Chapter 14

Ethanol Consumption Affects Neuronal Function: Role of the Mitochondria

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Abstract

Ethanol is a licit drug consumed by a large part of the population, from adolescence to adulthood. High ethanol consumption is a public health problem due to its addictiveness and the risk it produces of developing other diseases, including cardiovascular, hepatic, and mental pathologies. Different patterns of ethanol consumption and its toxic effects in the brain have been reported. Current studies suggest to mitochondria, one of the principal mediators for ethanol neurotoxicity. In this chapter, we will review the effects of ethanol on neurons in different scenarios of ethanol consumption and its relation with mitochondrial function. Finally, we will propose a mechanism of ethanol toxicity in which the mitochondria are the main mediator and in which the mitochondrial alterations correlate with the severity of ethanol consumption. Thus, improving mitochondrial health of brain cells could be considered as a potential therapeutic target to treat ethanol-associated disorders.

Keywords: ethanol, mitochondria, oxidative stress, neurodegeneration

1. Introduction

Ethanol is the most licit addictive drug worldwide, and its consumption in excess is the third leading cause of death in the world [1]. Global ethanol consumption is 6.2 L of pure ethanol per person, with 15 years or older, which corresponds to 13.5 g of pure ethanol per day [2, 3]. Ethanol abuse is a health concern, which can lead to problems associated with alcoholism and increases the risk factor for other diseases such as cardiovascular disease, cirrhosis, dementia, and depression [4]. Socially, ethanol abuse can trigger other problems as well as violence, low productivity at work, traffic accidents, and crime [5].

Excessive ethanol use results in brain intoxication, leading to motor and behavior alterations, and eventually to death as a consequence of the depressive effects on the central nervous system (CNS) [6]. These effects result in simultaneous alterations in neuronal circuits including the prefrontal cortex, which controls behavior [7]; the cerebellum, which regulates movement and coordination [8]; the frontal lobe, which controls emotions [9]; the reticular activating system, which determines the sleep-wake cycle [10]; the hippocampus, which mediates learning and memory [8]; and the medulla, which controls vital functions [6]. Ethanol intoxication induces cellular damage and neuronal death [11]. The precise mechanism participating in ethanol toxicity in the brain is unknown. However, at the cellular level, ethanol impairs the neurotransmitters signaling [12]. Also, ethanol promotes reactive oxygen species (ROS) production [13] and activates inflammatory processes [14]. Altogether these events could be responsible for ethanol-induced damage in the brain.

Mitochondria are dynamic organelles, which regulate the production of ATP, redox balance, and calcium homeostasis in the neuron [15]. Interestingly, many effectors described in ethanol toxicity are directly or indirectly related to mitochondria. Mitochondria are the main source of ROS in the brain, and they are mainly affected by the oxidative damage induced by ethanol intoxication [16]. Likewise, dysfunctional mitochondria play a role in inducing proinflammatory events [17]. Finally, during synaptic process, ATP production and calcium buffering capacity produced by mitochondria are critical [18, 19]; therefore, mitochondrial injury may have severe consequences on neuronal communication.

In fact, evidence suggests that ethanol produces catastrophic changes in the mitochondria of organs such as liver [20] and heart [21], and over the last decade, many studies have reported the toxicity of ethanol to the brain's mitochondria [22, 23]. Briefly, ethanol increases ROS production [23], alters mitochondrial respiration [23, 24], impairs ATP production [22, 25], and eventually induces cell death by opening the mitochondrial permeability transition pore (mPTP) [26], observed both *in vitro* and *in vivo* [27].

In this chapter, we will discuss the effects of ethanol toxicity in the brain, focusing on the mitochondria. We will describe the specific ethanol-induced alterations to mitochondrial integrity, dynamics, and bioenergetics in different scenarios of ethanol exposure including:

1. Acute ethanol toxicity, corresponding to the consumption of high ethanol concentrations for a short period, spanning over hours, and even days [26].

2. Hangover, a common term to describe the physical effects following excessive ethanol consumption. Veisalgia is the uncommonly used medical name for this condition [25].

3. Chronic ethanol consumption, a condition where ethanol intake lasts 3 months or more. High concentrations of ethanol consumed over time can trigger ethanol use disorder (AUD), commonly called alcoholism [23].

4. Ethanol withdrawal, a condition observed in individuals who have consumed a high amount of ethanol for a prolonged period followed by cessation in ethanol intake. Common symptoms are anxiety and shakiness, seizures, and eventually death [22].

5. Binge drinking ethanol consumption characterized by a short period of heavy ethanol consumption followed by a period of abstinence and by intermittent ethanol intake [28].

Finally, we will discuss evidence suggesting that mitochondrial dysfunction is a potential mechanism by which ethanol promotes neurotoxicity, placing ethanol intoxication as a mitochondrial disease.

2. Patterns of ethanol consumption: mitochondrial alterations

2.1. Acute ethanol consumption

Acute ethanol consumption refers to a high ingestion of ethanol at a rate faster than that at which the body can metabolize it. Acute ethanol intoxication leads to brain injury, resulting in significant alterations of brain structure and function, and induces neuronal apoptosis and neurodegeneration in mouse, rat, and cellular models [11, 29–33].

Although several studies have tried to explain how acute ethanol administration induces brain injury, data on pathophysiology and underlying molecular and cellular mechanisms are still insufficient [34]. One of the possible theories was shown *in vitro*. Acute ethanol exposure induces neuronal apoptosis and changes to the neuronal structure, which could be related to the development of mature synapses and lead to a deficit in brain development [35]. Another alternative is that ethanol triggers inflammatory processes by activating toll-like receptor (TLR4) signaling and down-regulating autophagy pathways that trigger cell death [36]. However, and most importantly, oxidative stress appears to be the main mechanism for explaining brain injury mediated by ethanol. Oxidative stress is significantly increased following ethanol administration in several animal and cellular models [32, 37–39].

Rats exposed to ethanol through gavage administration showed an increase in inflammatory and oxidative stress markers in the brain 1 h post ethanol exposure [38]. Also, pre-treatment for 3 days with 150 mg/kg of antioxidant vitamin E decreased inflammation, an effect that is not observed with other antioxidants such as *N*-acetylcysteine (NAC) or selenium [38]. Similarly, ethanol-related increases in ROS generation are a prime factor in ethanol neurotoxicity. Primary cortical neurons treated with 2.5 mg/mL ethanol for 24 h elicit a rapid onset of oxidative stress, which resulted in mitochondria-dependent apoptotic cell death both in cultured fetal rat cortical neurons and during embryonic development [32, 39]. Also, ethanol downregulates protective cellular antioxidant content in this neuronal model, thus seriously disturbing the cellular redox state [32]. Indeed, pretreatment with NAC increased cellular glutathione and prevented apoptosis, suggesting that oxidative stress precedes a cascade of events mediated by mitochondria. Prevention of apoptosis with NAC antioxidant supports the role of oxidative stress in neuronal death [32].

The mitochondria appear to be a major target of ethanol toxicity in the brain. Some studies suggest that disturbances of the integrity of the mitochondrial membrane are essential for ethanol-induced cell death in mitochondria isolated from ethanol-exposed fetal brains [40].

For example, ethanol treatment for 24 h decreased ATP production and apparently impaired mitochondrial function [41]. Also, ethanol treatment reduced peroxisome proliferator-activated receptor-gamma coactivator 1alpha (PGC-1α) promoter activity and expression. PCG-1α [41] is a transcriptional coactivator of peroxisome proliferator-activated gamma receptors (PPARγ) that regulates energy metabolism and mitochondrial biogenesis in the brain [42, 43]. These ethanol-mediated changes in PCG-1α could be involved in mitochondrial dysfunction and oxidative damage. In contrast, the overexpression of PCG-1α has shown protective effects against ethanol-induced neuronal death and toxicity [41].

Several reports indicate that acute ethanol induces neuronal death [44]. Bax is a proapoptotic protein that when it is activated, it is translocated to the mitochondrial membrane. In primary neuronal cultures, Bax dimerizes with protein inhibitors of apoptosis, such as Bcl-2 and Bcl-xL, in response to ethanol exposure, leading to cell death [44]. Ethanol exposure disrupts the mitochondrial membrane potential, increases ROS production, and finally induced apoptosis in cerebellar granule cells [45–48]. Interestingly, Bax could also interact with the mPTP [49], a high-conductance mitochondrial channel, involved in the mitochondrial permeability to ions and small solutes [50–52]. These result in a reduction in mitochondrial potential [53], dysregulated calcium homeostasis [54], increased ROS formation [55], decreased ATP production [56], and eventually lead to neuronal death [53].

Interestingly, ethanol treatment did not induce the mPTP opening in the isolated mitochondria from mice brain, suggesting that ethanol does not affect mitochondrial health directly in neurons [26]. Further studies showed that ethanol administration transiently decreased the oxygen consumption rate of the mouse brain, an effect that disappeared 24 h after the ethanol treatment ceased [26]. However, this impairment of mitochondrial respiratory function could contribute to spatial learning and memory impairment observed in young mice [57], and to the deficit in the nociceptive response, showed in infant mice [26]. Interestingly, mice treatment with mPTP inhibitors, such as cyclosporine A and nortriptyline, before the first ethanol injection improved the behavior of ethanol-exposed animals [26], highlighting the importance of mPTP opening in acute ethanol intoxication, despite that probably is an indirect effect.

All these evidences indicate that acute ethanol exposure induces an increase in the production of ROS that finally could lead to mitochondrial dysfunction through the opening of mPTP.

2.2. Hangover

In humans, a hangover is a physiological state that involves an unpleasant next day sensation following an evening of excessive ethanol consumption [58]. Hangover begins when ethanol is absent in plasma and is characterized by symptoms such as headaches, nausea, diarrhea, fatigue, tremor, combined with decreased functional, cognitive, or visual-spatial skill performance [59, 60]. This state has grave implications for activities such as job performance and driving [61, 62]. In different animal models, the hangover has been studied to provide insights into the physiological and behavioral changes that occur in this period [63, 64]. Some of these studies have reported decreased locomotor activity in adult rats [65].

Acetaldehyde, an ethanol metabolite, is the leading cause of hangover [66]. Acetaldehyde causes steatohepatitis, a condition characterized by inflammation of the liver with fat accumulation in this organ, hepatic cirrhosis, and downregulation of aldehyde dehydrogenase 2 (ALD2) expression due to mitochondrial dysfunction [67]. In the brain, an association between hangover and mitochondrial dysfunction has been proposed [68–70].

During the hangover state, alterations like the impairment in motor performance have been associated with mitochondrial dysfunction [68]. In mice studies using the ethanol hangover onset protocol, animals showed impaired motor performance that could be the result of disturbed motor control [68]. Because motor performance is associated with the cerebellar function, the effects of the hangover were evaluated in cerebellum. Mitochondria isolated from the cerebellum of AHO mice revealed that at the onset of ethanol hangover, malate-glutamate- and succinate-supported oxygen uptake is increased, accompanied by the decreased activity of mitochondrial I–III and IV respiratory complexes and reduced mitochondrial membrane potential [69]. Additionally, the opening of mPTP was reported [69]. Furthermore, the activity of antioxidant enzymes was also differentially affected; superoxide dismutase (SOD) and the monoamine oxidase (MAO) enzyme showed increased activity [69], whereas both catalase (CAT) and glutathione peroxidase (GPx) had decreased activity [69]. In this same context, neuronal nitric oxide synthase (nNOS) expression was reduced [69], indicating a specific effect of ethanol against oxidative defenses in hangover.

In the brain cortex, isolated mitochondria had increased hydrogen peroxide (H_2O_2) levels in ethanol hangover conditions [68]. Moreover, mitochondrial activity of I–III, II–III, and IV respiratory complexes and the membrane potential are reduced in hangover [68]. Also, in mitochondria from the brain cortex, NO production and NOS expression were decreased [68], and synaptic mitochondrial function was significantly affected [68]. Finally, mitochondria from the brain cortex of hangover mice are more prone to suffer damage, due to the opening of mPTP with dramatic consequences to neural cell survival [68].

Interestingly, hangover provokes an imbalance in cellular redox homeostasis in isolated mitochondria in AHO mice brain cortex [70]. Decreased activity of both CAT and SOD enzymes accompanied by increased MAO activity was reported in mitochondria from both nonsynaptic extracts and synaptosome, whereas the GSH/GSSG ratio was decreased only in synaptosome mitochondria, with a reduction in both GPx and glutathione reductase (GR) and an increase in glutathione S-transferase (GST) [70]. Also, H_2O_2 levels were higher in both mitochondrial pools [70], and treatment with diphenyl (a MAO inhibitor) prevented this increase in AHO in both non synaptic and synaptic mitochondria [70]. Altogether, these results show that ethanol hangover produces an imbalance in mitochondrial redox state, indicated by an overproduction of ROS and a decrease of antioxidant agents [70]. This evidence is consistent with previous studies that described oxidative stress as a key element of the hangover syndrome and suggests that antioxidants could suppress the toxicity caused by ethanol [71]. In fact, the importance of the antioxidant imbalance was confirmed by the administration of natural products to treat hangover mainly in liver and brain [72].

In line with the anterior, melatonin pretreatment prevented the impairment of motor performance and mitochondrial function during ethanol hangover [73]. Melatonin is a hormone mostly secreted by the pineal gland during the night in mammals [74]. Melatonin and its metabolites are endogenous free-radical scavengers and antioxidants [75–77]. The effects of melatonin pretreatment in AHO were evaluated, and it was reported that melatonin improved motor coordination [73] and partially prevent the decrease in malate-glutamate-dependent oxygen uptake [73]. Interestingly, decreased mitochondrial potential and overproduction of H_2O_2 and NO induced by ethanol hangover also were prevented by melatonin [73].

In summary, ethanol hangover affects ROS production and reduces the antioxidant activity in different brain regions. These events could lead to mitochondrial membrane depolarization and dysfunction of the respiratory activity, reducing the ATP production and favoring the opening of mPTP. ATP deficiency in the cortex and cerebellar synapses could be the cause of deterioration of motor coordination. More studies are necessary to understand the real implications of mitochondria on brain function during hangover state.

2.3. Chronic ethanol consumption

The chronic ethanol consumption severely affects mental and physical health, including the development of alcohol dependence and neurological, cardiovascular, and hepatic diseases, among others [24, 78–80]. This type of ethanol intake is characterized by the difficulty of controlling the ingestion of ethanol. In this condition, the person is highly dependent and consumes it whenever possible, ingesting highly dangerous levels despite the negative consequences [81–83]. High ethanol consumption can lead to serious health, mental, and social problems [84, 85]. Among these alterations, the alcohol use disorder (AUD) [78, 86] is defined by the diagnostic and statistical manual of mental disorders (DSM-V), as a pattern of habitual drinking of excessive amounts of ethanol over a prolonged period [78].

Chronic ethanol consumption or AUD produces neurobiological changes that lead to an increase in unsafe behaviors, impulsivity, and anxiety [78]. Researchers have shown that regular consumption of alcoholic beverages damages the CNS, affecting cerebral activity, motor coordination, and behavior and developing neurodegeneration and neurocognitive deficits through exacerbated oxidative stress and excitotoxicity [24, 87, 88].

At the cellular level, ethanol induces activation of inflammatory and degenerative processes in the brain [88, 89]. Ethanol affects neuronal communication by altering the synthesis, release, and signaling of neurotransmitters [90], including glutamate, dopamine, and GABA [88, 90, 91]. Studies performed in human alcoholics have demonstrated that alterations in dopamine signaling induced by chronic ethanol consumption produce alterations in the prefrontal cortex, resulting in deficits in executive functions. Altogether, these affect the quality of life and also increase the probability of relapse [91, 92].

Recent studies in rat brain have shown that common alterations due to chronic ethanol consumption, such as neuropathies and neurocognitive deficits, are associated with mitochondrial dysfunction [23, 24, 85]. Ethanol is toxic, and the most vulnerable organs are the liver

and the brain [93]. Ethanol is metabolized to acetaldehyde by the enzyme alcohol dehydrogenase and cytochrome P450 2E1. Acetaldehyde is highly toxic to nerve cells as it promotes oxidative stress and apoptotic events [84, 93], and its generation and accumulation cause brain damage [23, 94]. In the brain, acetaldehyde is capable of interacting with neurotransmitters and generates an environment of oxidative stress, which affects cells, organelles, and biomolecules with the mitochondria being the first affected organelle [23, 94].

In rats, chronic ethanol consumption causes alterations in mitochondrial function in the brain, increasing the ROS and reactive nitrogen species (RNS) production through the activity of NADPH oxidase and NOS and altering the electron transport chain activity, which leads to decreased ATP production [23, 24, 84, 94, 95]. This leads to the peroxidation of fatty acids, resulting in increased oxidative damage [95]. The brain is the most vulnerable organ to oxidative injury, due to the high concentration of lipids in the neural membranes, especially the polyunsaturated fatty acids of the phospholipids [23, 94]. These can be converted into hyperperoxidized lipids, which are unstable and quickly decompose to form new free radicals [23, 94, 95]. Chronic ethanol exposure also results in decreased antioxidant capacity, by reducing SOD, CAT, GPx, and GR activities [90, 94, 95]. This altered redox state leads to alterations in Ca^{+2} homeostasis, increasing cytoplasmic Ca^{+2} levels and mitochondria uptake, which promotes ROS formation and destabilizes the electron transport chain [90].

Interestingly, mice treated chronically with ethanol for 7–8 weeks present an altered glucose metabolism and transport in the brain [24]. Ethanol causes hypoglycemia, limiting the availability of glucose for the ATP formation. Also, it produces alterations in the expression of carnitine palmitoyltransferase (cPT) 1 and 2, an internal and external membrane mitochondrial enzyme, respectively, that regulate β-oxidation and ATP production [24]. Furthermore, in a mouse model of chronic ethanol consumption treated with acetyl-L-carnitine (ALC), a cofactor of cPT1 and cPT2, mitochondria health was reestablished increasing the oxidative phosphorylation and improving ATP production in mitochondria isolated from the brain [24]. Complementary studies showed that chronic ethanol consumption decreased the expression of the mitochondrial complexes I and V (ATP synthase) [24]. Additionally, chronic ethanol intake significantly increased the release of cytochrome-c [24], suggesting that ethanol affects the physical properties and functions of the mitochondria.

Excessive ethanol consumption also represents a risk during the pregnancy, which can result in fetal alcohol syndrome (FAS) [96]. Brain of prenatal ethanol-exposed fetuses showed increased apoptosis, possibly as a consequence of impaired function of mitochondria [96], increased ROS [97–99], and reduced activity of electron respiratory chain, which lead to decreased ATP production [96]. This could contribute to the malformations and developmental abnormalities observed previously in the fetuses with FAS [100].

Altogether, these studies indicate that chronic ethanol intake affects the mitochondrial structure and function, promoting the ROS production, altering substrate transport through the mitochondrial membrane, expression of the electron transport chain complex I and V, and ATP synthesis [24]. In conclusion, excessive ethanol consumption affects cognitive function and damages brain cells by a mechanism that involves mitochondrial dysfunction.

2.4. Ethanol withdrawal

When ethanol consumption stops or is abruptly reduced after a prolonged period of elevated ingestion, a state of physical ethanol dependence characterized by the appearance of neurological manifestations is acquired [87, 101]. The symptoms include tremors, hallucinations, arrhythmias, seizures, and later delirium tremens [86, 87, 102]. The severity of symptoms is determined mainly by the degree of ethanol intake, time of ethanol consumption, and the previous history of ethanol withdrawal [101].

As it was described in the previous section, ethanol produces structural and molecular changes in the brain. It exerts a potent effect on synaptic plasticity and dendritic spine formation in specific brain regions, providing a neuroanatomical substrate for the pathophysiology of alcoholism [82]. Dendritic spines are specialized protrusions on neuronal dendrites, considered the postsynaptic region of the excitatory synapses [103]. These structural changes induced by ethanol could affect the organization and function of the neural network and could explain the behavioral and cognitive alterations in alcoholic individuals [82]. Several studies have shown that abstinence from chronic ethanol consumption, and especially repeated withdrawal, causes an abrupt decline in the number of dendritic spines in the amygdala and other important regions of the brain such as the prefrontal cortex, accompanied by an increase in behaviors similar to anxiety [82, 92].

In synapses, the synaptic mitochondria play a fundamental role in providing the ATP necessary for diverse functions including vesicle exocytosis and neurotransmitter release, restoration of membrane potential through active transport, and recycling of receptors and neurotransmitters [18, 104]. The alterations described in the mitochondria of the cerebellum are of particular interest because ethanol intoxication triggers altered movement of lower limbs and the lack of voluntary coordination of muscles [105].

Ethanol withdrawal generates an excitatory effect in neurons from the cerebellum [106]. Ethanol promotes the activation of inhibitory GABA neurotransmission [107]; however, when ethanol concentrations are completely reduced, the excitatory transmission is overactivated [108]. Excitatory activation is mediated by the overly release of glutamate, which in turn activates its receptors such as α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) and *N*-methyl-D-aspartate (NMDA) [108]. The binding of glutamate to its receptors in neurons leads to a drastic increase of Ca^{2+} entry, inducing excitotoxicity that eventually leads to neuronal death [109]. In fact, the Ca^{2+}concentrations in a synaptosomal membrane fraction obtained from rat cerebellum were greater during ethanol withdrawal episodes compared with the period of ethanol consumption [110, 111].

The excessive uptake of Ca^{2+} by the neurons results in an increased in the Ca^{2+} uptake by the mitochondria, which in turn leads to overproduction of ROS and a decreased antioxidant response [22]. In a rat model of ethanol withdrawal, lipid peroxidation and mitochondrial protein oxidation is increased in the cortex, hippocampus and more drastically in the cerebellum [112]. During ethanol withdrawal, ATP production also is decreased in neurons from the cerebellum [113]; resulting in reduced cellular energy in this brain region. The high Ca^{2+} concentrations in the mitochondria can ultimately lead to prolonged

opening of mPTP [114, 115]. Therefore, ethanol withdrawal toxicity is probably mediated by mitochondrial dysfunction, which implicates an imbalance in the redox state, decreased ATP production, and prolonged mPTP opening due to high Ca^{+2} overload in the mitochondria.

2.5. Binge drinking

Binge drinking is a recurrent pattern of ethanol consumption, characterized by a short period of heavy ethanol consumption followed by a period of abstinence, which continue with intermittent high ethanol consumption [116]. Binge drinking is common during adolescence [117] and relatively little is known about its effects and potential toxicity mechanisms. Binge drinking is described as the intake of four drinks in women and five drinks in men in a period less than 2 h [118]. It is of great interest to understand the damaging effects of binge drinking in the brain [119], mainly because the adolescent brain is more sensitive to ethanol toxicity than that of the adult brain [120, 121].

Rats trained to self-administration of binge-like ethanol is associated with the development of depression-like symptoms [122], which could be a result of decreased survival and differentiation of neural progenitor cells in the hippocampus, finally resulting in decreased adult neurogenesis [122]. Additionally, ethanol binge drinking induces neuroinflammation, such as indicated by the activation of innate immune receptors TLRs and by an increase in the expression of the inflammatory cytokines TNFα and IL-1β, and demyelination suggested by significantly reducing the levels of the myelin proteins myelin basic protein (MBP) and myelin oligodendrocyte glycoprotein (MOG) in the prefrontal cortex [123]. Also, adolescent binge-like ethanol consumption produced alterations to cognitive processes associated with the prefrontal cortex in adulthood [124]. Changes in executive functions, reduced anxiety, and disinhibition in the exploratory behavior were observed after ethanol exposure [124]. In addition, increase in the resistance to the extinction of ethanol-seeking behavior in adult rats trained to self-administer ethanol [124]. Studies in rats that simulate the adolescent binge drinking pattern also reported memory and motor alterations [125].

In other murine model of binge drinking, rats pups were exposed to ethanol consumption at postnatal day 25 (PND25) (3.0 g/kg), during 2 consecutive days, with gaps of 2 days without injections, during 2 weeks [28]. Then, 24 h or 20 days after the final ethanol administration, the animals were used to evaluate cognitive and motor functions. The results revealed that binge drinking treatment impaired conditional discrimination learning, motor learning, and recognition memory during adolescence and in the adult stage [28]. Additionally, binge drinking ethanol treatment increases cyclooxygenase (COX-2) and inducible NOS (iNOS) levels and cell death in the neocortex, hippocampus, and cerebellum 24 h after administration. These effects, as well as the cognitive alterations, were prevented by the administration of indomethacin, a COX-2 inhibitor [28]. Interestingly, new reports have showed that mitochondrial dysfunction promotes brain inflammatory response, which in turn could induce an additional mitochondrial damage in a positive feedback loop induced of toxicity induced by ethanol, which could be reverted by the indomethacin treatment [17].

Mitochondrial alterations associated with binge alcohol drinking has not being described. For example, binge ethanol exposure in early postnatal stage induced defects in insulin and IGF signaling, resulting in impaired motor abilities [126]. Most important, deficiencies in insulin/IGF-1 signaling are associated to oxidative stress, DNA injury, loss of mitochondrial function, and apoptosis [126]. In other studies, rats submitted to binge alcohol drinking protocol showed an increase in the ventricular volume of the brain; reduced levels of N-acetyl aspartate (NAA) and creatine and increased choline-containing compound in the dorsal hippocampus, effects that were recovered after 7 days of alcohol treatment [127]. These metabolic changes could suggest transient deficiencies of mitochondrial NAA production, resulting finally in an impaired energy production of the brain immediately after binge ethanol consumption [127].

Currently, the most complete study associated to mitochondrial alterations corresponds to our findings [128]. In our study, adolescent rats PND25, were exposed to binge-like ethanol consumption using the same protocol described previously [128]. The rats were euthanized at 1, 3, or 7 weeks after treatment. Our results show that binge ethanol pretreatment (BEP) triggers alterations in hippocampal cell structure and function [128]. We found increase in oxidized proteins at 1-week posttreatment, which was subsequently restored at 3 and 7 weeks post BEP [128]. Additionally, proteins participating in the regulation of mitochondrial dynamics were affected. Mitochondrial dynamics involves fission and fusion events [129]. Fusion is mediated by the dynamin-related GTPases mitofusins (Mfn1 and Mfn2) and optic atrophy 1 (OPA1), which induce fusion of mitochondrial membranes [130]. Fission is performed by dynamin-related protein 1 (Drp1), which is recruited by the Fis1 protein [129] to the mitochondrial membrane to constrict mitochondria inducing its division [130]. BEP altered Drp1, Fis1, Mfn1, Mfn2, and Opa1 proteins levels [128], suggesting that binge-like ethanol consumption favors an pro-fission state at 1–3 weeks post BEP, and imbalance between fusion and fission proteins was restored but animals with 7 weeks after ethanol treatment showed reduced both mitochondrial fusion and fission processes compared to saline treated animals [128].

Interestingly, a decrease in ATP production was also reported in our study at 3 and 7 weeks post BEP [128], indicating a specific bioenergetics mitochondrial failure induced by binge ethanol exposure that persists over time. Finally, we observed a delayed increase in the expression of inflammatory markers such as NF-κB, Iba1, and GFAP, 7 weeks post BEP [128], suggesting a late inflammatory response due to adolescent ethanol consumption. Despite the damage present in the hippocampus of BEP rats, we detected the activation of a mechanism that could possibly protect the brain. These mechanisms include decreased expression of proteins that are key to mPTP formation, such as voltage-dependent anion channel (VDAC) and cyclophilin D (Cyp-D) [128], and the increased levels of nuclear factor erythroid-2 related factor 2 (Nrf2) [128], a transcription factor that is activated under stress conditions regulating the expression of antioxidant, anti-inflammatory, and detoxification enzymes that prevent mitochondrial impairment [131]. The increased Nrf2 levels were mainly observed at 3 and 7 weeks [128] suggesting that this signaling pathway could be involved in restoring redox balance.

In summary, our and others studies about adolescent ethanol consumption indicate that adolescent binge-like ethanol exposure results in severe structural and functional alterations in the brain that persist until the adult stage, affecting mainly to mitochondria. The mitochondria plays a key role in binge ethanol toxicity, since it alters the redox balance of the cell and reduces ATP availability to vital functions such as synaptic communication.

3. Mitochondrial impairment as a possible mechanism of ethanol toxicity in the brain

In the previous sections, we described the effects of ethanol on neuronal cells, focusing our attention on alterations to the mitochondria. In all patterns of ethanol consumption described above, we reported changes associated with mitochondrial injury [11, 27, 132], therefore strongly suggesting that mitochondria is an important mediator of ethanol neurotoxicity and could be considered as a potential therapeutic approaches for treating ethanol-associated disorders. In this section, we will summarize the effects of ethanol linked to mitochondrial function, and finally, we propose a mechanism in which mitochondrial impairment plays a central role in the neurotoxicity induced by ethanol.

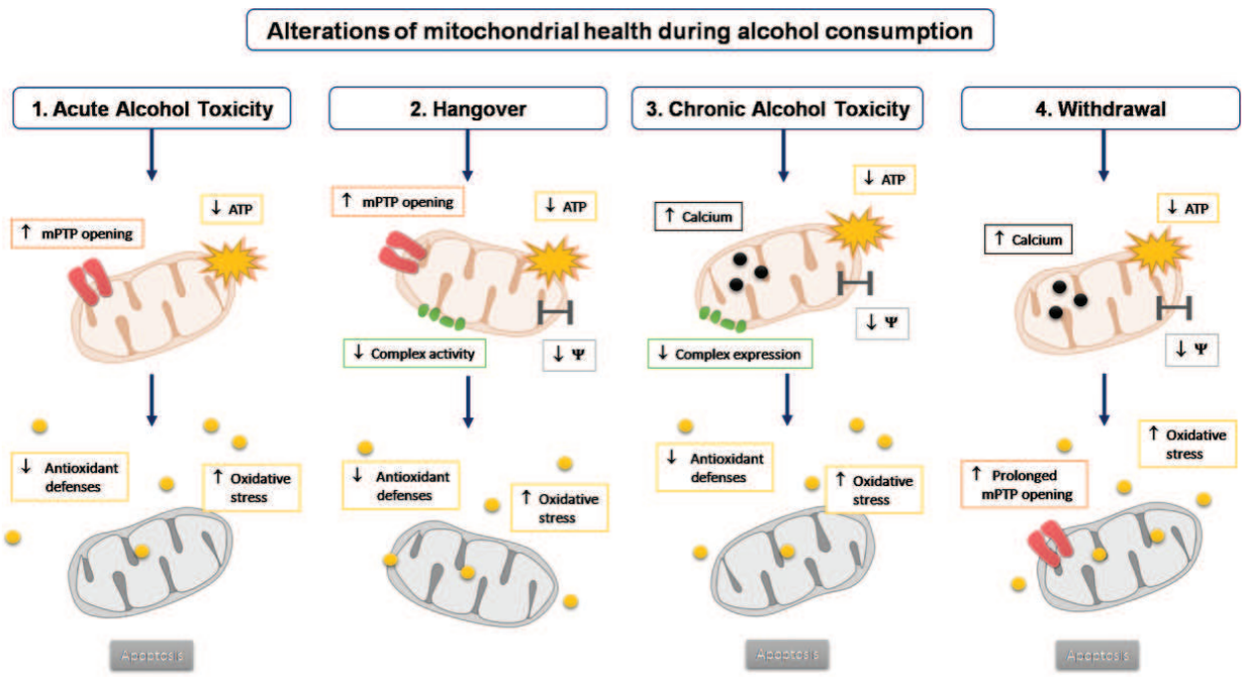

Figure 1. Alterations of mitochondrial health during ethanol consumption. A representative scheme is summarizing the mitochondrial alterations described in the different patterns of ethanol consumption. The severity of mitochondrial dysfunction is associated with the amount and time of ethanol exposure, starting with an imbalance in redox response, accompanied by decreased ATP production and the opening of the mPTP (1. Acute ethanol toxicity). Additionally, decreased activity of the electron transporter chain and the loss of mitochondrial membrane potential are observed (2. Hangover); following of high Ca^{+2} concentrations (3. Chronic ethanol toxicity) that lead finally to prolonged opening of mPTP that eventually triggers neuronal death (4. Withdrawal).

Figure 1 summarizes the mitochondrial alterations described after the different patterns of ethanol consumption (**Figure 1**). Acute ethanol exposure triggers mitochondrial toxicity, increasing ROS production and decreasing antioxidant defenses [38]. In turn, this leads to reduced ATP production [41] and finally to the opening of mPTP [26]. All these events could eventually lead to apoptotic neuronal death [32, 39] (**Figure 1.1**). After ethanol consumption, a hangover condition can be produced [58]. In this state, the mitochondrial alterations described in acute ethanol conditions are also accompanied by a period of reduced activity of the electron respiratory chain complexes [69] and a loss of mitochondrial membrane potential [69] that suggests a major state of mitochondrial dysfunction (**Figure 1.2**).

When ethanol consumption implicates the ingestion of a high amount of ethanol on repeated occasions and cannot be controlled, this is considered a pattern of chronic ethanol consumption [81–83]. This condition is pathological, and therefore the mitochondrial alterations are also more complex. In addition to redox imbalance [23, 94], deficiency in ATP generation and a loss of mitochondrial membrane potential can be detected by a reduction in the expression of respiratory complexes and increased levels of mitochondrial calcium [23, 94, 95]. Altogether, these events lead to severe loss of mitochondrial function (**Figure 1.3**). Chronic ethanol consumption implicates the development of addictive behaviors; therefore, in the absence of ethanol, those affected present ethanol withdrawal symptoms [87, 101]. In this state, the mitochondrial effects already described in chronic consumption are

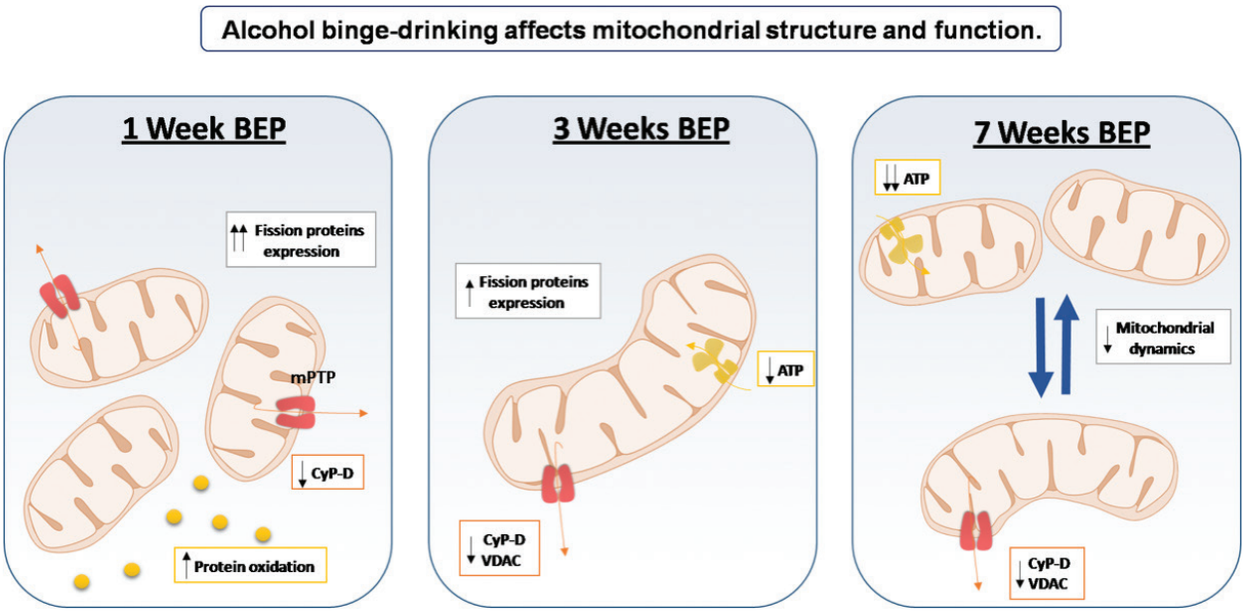

Figure 2. Ethanol binge-drinking affects mitochondrial structure and function. Binge-like ethanol consumption during the adolescence induces changes in the structure and function of the mitochondria that persist on time until adulthood. One week after binge ethanol pretreatment (BEP), rat hippocampus has increased expression of fission proteins and protein oxidation, accompanied by reduced expression of Cyp-D. Three weeks after BEP, addition is possibly observed decreased ATP production and reduced expression of VDAC. Finally, at adulthood (7 weeks post BEP), the levels of both fission and fusion proteins suggest decreased mitochondrial dynamics, and the deficiency in ATP production is more severe.

aggravated, mainly by the high amount of Ca^{+2} that enters to the mitochondria [110, 111], which leads to a prolonged opening of mPTP [114, 115] and neuronal death [22] (**Figure 1.4**). In summary, the mitochondrion plays a central role in all these patterns of ethanol consumption, and its alterations gradually increase according to the amount and time of ethanol exposure.

Binge drinking ethanol is associated with the adolescent population [116]. Diverse molecular alterations have been reported; however, the cellular mechanism involved in this process are unknown [11]. We recently indicated that adolescent binge ethanol consumption triggers cellular damage by a mechanism that probably involves the mitochondria [128]. Also, the mitochondrial alterations persist over time until adulthood [128]. **Figure 2** summarizes the mitochondrial changes induced by adolescent binge ethanol exposure. One week after BEP, the mitochondria showed increased protein oxidation, reduced expression of Cyp-D, and increased expression of proteins involved in mitochondrial fission, suggesting a mitochondrial pro-fission state [128]. Then, 3 weeks after BEP challenging the mitochondrial pro-fission state is less severe, indicated by a lower reduction in the expression of fission proteins; however, a decrease in VDAC protein, and a significant reduction of ATP, is also observed [128]. Finally, during adulthood, the balance in the fission/fusion state is restored, but the dynamics are decreased compared with the control condition. Also, the ATP deficiency persists and becomes even more drastic [128]. Nevertheless, the reduced expression of VDAC and Cyp-D suggests the activation of a protective mechanism that could prevent the opening of mPTP [128]. Altogether, these alterations indicate that mitochondria have an important role in the binge ethanol toxicity in the hippocampus of adolescent rats.

4. Future perspectives

We propose that the mitochondrion is the main mediator of ethanol neurotoxicity where mitochondrial alterations reveal the severity of ethanol toxicity. The initial effect of ethanol exposure implicates an imbalance in the cellular redox state, followed by changes in the respiratory complexes from the electron transport chain that leads to the reduction in ATP production and the opening of mPTP. Persistent ethanol consumption also induced the loss of mitochondrial membrane potential and increased Ca^{+2} entries into the mitochondria that provoke the prolonged opening of mPTP and finally promote neurodegeneration. Interestingly, these mitochondrial alterations ethanol-associated may could occur mainly in glial cells, inducing inflammation and interfering with the glial-neuronal communication in specific brain areas [119]. The hippotalamus, important to ethanol dependence, and the hippocampus, associated to learning and memory, are particularly vulnerable; possibly due to the downregulation of melanocortin system induced by ethanol [119]. Therefore, the description of all these events highlights the importance of maintaining the function of the mitochondria to prevent the harmful effects of ethanol consumption and propose a new potential treatment for the pathological condition related to ethanol use and abuse.

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