Ethanol and adult CNS neurodamage: oxidative stress, but possibly not excitotoxicity

Michael A. Collins[1], Edward J. Neafsey[1]

[1]*Department of Molecular Pharmacology and Therapeutics, Loyola University Chicago, Stritch School of Medicine, Maywood IL 60153*

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1. ABSTRACT

Evidence from experiments with adult rodents chronically treated with ethanol *via* either repetitive binges or continuous intake/exposure supports the occurrence of brain oxidative stress and, at least in binge intoxication/rat models, its essential causative role in neurodamage. However, pharmacological antagonism experiments reveal that N-methyl-D-aspartate (NMDA) receptor-dependent excitotoxicity is not responsible for adult mammalian brain neurodegeneration caused by repetitive binge ethanol intoxication and withdrawals. Since NMDA receptor antagonists apparently are untested with respect to neuronal death/loss in continuous intake/ingestion rodent models, e.g., ethanol/water or ethanol/liquid diets, it is therefore erroneous to assert, as is often done, that excitotoxicity is an important mechanism for ethanol-induced adult mammalian brain (neuronal) damage. Alternatively, results from several laboratories indicate that neurodegeneration due to chronic binge ethanol exposure/withdrawal may be dependent on redox transcription factor signaling and neuroinflammatory/oxidative stress pathways (increased arachidonic acid mobilization and pro-inflammatory cytokines; decreased anti-inflammatory cytokines) downstream of microglial/astroglial activation and moderate yet significant brain edema.

2. INTRODUCTION

Excitotoxicity is characterized by calcium overload and oxidative stress that are principally related to glutamate-dependent overstimulation of the extrasynaptic (as opposed to synaptic) N-methyl D-aspartate (NMDA) receptor(s) (NMDAR) (1). It is considered a fundamental mechanism of neurodegeneration in the widespread acquired brain damage circumstances of stroke and traumatic injury (1, 2). Excitotoxic mechanisms also are apparently important in neurodamage arising from micronutrient deficiencies (3) and environmental neurotoxins (e.g., pesticides, organometallics) (4). Within the domain of chronic neurodegenerative diseases, excitotoxicity has been linked to Alzheimer's and Parkinson's diseases, amylotropic lateral sclerosis, and multiple sclerosis (5). In the many *in vivo* investigations of acquired brain damage during the past three decades, the *sine qua non* for excitotoxicity has been neuroprotection *via* pharmacological antagonism of NMDAR.

An explicit mechanism for chronic ethanol-dependent neurodegeneration in the mature brain has been elusive, but oxidative stress is a likely key component. Largely based on *in vitro* reports, excitotoxicity has been assumed to be upstream of (and responsible for) oxidative

stress and neurodamage *in vivo* in adults. This article reviews adult rodent models of chronic ethanol exposure or intoxication for which there is evidence for or indications of brain oxidative stress. It further considers the (lack of) experimental evidence for excitotoxicity as a critical mechanism to explain incipient oxidative stress and related pathways leading to neurodegeneration in chronic ethanol exposure models. Finally, it argues that non-excitotoxic neuroinflammatory mechanisms related to glial activation, brain edema, redox alterations and possibly pro-inflammatory cytokines may be central to the neurodegeneration, at least with (sub)chronic binge intoxication models. It is important to emphasize that ethanol-induced brain injury, sometimes with malnutrition, reasonably underlies alcoholic dementia, which in some situations is nearly as prevalent as vascular (ischemic) dementia (6). A clearer understanding of ethanol's brain damage pathways could stimulate translational approaches to minimizing the progression to dementia associated with chronic alcoholism.

3. OXIDATIVE STRESS IN RODENT BRAIN DUE TO CHRONIC ETHANOL EXPOSURE

3.1. Essentials of brain oxidative stress related to ethanol

Brain oxidative stress consists of an imbalance of cellular reactive oxygen species (ROS, e.g., superoxide, hydroxyl radical, hydrogen peroxide, lipid peroxides) and perhaps reactive nitrogen species (RNS such as nitric oxide and derived peroxynitric acid) that can initiate macromolecular (per)oxidation and fragmentation, mitochondrial damage, and neurocellular degeneration. Considering ethanol, excitotoxic NMDAR overstimulation is but one of multiple sources for excessive oxidative stress. Other sources include (but are not limited to) mitochondria, which could "leak" ROS due to damage by ethanol or its metabolites; NADPH oxidase (NOX), which is highly localized in glial cells and can be activated by ethanol (7); a multitude of oxidative/peroxidative/epoxidative routes for membrane polyunsaturated fatty acids (especially arachidonic acid or AA) released from phospholipids by ethanol-potentiated phospholipase A2 (PLA2) activity (8); ethanol-dependent induction of monoamine oxidases (9) and possibly other amine oxidases that produce hydrogen peroxide; and oxidation of ethanol by cytochrome P450 2E1 (producing not only ROS but hydroxyethyl radicals as well), catalase, and various (per)oxidases. Additionally, not to be overlooked is reduced/impaired removal of ROS/RNS, should chronic ethanol exposure directly or indirectly cause depletion/inhibition of endogenous antioxidants, scavengers, and/or antioxidant enzyme systems. On this point, extracellular glutamate (at times shown to be released/elevated by ethanol (10)) can cause oxidative stress in a non-receptor manner with certain neuronal cell lines by blocking cystine transporters and interfering with glutathione synthesis (termed oxidative glutamate toxicity) (11), but this pathway's role *in vivo* is relatively unproven.

3.2. Chronic ethanol and brain oxidative stress *in vivo*

There is a robust literature indicating that chronic or sustained ethanol exposure can increase oxidative stress in many organs of adult animals (12). Brain oxidative stress changes and possible mechanisms due to ethanol were reviewed a decade ago (13). Here we summarize those with an exclusive *in vivo* focus and find there is a plethora of experimental reports since 1980 that examine the evidence for brain oxidative stress in chronic ethanol-exposed adult rats and mice (Table 1). These studies establish that, despite differing levels and types of ethanol exposures from as short as 2 weeks to as long as 18 months, and assays in whole brain or specific brain regions with different rodent models of presumably differing adult ages (where stated) for a variety of known indicators/markers, oxidative stress is a consistent result or consequence. (A few early analyses with rodents given chronic ethanol were negative, possibly because oxidative changes were possibly obscured by whole brain assays (14, 15)). The most common model has been male rats, although in several instances females were used, and a few studies used male and female mice. With one exception, chronic ethanol administration or exposure was either of two protocols: continuous intake (in water or liquid diets), or repetitive (usually daily) "binging" via intraperitoneal or intragastric routes. Maximum blood ethanol levels (BELs) in continuous intake typically would not exceed 25-30 mM, but in the binge model could range from 70 to 100 mM, depending on the g/kg dose administered. The exception to these exposure routes in Table 1 was an ethanol inhalation model, discussed further below, which achieved relatively high BELs.

The most common oxidative stress assessments in Table 1 relied primarily on indirect markers or indicators—e.g., changes in antioxidant systems such as glutathione (GSH), its synthesizing enzymes (16), and oxidized GSSG, and increases in end-products of lipid peroxidation such as malondialdehyde (usually measured by the thiobarbituric acid reacting substances [TBARS] method, but sometimes more specifically by HPLC); however, in the initial studies by the Portuguese laboratories, regional lipofuscin accumulation (17, 18), or of oxidized proteins (protein carbonyls, PCO) indicated oxidative stress. Measured in some studies were alterations, usually increases, in redox-associated or oxidative stress-generating enzymes such as cytochrome P450 2E1 (CYP450 2E1), superoxide dismutase (SOD), nitric oxide synthase (NOS), cyclooxygenase-2 (inducible COX, which converts AA to proinflammatory prostaglandin precursors) and NOX; decreases in glutamine synthetase (GluS, an astroglial enzyme exquisitely sensitive to ROS damage) also have been measured. However, more direct measurements of ROS were done in several of the ethanol studies, e.g., brain superoxide (19), or hydroxyl radicals after trapping with salicylate (20). It is notable that in a number of the studies (Table 1), administration of an antioxidant, a dietary polyphenolic flavonoid, a specific amino acid or a neuroprotective steroid was effective in reducing or preventing the changes in brain oxidative stress, providing further evidence for a "hyper-oxidative" link to ethanol exposure or its withdrawal.

3.3. Oxidative stress relationships to withdrawal and neurodegeneration

The issue of the importance of the ethanol withdrawal phase to increased oxidative stress was the

Chronic ethanol and adult brain neurodegeneration

Table 1. Brain oxidative stress due to chronic ethanol (EtOH) in adult rodents

EtOH Exposure Model	Evidence/indicator(s) of oxidative stress	Reference
Male SD rats; ~6% EtOH/liquid diet for 4-14 weeks	Brain GSH depletion; increased GSH-synthesizing enzymes during EtOH intake and WD	(16)
Male SD rats; 20% EtOH/H$_2$O intake *ad libitum* for 1-18 months	Increased cerebellar lipofuscin (lipid peroxidation end-product)	(17)
Male SD rats; 20% EtOH/H$_2$O intake *ad libitum* for 1-18 months	Increased hippocampal lipofuscin deposition	(18)
Male SD rats; 20% EtOH/H$_2$O intake *ad libitum* for 6-12 months	Increased cerebellar and hippocampal lipofuscin; blocked by piracetam, a nootropic and possible antioxidant	(63)
Female (intact) Wistar rats; EtOH/liquid diet for 8 weeks	Decreased brain GSH/GSSG, increased microsomal CYP450 2E1	(64)
Male SD rats; 5-7% EtOH/liquid diet for 14 days	Decreased brain GSH and SOD; not prevented by MK801 co-treatment	(32)
Male Wistar rats; 5% EtOH/liquid diet for 40 days	Increased brain lipid peroxidation products and nuclear DNA breaks	(65)
Male SD rats; 10% EtOH/H$_2$O intake *ad libitum* for 4-8 weeks	Increased cerebellar GSH-S-transferase and reduced vitamin E content	(66)
Male SD rats; EtOH vapor chamber (>300 mg/dl BELs)	Increased brain hydroxyl radicals and PCO, decreased GluS; correlations with WD seizures	(20)
Male Wistar rats; 9.7 g/kg/d i.g. EtOH 1x daily for 1 month (?)	Increased brain TBARS, decreased brain GSH; Prevented by curcumin	(67)
Male Wistar rats: 6% EtOH/liquid diet for 20 months	Decreased GSH/GSSG and GSHR, increased ROS-induced brain luminescence; prevention by NAC	(68)
Male SD rats, 5% EtOH/liquid diet for 2 months	Increased dentate granule cell COX2 expression; amelioration by grape polyphenol administration	(69)
Male Wistar rats; 10-35% EtOH/H$_2$O intake *ad libitum* for 20 days	Decreased cerebral GSH; increased TBARS; partial prevention by vitamin E supplementation	(70)
Male laka mice; 2 g/kg/d i.g. EtOH 1x daily for 24 days	Increased forebrain TBARS, decreased GSH, SOD and catalase; normalized by free radical scavenger, quercetin	(71)
Male Wistar rats; 3g/kg i.g. EtOH 2x daily for 28 days	Increased brain TBARS and LHP; protection by taurine administration	(72)
Female Wistar rats; 5% EtOH/liquid diet for 5 months	Increased cortical iNOS and COX2 expression	(73)
Female (ovx) SD rats; 7.5% EtOH/liquid diet for 5 weeks	Elevated cerebellar and hippocampal TBARS during EtOH withdrawal; prevented by estradiol	(74)
Male Wistar rats; 20% EtOH/H$_2$O *ad libitum* for 6 months	Increased hippocampal and cerebellar lipofuscin; prevented by grape seed flavonoids	(75)
Male Wistar rats; 2g/kg/d i.p. EtOH 1x daily for 45 days	Increased regional MDA and 4-hydroxyalkenols; prevented by melatonin co-treatment	(76)
Male Wistar rats; 5% EtOH/liquid diet for 4 weeks	Decreased brain antioxidant enzymes; increased LPO indicators; partial protection by green tea supplementation	(77)
Male SD rats; EtOH/liquid diet for 21-28 weeks (av. ~12 g/kg/d EtOH intake)	Microarray study: increased expression of oxidative stress-related genes in nucleus tractus solitarius	(78)
Male Wistar rats; 2.5g/kg i.g. EtOH 1x on alternate days for 90 days	Increased regional PCO, decreased GSH; partially prevented by antioxidant suppl. mixture	(79)
Male Wistar rats; 3g/kg/d i. p. EtOH 1x daily for 6 weeks	Increased brain TBARS; prevention by resveratrol supplementation	(80)
Male Long-Evans rats; <10% EtOH/ liquid diet for 9 weeks	Increased regional brain NOX expression; increased LPO, HNE and DNA damage	(23)
Male SD rats; 6.4% EtOH/liquid diet for 18-22 days	Increased hippocampal TBARS, decreased GSH; prevented by ebselen antioxidant	(81)
Male Balb/C mice; 8% EtOH/H$_2$O *ad libitum* for 15 days	Increased brain GSSG/GSH, TBARS, catalase; prevented by vitamin E supplementation	(82)
Male Wistar rats; 6g/kg/d i.g. EtOH 2x daily for 45 days	Increased TBARS and LHP; increased GSSG/GSH; prevention by high PUFA/PL soybean extract	(22)
Male SD rats; 6.5% EtOH/liquid diet for 5 weeks	Increased brain regional superoxide and PCO; partial prevention by intermittent hypoxia	(19)
Male Wistar rats; 10 g/kg/d i.g. EtOH 1x daily for 10 weeks	Increased cortical & hippocampal nitrite, TBARS, and lipid peroxides, reduced SOD activity and GSH; all largely prevented by vitamin E and tocotrienol	(83)
Male Wistar rats; 2g/kg/d i.p. EtOH 1x daily for 14 days	Increased brain MDA and PCO; prevention by carnosine administration	(84)
Female C57/Bl mice; 10% EtOH/H$_2$O *ad libitum* for 5 months	Increased cortical COX2 and iNOS	(85)
Male C57/Bl mice; 4% EtOH liquid diet for 8 weeks	Increased cerebrocortical iNOS, nitrotyrosine-protein adducts, 4-HNE and ROS, increased glial NOX; partial neutralization by acetyl-L-carnitine	(21)

Abbreviations: SD, Sprague Dawley; i.g., intragastric; DNQX, AMPA/kainate receptor antagonist; COX2, cyclooxygenase-2; ERC, entorhinal cortical; DGC, dentate granule cell; GSHR, glutathione reductase; HNE, 4-hydroxynonenal; iNOS, inducible nitric oxide synthase; LHP, lipid hydroperoxides; nNOS, neuronal nitric oxide synthase; NAC, N-acetylcysteine; NFkB, nuclear factor kappa beta; BHT, butylated hydroxytoluene; PCO, protein carbonyls; BB, blueberry extract; TGF-β, transforming growth factor-β

main intent of some of the studies (Table 1). Vallett *et al.* (20), using a vapor phase model, argued that chronic ethanol inhalation would produce a more reliable model of dependence (withdrawal seizures) than other models. Some of their oxidative stress measures were significantly higher early after withdrawal from ethanol vapor, and others some

Table 2. Binge ethanol (EtOH) exposure and neurodegeneration in adult rats: protection studies with receptor/channel antagonists/agonists, antioxidants, or diuretics

EtOH Exposure Model	Evidence For or Against Neuroprotection	Reference
Male SD; 6-12 g/kg/d i.g. EtOH 3x daily for 4 days	MK801 did not protect against ERC neurodegeneration	(35)
Male SD; 6.5-12 g/kg/d i.g. EtOH 3x daily in liquid nutrient for 4 days	iNOS and nNOS inhibitors failed to prevent ERC & DGC neurodegeneration	(39)
Male SD; ~9 g/kg/d i.g. EtOH 3x daily in liquid nutrient for 4 days	MK-801, DNQX or nimodipine did not reduce ERC & DGC neurodegeneration	(38)
Male SD; ~5g/kg/d i.g. EtOH 1x daily for 7-8 days	MK-801 did not prevent ERC & DGC neurodegeneration; neuroprotection by furosemide diuretic/antioxidant	(40)
Male SD; 9-12 g/kg/d i.g. EtOH 3x daily for 4 days	MK-801, memantine or nimodipine were not neuroprotective in ERC & DGC; antioxidants cannabidiol, BHT, vitamin E and furosemide were neuroprotective	(41)
Male SD; 8-12 g/kg/d i.g. EtOH 3x daily in liquid nutrient for 4 days	BHT antioxidant prevented elevations in oxidative stress indicators (NFkB; COX2) and ERC & DGC neurodamage; ebselen, vitamin E and BB did not neuroprotect	(49)
Male SD; 3.5-6.5 g/kg/d i.g. EtOH 1x daily in liquid nutrient for 8 days	Acetazolamide (diuretic and aquaporin-4 inhibitor) protected against ERC & DGC neurodamage	(51)
Male Wistar; 8-12 g/kg/d i.g. EtOH 3x daily in 6% sucrose/14.7% milk powder for 4 days	Metabotropic GluR II agonist reduced ERC neurodegeneration, concomitant with preventing deficits in TGF-β and cognition	(60)

Abbreviations: SD, Sprague Dawley; i.g., intragastric; DNQX, AMPA/kainate receptor antagonist; COX2, cyclooxygenase-2; ERC, entorhinal cortical; DGC, dentate granule cell; GSHR, glutathione reductase; iNOS, inducible nitric oxide synthase; nNOS, neuronal nitric oxide synthase; NAC, N-acetylcysteine; NFkB, nuclear factor kappa beta; BHT, butylated hydroxytoluene; BB, blueberry extract; TGF-β, transforming growth factor-β

12-24 hr following exposure, lending support to their hypothesis that there are multiple components or sources of [increased] oxidative stress which "peak and decline at different time points and potentially interact with one another" (20). Jung and coworkers, utilizing an ethanol/liquid diet for 5 weeks, also showed increased brain oxidative stress in adult rats that was further increased during withdrawal (19). These findings with two different models can be contrasted with the subchronic binge ethanol intoxication model (discussed below), where neurodamage in several brain regions was highest within ~8 hrs after the last repetitive ethanol dose rather than at longer withdrawal times, when seizures are believed to be of highest intensity.

Most of the studies in Table 1 acknowledged the probable importance of oxidative stress induction to neurodegeneration, but few specifically measured or assessed brain neuronal damage or loss due to ethanol, nor the effect of antioxidants on neurodegeneration. However, recently Rump *et al.* (21) used immunocytochemistry to show that striatal (dopaminergic and cholinergic) neurons were reduced by chronic ethanol ingestion, and that acetyl-L-carnitine, a pleiotropic antioxidant, blocked the reductions. Also, indications of brain damage were evident in several other oxidative stress reports— Jayaraman *et al.* (22) noted regional brain weight losses that likely reflect ethanol-dependent brain tissue atrophy in concert with increased oxidative stress, with both being normalized by administration of putatively neuroprotective soybean phosphatidylcholine extract; also, increased DNA damage was assumed by Cohen *et al.* (23) to reflect brain damage. In these instances the *in vivo* changes could reflect loss/damage of glia as well as neurons. It is also likely that brain glia are among the key sources of ethanol-induced ROS or RNS, especially with respect to cellular NADPH oxidase (NOX) (24).

4. EVALUATIONS OF EXCITOTOXICITY IN ETHANOL-INDUCED NEURODAMAGE

Excitotoxicity has been often invoked as a mechanism responsible for the brain oxidative stress and neurodegeneration in chronic ethanol-exposed rodents as well as in alcoholics (25-27). The possibility seems to follow from findings with ethanol-exposed fetal/adolescent brain slice cultures indicating that excitotoxic antagonists can be neuroprotective (28, 29), from data showing increased extracellular glutamate or other NMDAR agonists in certain ethanol experiments (for review see (10)), and from neurobehavioral or receptor density studies with ethanol *in vivo* and *in vitro* (30, 31). However, unlike stroke, trauma and other acquired brain injuries, available pharmacological antagonist studies of ethanol-induced neurodegeneration with adult rats (Table 2) provide substantive evidence against the key involvement of excitotoxicity, at least with subchronic/chronic binge intoxication. NMDAR also may not be a significant source of brain oxidative stress in adult rats ingesting ethanol in liquid diets, based on the results of Bondy & Guo (32) in which an NMDAR antagonist failed to alter the depletion of brain GSH and SOD.

4.1. Neurodegeneration due to binge ethanol: the model(s) and evidence against excitotoxicity

Specifically addressing excitotoxicity's role in neurodamage (Table 2), several reports to date describe unsuccessful attempts to reduce or block chronic ethanol-induced brain neurodegeneration with glutamatergic receptor antagonists, calcium channel blockers, or nitric oxide synthase inhibitors. The last two pharmacological approaches are based on the mechanistic links between excitotoxicity, neuronal calcium channel opening, and excessive nitric oxide generation (1, 2). Unlike brain oxidative stress studies (Table 1) that encompass a range of

chronic ethanol exposure models, neurodegeneration studies (Table 2) have been restricted to a repetitive binge intragastric (i.g.) approach with male adult rats. This model originated from withdrawal symptom (seizures) studies with rats intubated 3-4 times daily with ethanol (8-12 g/kg/d) over a 4-day interval—the so-called Majchrowicz technique (33). A subsequent abstract from that NIH laboratory summarized that the binge treatment caused surprisingly regionalized neuronal death in regions of the temporal cortex (esp. layer 3 entorhinal cortical [ERC] neurons) and hippocampal dentate granule cells (DGC), as determined with sensitive deOlmos cupric-silver neurodegeneration staining (34).

As we indicated in an initial abstract (35), our exploratory experiments investigating excitotoxicity's role with the above binge ethanol/ rat model found that co-treatment with MK-801 (dizocilpine, a potent noncompetitive NMDAR antagonist) failed to prevent ERC neurodamage. Modifying the binge intoxication protocol with nutritional supplement and subcutaneous intragastric cannulae, we pursued studies with the model and reported that, in addition to prominent ERC pyramidal neuron and DGC damage, neurodegeneration was apparent in olfactory bulb glomeruli neurons along with associated olfactory (piriform and perirhinal) cortical pyramidal cells (36); accordingly, the suggestion was that brain damage of this nature in chronic alcoholics might explain reported olfactory deficits, in addition to aspects of dementia/cognitive decline (e.g., hippocampus and entorhinal cortex are critical regions for memory function). Indeed, later studies from the Crews laboratory demonstrated learning and memory impairments with this rat binge intoxication model (37). Our findings further indicated that the amount of neurodegeneration peaked as early as ~8 hr after the last binge ethanol treatment rather than later at 24-36 hr, when induction of withdrawal seizures is maximal (33). This places into question an oft-stated assumption that (full-blown) withdrawal after chronic ethanol is a requirement for extensive neurodegeneration. Also, it was noted that the apparent absence of degenerating neurons in the hippocampal CA1-CA3 regions (36), normally very susceptible to NMDAR-mediated insult, was inconsistent with excitotoxicity being a significant mechanism.

In further pharmacological assessments of possible excitotoxicity-associated mechanisms, we found that, in addition to MK-801, the agents DNQX, an antagonist of glutamatergic AMPA/kainic acid receptors as well as the NMDAR glycine co-agonist site, and nimodipine, a neuronal calcium channel blocker, lacked neuroprotective effects against binge ethanol-induced ERC and DGC degeneration (38). Also, two NO synthase (NOS) inhibitors were administered during the binge intoxication protocol (39): nitroarginine methyl ester, a general NOS inhibitor, which was delivered icv via minipump (its peripheral co-administration with binge ethanol was found to cause ~100% mortality), and 7-nitroindazole, a relatively specific inhibitor of neuronal NOS *in vivo*, given intraperitoneally. In these experiments neither inhibitor was neuroprotective; in fact, NOS inhibition significantly worsened the binge ethanol-induced neurodamage in one or more brain regions. Additionally, histochemical

localization of NOS (as NADPH diaphorase) did not coincide with areas of neurodegeneration. In later experiments, we modified the gavage intoxication protocol to ~5 g/kg ethanol once daily for 7-8 days and found that ERC and DGC degeneration induced by this modification likewise was not prevented by MK-801 administration (40). Importantly, the NIH laboratory of Eskay and coworkers also examined the possibility of excitotoxicity with the Majchrowicz 4-day repetitive binge ethanol technique, modified with nutritional supplement and intragastric cannulae (41). Their results confirmed findings with MK-801 and further showed that concurrent treatment with memantine, an uncompetitive NMDAR antagonist with therapeutic applications (42), did not significantly protect against ethanol-induced ERC and DGC neurodegeneration.

4.2. Possible excitotoxicity in other chronic ethanol models

It is still plausible that excitotoxicity is an important pathway leading to neurodegeneration in adult rodent models during/after long-term ethanol ingestion (water or liquid nutrient), in which the repetitively high BELs from i.g. or i.p. binge administration and the sharp contrast of relatively abrupt withdrawals are absent. There are supportive indications; for example, memantine prevented the robust neurobehavioral deficits in adult rats withdrawn from 6 wks of 20% ethanol intake in water (43). Furthermore, upregulation of hippocampal and cortical NMDAR subunits comcomitant with increased apoptosis was observed in adult rats after 12 weeks of 10% ethanol ingestion (44). In contrast, brain NMDAR subunit upregulation, as indicated by labeled antagonist binding, is negligible in the Majchrowicz binge ethanol model (45), and, as indicated above, NMDAR antagonists are ineffective neuroprotectants. Furthermore, reiterating an earlier point, NMDAR antagonism did not prevent the apparent brain oxidative stress in adult rats during chronic ethanol/liquid diet intake (32), so the significance of ethanol-induced excitotoxicity in any adult mammalian model remains a question.

However, after chronic ethanol exposure in a non-mammalian model such as adult drosophila, excitotoxicity as well as apoptosis both appear to be significant (46). This bears upon a further possible difference between chronic intake and binge intoxication in mammalian models, in that the apoptotic mode of cell death for brain neurons appears minimal in the adult rat binge intoxication model (47). It is possible this represents an acknowledged dose dependency of toxicant-induced cell death modes (48), for which, with some toxicants, low dose (and with ethanol, extended duration) exposure could promote apoptosis, whereas higher concentrations (combined with repetitive withdrawals in ethanol's case) might cause mainly necrosis. Clearly, further studies are needed to clarify this basic issue.

5. OXIDATIVE STRESS AND NEURODEGENERATION IN ETHANOL BINGE INTOXICATION: NONEXCITOTOXIC NEUROINFLAMMATION

In addition to confirming our laboratories' findings of an apparent absence of excitotoxicity-induced

neuronal death, the above-mentioned binge intoxication study with adult rats by Hamelink *et al.* (41) established a clear link between oxidative stress and neurodamage by demonstrating that several antioxidants significantly neuroprotected against the ERC and DGC neurodegeneration. These included butylated hydroxytoluene (BHT), vitamin E, cannabidiol, and furosemide (a diuretic shown by these researchers to be a good antioxidant). Pursuing the view that redox pathways are critical determinants of neurodamage in this binge model, Crews *et al.* (49) confirmed the neuroprotective effects of BHT. However, this study failed to confirm Hamelink *et al.* (41) regarding vitamin E neuroprotection in the model; dissimilarities in vitamin E bioavailability due to the different vehicles used was a suggested explanation. Also, for unknown reasons, neither ebselen, a superoxide dismutase mimetic, nor blueberry extract, which has recognized antioxidant effects, was an effective neuroprotectant. Importantly, the Crews *et al.* report associated binge ethanol damage and its prevention by BHT with a critical redox transcription factor, NFkβ (49). Other reports from this laboratory further have linked NFkβ to production of pro-neuroinflammatory brain cytokines such as TNFα in binge ethanol exposure and withdrawal (50).

Two additional neuroprotectants in the binge ethanol model *in vivo* as well as in organotypic rat brain slice cultures are the diuretics, furosemide and acetazolamide (40, 51)—the use of which prevented the modest but still substantial brain edema generated in these models. Furosemide is also an antioxidant, but acetazolamide has negligible antioxidant potential (51); furthermore, the latter diuretic has recently been shown to inhibit aquaporin-4 (52), a major water transporter in astroglia. Aquaporin-4 is upregulated in (and responsible for) cytotoxic brain edema in general and is increased in binge ethanol exposure *in vitro* (51). Also, *in vivo* binge ethanol studies show it is significantly increased in adult rat hippocampus (Collins et al., Abst. Soc. Neurosci. 2011). Furthermore, brain edema links with (and is a component of) neuroinflammation in several ways, perhaps the most germane being *via* activation of PLA2 isoforms and release of ROS-generating AA (53, 54); conversely, ROS are able to promote more brain edema (55). Indeed, aquaporin-4 itself may have an associated proinflammatory role (87). In chronic ethanol models the neuroinflammatory processes indicated by increased ROS, NFkβ and proinflammatory cytokines (50) could also be interacting with or dependent on the aforementioned edema-PLA2-AA-ROS cascades; proinflammatory cytokines have been reported to be both upstream (56) and downstream (57, 58) of PLA2 activation, while AA, through ROS, can activate potentially upstream NFkB pathways (59).

Most recently, a study with the binge ethanol intoxication model by Cippitelli *et al.* (60) demonstrated that central activation of metabotropic glutamate receptors (group II mGluR) with LY379268 agonist blocked degeneration of neurons in the ERC, in concert with prevention of deficits in neuroprotective brain TGF-beta and cognitive function. Such protection might appear to conflict with the interpretation that excitotoxicity is not involved, since the LY agonist has been reported to be protective against NMDA-induced neurotoxicity *in vivo* (61). However, these authors indicated that LY neuroprotection versus NMDA appears more complicated than suppressing NMDAR overactivation; furthermore, Lee *et al.* (62) found that activation of group II mGluR protects against ROS (hydrogen peroxide)-induced neurodamage, a fact consistent with reduction of oxidative stress/neuroinflammatory pathways as a component of the neuroprotective action of the LY agonist during binge ethanol intoxication.

6. PERSPECTIVE

Available evidence fails to support excitotoxicity in adult brain as a prominent neurotoxic mechanism promoted by repetitive binge ethanol treatment, and its role in neurodamage acquired in mammalian models using chronic ingestion remains to be unequivocably demonstrated. Rather, there are considerable indications that a cross-communicating array of neuroinflammatory (glial-neuronal) pro-oxidant pathways are initiated by chronic (esp. binge) ethanol exposure and withdrawal(s). An inclusive model or diagram is beyond the scope of this article, but the aspects of neuroinflammation discussed here provide a basis for further chronic ethanol intoxication/withdrawal studies with different mammalian models that result in neurodegeneration and cognitive impairment. Results from such studies could foster translational approaches to minimizing the impact of a "silent epidemic of dementia" (86) putatively incurred worldwide due to chronic alcoholism.

7. ACKNOWLEDGEMENTS

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Send correspondence to: Michael A. Collins, Department of Molecular Pharmacology and Therapeutics, Loyola University Stritch School of Medicine, 2160 S. First Avenue, Maywood IL 60153 USA. Tel: 708-216-4560. Fax: 708-216-6289. E-mail: mcollin@lumc.edu