Biochemical and Neurotransmitter Changes Implicated in Alcohol-Induced Brain Damage in Chronic or ‘Binge Drinking’ Alcohol Abuse

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Abstract — The brain damage, which occurs after either chronic alcoholization or binge drinking regimes, shows distinct biochemical and neurotransmitter differences. An excessive amount of glutamate is released into specific brain regions during binge drinking (in excess of 4- to 5-fold of the normal basal concentration) that is not evident during periods of excessive alcohol consumption in chronic alcohol abusers. Increases in glutamate release are only observed during the initial stages of withdrawal from chronic alcoholism (∼2- to 3-fold) due to alterations in the sensitivities of the NMDA receptors. Such changes in either density or sensitivity of these receptors are reported to be unaltered by binge drinking. When such excesses of glutamate are released in these two different models of alcohol abuse, a wide range of biochemical changes occur, mediated in part by increased fluxes of calcium ions and/or activation of various G-protein-associated signalling pathways. Cellular studies of alveolar macrophages isolated from these two animal models of alcohol abuse showed enhanced (binge drinking) or reduced (chronic alcoholization) lipopolysaccharide (LPS)-stimulated NO release. Such studies could suggest that neuroadaptation occurs with the development of tolerance to alcohol’s effects in both neurotransmitter function and cellular processes during chronic alcoholization that delay the occurrence of brain damage. In contrast, ‘binge drinking’ induces immediate and toxic effects and there is no evidence of an increased preference for alcohol as seen after withdrawal from chronic alcoholization.

INTRODUCTION

Chronic alcoholism is the popular term for two disorders: alcohol abuse and alcohol dependence. The key element of these disorders is that a person’s use of alcohol has repeatedly caused problems in his or her life. Alcohol abuse can result in a number of problems that include memory disorders, liver disease, high blood pressure, muscle weakness, heart problems, anaemia, low immune function, disorders of the digestive system and pancreatic problems. This will occur after many years of excessive alcohol intake. Alcoholism can also lead to a number of personal problems, including depression, unemployment, family problems and child abuse [reviewed in many papers, for example Adachi et al. (2003) and Harper (2007)]. The cost to the economy of the use and abuse of alcohol in the UK may well be in excess of 20 billion pounds.

However, recent media attention has been directed towards ‘binge drinking’ or heavy episodic drinkers (HED) in the younger generation, 15–21 years, where early neurochemical changes associated with brain damage are evident, particularly in the corticolimbic region (Obernier et al. 2002a, 2002b; Crews et al., 2004), and occur after a relatively short time of such alcohol abuse. This leads to differences in both mood and cognitive performance, e.g. changes in memory (Townsend and Duka, 2005), an increased risk of dementia (Jarvenpaa et al., 2005) as well as susceptibility to developing chronic alcoholism (Bates and Labouvie, 1997). It is estimated that binge drinking costs the UK economy £20 billion a year, with 17 million working days lost to hangovers and drink-related illness each year. This results in costs of £6.4 billion to employers and an annual cost of alcohol harm to the National Health Service in England of £2.7 billion. Furthermore, it has recently been mentioned in a report from Denmark that ‘binge drinking’ three or more times during pregnancy is associated with an increased risk of stillbirth (Strandberg-Larsen et al., 2008). Urgent action is therefore needed to comprehend the aetiology and pathogenesis of the binge drinking culture, as well as to educate individuals on the dangers of such drinking.

The underlying neurochemical changes in binge drinking may be mediated by an imbalance between inhibitory and excitatory amino acids and/or changes in monoamines release, which could drive the excessive drinking behaviour. Biochemically, alteration in phosphorylation possibly occurs, which could lead to changes in the mediators of the inflammatory response. In addition, there are clear and distinct differences between binge drinking and chronic alcoholism in behavioural characteristics, for example, in the number of visits to the Hospital Emergency Departments for alcohol-related injuries (Gmel et al., 2007). It is noteworthy that no alcohol-induced preference or severe withdrawal symptoms occur in binge drinkers after cessation of alcohol.

In this present review, data will be presented on our current understanding of neurochemical and biochemical changes that occur in ‘binge drinking’ individuals. Various signalling pathways will be presented, which may be responsible for the reinforcing and toxic effects of ‘binge drinking’. Finally the action of ethanol on the immune system is discussed, with phagocytic cells being utilized to investigate the differing action of chronic alcoholization and binge drinking on the immune response.

Inhibitory and excitatory amino acids

Glutamate. Glutamate, when released into the synaptic cleft, exerts its action by binding to several classes of glutamate receptors, N-methyl-D-aspartic acid (NMDA), alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) and metabotropic glutamate receptor (mGlu), which are involved in a multitude of functions including learning and its modulation of consolidation and/or recall (Riedel et al., 2003), by activating G-proteins leading to increased phospholipase C, diacylglycerol (IP3 DAG) and calcium-dependent protein kinases (Fig. 1).

Excessive glutamate release is a major cause of neuronal cell death, possibly involving two pathways. Firstly, excitotoxicity that occurs through the activation of glutamatergic receptors (Choi, 1988; Michaels & Rothman, 1990), causing Ca2+ ion release into the cytoplasm, which in turn activates a range of cellular processes that can lead to cell death. This excitotoxicity involves the excessive release of glutamate in the synaptic cleft, which acts independently of other neurotransmitters to cause cell death. Glutamate is released from nerve terminals and acts through glutamate receptors, which belong to one of three different superfamilies of ionotropic receptors: the ionotropic glutamate receptors (iGluRs), metabotropic glutamate receptors (mGluRs) and a group of heteromeric receptors (GluR-B). Glutamate is released into the synaptic cleft, where it binds to specific receptors on the postsynaptic cell, resulting in a change in the permeability of ion channels, which in turn changes the membrane potential and leads to changes in neuronal function.

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**Fig. 1.** Signalling pathways activated by neurotransmitters binding to their respective receptors. cAMP—adenosine 3',5'-cyclic monophosphate; CREB—cyclic AMP response element-binding protein; CBP—calmodulin-binding peptide; IP$_3$R1—inositol 1, 4, 5-trisphosphate receptors; DARPP32—dopamine and cAMP regulated phosphoprotein (MW 32 kD); PLC—phospholipase C; IP$_3$—inositol trisphosphate; PP1—protein phosphatase-1.

**Fig. 2.** The release of glutamate into the synaptic cleft will stimulate AMPA receptors inducing sodium (Na$^+$) fluxes and NMDA receptors stimulating calcium fluxes Ca$^{2+}$. Such calcium fluxes will bind to calcium-binding proteins including calmodulin to activate nitric oxide synthase to enhance nitric oxide, NO, production. Calcium fluxes will also stimulate phospholipases to generate reactive oxygen species, ROS.

influx, with NMDA-mediated generation of nitric oxide (NO), mitochondrial depolarization, Na$^+$ influx leading to an unsustainable increase in ATP demand, microtubule depolymerization, mitochondrial collapse and dendritic beading (reviewed by Greenwood et al., 2007) (Fig. 2). Secondly, oxidative glutamate toxicity, that is mediated via a series of disturbances to the redox homeostasis of the cell (Murphy et al., 1989; Choi, 1992). Several studies have identified an increased glutamate release in ‘binge drinking’ animals in NAC during ethanol ingestion; e.g. the scheduled high alcohol consumption murine model (SHAC) showed enhanced glutamate release in mice administered a 5% alcohol solution on six occasions followed by an i.p. injection of 1.5 or 2 g/kg ethanol (Szumlinski et al., 2007), and the rat model administered 3 g ethanol/kg two times a week for 4 weeks followed by 5 days of abstinence, and then a further challenge with 3 g/kg (Lallemand et al., 2008) (Fig. 3). In a study of the anterior cingulated brain region of 13 recently abstinent young alcoholics, proton magnetic resonance spectroscopy and magnetic resonance imaging identified a significant increase in the glutamate to creatine ratio (Lee et al., 2007). Furthermore in this latter study such alterations in glutamate correlated with altered short-term memory function. It could be argued that such an increase in glutamate release was related to the priming acute dose of ethanol administered immediately prior to the microdialysis experiment of binge drinking animals. However, earlier studies showed that acute administration of either 2 g/kg or 3 g/kg ethanol to naïve rats elicits a decrease (Moghaddam and Bolinao, 1994; Yan et al., 1998) or no change (Dahchour et al., 1994) in glutamate release into the NAC. In contrast, in specific rat strains, i.e. Lewis but not Fischer rats (Selim and Bradberry, 1996), and low alcohol sensitive rats (Dahchour et al., 2000) there were increases in glutamate release into the NAC after 1 g/kg
or 2 g/kg ethanol but of much smaller magnitude to that observed in these binge drinking animal models. It is noteworthy that alcohol-preferring rats show greater region-specific brain damage after a ‘binge-drinking’ regime than its genetic non-alcohol-preferring rat line. Such findings indicate that there is a genetic component that possibly contributes to the brain damage that occurs in ‘binge drinking’ individuals (Crews and Braun, 2003). No increases in NMDA receptor density are reported in binge drinking models (Rudolph and Crews 1996). Furthermore, glutamate antagonists, such as MK801 (NMDA antagonist), nimodipine (voltage-gated Ca\(^{2+}\) channel), DNQX (AMPA antagonist and NMDA antagonist at glycine site), do not protect against brain damage in a ‘binge drinking’ animal model [reviewed in Crews et al. (2004)]. Additional evidence, which indicated that glutamate release may have an effect on NMDA receptors, was shown in recent microdialysis experiments in the ‘binge drinking’ rat model, where significant decreases in arginine release were assayed after both 2 and 3 g/kg ethanol (Lallemand et al., unpublished results) (Fig. 4). This could indicate the utilization of arginine for the formation of the signalling molecule NO mediated via the Ca\(^{2+}\)/calmodulin pathway and NO synthases (Fig. 2). In one previous study

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where chronically alcoholized rats were repeatedly withdrawn from ethanol (Dahchour and De Witte, 2003), a significant reduction in arginine hippocampus microdialysate content was also noted but only after the third withdrawal. It is reported that ‘binge-drinking’ individuals, with compensated alcoholic cirrhosis, show a transient significant increase of serum nitrite and nitrates after an 80 g dose of ethanol (Oekonomaki et al., 2004) that might be indirect evidence for an increase in NO production in certain tissues, particularly the liver and brain.

No changes in the basal concentration of glutamate are apparent in various brain regions during the period of chronic alcohol intoxication (Dahchour and De Witte, 1999, 2003) despite the fact that blood alcohol levels are high, ~2 g/l. However, between 3 and 5 h after the commencement of detoxification from ethanol, there is an enormous increase in glutamate release (due to changes in the sensitivity of NMDA receptors) (Chandler et al., 1993), both NMDAR1 and NMDAR2B polypeptide levels being increased in numbers but decreased in sensitivity during the chronic alcoholization stage (Kumari and Anji, 2005) in the nucleus accumbens (Dahchour and De Witte, 1999), hippocampus (Dahchour and De Witte, 2003) and the striatum (Rossetti and Carboni, 1995). Such excesses of glutamate release during these initial stages of detoxification cause significant behavioural disturbances as well as alcohol craving. Elevations in the synthesis and release of the polyamines spermidine and spermine (modulators of NMDARs) contribute to the increased activity of the receptor during ethanol withdrawal (Gibson et al., 2003). Such adaptive changes play an important role in the development of alcohol dependence as well as alcohol withdrawal.

Gamma-amino-butyric acid (GABA). GABA is a major inhibitory neurotransmitter, which binds to GABA<sub>A</sub> receptors, thereby hyperpolarizing the cell membrane and inhibiting neural activity. Alcohol modulates GABA function, such that in certain brain regions ethanol will increase GABA release (Carta et al., 2004; Hanchar et al., 2005), possibly by inhibiting its degradation. Ethanol will alter GABA-gated current, which is dependent on the time course of exposure and its concentration (Smith and Gong, 2007). Reducing GABA<sub>A</sub> activity decreases the signs of alcohol intoxication and also alcohol’s anti-anxiety effects by GABA. As yet, there have been no studies of the effect of binge drinking on GABA release in different brain regions.

Dopamine. The dopaminergic mesolimbic system plays a significant part in the motivational and reinforcement mechanisms related to behaviour. Alcohol increases dopaminergic transmission in the mesolimbic pathway and increases the firing rate of dopaminergic neurons (Heidbreder and De Witte, 1993) enhancing dopamine release, which may be mediated by an increase in the endocannabinoid tone (Cheer et al., 2007). Direct evidence of a role for dopamine in ethanol reward comes from the finding that rats that operantly self-administer ethanol will stimulate its release both in the NAC (Weiss et al., 1993) and the ventral tegmental cell body region of the meso-accumbens dopamine reward pathway (Gatto et al., 1994). This process can be modified by pharmacological agents that interact with dopamine neurotransmission (McBride et al., 1990). During chronic abuse of alcohol, larger amounts of alcohol may need to be consumed to evoke dopamine release, in order to substantiate the pleasurable effects of alcohol intake. During alcohol withdrawal, dopamine release will be reduced, dramatically reducing the firing of related neurons leading to dysphoria, malaise and depression. Studies are currently underway in our laboratory to investigate changes in hippocampal dopamine release during ‘binge drinking’ in the rat model.

Serotonin. Serotonin plays a role in the regulation of mood, eating arousal, sleep pain and many other behaviours (Carlson, 1998). Alcohol increases serotonin release in the CNS affecting emotion, mood and thinking. There are several types of serotonin receptors, 5-HT<sub>1A</sub>, 5-HT<sub>1B</sub>, 5-HT<sub>2</sub>, and 5-HT<sub>3</sub>, each of which has its own specific influence on behaviour related to the consumption of alcohol (Lovinger, 1999). The 5-HT<sub>3</sub> receptor, a ligand-gated ion channel, has been localized to several regions of the brain and appears to be involved in many neuronal functions including responses to alcohol and other drugs of abuse. There is an extensive and growing literature indicating that 5-HT<sub>3</sub> receptors are involved in several facets of alcohol-seeking behaviour, alcohol intoxication and addiction. In addition, there is strong evidence that ethanol alters the function of the 5-HT<sub>3</sub> receptor, possibly through actions on its receptor protein (Rodd et al., 2007). In a recent study (Szumlinski et al., 2007), it was shown that repeated alcohol consumption in mice (i.e. presentation of 5% alcohol every third day for 18 days for increasing time periods) deregulates serotonin function within the nucleus accumbens, by reducing the extracellular concentration of serotonin.

Opioid system. The opioid system includes endorphin, enkephalin and other endogenous substances that modulate pain, mood reinforcement and response to stress. Endorphin and enkephalin are related to reinforcement from alcohol. Alcohol administration enhances endogenous opioid activity in both rats and man, by increasing opioid release, after in vitro brain slices and of blood levels in humans in vivo. In occasional alcohol users, alcohol-induced dopamine release may produce reinforcement as this is regulated by opioids (Herz, 1997). In contrast, chronic alcohol abuse leads to reduced brain levels of endorphin, which contribute to the negative emotional states that accompany alcoholic withdrawal, as well as increasing the CNS expression of inhibitory G-proteins, thereby reducing adenylyl cyclase (Wand et al., 1993). There are few reports of the effects of ‘binge drinking’ on opioid function in adolescent or mature rodents, although binge-like exposure in newborn rats increases the number of apoptotic β-endorphin neurons in the arcuate nucleus of the hypothalamus (Sarkar et al., 2007).

Taurine. Some investigators have suggested that binge drinking intoxicated rats showed significant cortical oedema (Crews et al., 2004), which could be reduced by the administration of diuretics such as furosemide and acetazolamide. No significant change in the basal levels of taurine was observed in binge drinking rats, which might have been apparent if oedema was occurring since taurine functions as an osmoregulator (Lallemand et al., 2008). Small transient changes in the taurine microdialysis content were evident after ethanol administration to ‘binge drinking’ rats. These were comparable to the pattern of responses seen after acute intraperitoneal ethanol injections (Dahchour et al., 1994), which are attributed to ethanol-induced osmolarity changes.

Biochemical changes
Chronic alcohol abuse and ‘binge drinking’ induce a wide range of biochemical changes in the brain that are related to changes
on the intricate signalling pathways (Hoek and Kholodenko, 1998; Pandey, 1998), such as NO synthesis, protein phosphorylation and the action and release of trophic factors, MAPK and NFκB pathways. The generation of free radicals in the brain, generated by the inducible MEOS system (Sohda et al., 1993) or acetaldehyde toxicity, (Heap et al., 1995) may contribute to the alcohol-induced brain damage (Pratt et al., 1990), which will be minimized if adequate cytoprotection can be upregulated, e.g. reduced glutathione, in the specific brain regions affected.

Changes in phosphorylation. Cellular signalling events rely heavily on protein phosphorylation (with protein kinases initiating phosphorylation while protein phosphatases will dephosphorylate), thereby activating and inactivating signal transduction cascades.

Neurotransmitters interact with specific cell surface receptors that are coupled to the stimulatory G protein Gs, thereby activating cAMP, which results in phosphorylation of the transcription factor to the cyclic AMP responsive element-binding protein, CREB (Fig. 1). This latter protein serves as both an up- and down-stream molecular target for the action of brain-derived neurotrophic factors by inducing key genes that improve neuronal vitality, growth and resistance to insults (Walton and Dranow, 2000; Mantamadiotis et al., 2002). CREB requires phosphorylation to initiate transcription of pro-survival neuronal factors (Fig. 1). Both ‘binge drinking’ and chronic alcohol intoxication reduce the amount of phosphorylated CREB immunoreactivity in the hippocampal dentate gyrus during intoxication (Yang et al., 1998; Bison and Crews, 2003). In contrast, an acute ethanol challenge, 3 g/kg, induced an increase in the phosphorylated form of CREB in the rat cerebellum.

Changes in phosphorylation of the protein phosphatase inhibitor DARPP-32 may play an important role in reducing ethanol inhibition of NMDA receptors. In a recent study of cultured neurons and brain slices, Maldve et al. (2002) showed that activation of dopamine D1 receptors may over-ride the alcohol-induced inhibition of NMDA receptors in the NAC. This was dependent on a series of intracellular signalling events involving the activation of adenylate cyclase, the subsequent activation of the cyclic AMP-dependent protein kinase A (PKA) and phosphorylation of the protein phosphatase inhibitor DARPP-32 (Fig. 5). Activation of D1 receptors also increased phosphorylation of the NR1 subunit of the NMDA receptor, presumably because phosphorylation of DARPP-32 inhibits the protein phosphatase PP-1 that normally removes the phosphate group at serine 897 within the NR1 protein. Such interactions between alcohol, dopamine and glutamate may be important in the development of ‘binge drinking’, with D1 receptors and DARPP-32 mediating such effects.

Transcription factors were the first signalling proteins to be identified as redox sensitive, which are regulated through sensitive cysteine residues, that need to be reduced for activity. The transcription factor NFκB (NFκB) is sequestered in the cytoplasm where it is associated with a member of the IκB family of inhibitory proteins (Fig. 6). For the activation of NFκB, rapid phosphorylation of its inhibitory subunit, IκB, by specific IκB kinases is necessary. IκB is then released from NFκB to be ubiquinated and degraded by proteosomes. NFκB is then translocated to the nucleus where it mediates transcriptional initiation of a number of cytokines. Originally it was thought that NFκB activation was due to the production of reactive oxygen species (Schreck et al., 1992), but later studies revealed that, in vitro, such NFκB activation by exogenous H2O2 was cell specific (Bowie and O’Neill, 2000). Although one study did show some benefit of specific anti-oxidants, i.e. furosemide and butylated hydroxytoluene, in preventing neurodegeneration in binge drinking animal models (Crews et al., 2004), no results for NFκB activation were presented. In our previous studies of NFκB activation in the cortex of chronically alcoholized rats (Ward et al., 1996), it was evident that this transcription factor was severely down regulated by comparison to the high activation of NFκB after an acute ethanol dose. Such neuroadaptations may not occur in the binge drinking model, since it was noted that there was excessive generation of ROS, particularly hydroxyl radicals, during the binge drinking regime as well as after further acute ethanol challenges (Lallemand et al., 2008).

Glial and macrophages
Glial cells play an important function in the brain, nurturing neurons and facilitating neuronal activity. Four different types...
of glial cells exist, e.g. oligodendrocytes, ependymal cells, astrocytes and microglia. Microglial cells are closely related to monocytes and macrophages (Stoll and Jander, 1999) and play a pivotal role in CNS immunity. Glial cells, including astrocytes, are essential for the regulation of released glutamate and its conversion to glutamine through the enzyme glutamine synthetase. Activated microglia secretes neurotoxic inflammatory cytokines and mediators such as tumour necrosis factor (TNFα), and NO, which may initiate or amplify the neuroinflammatory responses. The activation of microglial cells may be aimed at initially protecting the neurons but latterly could induce neuronal destruction. Deregulation by ethanol of the inflammatory activation signalling of microglia may contribute to the derangement of CNS immune and inflammatory responses. Chronic ethanol consumption did not alter the density of microglial cells that reside in the molecular layer of the cerebellar cortex (Dlugos and Pentney, 2001) while intermittent ethanol exposure increased the number of cerebellar microglia, indicating microgliosis (Riikonen et al., 2002). Therefore, understanding the regulatory processes of such neuro-inflammation is pivotal to understanding the mode of neuropathology in mal models of alcohol toxicity, i.e. chronic alcohol intoxication and 'binge drinkers'.

Many intracellular signalling pathways are mobilized by microglial activation and include NfκB, MAPK, PKA, PKC, JAK-STAT, Nos and TLRs (Fig. 7). Many of the glial signalling pathways are affected by ethanol, e.g. NfκB, MAPKs and JAK-STAT, although there is no consensus as to whether ethanol suppresses, enhances or has no effect [reviewed by Suk (2007)]. The cell type, ethanol concentration dose and timing of exposure may be important factors to consider.

Among the many inflammatory mediators produced by activated phagocytic cells, i.e. macrophages and microglia, NO production has been widely regarded as representative of inflammatory activation. Microglia-derived NO will exert direct toxicity towards neurons (Liu et al., 2002). Exposure of the immortalized cell line BV-2 microglia cells to ethanol, 10 or 100 mM, significantly reduced the LPS-induced NO production. Ethanol also inhibited NfκB signalling (Lee et al., 2004), although the mechanism did not involve changes either in the ability of NfκB to bind to its cognate DNA sequences or IκB degradation. This would indicate that the site of ethanol’s action is between DNA binding of NfκB and the transactivation of target genes. One of the co-activators involved in this process, p300, may be the site of ethanol’s action. Importantly IFNγ signalling in microglia is not affected by ethanol; neither STAT1 nor IRF-1 (constituting the main components of the IFN-γ signalling pathway) is altered after ethanol administration. In our previous studies, alveolar macrophages (which are the functional equivalent of brain microglia) isolated from binge drinking rats (EtOH) or chronically alcoholized rats, after incubation with stimulants, either lipopolysaccharide (LPS) alone or together with interferon gamma (INFγ).
concentrations, >100 mM (Militante et al., 1997; Davis and Syapin 2004, respectively). However, COX-2 expression and the resulting PGE2 production was increased by ethanol in rat astrocyte cultures (Luo et al., 2001). Further studies are needed to identify the specific effects of ethanol, when administered acutely, chronically or intermittently, on the intracellular signal transduction that may be different among the glial cell population.

Considerable research efforts are necessary to ascertain how the various signalling pathways are altered by alcohol when administered either chronically or intermittently. The results of such studies will enable pharmaceutical intervention to be made to prevent the onset and development of alcohol-induced brain damage. There is little doubt that the problem of binge drinking, particularly by adolescents, needs to be addressed urgently, to prevent cognitive impairment, which could lead to irreversible brain damage.

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