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Ethanol Toxicity in the Brain: Alteration of Astroglial Cell Function

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1. Introduction

Ethanol consumption has for a long time been associated with brain damage. Experimental studies and necropsy examinations of chronic alcoholics have shown a variety of structural and functional alterations in the neurons as well as in the glial cells. Such alterations are seen also in children with the alcoholic foetal syndrome. Ethanol is known to be a teratogen. Its abusage can result as dysfunction of the central nerve system (CNS), growth deficiency and facial malformation in the fetus, and behavioural, learning, sensory and motor disabilities (Barret et al., 1996; González & Salido, 2009; Šarc & Lipnik-Štangelj, 2009a). Chronic ethanol consumption in the adult is also intimately associated with brain atrophy. Accumulating evidence indicates that ethanol-induced neurobehavioral dysfunctions may be related to disruptions in the patterns of neuronal and glial developments such as depression of neurogenesis, aberrant migration of neurons and alterations in late gliogenesis and neurogenesis. These changes can further reduce the populations of cortical neurons and glial cells, trigger the biochemical alterations in glial cells and deleterious consequences for neuronal-glial interactions, and eventually lead to damage or apoptosis of these cells (González & Salido, 2009; Šarc & Lipnik-Štangelj, 2009b; Sofroniew & Vinters, 2010).

As the most abundant type of glial cells in the brain, astrocytes provide metabolic and trophic support to neurons, modulate synaptic activities and have a strong capacity to scavenge oxidants and suppress cellular apoptosis. However, when the capacity of cells to eliminate the oxidants is overwhelmed, overproduction of reactive oxygen species (ROS) can cause morphological and functional alterations in the cells, including cellular Ca^{2+} homeostasis and some active molecules tightly associated with neuronal activity (Allansson et al., 2001; Halassa et al., 2007; Sofroniew & Vinters, 2010).

Although astrocytes are more resistant than neurons to the oxidative and neurotoxic stresses and to the chemical and toxic damages in the surrounding environment, any impairment of astrocytes can dramatically affect neuronal functions. The ethanol-induced detrimental alterations of astrocytes would lead to perturbances in neuron–astroglia interactions and developmental defects of the brain (González & Salido, 2009; Šarc & Lipnik-Štangelj, 2009b). Given this important role of astroglial cells in neuronal functioning, they have become a significant object of toxicological evaluation.

2. Astrocytes in the central nerve system

Central nerve system is a complex network, constitutes from several types of cells. Besides neuronal cells, where the information is received, integrated and sent as an output signal, there are several other cell types in the CNS. Oligodendrocytes are specialized for the myelin formation, astrocytes have multiple support functions to neurons, and microglial cells play an important role in defence and inflammation, and act as scavengers when tissue is destroyed. Some other types of cells in the CNS are ependimal cells, which are epithelial cells that line brain ventricles and central canal of spinal and assist in secretion and circulation of cerebral spinal fluid, and endothelial cells which create a blood-brain barrier (González & Salido, 2009).

Glial cells were discovered by the pathologist Rudolf Virchow in 1856. They represent the majority cell population in the CNS. There is a number between 12 and 15 billion neurons in cerebral cortex and about a billion neurons in spinal cord, whereas there are 10 to 50 times more glial cells than neurons in the CNS. When they were discovered, glial cells have been recognised as brain glue. They surround neurons and hold them in place. Later it has been realized that glial cells play a number of other functions in the brain. Astrocytes are the most abundant type of glial cells, and present numerous projections that anchor neurons to their blood supply (Braet et al., 2001; González & Salido, 2009; Grafstein et al., 2000; Haydon, 2001).

2.1 Molecular aspects of astrocyte function

Astrocytes signal each other using Ca^{2+} ions (Verkhratsky et al., 1998). This type of cell-to cell communication has been termed "calcium excitability" that occurs as transient or prolonged elevations in intracellular concentration of Ca^{2+} ions. It can be spontaneous or triggered in response to specific neurotransmitters (Araque et al., 2001; Cornell-Bell et al., 1990). The membrane potential of glia is relatively stable, and although they can express voltage-gated channels (Verkhratsky et al., 1998), they exhibit little or no fluctuation in membrane potential.

Astrocytes respond to a variety of extracellular stimuli by raising intracellular concentration of Ca^{2+} ions that modulates different intracellular processes like differentiation, cytoskeleton reorganisation, and secretion of neuroactive molecules (Araque et al., 1998; Sofroniew & Vinters, 2010; Verkhratsky & Kettenmann, 1996). A rise in intracellular concentration of Ca^{2+} ions, localize to one part of an astrocyte can propagate through-out the entire cell, and Ca^{2+} resposes may be transmitted from one astrocate to others, leading to regenerative Ca^{2+} signal that spread within astrocyte networks (Cornell-Bell et al., 1990; Fam et al., 2000). This cell-to-cell communication could effectively signal to neurons, endothelial or other cell type in the CNS. Obviously, Ca^{2+} signalling in astrocytes is complementary to and interacts with signalling in vascular brain cells (Leybaert et al., 2004) and electrical signalling in neurons (Araque et al., 1999; Parpura et al., 1994). Besides calcium excitability, there are also other mechanisms for transmitting signals between astrocytes, such as releasing of diffusible extracellular messengers. Extracellular release of neurotransmitters like glutamate or adenosine triphosphate (ATP), and consequent activation of specific receptors on neighbouring astrocytes, may also mediate Ca^{2+} wave propagation (Bowser & Khakh, 2007). In addition, astrocytes are able to release other signalling molecules like D-serine and eicosanoids, and more than one of describing mechanisms for neurotransmitter release does

operate within astrocytes (Araque et al., 2001; Fellin et al., 2004; Gonzales et al., 2006a; Malarkey & Parpura, 2008; Montana et al. 2006).

Released messengers, in turn, activate Ca^{2+} entry or Ca^{2+} release from intracellular stores by acting on ionotropic and metabotropic receptors, respectively. By this way, ATP and glutamate are the major active neurotransitters involved in the cell-to-cell communication of Ca^{2+} signals in astrocytes and other cell types in the CNS (Bowser & Khakh, 2007; Percea & Araque, 2007). Another putative intercellular signalling molecule for cel-to-cell communication is nitric oxide which is synthesized by enzymatic oxidation of L-arginine by nitric oxide synthase (Willmott et al., 2000). Nitric oxide (NO) activates guanylilcyclase and increases cytoplasmic cyclic guanosine monophosphate (cGMP) signalling cascades (Galione et al, 1993).

Communication between astrocytes thus seems to rely on any communication systems and signalling molecules, which act in parallel or display regional and cellular specialisation. From this point of view, there is a bidirectional signal communication system within the CNS, which might be mainly carried out by extracellular messengers, released from any type of cells. Because of their close apposition to neurons, signalling molecules released by astrocytes can modulate synaptic transmission and neuronal excitability, as well as neuronal plasticity and survival. Even it could be possible that astrocytes could play roles in higher cognitive functions like learning and memory. It is not therefore estrange that an alteration in Ca^{2+} signalling, and hence in the function of astrocytes, could affect synaptic activity and plasticity and bran homeostasis (Gonzalez & Salido, 2009).

2.2 Release of intercellular messengers

Close physical relationship between astrocytes and neurones provides an opportunity for many functional interactions. There is a bidirectional signalling pathway between astrocytes and neurons on one side, and astrocytes and blood vessels on the other, which opens the possibility to an exchange of a huge amount of information in the CNS. There are several mechanisms that have been suggested to underline the release of signalling molecules from astrocytes: reverse operation of glutamate transporters, volume-regulated anion channels, gap-junctional hemichannels, diffusional release through purinergic receptors and Ca^{2+}-dependent exocytosis (Araque et al., 2001; Haydon & Carmignoto, 2006; Montana et al., 2006; Parpura et al., 2004). Among the different molecules released, two major signalling messengers, released by astrocytes, are ATP and glutamate (Gonzalez & Salido, 2009).

The mechanisms, by which astrocytes release ATP, appear to be diverse, employing vesicular release, connexion hemi-channels, cystic fibrosis transmembrane regulator, or the P-glycoprotein (Braet et al., 2004). On the other hand, astrocytic glutamate release can be carried out through connexion hemi-channel, excitatory amino acid transporters (EAAT), anion transporter, via $P2X_7$ receptor channels or exocytosis. Depending on the mechanism employed, ATP and/or glutamate release by astrocytes can be Ca^{2+}-dependent or independent (Bowser & Khakh, 2007; Braet et al., 2004).

Besides the mechanisms for ATP and/or glutamate release from astrocytes, exocytosis constitutes the mechanism that has recently received special attention, since it was initially considered to occur only in neurons (Bowser & Khakh, 2007; Fellin et al., 2006; Gonzalez et al., 2006a; Perea & Araque, 2007).

2.3 The role of astroytes in the central nerve system

2.3.1 Astrocytes and development of central nerve system

The developmental generation of astrocytes tends to occur after the initial production of neurons in many CNS regions (Sofroniew & Vinters, 2010). During development of the brain, astrocytes (radial glia) take part in guiding the migration of developing axons and certain neuroblasts (Powel & Geller, 1999). In addition, substantive evidence is accumulating that astrocytes are essential for the formation and function of developing synapses by releasing molecular signals such as thrombospondin (Barres, 2008; Christopherson et al., 2005). Astrocytes appear also to influence developmental synaptic pruning by releasing signals that induce expression of complement C1q in synapses and thereby tag them for elimination by microglia (Barres, 2008).

2.3.2 Blood-brain barrier and regulation of blood flow

Together with brain microvascular endothelial cells astrocytes create the blood-brain barrier that protects the brain from toxic substances in the blood, supplies the brain tissues with nutrients, and filters harmful substances from the brain back to the bloodstream, enabling the proper environment in the CNS. Astrocytes may regulate endothelial cell metabolism, and vasoconstriction and vasodilatation by producing substances with angiogenic properties, such as endothelial growth factor (Proia et al., 2008), ATP (Leybaert et al., 2004), and arachidonic acid, prostagladins and nitric oxide, (Gabryel et al., 2007; Sofroniew & Vinters, 2010), that can increase or decrease CNS blood vessel diameter and blood flow in a coordinated manner. Moreover, astrocytes may be primary mediators of changes in local CNS blood flow in response to changes in neuronal activity (Koehler et al., 2009). Thus, astrocytes play important functions at the level of arterioles where blood flow is controlled, at the level of capillaries where blood-brain barrier is located and at the level of blood immune cells (Leybaert et al., 2004).

2.3.3 Energy, metabolism and homeostasis

Astrocytes play a number of other functions which are crucial for the maintenance of homeostasis and neuronal function. They provide energy supply to neurons and coordinate metabolic reactions. Astrocytes are the principal storage sites of glycogen granules in CNS. The greatest accumulation of astrocytic glycogen occurs in areas of high synaptic density, and its utilisation can sustain neuronal activity during hypoglicemia and during periods of high neuronal activity (Sofroniew & Vinters, 2010).

Astrocytes regulate the external chemical environment by removing excess ions notably potassium, regulate brain cell volume, and participate in recycling neurotransmitters released during synaptic transmission by expressing high levels of transporters for neurotransmitters such as glutamate, GABA, histamine and glycine, that serve to clear the neurotransmitters from the synaptic space. Astrocytes also represent the major site for the detoxification or bioactivation of neurotoxins (Perdan et al., 2009).

2.3.4 Synapse function

There is accumulating evidence that astrocytes play direct roles in synaptic transmission through the regulated release of synaptically active molecules including glutamate, purines

(ATP and adenosine), gamma-aminobutyric acid (GABA), and D-serine. The release of such gliotransmitters occurs in response to changes in neuronal synaptic activity, involves astrocyte excitability as reflected by increases in intracellular concentration of Ca^{2+} ions, and can alter neuronal excitability (Halassa et al., 2007; Perea et al., 2009). Such evidence has given rise to the 'tripartite synapse', which posits that astrocytes play direct and interactive roles with neurons during synaptic activity in a manner that is essential for information processing by neural circuits (Araque et al., 1999; Halassa et al., 2007; Perea et al., 2009).

2.3.5 Immune response

Astrocytes importantly contribute to creation of immune response in the brain. They are an important source of several cytokines and neurotrophic factors in the CNS that have a crucial immunoregulatory role and also promote neuronal survival and neurite growth (Lipnik-Štangelj, 2006). Moreover, cytokines have an impact on neurotoxicity, synaptic transmission and synaptic plasticity in the brain (Allan & Rothwell, 2001). Activation of astrocytes leads to up-regulation of pro-inflammatory cytokines like interleukin-1 beta (IL-1beta), tumour necrosis factor alpha (TNF-alpha), interleukin-6 (IL-6), and inducible nitric oxide synthase (iNOS), and cyclooxygenase 2 (COX2) (Gonzalez & Salido, 2009; Sofroniew & Vinters, 2010).

Research into the actions of IL-1beta in the brain initially focused on its role in host defence responses to systemic disease. IL-1beta can also elicit an array of responses which could inhibit, exacerbate or induce neuronal damage and death (Gonzalez & Salido, 2009).

TNF-alpha has an important function in neurotoxicity, synaptic transmission and synaptic plasticity. It influences homeostatic synaptic scaling by inducing the insertion of AMPA receptors at post-synaptic membranes (Stellwagen & Malenka, 2006). In addition, TNF-alpha may have a pivotal role in augmenting intracerebral immune responses and inflammatory demyelination due to its diverse functional effects on glial cells, such as oligodendrocytes and astrocytes themselves (Šarc et al., 2011).

Unlike TNF-alpha, which is a prototypical pro-inflammatory cytokine, IL-6 affects inflammation and neuronal regeneration via a number of mechanisms. In this sense, besides its immunoregulatory role, IL-6 can also promote neuronal survival and neurite growth. IL-6 can be induced by a variety of molecules including IL-1beta, TNF-alpha, transforming growth factor-beta and prostaglandins, and many other mediators such as beta-amyloid, interferon-g and IL-4 can potentiate these primary inducers, highlighting the complex nature of IL-6 modulation (Šarc et al., 2011; Gonzalez & Salido, 2009).

2.4 Reactive gliosis and glial scar formation

After brain injury, such as a stroke or trauma, astrocytes become reactive, and can undergo to profound proliferation, forming gliosis near or at the site of damage. Astrocyte activity is marked by hypertrophy, resulting in an expression of protein such as glial fibrillary acidic protein (GFAP), adhesion molecules and antigen presenting capabilities, including major histocompatibility antigens. Reactive astrocytes represent an obstacle preventing establishment of normal neural contact and circuitry. On the other hand, reactive astrocytes produce a myriad of neurotoxic substances in various brain pathologies (Mori et al., 2006).

Although reactive astrogliosis is used widely as a pathological hallmark of diseased CNS tissue, definitions of reactive astrogliosis can vary considerably among authors and there are no widely accepted categories of intensity or severity. Recently proposed definition encompasses four key features: (1) reactive astrogliosis is a spectrum of potential molecular, cellular and functional changes in astrocytes that occur in response to all forms and severities of CNS injury and disease including subtle perturbations, (2) the changes undergone by reactive astrocytes vary with severity of the insult along a gradated continuum of progressive alterations in molecular expression, progressive cellular hypertrophy, and in severe cases, proliferation and scar formation, (3) the changes of reactive astrogliosis are regulated in a context-specific manner by inter- and intracellular signalling molecules, (4) the changes undergone during reactive astrogliosis have the potential to alter astrocyte activities both through gain and loss of function that can impact both beneficially and detrimentally on surrounding neural and non-neural cells. Of particular interest as regards function of reactive astrocytes, is recent evidence that reactive astrogliosis and glial scar formation play essential roles in regulating CNS inflammation (Sofroniew, 2009).

In response to different kind of stimulation, reactive astrocytes can make many different kinds of molecules with either pro- or anti-inflammatory potential (John et al., 2003). There is a normal process of reactive astrogliosis and glial scar formation that exerts various beneficial functions including protecting neural cells and function, restricting the spread of inflammation, and promoting tissue repair.

On the contrary, in a manner analogous to inflammation, reactive astrogliosis also has the potential to exert detrimental effects. For example, reactive astrocytes can be stimulated by specific signalling cascades to gain of detrimental effects such as exacerbating inflammation via cytokine production (Brambilla et al., 2009), producing neurotoxic levels of ROS (Hamby et al., 2006), releasing potentially excitotoxic glutamate (Takano et al., 2005), potential contribution to seizure genesis (Tian et al., 2005), compromising blood brain barrier function due to VEGF-production (Argaw et al., 2009), causing cytotoxic edema during trauma and stroke (Zador et al., 2009), and contributing to chronic pain (Milligan et al., 2009).

2.4.1 Molecular mechanisms of reactive gliosis and scar formation

Many different types of intercellular signalling molecules are able to trigger reactive astrogliosis or to regulate specific aspects of reactive astrogliosis, including large polypeptide growth factors and cytokines such as IL-1, IL6, IL-10, TNF-alpha, tumour growth factor beta (TGF-beta), mediators of innate immunity such as lipopolysaccharide and other Toll-like receptor ligands, neurotransmitters such as glutamate and noradrenalin, purines such as ATP, ROS including nitric oxide (NO), hypoxia and glucose deprivation, products associated with neurodegeneration such as beta-amyloid, molecules associated with systemic metabolic toxicity such as NH4, and regulators of cell proliferation such endothelin-1, as reviewed in detail elsewhere (Sofroniew, 2009). Such molecular mediators of reactive astrogliosis can be released by all cell types in CNS tissue, including neurons, microglia, oligodendrocyte lineage cells, pericytes, endothelia, and astrocytes, in response to all forms of CNS insults, ranging from subtle cellular perturbations that release some of the specific factors just listed, to cell stretching as might be encountered during acceleration/deceleration CNS injury and which

releases ATP, to intense tissue injury and cell death that release various intracellular molecules that signal intense tissue damage (Sofroniew, 2009).

It is becoming clear that different molecular, morphological, and functional changes in reactive astrocytes are specifically controlled by inter- and intra-cellular signalling mechanisms that reflect the specific contexts of the stimuli and produce specific and gradated responses of reactive astrogliosis. For a long, reactive gliosis and scar formation have been recognized as the main impediment to functional recovery after CNS injury or disease. This absolutely negative viewpoint of reactive astrogliosis is no longer tenable and it is now clear from many different lines of experimental evidence that there is a normal process of reactive astrogliosis that exerts essential beneficial functions and does not do harm. As reviewed in detail elsewhere, many studies using transgenic and experimental animal models provide compelling evidence that reactive astrocytes protect CNS cells and tissue by uptake of potentially excitotoxic glutamate, protection from oxidative stress via glutathione production (Dringen et al., 2000), neuroprotection via adenosine release (Lin et al., 2008), protection from NH4 toxicity (Rao et al., 2005), neuroprotection by degradation of amyloid-beta peptides (Koistinaho et al., 2004), facilitating blood brain barrier repair, reducing vasogenic edema after trauma (Bush et al., 1999), stroke or obstructive hydrocephalus, stabilizing extracellular fluid and ion balance and reducing seizure threshold (Zador et al., 2009), and limiting the spread of inflammatory cells or infectious agents from areas of damage or disease into healthy CNS parenchyma (Bush et al., 1999; Voskuhl et al. 2009).

3. Ethanol in the central nerve system

The deleterious effects of ethanol in CNS could result either from a direct toxic effect of ethanol or from an indirect effect involving its metabolites and/or ROS generation. Ethanol can induce several cellular reactions which result in a modification of cellular redox status that can severely affect the cell's capacity to be protected against the endogenous production of ROS (Gonthier et al., 2004). The consequences derived from the effects of ethanol on cellular structures would end in a morphological and functional impairment of cellular physiology. Among brain cells, astrocytes seem less vulnerable than neurons, but their impairment can dramatically affect neurons because of their protective role towards neurons.

3.1 Ethanol metabolism in the brain

In the CNS, astrocytes represent the major cellular localisation of ethanol metabolism, and have been postulated to protect neurons from ethanol-induced oxidative stress (Watts et al., 2005). The exact enzymatic mechanism responsible for ethanol oxidation in the brain is not clear yet.

Ethanol is normally metabolised in the liver to acetaldehyde by the alcohol dehydrogenase reaction, and acetaldehyde can be further metabolised to acetic acid via aldehyde dehydrogenase reaction. The last step in the pathway is the conversion of acetic acid to acetyl-Co-A. Although theoretically the activity of the latter enzyme is high enough to cope with the rate at which ethanol is oxidized by alcohol dehydrogenase, there is a limit to the rate at which the reaction can continue and can therefore lead to accumulation of acetaldehyde, which is toxic for most tissues, including CNS. Thus, there is always a build

up of acetaldehyde which passes out from the liver into the blood, and this acetaldehyde is responsible or some of the unpleasant symptoms of alcohol excess. Once in the bloodstream, the acetaldehyde can also cross the blood-brain barrier and attack the CNS.

In the brain, ethanol can be metabolized by catalase, cytochrome P450 2E1, and alcohol-dehydrogenase, with catalase, playing a pivotal role among the others (Gonzalez et al., 2007).

On the other hand, ethanol also induces up-regulation of antioxidant defences by increasing the enzymatic activities of superoxide-dismutase, catalase, and glutathione-peroxidase (Eysseric et al., 2000; Rathinam et al., 2006). The expression of heat shock proteins like HSP70 (Russo et al., 2001), which have a protective and stabilizing effect on stress-induced injury, is also induced by ethanol. Altogether, this would confer to astrocytes a survival advantage preventing oxidative damage.

3.2 Ethanol influence on astrocyte function

Ethanol has several targets in astrocytes and other cell types, impairing cellular redox status, cell growth and differentiation, interfering with the stimulatory effect of trophic factors or altering the expression of cytoskeletal proteins. In addition, ethanol induces astroglial activation, associated with up-regulation of several pro-inflammatory cytokines, that contribute to neuroinflammation, neurodegeneration and cell apoptosis (Alfonso-Loeches et al., 2010; Šarc & Lipnik-Štangelj, 2009).

3.2.1 The effects of ethanol on developing central nerve system

Ethanol is a known teratogen and has been implicated in the etiology of human fetal alcohol syndrome, which is characterized by distinct craniofacial abnormalities such as microcephaly, agnathia, and ocular aberrations. Prenatal ethanol exposure induces functional abnormalities during brain development affecting neurogenesis and gliogenesis. Thus, ethanol cases a number of changes in several neurochemical systems. Astrocytes are predominant source of postnatal retinoic acid synthesis in the cerebellum, and this acid shows teratogenic effects responsible for the fetal alcohol syndrome.

McCaffery et al. (2004) showed that ethanol could stimulate retinoic acid synthesis leading to abnormal embryonic concentrations of this morphogen and, thus, ethanol could represent a major cause of fetal alcohol syndrome. Additionally, increased sensitivity of glutamate receptors and enhanced trans-membrane transport of glutamate has been observed in the presence of ethanol. This was in relationship to the increase in the expression of the excitatory amino acid transporters EAAT1 and EAAT2. Thus, glutamatergic system is affected by ethanol, which can be viewed as a maladaptive process that disposes the developing brain to fetal alcohol syndrome (Zink et al. 2004).

Furthermore, ethanol affects the synthesis, intracellular transport, distribution, and secretin of N-glicoproteins in different cell types, including astrocytes and neurons (Braza-Boils t al., 2006). Glicoproteins, such as adhesion molecules and growth factors, participate in the regulation of nervous system development. Thus, the alteration in the glycosylation process induced by ethanol could be a key mechanism involved in the teratogenic effects of ethanol exposure on brain development. Further studies by Martinez et al., (2007) showed that long-term ethanol treatment substantially impairs glycosylation and membrane trafficking in

primary cultures of rat astrocytes. Ethanol reduced endogenous levels of acive RhoA due to an increase in the activity of small Rho GTP-ases, reduced phosphoinositides levels and induced changes in the dynamics and organization of the actin cytoskeleton.

Ethanol presents as well morphological effects on the developing adolescent brain. There were clear effects immediately and long after drinking cessation of a chronic ethanol administration on two neurotransmitter systems (the serotoninergic and nitrergic), which decreased, and the astrocytic cytoskeleton and neuron, which increased and decreased, respectively (Evrard et al., 2006). The authors concluded that drinking cessation can partially ameliorate the ethanol-induced morphological changes on neurons and astrocytes but cannot fully return it to the basal state.

3.2.2 The effects of ethanol on cholesterol homeostasis

Cholesterol is an essential component of cell membranes and plays an important rule in signal transduction. There are evidences that cholesterol homeostasis may be affected by ethanol, and this may be involved in neurotoxicity (Guizzetti & Costa, 2007). Indeed, the pathogenesis of Alzheimer's disease has been linked to altered cholesterol homeostasis in the brain. Several functions are carried out by cholesterol and are important for brain development, such as glial cell proliferation, synaptogenesis, neuronal survival and neurite outgrowth. In addition, the brain contains high level of cholesterol, mostly synthesized in situ. Furthermore, astrocytes produce large amounts of cholesterol that can be released by these cells and utilized by neurons to form synapses (Gonzalez & Salido, 2009).

3.2.3 The effects of ethanol on synaptic structure

It has been shown that chronic ethanol consumption affects the synaptic structure. The density of dendritic spines was found lower in the nucleus accumbens, and depicted an up-regulation of a subunit of the NMDA receptor. The up-regulated NMDA receptor subunit is a splice variant isoform which is required for membrane-bound trafficking or anchoring into a spine synaptic site. These changes, evoked by ethanol, demonstrated an alteration of micro circuitry for glutamate reception (Zhou et al., 2007).

Adermark and Loviger (2006) showed that ethanol inhibits a Ca^{2+}-insensitive K^+ channel activity, and affects gap junction coupling, demonstrating that astrocytes play a critical role in brain K^+ homeostasis, and that ethanol effects on astrocytic function could influence neuronal activity.

Finally, despite most of the investigations on the effects of ethanol have been performed following its addition to tissue or cell cultures, an interesting study has shown excessive activation of glutamatergic neurotransmission in the cerebral cortex following ethanol withdrawal and its contribution to significant behavioural disturbances and to alcohol craving. These effects were related to the activity of the enzyme glutamine synthetase, which converts released glutamate to glutamine (Miguel-Hidalgo, 2006).

3.2.4 Ethanol and glial oxidative stress

Brain tissue is particularly vulnerable to oxidative damage, possibly due to its high consumption of oxygen and the consequent generation of high quantities of ROS during

oxidative phosphorylation. In addition, several regions of the brain are rich in iron, which promotes the production of ROS. On the other hand, the brain counts wit relatively poor levels of antioxidant enzymes and antioxidant compounds. ROS increase intracellular concentration of Ca^{2+} ions, inhibit response of astrocytes to physiological agonists, and stimulate glutamate secretion, which in excess is neurotoxic (Gonzalez et al., 2006a). Although glutamate is the principal excitatory neurotransmitter in the mammalian brain, high levels of this neurotransmitter lead to excitotoxic neuronal death, mediated by Ca^{2+} influx, principally through NMDA-gated channels (Bambrick et al., 2004).

Ca^{2+} signalling is an important medium for neuron-glia interaction, in the sense that neuronal activity can trigger Ca^{2+} signals in glial cells and vice versa. Due to its critical importance for the cellular functions, resting intracellular concentration of Ca^{2+} ions is tightly controlled, and abnormalities in Ca^{2+} regulation lead to impairment of cellular physiology. Ca^{2+}-ROS interplay can be considered as a push-pull relationship. An elevated level of intracellular concentration of Ca^{2+} ions can lead to excessive ROS production, whereas excessive ROS production can lead to cytosolic Ca^{2+} overload (Gonzalez & Salido, 2009). Acute exposure of astrocytes to ethanol increases intracellular concentration of Ca^{2+} ions, probably due to inhibition of plasma membrane Ca^{2+}-ATPase activity (Sepulveda & Mata, 2004). Other changes, evoked by ethanol are cell swelling, and transformation of actin cytoskeleton (Allansson et al., 2001).

Mitochondria represent the major source of intracellular ROS, and Ca^{2+} uptake into the organelle can lead to ROS generation (Gonzalez et al., 2006b; Granados et al., 2004). Ethanol-evoked ROS production takes place in the mitochondria, and accumulated mitochondrial ROS can be released to the cytoplasm leading to damage of different transport mechanisms, ion channel modification, lipid peroxidation, and DNA damage. Furthermore, damage to mitochondrial metabolism may generate additional damaging radial species, thus activating cellular death pathways (Gonzalez et al., 2006a).

Ethanol evokes a dose-dependent increase in glutamate secretion by an exocytosis mechanism, which was dependent on Ca^{2+} mobilisation. The secretory effect of ethanol is reduced in the presence of antioxidants, therefore indicating the participation of ROS in ethanol-evoked glutamate secretion by astrocytes. Glutamate and the attendant increase in intracellular Ca^{2+} play crucial role in triggering excitotoxic cell death in neighbouring cells (Molz et al., 2008). Because astrocytes are the major regulators of glutamate homeostasis, their death can cause and/or aggravate diseases of the CNS.

3.2.5 Ethanol, inflammation and immune response

Ethanol is able to activate glial cells, which is a critical event in the neuroinflammatory processes. Chronic ethanol intake enhances inflammatory mediators like COX-2, and iNOS in rat cerebral cortex and cultured astrocytes. Astrocytes undergo actin cytoskeleton disorganisation, and there is a stimulation of both, interleukin receptor-associated kinase (IRAK)/extracellular signal-regulated kinases (ERK)/nuclear factor-kappaB (NF-kappaB) pathway and the COX-2 expression, which are associated with the inflammatory responses (Guasch et al., 2007).

Ethanol-induced glial activation is also associated with changes in the expression of inflammatory cytokines like IL-1alpha, TNF-alpha, IL-6. Notably, an increased expression of

the pro-inflammatory cytokine MCP-1 (monocyte chemoattractant protein 1) and microglial activation as well as astrogliosis have been demonstrated by postmortem analyses in alcoholic brains (Gonzalez & Salido, 2009; He and Crews, 2008).

Besides ethanol, its primary metabolite acetaldehyde is also able to modulate TNF-alpha and IL-6 secretion from cultured astrocytes. Both compounds showed a biphasic, hormetic effect on the IL-6 secretion after the acute as well as after the long-term exposure. It has been shown that long-term exposure to ethanol and acetaldehyde is more toxic than an acute exposure. The maximum stimulation was reached for 50 mM ethanol and 1 mM acetaldehyde after chronic exposure. In contrast, both compounds reduced the TNF-alpha secretion, where the effect was concentration dependent. Acetaldehyde showed to be more potent toxin than ethanol, and the ethanol's toxicity in the brain is at least partially due to its primary metabolite, acetaldehyde (Šarc et al., 2011).

Inflammation is primarily a protective response of the target organism to a noxis. On the other hand, excessive or long-lasting inflammation is often followed by degenerative processes. The stimulatory effect of ethanol and acetaldehyde on IL-6 secretion seems to be involved in both neuroregenerative and survival processes as well as in neurodegeneration. The obtained hormetic dose-response relationship indicates that higher concentrations and long-term exposure could lead in a neurodegenerative direction whereas low concentrations may act as neuroprotective. Unlike TNF-alpha, which is responsible for the induction of multiple pro-inflammatory genes, IL-6 often fails to induce these genes. Moreover, IL-6 can down-regulate the expression of TNF-alpha, which correlate with the data, where the first significant decrease in the TNF-alpha level was found at the highest level of IL-6 after a long-term exposure to ethanol (Šarc et al., 2011).

3.2.6 Ethanol and glial cell death

Apoptosis or programmed cell death is a form of cell death that occurs in multicellular organisms. Apoptosis is a tightly regulated process which engages multiple cell signalling pathways, and involves the altruistic suicide if individual cells in favour of the organism. This process is desirably during organism development and morphological changes, especially at the embryonic stage, as well as during the activation of the immune system. However, defects in apoptosis can result in cancer, autoimmune diseases and neurodegenerative disorders. Studies on Ca^{2+} signalling in apoptosis showed that ethanol potentiates apoptotic cell death induction by thapsigargin, caffeine, and the protonophore, which separately caused similar increases in Ca^{2+} levels, and also induces similar apoptotic death. These effects of ethanol are concentration and time-dependent (Hirata et al., 2006).

The effect of ethanol on the induction of apoptosis in astrocytes, and the formation of ceramide as apoptotic signal was investigated by Schatter et al. (2005). Ethanol induced nuclear fragmentation and DNA laddering, and inhibited phospholipase D-mediated formation of phosphatidic acid, which is a mitogenic lipid messenger. The authors concluded that ethanol induced glial apoptosis during brain development via formation of ceramide. Further studies have shown that astrocytes exposed to ethanol, undergo morphological changes associated with anoikis, a programmed cell death induced by loss of anchorage. Astrocytes depicted peripheral reorganisation of both, focal adhesions and actin-myosin system, cell contraction, membrane blebbing and chromatin condensation (Gonzalez & Salido, 2009).

Recently, it has been shown that ethanol affect intracellular trafficking. In fact, ethanol could interfere with nucleoplasmic transport in astrocytes, in such a way that ethanol induces a delay in both import and export of proteins to the nucleus (Marin et al., 2008).

Neurodegeneration, brain injury, and neuroinflammation are associated not only with increased cell apoptosis but also with the activation of a key proteolytic enzyme in this process, caspase-3. Immunohistochemical findings in mice, fed chronically with ethanol, reveal that inflammatory processes occur concomitantly with caspase-3 activation, suggesting an increase in programmed cell death. Moreover, it seems that the alcohol-induced toll-like receptor 4 (TLR4) response triggers both, inflammatory processes and apoptosis. A recent study suggests that the TLR4 response can also induce oxidative stress and neuronal injury, which agrees with a role of TLR4 in ethanol-induced brain damage and possibly in neurodegeneration (Alfonso-Loeches et al., 2010).

It has been shown that ethanol can activate or inhibit TLR4 by interacting with membrane lipids. Low/moderate ethanol concentrations (10–50 mM, in the range found in the blood of social drinkers and alcoholics) are capable of promoting translocation and clustering of TLR4 and a surface marker protein CD14, and the signalling molecules, like interleukin receptor-associated kinase (IRAK) and extracellular signal-regulated kinases (ERK), into the lipid rafts (Blanco et al., 2008; Fernandez-Lizarbe et al., 2008). Conversely, high ethanol concentrations or lipid raft-disrupting agents (streptolysin-*O* or saponin) inhibit ethanol-induced activation of the TLR4 signalling pathway (Blanco et al., 2008; Fernandez-Lizarbe et al., 2008). However, the molecular mechanism of ethanol interactions with TLR4 remains unknown.

4. Conclusion

Astrocytes are essential for maintaining a healthy and well-functioning brain. They face the synapses, send end-foot processes that enwrap the brain capillaries, and form an extensive network interconnected by gap junctions. They have the potential to impact on essentially all aspects of neuronal function through regulation of blood flow, provision of energy substrates, or by influencing synaptic function and plasticity. Moreover, astrocytes also protect and aid the brain in the functional recovery from injuries. The activation of glial cells in the CNS is the first defence mechanism against pathological abnormalities that occur in neurodegenerative diseases.

Ethanol has an extensive array of actions on astrocytes, transforming them into activated, potentially injurious cells with negative consequences to neuronal function and survival, and to brain function.

Therefore, it is a pivotal solution to seek molecular mechanisms and molecules that may inhibit or attenuate ethanol-induced neurotoxicity in astrocytes, thus offering an alternative strategy to prevent or treat neurodevelopmental disorders and mental retardation caused by ethanol.

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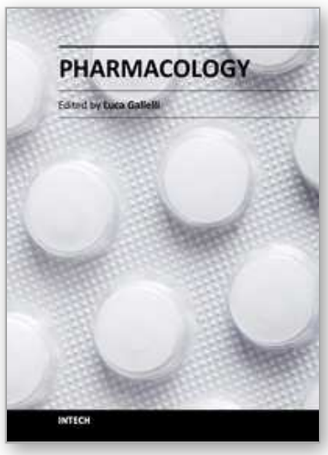

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The history of pharmacology travels together to history of scientific method and the latest frontiers of pharmacology open a new world in the search of drugs. New technologies and continuing progress in the field of pharmacology has also changed radically the way of designing a new drug. In fact, modern drug discovery is based on deep knowledge of the disease and of both cellular and molecular mechanisms involved in its development. The purpose of this book was to give a new idea from the beginning of the pharmacology, starting from pharmacodynamic and reaching the new field of pharmacogenetic and ethnopharmacology.

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