smaller when the dihydropyridine calcium

channel antagonist, nitrendipine, was given chronically with the ethanol, even though the amount of ethanol drunk was unaltered. The effects of the *in vivo* ethanol treatment were also reduced when nitrendipine was added to the bathing medium. No significant effects of bath-applied nitrendipine were seen in slices from control animals on the thresholds for eliciting population spikes, paired pulse potentiation or input/output data for population spike area, amplitude and e.p.s.p. slope. Chronic administration of ethanol therefore appeared to alter the actions of nitrendipine on the field potentials.

These results parallel the effects of nitrendipine treatment on the behavioural manifestations of withdrawal (Whittington & Little, 1988; Whittington *et al.*, 1991). We have previously demonstrated that concurrent nitrendipine treatment also prevented the development of ethanol tolerance (Dolin & Little, 1989). Such treatment also prevented the increase in central dihydropyridine binding caused by chronic ethanol administration (Dolin *et al.*, 1988; Dolin & Little, 1989). Chronic administration of nitrendipine therefore decreased the results of long-term ethanol administration as seen by three different techniques: behaviour, field potential recording and receptor binding. In addition, Brennan *et al.* (1990) showed a correlation between the sensitivity of mouse strains to ethanol withdrawal and the extent of the increases in dihydropyridine binding. Changes in neuronal dihydropyridine-sensitive

Figure 5 (a) The effects of chronic nitrendipine treatment on the changes caused by withdrawal from chronic ethanol treatment *in vivo*, in input/output curves for field excitatory postsynaptic potentials (e.p.s.p.) slope against stimulus intensity. The graph illustrates the changes seen 7 h after ethanol withdrawal. The chronic nitrendipine treatment prevented the decrease in maximum response seen after ethanol withdrawal at this time. Data expressed as mean with s.e.mean shown by vertical bars. ($\square$) Controls; ($\blacktriangle$) chronic ethanol treatment; ($\blacksquare$) chronic ethanol plus nitrendipine treatment. (b) Effects of bath application of nitrendipine, $10\text{ }\mu\text{M}$, on changes, caused by withdrawal from chronic ethanol treatment, in input/output curves for field e.p.s.p. slope against stimulus intensity. The graph illustrates the changes seen 7 h after ethanol withdrawal. Nitrendipine prevented the decrease in maximum response seen after ethanol withdrawal at this time. Data expressed as mean with s.e.mean shown by vertical bars. ($\square$) Controls; ($\blacktriangle$) chronic ethanol treatment; ($\bullet$) chronic ethanol treatment, nitrendipine, $10\text{ }\mu\text{M}$, in perfusion medium.

calcium channels may therefore be involved in ethanol dependence.

The actions of nitrendipine in decreasing all the changes in the field potentials appeared to be qualitatively similar whether it was given chronically during the ethanol treatment or 'acutely' in the bathing medium, although the effects of the latter were rather small. We have previously shown that the calcium channel antagonist, PN 200-110, added to the perfusion medium, completely prevented the changes in the hippocampal slices after chronic ethanol treatment, in a stereospecific manner (Whittington & Little, 1989; 1991). The brain concentrations of nitrendipine, measured during the chronic treatment (Whittington *et al.*, 1991), were in the range 200–260 nM (this is an approximate calculation assuming equal distribution throughout the CNS). The effects of chronic and acute treatment cannot be compared directly as the action of chronic nitrendipine appears to involve an adaptive response. It is unlikely that the action of the chronically administered nitrendipine was due to the presence of the compound in the tissue at the time of recording because the nitrendipine was removed from the drinking fluid 24 h before the preparation of the slices and very little was present in the brain at the time of preparation of the tissues (Whittington *et al.*, 1991).

In our previous study of the changes in field potentials in the hippocampal slice during ethanol withdrawal, we concluded that possible mechanisms for these changes could include decreases in feedback inhibition, increased ability of

Figure 6 (a) Lack of effect of nitrendipine, $10\text{ }\mu\text{M}$ applied in the perfusion medium, on single population spike thresholds in tissues from control animals (mean with s.e.mean shown by vertical bars). Nitrendipine did not cause any changes in the thresholds for eliciting single population spikes ($P > 0.01$). ($\square$) Controls plus vehicle; ($\blacktriangle$) controls plus nitrendipine. (b) Lack of effect of nitrendipine, $10\text{ }\mu\text{M}$ applied in the perfusion medium, on multiple population spike thresholds in tissues from control animals (mean with s.e.mean shown by vertical bars). Nitrendipine did not cause any changes in the thresholds for eliciting multiple population spikes ($P > 0.01$). ($\square$) Controls plus vehicle; ($\blacktriangle$) controls plus nitrendipine. (c) Lack of effect of nitrendipine, $10\text{ }\mu\text{M}$ applied in the perfusion medium, on paired pulse potentiation in tissues from control animals (mean with s.e.mean shown by vertical bars). Nitrendipine did not cause any changes in paired pulse potentiation ($P > 0.1$). ($\square$) Controls plus vehicle; ($\bullet$) controls plus nitrendipine.

pyramidal cells to generate action potentials for a given field e.p.s.p. size and possibly changes in intracellular calcium concentrations (Whittington & Little, 1990). The present study suggests that alterations in dihydropyridine-sensitive calcium channels might be the underlying cause of these changes. It is appreciated, however, that intracellular measurements will be necessary before definitive conclusions can be drawn concerning the selectivity of the doses and concentrations of nitrendipine used in the present study. These are currently in progress.

We suggest therefore that the effects of chronically administered nitrendipine were due to an adaptive response to the presence of the compound in the central nervous system, preventing the action of ethanol on the dihydropyridine-sensitive channels. Panza *et al.* (1985) demonstrated that the long term intake of a dihydropyridine calcium channel antagonist could result in down-regulation of central dihydropyridine-sensitive binding sites. This study used considerable higher doses (280 mg kg^{-1} daily) than the present experiments. We have found that doses in the range that we use (50 mg kg^{-1} daily) did not cause down-regulation of dihydropyridine-sensitive sites when given alone, but did prevent the up-regulation caused by chronic ethanol adminis-

tration (Dolin *et al.*, 1988; 1989; Whittington & Little, 1991; Whittington *et al.*, 1991).

Although high affinity binding sites have been demonstrated on neurones in the CNS (Glossman *et al.*, 1982), calcium channel antagonists have only weak effects on normal mammalian neurones. The results in the present paper demonstrating a lack of effect of nitrendipine on any aspect of the field potentials in hippocampal slices from control animals are in agreement with the literature. Some actions of dihydropyridines on neurones have been demonstrated by voltage clamp techniques, for example, Docherty & Brown (1986) showed that nimodipine at 0.05–10 μM decreased calcium currents in hippocampal CA1 pyramidal cells. Nifedipine at 1 μM to 10 μM did not modify presynaptic calcium entry in hippocampal slices, but decreased the calcium concentration changes thought to be associated with postsynaptic responses (Louvel *et al.*, 1986; Jones & Heinemann, 1987). Low micromolar concentrations of dihydropyridines have been shown to affect subtypes of calcium channels other than the 'L' subtype for which they were originally thought to be selective (Takahashi *et al.*, 1989; Takahashi & Akaïke, 1990). Grover & Teyler (1990) have demonstrated a dihydropyridine-sensitive component of transmission in CA1 pyramidal cells, seen only at very high frequency stimulation.

The extent of the protective action of nitrendipine differed slightly for the various electrophysiological measurements. The decreases in the thresholds for eliciting population spikes after chronic ethanol treatment were only partially prevented by the chronic nitrendipine treatment, but the shifts to the left of the input/output curves were almost completely abolished. The initial increase in population spike threshold, seen in slices from ethanol-treated mice was not affected by nitrendipine treatment. This increase may indicate that residual levels of ethanol were present in the slice during the initial stages of recording, or that the presence of ethanol in the brain during dissection affected the re-establishment of synaptic activity in the slice during the first minutes of the recording period.

The decreases in maximal field e.p.s.p. slopes, after chronic ethanol treatment, were completely prevented by nitrendipine. This effect of ethanol withdrawal may indicate a decrease in maximum e.p.s.p. size at the site of generation on apical dendrites. Alternatively, the maximum e.p.s.p. size may have been reduced by alteration in the shape of the field potential, i.e. whether or not a population spike was present. In the latter case, the changes would have been produced by effects at the recording site, the cell soma. The increases in field e.p.s.p. slope in response to lower levels of stimulation seen after the ethanol treatment were little affected by nitrendipine.

The nitrendipine treatment schedule used in this study totally prevented the behavioural withdrawal syndrome (see Whittington *et al.*, 1991, preceding paper). Comparable

amounts of ethanol were taken in during both sets of experiments, so the severity of withdrawal would be expected to be similar. These results suggest that the input/output curves may be a better model of the withdrawal syndrome than the thresholds and there may be different components to the changes in field e.p.s.p. slope at low stimulation levels than in the responses to the rest of the stimulation range.

The hippocampal slice was chosen for this study because of the extent of knowledge about the neuronal pathways and the demonstration of high concentrations of dihydropyridine binding sites (Glossman *et al.*, 1982). It is possible, however, that the first stages of the behavioural withdrawal syndrome occur lower in the brainstem (Hunter & Walker, 1978). Many of the changes seen after chronic ethanol could be explained by increased numbers of dihydropyridine-sensitive calcium channels. These sites have recently been shown to be located on cell bodies and dendrites of hippocampal pyramidal CA1 pyramidal cells and are found in high density at the base of major dendrites (Westenbroek *et al.*, 1990). They are thought to contribute to the large local calcium currents in this area. These currents will play a part in controlling the transmission of stimuli from dendrites to soma. Increases in their number would be likely to increase this transmission, thus causing hyperexcitability.

It is not yet certain whether the chronic ethanol treatment used in this study caused any anatomical neuronal damage. This is currently under investigation. Longer ethanol treatment schedules have been shown to cause neuronal loss (Lee *et al.*, 1981). While the decreases in the maximum field e.p.s.p. responses could have been explained by loss of neurones, it seems unlikely that this could have been reversed by the bath application of nitrendipine, as was seen.

Littleton and co-workers, using cell cultures, have recently shown that the upregulation of calcium channels after chronic ethanol treatment may involve the inositol lipid-protein kinase C system (Brennan & Littleton, 1990) and that the synthesis of new protein is required (Harper *et al.*, 1989). The prevention of this upregulation by chronic dihydropyridine treatment, demonstrated in our studies, was seen also in the cell cultures. Our results therefore show that chronic ethanol treatment causes an increase in the number of dihydropyridine binding sites and an enhanced functional activity of neuronal calcium channels, and that this, in whole or in part, is the cause of withdrawal hyperexcitability and can be prevented by concurrent dihydropyridine administration.

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