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Ethanol modulates *N*-methyl-D-aspartate-evoked arachidonic acid release from neurones

Molin Navamani, Michael Morgan, Robert J. Williams *

Biochemical Neuropharmacology Group, Division of Biochemistry and Molecular Biology, UMDS, Guy's Medical School, London Bridge,London SE1 9RT, UK

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Abstract

Glutamate-evokes a Ca^{2+}-dependent release of arachidonic acid from cultured neurones via the activation of NMDA and AMPA receptors. In this study we investigated whether exposing cultured striatal neurones either acutely or chronically to ethanol would modify these responses. Acute ethanol (100 mM, 15 min) inhibited the liberation of arachidonic acid evoked by a maximally effective concentration of glutamate, an affect which appeared to be mediated primarily by a reduction in NMDA receptor responsiveness. In contrast, chronic ethanol exposure caused a dose-dependent increase in the glutamate, *N*-methyl-D-aspartate (NMDA) and α-amino-3-hydroxy-5-methylisoxazole-4-propionate (AMPA) evoked release of arachidonic acid, although ethanol was less potent at the AMPA response. Basal responses were not altered by acute or chronic ethanol and the concentrations of ethanol employed were not toxic. Chronic ethanol (100 mM, 48 h) increased NMDA-mediated neuronal damage at sub-maximal concentrations of the agonist, suggesting that an enhanced mobilisation of arachidonic acid may underly the potentiated excitotoxic neuronal loss observed following exposure to ethanol.

Keywords: Ethanol; Glutamate; Excitotoxicity; Arachidonic acid; Striatal neuron

1. Introduction

L-glutamate, the principal excitatory neurotransmitter in the central nervous system (CNS), mediates synaptic transmission via the activation of NMDA and non-NMDA receptor subtypes. By acting on ionotropic and metabotropic receptors, glutamate has been implicated in complex neuronal functions such as learning, memory and synaptic plasticity (Collingridge and Singer, 1990). In addition to this physiological role, glutamate is also potentially neurotoxic and a sustained activation of glutamate receptors is believed to be an important initial step in neuronal degeneration which occurs following ischaemia, hypoglycaemia and cerebral trauma (Meldrum and Garthwaite, 1990). The precise mechanisms underlying this neuronal loss are unclear although potentially damaging reactive oxygen species are generated during neuronal degeneration (Coyle and Puttfarcken, 1993) and following the activation of NMDA receptors (Lafon-Cazal et al., 1993; Reynolds and Hastings, 1995; Dugan et al., 1995; Gunasekar et al., 1995). One potential source of ROS is through the metabolism of the multifunctional signalling molecule, arachidonic acid.

Glutamate evokes a Ca^{2+}-dependent release of arachidonic acid from neurones via the activation of NMDA receptors (Dumuis et al., 1988; Lazarewicz et al., 1988), by the joint stimulation of AMPA and metabotropic receptors (Dumuis et al., 1990; Williams et al., 1995) or through the activation of AMPA receptors alone in the presence of cyclothiazide (Williams and Glowinski, 1996). The mobilisation of this fatty acid within the CNS is of considerable importance as arachidonic acid is a potent modulator of glutamatergic neurotransmission (Miller et al., 1992) and a potential mediator of both long term potentiation (Williams et al., 1989) and long term depression (Linden, 1995). Significantly, from a pathological viewpoint, elevated levels of arachidonic acid are observed following cerebral ischaemia, hypoglycaemia and electroconvulsive shock (Bazan, 1970). Arachidonic acid potently inhibits

* Corresponding author. Tel.: +44-171-9552979; fax: +44-171-9554191; e-mail: r.williams@umds.ac.uk.

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Na^+-dependent glutamate uptake into synaptosomes and astrocytes (Chan et al., 1983; Barbour et al., 1989; Volterra et al., 1992) and thereby potentially induces neuronal damage via an overactivation of glutamate receptors. Thus, modification of the arachidonic acid cascade can potentially affect both the physiology and pathophysiology of the CNS.

Ethanol is a potent modulator of synaptic transmission and its intake during gestation can profoundly damage the development of the nervous system. Furthermore, studies in both animals and humans have shown that chronic ethanol ingestion and or ethanol withdrawal can induce neurotoxicity (Walker et al., 1980; Charness et al., 1989). The precise cellular mechanisms involved in these processes are unclear. A large number of studies have suggested that NMDA receptors may represent an important site of action for ethanol within the CNS (Tsai et al., 1995; Tabakoff and Hoffman, 1996; and references therein). Ethanol, acutely, inhibits NMDA-induced ion currents (Lovinger et al., 1989, 1990), Ca^{2+} influx, cGMP production (Hoffman et al., 1989) and NMDA- mediated elevation of intracellular Ca^{2+} (Dildy-Mayfield and Leslie, 1991), and reduces NMDA-evoked neurotoxicity (Takadera et al., 1990; Lustig et al., 1992; Chandler et al., 1993). In contrast, chronic exposure to ethanol enhances NMDA receptor function (Iorio et al., 1992; Hu and Ticku, 1995) increases the number of NMDA binding sites (Grant et al., 1990; Gulya et al., 1991; Hu and Ticku, 1995) and potentiates NMDA-induced neurotoxicity (Iorio et al., 1993; Chandler et al., 1993; Ahern et al., 1994). The pathways leading to increased excitotoxicity which lie downstream of an enhanced NMDA-evoked elevation in intracellular Ca^{2+} are unknown. In this study, therefore, we investigated whether acute and chronic exposure to ethanol would modify Ca^{2+}-dependent glutamate-evoked arachidonic acid release from cultured neurones and thus provide a possible mechanism to explain some of the previously observed physiological and pathophysiological effects of ethanol within the CNS.

2. Materials and methods

2.1. Chemicals

Chemicals were purchased from the following sources; L-glutamate, NMDA, veratridine, MTT (3-[4,5-dimethyliazol-2-yl]-2,5-diphenyltetrazolium bromide; Thiazolyl blue), poly-L-ornithine (MW 40,000), trypan blue, fatty acid free bovine serum albumin, indomethacin, nordihydroguaiaretic acid, from Sigma; AMPA and MK 801 from Tocris Cookson (Bristol), cyclothiazide from RBI; Dulbecco's modified Eagle's medium and F-12 nutrient from GIBCO BRL and [^{3}H]arachidonic acid (200 Ci/mmol) from Amersham (UK).

2.2. Cell culture

Primary cultures of mouse striatal neurones were prepared essentially as previously described (El Etr et al., 1989). Briefly, striata were dissected from 14–16 day old Swiss mouse embryos (NIH, Harlan) and mechanically dissociated using a fire-polished Pasteur pipette in phosphate-buffered saline supplemented with glucose (33 mM). Cells were plated into 24-well Nunc® multi-well plates that had been coated previously overnight with 15 μg/ml poly-L-ornithine and then with culture medium supplemented with 10% bovine calf serum (GIBCO BRL) for 2 h. Following removal of the final coating solution cells were seeded in a serum-free medium composed of a mixture of Dulbecco's modified Eagle's medium and F-12 nutrient (1:1) supplemented with 33 mM glucose, 2 mM glutamine, 6.5 mM sodium bicarbonate and 5 mM HEPES (pH 7.4), 100 μg/ml streptomycin and 60 μg/ml penicillin. A mixture of hormones and salts composed of insulin (25 μg/ml), transferrin (100 μg/ml), putrescine (60 μg/ml), progesterone (20 nM) and sodium selenate (30 nM) (all from Sigma) was also added to the culture medium. Cells were cultured at 37°C in an humidified atmosphere of 94% air and 6% CO_2. After 5–10 days in culture cells were defined immunocytochemically as described previously (El Etr et al., 1989), as neurones devoid of detectable glial cells.

2.3. Ethanol treatments

For chronic treatments ethanol (10–100 mM) was added directly to the culture medium. In order to maintain the ethanol concentration, media were replaced every 24 h with fresh media (containing the appropriate ethanol concentration), since ethanol has been reported to evaporate at a rate of approximately 10% of its concentration per day under these conditions (Hu and Ticku, 1995). The media on control cells was also changed in parallel every 24 h. For the purpose of this study a *chronic treatment* is defined as exposure to ethanol for $\geq$ 48 h.

2.4. Arachidonic acid release

Arachidonic acid release was performed essentially as described previously (Williams et al., 1995). Cells were cultured overnight with 1 μCi/ml of [^{3}H]arachidonic acid which was added directly to the culture medium. Unincorporated label was removed by three successive washes with buffer (145 mM NaCl, 5.5 mM KCl, 1.1 mM $MgCl_2$, 1.1 mM $CaCl_2$, 5.5 mM glucose, 20 mM HEPES, pH 7.4), containing fatty acid-free bovine serum albumin (1 mg/ml). Cells were then preincubated in Mg^{2+}-free buffer for 10 min at 37°C. The preincubation buffer was removed and the cells were then exposed to agonists also in Mg^{2+}-free buffer for 15 min at 37°C either in the presence (*acute*

treatment) or absence of ethanol. At the end of the incubation period media were centrifuged at 300 *g* for 5 min to remove dislodged cells and the radioactivity in the supernatants was then measured by scintillation counting. As arachidonic acid is poorly metabolised in primary cultured mouse neurones (Oomagari et al., 1991; Stella et al., 1995) the ^{3}H released was assumed to be essentially [^{3}H]arachidonic acid. Furthermore, the addition of indomethacin and nordihydroguaiaretic acid which are inhibitors of cyclooxygenase and lipoxygenase, respectively, did not significantly reduce the levels of ^{3}H released into the supernatant either in the presence or absence of ethanol (data not shown). This suggested that ethanol was probably not altering arachidonic acid metabolism under our conditions.

2.5. Neuronal viability

The effect of ethanol on neuronal viability was assessed by a colourimetric assay based on the cleavage of the tetrazolium salt MTT into a blue coloured formazan product by mitochondrial succinate dehydrogenase (Denizot and Lang, 1986). Following exposure to ethanol for the defined period, neurones were washed three times with buffer, as described for arachidonic acid release experiments, and then incubated for 2 h at 37°C in buffer containing MTT (0.5 mg/ml). After the incubation the buffer was carefully removed and the blue formazan product was solubilised in 0.5 ml of 100% dimethyl sulphoxide (DMSO). The absorbance of each well was measured at 570 nm.

The effect of ethanol on neuronal membrane integrity was periodically assessed by the ability of the cells to exclude trypan blue (as detailed in Sigma protocol).

2.6. Neurotoxicity

Cells (8–10 days in culture), previously exposed to ethanol for 48 h, were washed with 0.5 ml of a Mg^{2+}-free HEPES buffer (154 mM NaCl, 5.6 mM KCl, 3.6 mM $NaHCO_3$, 2.3 mM $CaCl_2$, 5.6 mM glucose and 5 mM HEPES, pH 7.4, previously equilibrated in the incubator for 1 h) and then incubated in the same buffer in the presence of NMDA for 25 min at 37°C in an humidified atmosphere of 94% air and 6% CO_2. Following the incubation period cells were washed twice in buffer and returned to the incubator in fresh culture medium for a further 24 h. The extent of neurotoxicity was then estimated by the MTT assay (as outlined above) and by measuring the amount of lactate dehydrogenase released from the cells into the media based on a lactate dehydrogenase (LDH) endpoint assay (Sigal et al., 1994).

2.7. Experimental design and statistical analysis

In all experiments, ethanol treated cells were grown in parallel with untreated controls. Arachidonic acid release, viability and excitotoxicity experiments were performed in triplicate. Owing to the variability in the absolute levels of arachidonic acid released from different cultures, data were normalised to show percent changes as compared with basal values, which was taken to be 100% in all cases. Data were analysed by unpaired two-tailed Student's *t* tests. A difference was considered to be statistically significant when $P < 0.05$.

3. Results

3.1. Effect of acute ethanol on glutamate, NMDA and AMPA-evoked arachidonic acid release

It has previously been shown that acute ethanol exposure selectively inhibits NMDA-mediated Ca^{2+} influx, therefore, we investigated whether or not acute ethanol would modify glutamate, NMDA or AMPA receptor-evoked arachidonic acid release from cultured striatal neurones. AMPA-stimulated arachidonic acid release was performed in the presence of cyclothiazide (30 μM) which unmasks a response in cultured neurones by inhibiting AMPA receptor desensitisation (Williams and Glowinski, 1996). The release of arachidonic acid, stimulated by maximally effective concentrations of either glutamate or NMDA, was modestly inhibited when 100 mM ethanol was present throughout the 15 min incubation (Fig. 1). Although AMPA receptor responsiveness was consistently slightly lower in the presence of ethanol the apparent reductions in the release of arachidonic acid were not significant. Acute ethanol did not modulate the non-stimulated basal release of arachidonic acid.

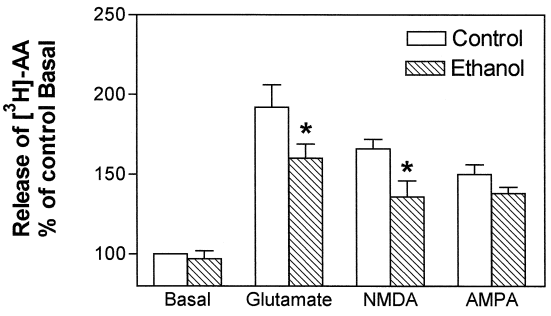
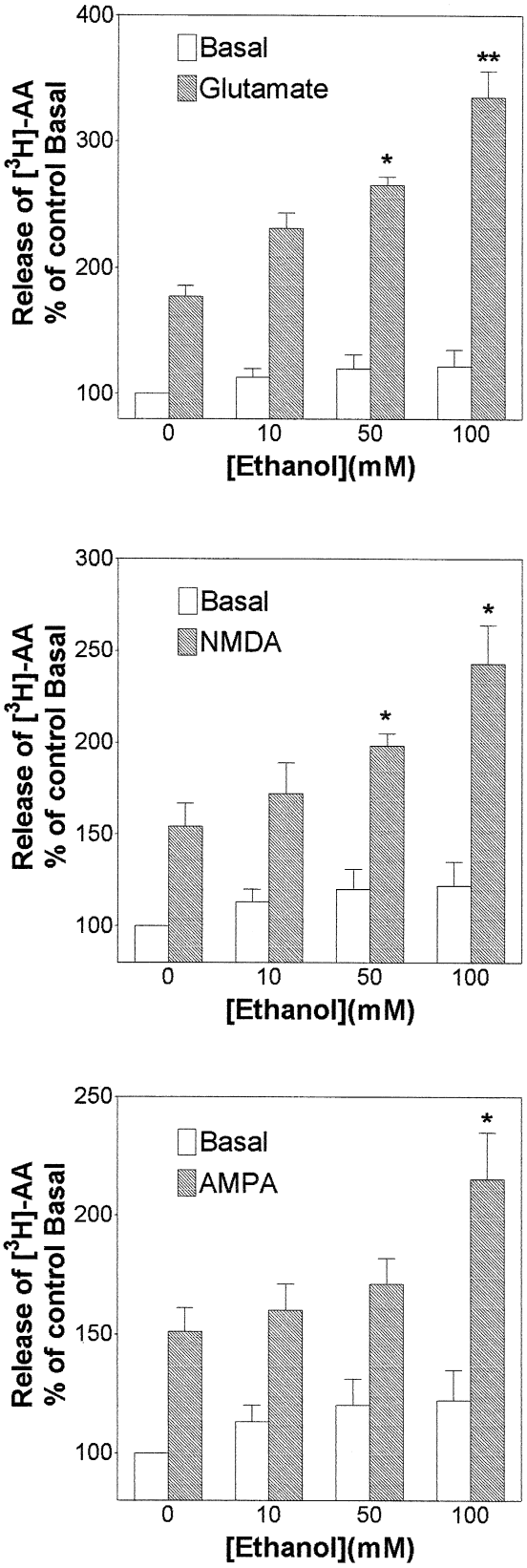


Fig. 1. Effect of acute ethanol on glutamate receptor-evoked [^{3}H]arachidonic acid release. Neurones were incubated for 15 min with maximally effective concentrations of glutamate (100 μM), NMDA (100 μM) or AMPA (30 μM, in the presence of cyclothiazide also 30 μM) in the presence or absence of ethanol (100 mM). The results are expressed as percent release of [^{3}H]arachidonic acid relative to basal values (no agonist present) estimated in the absence of ethanol (defined as 100%). Data are mean ± S.E.M. (bars) values of four independent experiments each performed in triplicate. Ethanol significantly inhibited the release of [^{3}H]arachidonic acid evoked by glutamate or NMDA (*) but not that induced by AMPA.



3.2. Effect of chronic ethanol on glutamate, NMDA and AMPA-evoked arachidonic acid release

Prolonged inhibition of the NMDA receptor by ethanol has been reported to result in the development of receptor supersensitivity leading to an increase in NMDA receptor function. To investigate whether or not cultured striatal neurones exposed to chronic ethanol for 48 h would have enhanced receptor responses cells were cultured in the presence of increasing concentrations of ethanol (10–100 mM) for 48 h, washed, and the glutamate-, NMDA- and AMPA-evoked liberation of arachidonic acid measured. Pre-exposure of neurones to ethanol concentration-dependently increased the amount of [^{3}H]arachidonic acid released in response to either glutamate (100 μM), NMDA (100 μM) or AMPA (30 μM in the presence of 30 μM cyclothiazide). Significant increases in responsiveness were observed at ethanol concentrations of $\geq$ 50 mM for glutamate or NMDA and $\geq$ 100 mM for AMPA (Fig. 2). The glutamate response measured following chronic exposure to 100 mM ethanol was strongly inhibited (76%, mean of three determinations) by the application of the NMDA receptor antagonist MK 801 (2 μM) but only weakly inhibited (18%, mean of three determinations) by the AMPA receptor antagonist CNQX (10 μM) suggesting that ethanol was acting primarily on the NMDA response. In order to establish if the observed potentiation was limited to glutamate receptor responsiveness alone, the effect of ethanol on veratridine (a Na^+ channel agonist)-stimulated arachidonic acid release was investigated. Veratridine (10 μM) evoked arachidonic acid release, measured in the presence of MK801 (2 μM) and CNQX (10 μM) was only slightly enhanced following exposure to chronic ethanol, from 119 $\pm$ 3% in the absence of ethanol to 131 $\pm$ 7% after a chronic pretreatment with 100 mM ethanol (data expressed as a percent of basal, taken as 100% $\pm$ S.E.M., $n = 3$). In contrast, the basal release of [^{3}H]arachidonic acid from unstimulated cells was not affected by chronic ethanol at any of the concentrations tested. Chronic ethanol (100 mM, 48 h) produced an increase in the maximal response to NMDA without causing a significant shift in the EC_{50} (Fig. 3).

Fig. 2. Effect of chronic ethanol on glutamate receptor-evoked [^{3}H] arachidonic acid release. Neurones were cultured in the presence or absence of increasing concentrations of ethanol for 48 h. Cells were then washed to remove residual ethanol and incubated for 15 min with maximally effective concentrations of glutamate (100 μM), NMDA (100 μM) or AMPA (30 μM, in the presence of cyclothiazide also 30 μM). The results are expressed as percent release of [^{3}H]arachidonic acid relative to basal values estimated in untreated control cells in the absence of agonist (defined as 100%). Data are mean $\pm$ S.E.M. (bars) values of three independent experiments each performed in triplicate. Increasing the concentration of ethanol in the chronic pretreatment significantly enhanced the release of [^{3}H]arachidonic acid evoked by glutamate, NMDA or AMPA ($^*P < 0.05$, $^{**}P < 0.01$).

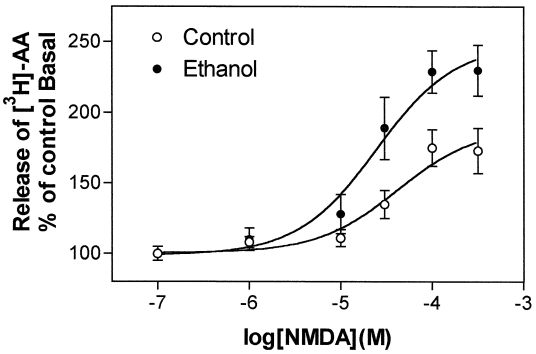


Fig. 3. Effect of chronic ethanol on the concentration–response curve for NMDA-evoked [^{3}H]arachidonic acid release. Neurones were cultured for 48 h in the presence or absence of 100 mM ethanol, washed and then incubated for 15 min with increasing concentrations of NMDA. The results are expressed as percent release of [^{3}H]arachidonic acid relative to basal values estimated in untreated control cells in the absence of NMDA (defined as 100%). Data are mean ± S.E.M. (bars) values of three independent experiments each performed in triplicate. Chronic ethanol significantly enhanced the release of [^{3}H]arachidonic acid evoked by NMDA at concentrations > 30 μM.

3.3. Chronic ethanol does not modify the uptake of arachidonic acid

The level of incorporation of [^{3}H]arachidonic acid into cultured neurones was the same in the presence or absence of 100 mM ethanol (Table 1), strongly suggesting that the increased liberation of arachidonic acid from these cells following exposure to chronic ethanol did not result from an enhanced uptake of label under these conditions.

3.4. Effect of ethanol on neuronal viability

To investigate whether the increased receptor-responsiveness was due to an alteration in neuronal viability following exposure of the cells to ethanol, MTT turnover and trypan blue exclusion experiments were performed. The MTT assay reflects mitochondrial succinate dehydrogenase activity and thus, is a general indicator of the respiratory status of the cells and an indirect measure of viable cell number. Exposure of the neurones to concentrations of ethanol up to 100 mM for 48 h did not adversely affect the respiratory capacity of the cells and indeed modest increases in MTT turnover were observed (Table 2). Similarly, the ability of cells to exclude trypan blue, a crude measure of membrane integrity, was not affected by exposure to chronic ethanol. However, a marked deterioration in membrane integrity and cellular morphology was observed when neurones were exposed to concentrations of ethanol of ≥ 200 mM for 72 h (data not shown).

Table 1
Chronic ethanol does not modify [^{3}H]arachidonic acid uptake

	DPM/100 μl
Control	49,596 ± 3,566
Ethanol	50,117 ± 7,239

Striatal neurones were cultured for 48 h in the presence or absence of 100 mM ethanol and [^{3}H]arachidonic acid (1 μCi/ml) was added to the media for the final 16 h. Cells were then washed three times to remove unincorporated label, solubilised with 0.5 ml of 0.5 M NaOH and 100 μl of the lysate was subjected to scintillation counting. Values are DPM/100 μl of cell lysate. Data represent means ± S.E.M. from 3 experiments each performed in triplicate.

Table 2
Effect of chronic ethanol on neuronal viability

[Ethanol] (mM)	MTT (% control)	% Trypan Blue + ve
0	100	9 ± 4
10	115 ± 6	8 ± 2
50	121 ± 9	12 ± 5
100	128 ± 14	10 ± 6

Striatal neurones were exposed to increasing concentrations of ethanol for 48 h. Cell viability was then assessed by the MTT assay, which is a measure of succinate dehydrogenase activity, and by the ability of the neurones to exclude trypan blue. Basal MTT turnover in untreated control cells was taken to be 100% and ethanol treated cells are expressed as a percentage of this. The values for trypan blue represent the number of positively stained cells in a field of 100 neurones. All data represent mean ± S.E.M. from 3 experiments.

3.5. Effect of chronic ethanol on NMDA-induced neurotoxicity

The ability of NMDA to induce cell degeneration in our neuronal cultures was investigated following chronic exposure to ethanol. A short exposure to NMDA dose-dependently induced modest neuronal degeneration as measured

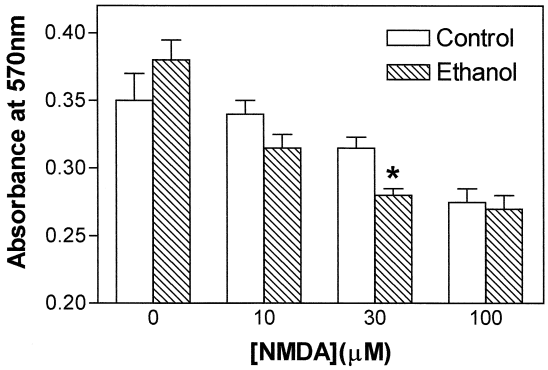


Fig. 4. Effect of chronic ethanol on NMDA-evoked neurotoxicity. Neurones were cultured in the presence or absence of 100 mM ethanol for 48 h. Media were removed and cells were then incubated in buffer (as described in Section 2) for 30 min with various concentrations of NMDA. Neurones were washed thoroughly with PBS to remove residual NMDA, fresh media were added and neurones were returned to the incubator for 18 h. Cell damage was estimated by the MTT assay. Results are absorbances measured at 570 nm. Data are mean ± S.E.M. (bars) values of three independent experiments each performed in triplicate. Neurones pre-exposed to chronic ethanol and then treated with 30 μM NMDA had significantly lower succinate dehydrogenase activity than cells treated with 30 μM NMDA but not pre-exposed to ethanol.

24 h later by the MTT assay. Pre-exposure to 100 mM ethanol for 48 h increased the degree of neuronal loss evoked by 30 μM NMDA but not that induced by higher concentrations of agonist (Fig. 4). Similar trends were observed when neurotoxicity was assessed by the amount of LDH released into the media (data not shown).

4. Discussion

Acute and chronic ethanol consumption produce perturbations of neuronal function that may account for clinical neurological conditions accompanying acute ethanol intoxication, abrupt withdrawal from ethanol, chronic ethanol abuse and damage to the developing foetus. A growing body of evidence now suggests that many of the neuropathological effects of ethanol are mediated through a modulation of the glutamatergic system (Tsai et al., 1995). Indeed, whilst acute ethanol exposure inhibits NMDA receptor responsiveness (Hoffman et al., 1989; Lovinger et al., 1989, 1990; Takadera et al., 1990; Dildy-Mayfield and Leslie, 1991; Morrisett et al., 1991), chronic ethanol treatment increases NMDA-receptor function and enhances glutamate and NMDA-induced neuronal death (Iorio et al., 1992; Chandler et al., 1993; Ahern et al., 1994; Iorio et al., 1993). However, although it is clear that chronic ethanol treatment potentiates NMDA-evoked elevation of intracellular Ca^{2+} in vitro, the subsequent downstream mechanisms underlying enhanced neuronal loss are unknown. NMDA mediates a Ca^{2+}-dependent mobilisation of arachidonic acid in neurones, probably via a stimulation of cytosolic phospholipase A_2, and activation of this cascade has been implicated in the processes leading to excitotoxic neuronal death (Lafon-Cazal et al., 1993; Gunasekar et al., 1995; Reynolds and Hastings, 1995). In this study, therefore, we investigated whether exposure of cultured neurones to either chronic or acute ethanol would modify glutamate and NMDA-evoked arachidonic acid release.

Acutely, ethanol inhibited the glutamate and NMDA-evoked release of arachidonic acid from striatal neurones which was consistent with previous reports of decreased NMDA receptor responsiveness in the presence of ethanol, although the extent of the inhibition was not dramatic. In contrast, exposing cultured striatal neurones chronically to ethanol substantially enhanced glutamate-evoked arachidonic acid release. This appeared to be mediated primarily by a large increase in NMDA receptor responsiveness but also by a modest increase in the AMPA response. Thus, the effects of ethanol were not completely selective for NMDA receptors. Interestingly, the NMDA response was more sensitive to the effects of ethanol than the AMPA response with significant increases in agonist-evoked arachidonic acid release observed at 50 and 100 mM ethanol, respectively. This may reflect different sensitivities of the NMDA and non-NMDA receptor subtypes to ethanol. A number of previous studies have also reported effects of ethanol on non-NMDA (AMPA/kainate) receptors (Lovinger, 1993; Jones and Lodge, 1993; Frohlich et al., 1994; Dildy-Mayfield and Harris, 1995) although this appears to depend critically on the brain region or cell type employed, the concentration of agonist used and the subunit composition of the receptor under investigation. Chronic ethanol did not alter the basal, non-receptor-evoked release of arachidonic acid from these cells suggesting that the observed modulatory effects of ethanol were restricted to agonist-stimulated events alone and were not due to increases in the tonic turnover of arachidonic acid. However, veratridine-evoked arachidonic acid, which was extremely modest under control conditions, was also slightly potentiated following chronic ethanol exposure, suggesting that the effects of ethanol were not entirely selective for glutamate responses. Nevertheless, it seems likely that the potentiated release of arachidonic acid was mediated principally by enhanced agonist-evoked increases in intracellular Ca^{2+} and subsequent increases in the activity of Ca^{2+}-dependent cytosolic phospholipase A_2.

Although the concentrations of ethanol employed in this study were in the high physiological to pharmacological range they have been widely used in many other similar investigations in vitro. Nonetheless we were concerned that the observed effects on arachidonic acid release perhaps resulted from non-specific deleterious effects of ethanol on the cultured cells and a loss of membrane integrity. Several pieces of evidence suggested that this was not in fact the case. Firstly, exposing the neurones to 100 mM ethanol for 48 h did not adversely affect the respiratory capacity of the cells, which is a reliable indicator of cell viability. In fact ethanol caused a modest increase in mitochondrial succinate dehydrogenase activity. Secondly, the same chronic treatment did not alter the ability of the neurones to exclude trypan blue which is, albeit a rather crude, measure of membrane integrity. Equally, the increased release of arachidonic acid could conceivably have resulted from an enhanced uptake of [^{3}H]arachidonic acid due to membrane disruption or altered membrane fluidity. This could be discounted as total labelling of the neurones was identical in either the presence or absence of chronic ethanol.

As previously reported in a number of other cell types, chronic ethanol also increased NMDA-receptor mediated neuronal damage in our cultured mouse striatal neurones. However, the largest increases in neurotoxicity following ethanol exposure were observed at sub-maximal concentrations of agonist, even though ethanol strongly stimulated the release of arachidonic acid in response to maximally effective concentrations of NMDA. This is consistent with a previous study which found that ethanol enhanced neuronal loss induced by low concentrations of NMDA (10–30 μM) but was ineffective against high concentrations of NMDA (Chandler et al., 1993). A likely explanation for this finding is that there is a threshold level of Ca^{2+} or arachidonic acid, required to initiate cell damage, which is

achieved at lower than normal concentrations of NMDA following exposure to ethanol. Given the multiple regulatory roles played by arachidonic acid in the brain, it is possible that an increased NMDA- or AMPA- receptor-evoked mobilisation of arachidonic acid may account for some of the pathophysiological effects of ethanol in the CNS associated with chronic alcohol abuse and the foetal alcohol syndrome.

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