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Inhibition of cholinergic muscarinic signaling by ethanol: Potential mechanism of developmental neurotoxicity and biological plausibility for the beneficial effects of choline supplementation

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Abstract

Central nervous system dysfunctions are among the most significant effects of *in utero* exposure to ethanol. Ethanol has been shown to affect neurons and glial cells, causing cell loss and impaired cell migration and maturation. Multiple mechanisms have been suggested to underlie the effects of ethanol, including interference with growth factors, cytokines, cell adhesion molecules and neurotransmitters. Here, we propose that a relevant mechanism of ethanol's developmental neurotoxicity may be its ability to inhibit the actions of acetylcholine in the developing nervous system mediated by activation of cholinergic muscarinic receptors. Acetylcholine has been shown to induce proliferation of astrocytes, to protect neurons against apoptotic cell death, and to foster astrocyte-neuronal interactions, thereby increasing neuritogenesis. By interfering with muscarinic receptor signal transduction pathways (mostly at the level of phospholipase D), ethanol inhibits all these effects of acetylcholine in the developing brain. Such action of ethanol may be responsible, at least in part, for some manifestations of developmental neurotoxicity, such as microencephaly, neuronal cell death and impaired neuronal differentiation. Among potential therapeutic interventions for fetal alcohol spectrum disorders, choline supplementation appears to be one of the most promising. The cholinergic hypothesis of ethanol's developmental neurotoxicity provides biological plausibility for the beneficial effects of choline. Indeed, by "potentiating" the cholinergic system during development (through increased synthesis of acetylcholine and phosphatidylcholine, and increased phospholipase D activity), choline would antagonize at least some of the deleterious effects of ethanol.

In utero exposure to ethanol can cause fetal alcohol spectrum disorders (FASD), whose most severe manifestation is fetal alcohol syndrome (FAS), characterized by growth retardation, facial dysmorphology, and neurodevelopmental abnormalities leading to long-lasting cognitive and behavioral deficits (Jones, 2011; Riley, Infante, & Warren, 2011; Streissguth, Landesman-Dwyer, Martin, & Smith, 1980). Animal studies and human observations indicate that exposure to ethanol during the brain growth spurt causes microencephaly, which is present in more than 80% of FAS children (Samson, 1986). A large number of studies have also shown that alcohol abuse during pregnancy causes loss of certain neurons (Cui, Terwai, Schneider, & Rubin, 1997; Ikonomidou et al., 2000), alterations in the elongation of axons and neurites (Miller, 1997; Miller, Astley, & Clarren 1999), and modifications of glial cells, in particular astrocytes (Costa, Yagle, Vitalone, & Guizzetti, 2004; Guerri, Pascual, & Renau-Piqueras, 2001; Miller & Robertson, 2003; Riley et al., 1995). For the past several years our laboratory has been pursuing the hypothesis that the signal transduction pathways activated by the neurotransmitter acetylcholine may represent a relevant target for the developmental neurotoxicity of ethanol. This review will briefly summarize the investigations carried out over the years to address this hypothesis, from the initial serendipitous observations to the mechanism-driven studies which have also uncovered novel actions of acetylcholine in the developing nervous system.

The cholinergic system and brain development

There is substantial evidence that acetylcholine may influence various aspects of brain development (Abreu-Villaca, Filgueiras, & Manhaes, 2011; Buznikov, 1984; Costa, 1993, 1996, 1998; Costa & Guizzetti, 1999; Lauder & Schambra, 1999; Weiss, Maness, & Lauder, 1998). Acetylcholine is believed to have non-transmitter effects during development, as it can regulate morphogenic cell movements during gastrulation, glial cell proliferation, and neuronal differentiation and survival (Lauder & Schambra, 1999; Lipton & Kater, 1989; Nguyen et al., 2001).

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Developing neurons may fire action potentials and trigger acetylcholine secretion from the axonal growth cone while the axon is still growing, and neuron-released acetylcholine may contribute to further axonal growth and formation of synaptic contacts (Yao, Rusch, Poo, & Wu, 2000). Furthermore, astrocytes may themselves release acetylcholine, which may act in an autocrine fashion (Wessler et al., 1997). Components of the cholinergic system, such as choline acetyltransferase and acetylcholine receptors, are present early in development, long before the appearance of synapses, and increase slowly with age (Costa, 1993; Balduini, Murphy, & Costa, 1987; Ladinsky, Consolo, Peri, & Garattini, 1972). Levels of choline, the rate-limiting factor in the synthesis of acetylcholine, are high in the neonatal rat brain (Ladinsky et al., 1972); this fact, together with the low activity of acetylcholinesterase, may explain why acetylcholine levels are high (80–90% of adult values) early postnatally (Coyle & Yamamura, 1976).

There are five distinct subtypes of muscarinic receptors, M1–M5; while the M2 and M4 subtypes are negatively coupled to adenylate cyclase, the other subtypes stimulate phospholipid hydrolysis (Hosey, 1992). In an earlier study we found that, unlike other neurotransmitter receptor systems, muscarinic receptor-stimulated phosphoinositide metabolism was much higher in brains from neonatal rats than in adult rats, despite a lower expression of muscarinic receptors (Balduini et al., 1987; Heacock, Fisher, & Agranoff, 1987). Muscarinic receptor density increases postnatally, reaching a plateau at about four weeks of age (Balduini et al., 1987). Phosphatidylcholine hydrolysis, due to phospholipase D (PLD) activation, is also enhanced in the neonatal rat brain (Costa, Balduini, & Reno, 1995; Zhao, Berse, Holler, Cermak, & Blusztajn, 1998). Though the exact molecular mechanism(s) of such enhanced muscarinic receptor stimulation of phospholipid metabolism are still not fully understood, similar effects are also present in brains from neonatal mice and human fetuses (Larocca, Rodriguez-Gain, Rashbaum, Weidenheim, & Lyman, 1994; Tan & Costa, 1995). These initial serendipitous findings, and the fact that muscarinic receptor formation precedes the development of pre-synaptic markers, suggested that muscarinic receptors may play a role in the regulation of neurocytomorphogenesis, neuritogenesis, synaptogenesis and glial cell proliferation (Costa, 1993, 1996, 1998). These considerations led to our initial hypothesis that the muscarinic receptor signal transduction system may represent a possible target for the developmental neurotoxicity of ethanol.

Ethanol, muscarinic receptors signal transduction and microencephaly

Initial *in vivo* studies showed that administration of ethanol from postnatal days (PND) 4–10, i.e., during the brain growth spurt (Dobbing & Sands, 1979), caused microencephaly and inhibited muscarinic receptor-stimulated phosphoinositide metabolism in cerebral cortex and hippocampus (Balduini & Costa, 1989). This exposure to ethanol did not alter the expression of muscarinic receptors (Balduini & Costa, 1989). Thus, an early and short-lasting effect of ethanol on muscarinic signaling was associated with modification of the nervous system (microencephaly), which is a long-lasting event. Further studies investigating the dose-and time-dependence of these effects showed that microencephaly and reduced phosphoinositide metabolism were only observed when ethanol was given on PND 6–8 or 8–10, but not earlier or later, despite similar blood ethanol concentrations (BAC; ~50 mM) (Balduini, Reno, Costa, & Cattabeni, 1994). Furthermore, only doses capable of inhibiting phosphoinositide hydrolysis would cause microencephaly (Reno, Tan, Balduini, & Costa, 1994). Additional studies carried out *in vitro* in rat brain slices showed that the sensitivity of muscarinic receptor-stimulated phosphoinositide hydrolysis to ethanol inhibition was maximal at PND 7–10; that it was more pronounced in cortex and hippocampus; that it was relatively specific for acetylcholine and muscarinic receptors compared to other neurotransmitters (e.g., norepinephrine, 5-hydroxytryptamine); and that it was due to ethanol itself, rather than to its conversion to acetaldehyde (Balduini, Candura, Manzo, Cattabeni, & Costa, 1991; Balduini & Costa, 1990; Tan, Castoldi, Manzo, & Costa, 1993). All together, these studies provided strong evidence of an association between the ability of ethanol to cause developmental neurotoxicity (microencephaly) and inhibition of muscarinic receptor signal transduction. However, a more meaningful causal relationship had yet to be shown.

Ethanol inhibits the mitogenic action of acetylcholine in glial cells

Prompted by an important paper by Ashkenazi, Ramachandran, and Capon (1989), we developed the hypothesis that acetylcholine may act as a mitogen in glial cells. This effect may indeed be relevant in terms of brain development, as proliferation of astrocytes is a major event occurring during the bran growth spurt, a period when muscarinic signaling is particularly enhanced (Balduini et al., 1987; Costa et al., 1995). Ethanol, by interfering with muscarinic receptor signaling, would inhibit the mitogenic action of acetylcholine in glial cells, and this would explain, at least in part, microencephaly seen upon ethanol exposure during the brain growth spurt (Balduini & Costa, 1989; Samson, 1986). Though ethanol-induced apoptotic cell death of neurons (Ikonomidou et al., 2000) would also contribute to a reduction in brain size, the very high glia/neuron ratio suggested that a decrease in astrocyte proliferation, leading to a reduced number of astrocytes, would play a most relevant role in ethanol-induced microencephaly (Costa & Guizzetti, 1999).

Initial experiments indicated that activation of M3 muscarinic receptors on astroglial cells (primary rat and human astrocytes, and a human astrocytoma cell line) increased DNA synthesis, and that ethanol (25–75 mM) inhibited this effect (Guizzetti & Costa, 1996; Guizzetti, Costa, Peters, & Costa, 1996; Guizzetti, Moeller, & Costa, 2003) (Table 1). Further studies were aimed at investigating the signaling pathway(s) involved in the mitogenic action of acetylcholine in astroglial cells, and at identifying the molecular target(s) of ethanol's antiproliferative action. Three major signaling pathways activated by M3 muscarinic receptors were found to be

involved in astroglial cell proliferation. A first pathway involves activation of phospholipase C, with a subsequent increase in intracellular calcium levels, and activation of novel PKC ε and of mitogen activated protein kinase (MAPK). This pathway appears to play a (minor) role in the mitogenic action of acetylcholine, and is affected only by relatively high (>100 mM) concentrations of ethanol (Catlin, Guizzetti, & Costa, 2000; Guizzetti & Costa, 2000a; Yagle, Lu, Guizzetti, Moeller, & Costa, 2001). A second pathway involves the sequential activation of PLD, formation of phosphatidic acid, activation of atypical PKC ξ, and activation of p70S6 kinase and of Nf-kB. Ethanol was found to target this pathway, by specifically inhibiting PLD (and, as a consequence, all downstream events) at concentrations of 25–50 mM (Guizzetti & Costa, 2000b, 2002; Guizzetti, Bordi et al., 2003; Guizzetti, Thompson, Kim, VanDeMark, & Costa, 2004). Similar findings were also obtained following *in vivo* administration of ethanol (Tsuji, Fattori, Abe, Costa, & Kobayashi, 2008; Tsuji, Guizzetti, & Costa, 2003). Table 2 shows the effects of *in vivo* ethanol administration (PND 4–7) on brain weight and levels of phospho-p70S6 kinase. A third pathway involves activation of phosphoinositide-3 kinase and subsequent activation of PKC ξ; this pathway was inhibited only by high concentrations of ethanol (Guizzetti & Costa, 2001, 2002). Inhibition of astrocyte proliferation by ethanol is consistent with the reduced number of glial cells found following *in vivo* ethanol exposure (Miller & Potempa, 1990; Perez-Torrero et al., 1997; Riley et al., 1995), and may contribute to ethanol-induced microencephaly (Samson, 1986). It should also be noted that activation of muscarinic receptors increases DNA synthesis in oligodendrocytes and in progenitor cells, whose proliferation may be affected by ethanol (Costa et al., 2001; Crews et al., 2003).

Ethanol impairs the trophic action of acetylcholine

Acetylcholine has also been shown to have a trophic effect in neurons by activating muscarinic receptors. Cerebellar granule cells maintained in nondepolarizing conditions undergo apoptosis, and this is antagonized by activation of muscarinic receptors (Yan, Lin, Irwin, & Paul, 1995). Ethanol has been shown to antagonize the anti-apoptotic action of acetylcholine in cerebellar granule neurons (Castoldi, Barni, Randine, Costa, & Manzo, 1998). Given that activation of muscarinic M3 receptors in cerebellar neurons and in other cells antagonizes apoptosis induced by a variety of stimuli (Giordano et al., 2009), inhibition of such action by ethanol during brain development may contribute to enhanced neuronal apoptosis, which is also elicited by inhibition of other trophic signals (Castoldi et al., 1998; Ikonomidou et al., 2000). The latter, particularly those mediated by activation of the NMDA (N-methyl D-aspartate) receptors, may be the most relevant (Ikonomidou et al., 2000).

Ethanol inhibits astrocyte-neuron interactions involved in neuritogenesis

Astrocytes are known to exert a profound effect on neuronal development, as they provide trophic support essential for neuronal survival and are involved in neuronal migration, axon and dendritic outgrowth, and synaptogenesis (Barres, 2008; Seth & Koul, 2008; Ullian, Christopherson, & Barres, 2004; Volterra & Meldolesi, 2005). Astrocytes express and release molecules that promote (e.g., fibronectin) or inhibit (e.g., neurocan) neurite outgrowth, thus playing an important role during brain development and regeneration after lesions (Pagani et al., 1991; Rauch, Feng, & Zhou, 2001). We hypothesized that activation of muscarinic receptors in astrocytes may increase the expression and release of permissive factors leading to neuronal differentiation, most notably neuritogenesis, and that by inhibiting muscarinic receptor signal transduction, ethanol would inhibit these effects. Rat cortical or hippocampal astrocytes incubated with carbachol and then co-cultured for 24 hours with rat hippocampal neurons stimulated neuritogenesis, as assessed morphologically (Guizzetti, Moore, Giordano, & Costa, 2008); length of the longest neurite (identified as the axon by Tau-1 staining) was increased by 2–3-fold, while length of minor neurites (identified by MAP-2 staining) and number of neurites per cell were increased by 2-fold.

The effect of carbachol was due to the activation of M3 muscarinic receptors in astrocytes, was not related to an increased number of astrocytes (as, under these

Table 1

Effect of ethanol on carbachol-induced proliferation of astroglial cells

	Carbachol	Ethanol (mM)			
Cell Type	**(1mM)**	**10**	**25**	**50**	**100**
Rat cortical astrocytes	100	50	30	18	3
Human fetal astrocytes	100	70	45	10	nd
1321N1 human astrocytoma	100	40	25	18	2

Note. Proliferation of astroglial cells was measured by ^{3}H-thymidine incorporation into DNA. Cells were treated for 24 h with 1 mM carbachol in the absence or presence of ethanol. Results are expressed as percentage of carbachol's effects. All data with ethanol are significantly different from carbachol. Stimulation of proliferation by carbachol (1 mM) ranged from 3- to 9-fold, depending on cell type. No cytotoxicity was observed in any experimental condition. Data are adapted from Guizzetti & Costa (1996) and Guizzetti, Bordi et al. (2003a).

Table 2

Effect of ethanol on brain weight and phospho-p70S6 kinase

		Ethanol (mg/kg)		
End Point	**Control**	**2**	**4**	**6**
Body weight (g)	16.4	16.9	16.2	12.9*
Brain weight (mg)	741.0	756.0	706.0*	627.0*
BAC (mM)	--	25.0	64.0	92.0
Phosho p70S6K (%)	100.0	50.0*	20.0*	5.0*

Note. Male rats were given ethanol or an equicaloric sucrose solution by gavage from PND 4 to 7 and were sacrificed 45 min after the last treatment. *Significantly different from control, $p < 0.05$. Data are adapted from Tsuji et al. (2003).

Table 3

Effect of ethanol on astrocyte-mediated neuritogenesis

		Ethanol (mM)		
End Point	**Control**	**25**	**50**	**100**
Average length of longest neurite (um)	58.0	57.0	53.0	53.0
Average length of minor neurites (um)	22.0	21.0	22.0	20.0
Number of neurites/cell	3.8	3.7	3.7	3.8
	+Carbachol			
Average length of longest neurite (um)	160.0	140.0*	70.0*	60.0*
Average length of minor neurites (um)	42.0	40.0	27.0*	24.0*
Number of neurites/cell	8.0	7.1*	4.5*	4.0*

Note. Rat cortical astrocytes were exposed for 24 h to ethanol (25, 50 or 100 mM) in the absence (control) or the presence of 1 mM carbachol. After wash-out, astrocytes were co-cultured with rat hippocampal neurons for an additional 24 h. Neurons were then stained with βIII-tubulin antibody for morphometric analysis. *Significantly different from carbachol alone, $p<0.05$. Data are adapted from Guizzetti et al. (2010).

experimental conditions, carbachol did not stimulate DNA synthesis (Guizzetti, Wei, & Costa, 1998)), and was mediated by multiple signaling pathways in astrocytes, including the PLD, PKC ξ, p70S6 kinase and NF-kB pathway, the PI-3K, PKÇ ξ, p70S6 kinase pathway, and the PKÇ ε, MAPK pathway (Guizzetti, Moore, VanDeMark, Giordano, & Costa, 2011). When astrocytes were incubated with carbachol in the presence of various concentrations of ethanol (25, 50, 75 mM), and then co-cultured with neurons, neuritogenesis was significantly inhibited (Table 3) (Guizzetti, Moore, Giordano, VanDeMark, & Costa, 2010). The effect of ethanol was due to inhibition of PLD, as it was mimicked by 1-butanol (a PLD inhibitor), but not by *tert*-butanol, an analog that does not undergo the transphosphatidylation reaction, and thus does not affect PLD activity (Guizzetti et al., 2010).

Conditioned medium from primary rat astrocytes was analyzed by shotgun proteomics, and 302 different proteins were identified by gene ontology analysis; of these, 133 (43%) were secreted (extracellular) proteins, most of which were involved in neuronal development (Moore, Costa, Shaffer, Goodlett, & Guizzetti, 2009). Exposure of astrocytes to carbachol increased the expression of the extracellular matrix (ECM) proteins fibronectin and laminin in these cells and in the medium; this was due in part to an increase in laminin and fibronectin mRNA levels, and in part to the up-regulation of plasminogen activator inhibitor-1 (PAI-1), an inhibitor of the proteolytic degradation of the ECM, which has neuritogenic properties on its own. The effects of carbachol on ECM proteins were due to activation of muscarinic M3 receptors, and were mediated by the same signaling pathways in astrocytes shown to be involved in carbachol-stimulated, astrocyte-mediated neurite outgrowth (Guizzetti et al., 2011). Inhibition of fibronectin activity (with a function-blocking antibody) strongly reduced the effect of carbachol on the elongation of all the neurites, while inhibition of laminin activity reduced the elongation of minor neurites only (Guizzetti et al., 2008). Ethanol (25–50 mM) decreased the levels of ECM proteins and of PAI-1 in astrocytes; this effect was also due to inhibition of PLD, as substantiated by the fact that phosphatidic acid, a product of phosphatidylcholine hydrolysis by PLD, increased fibronectin and laminin expression in astrocytes, and that these effects were not altered by ethanol (Guizzetti et al., 2010).

The direct effect of carbachol on hippocampal pyramidal neurons was also investigated. By activating M1, rather than M3, muscarinic receptors, carbachol increased the length of the longest neurite, but did not alter minor neurite length. This effect involved primarily activation of Erk 1,2

MAPK (VanDeMark, Guizzetti, Giordano, & Costa, 2009a). Ethanol inhibited the direct neuritogenic effect of carbachol by inhibiting MAPK activation, but only at concentrations of 50 mM or higher (VanDeMark, Guizzetti, Giordano, & Costa, 2009b).

To more closely mimic an *in vivo* situation, the effects of carbachol and of ethanol on neuritogenesis of hippocampal neurons were also investigated in a more complex system: the hippocampal slice in culture (Giordano, Guizzetti, Dao, Mattison, & Costa, 2011). Exposure of hippocampal slices to carbachol (1 mM for 24 h) induces neurite outgrowth in hippocampal pyramidal neurons (a 4.7-fold increase in the length of the longest neurite, a 3.7-fold increase in minor neurite length, and a 3.2-fold increase in the number of branches), while the number of neurites per cell is unchanged (Giordano et al., 2011). Ethanol (50 mM) inhibits all effects induced by carbachol. In agreement with earlier findings in astrocyte-neuron co-cultures, carbachol increases the levels of fibronectin and laminin, and these are inhibited by ethanol. The effects of carbachol on neurite outgrowth and ECM protein levels were both mediated by M3 receptors, suggesting that its neuritogenic effect is indirect, primarily due to activation of muscarinic receptors on astrocytes. Furthermore, fibronectin and laminin function-blocking antibodies antagonized the neuritogenic effect of carbachol, indicating again that it was due to a primary action on astrocytes (Giordano et al., 2011).

All together, these findings are supportive of previous observations of an effect of ethanol on neuronal differentiation upon *in vivo* exposure (Davies & Smith, 1981; Smith & Davies, 1990), and may explain contrasting results previously obtained *in vitro* in neurons (Bearer, Swick, O'Riordan, & Cheng, 1999; Zou, Rabin, & Pentney, 1993). Indeed, the primary effect of ethanol is due to inhibition of muscarinic receptor signaling in astrocytes, which in turn affects astrocyte-neuron cross-talks.

Choline supplementation and ethanol's developmental neurotoxicity

The studies summarized above have identified novel actions of acetylcholine in the developing nervous system, due to activation of muscarinic receptors, particularly in astrocytes, and have shown that by inhibiting muscarinic receptor signal transduction, ethanol antagonizes such developmental effects of acetylcholine. We believe that these mechanistic findings may provide biological plausibility for mechanism-driven therapeutic interventions, particularly for the use of choline.

During the past several years, a series of studies have provided ample evidence that choline supplementation (either prenatally or postnatally) attenuates several behavioral effects associated with prenatal or neonatal ethanol exposure. In an initial study, choline, given postnatally, was shown to ameliorate the effects of prenatal ethanol exposure on a discrimination learning task (Thomas, La Fiette, Quinn, & Riley, 2000). Similar results were also found in studies in which both choline and ethanol (under different paradigms) were given post-natally (Ryan, Williams, & Thomas, 2008; Thomas, Biane, O'Bryan, O'Neill, & Dominguez, 2007; Thomas, Garrison, & O'Neill, 2004). Choline also prevented ethanol-induced deficits in trace fear conditioning (Wagner & Hunt, 2006), but did not mitigate motor coordination deficits (Thomas, O'Neill, & Dominguez, 2004). Prenatal administration of choline also prevented effects of prenatal ethanol on brain weight, working memory tests, and most developmental behavioral measures (Thomas, Abou, & Dominguez, 2009; Thomas, Idrus, Monk, & Dominguez, 2010). The beneficial effects of choline are not due to alterations of ethanol's pharmacokinetics, as BAC is not changed by choline. Hypotheses formulated to explain the effects of choline are basically of two types (Thomas, Monk, Idrus, Otero, & Kelly, 2011): (1) choline, which contains three methyl groups, may influence DNA methylation, thus acting through epigenetic mechanisms, or may affect methylation of homocysteine to methionine (Hobbs, Cleves, Melnyk, Zhao, & James, 2005; Zeisel, 2011); and (2) choline may affect the developing cholinergic system. The latter hypothesis would be in concordance with the cholinergic hypothesis previously indicated. Choline is a precursor of the neurotransmitter acetylcholine, of the membrane phospholipid phosphatidylcholine (the substrate of PLD), as well as of sphyngomyelin, and of lyso-phosphatidylcholine (Wurtman, Cansev, Sakamoto, & Ulus, 2009). Additionally, choline may act as a direct agonist at nicotinic and muscarinic receptors (Costa, Kaylor, & Murphy, 1986; Costa & Murphy, 1984). Free choline readily passes the blood-brain barrier (Cornford, Braun, & Oldendorf, 1978), and *in vivo* pre- or post-natal choline administration has been shown to decrease acetylcholinesterase activity (which would lead to an increase in acetylcholine) (Cermak et al., 1999; Cermak, Holler, Jackson, & Blusztajn, 1998); increase PLD activity (Holler, Cermak, & Blusztajn, 1996); increase potassium-evoked release of acetylcholine in the hippocampus (Cermak et al., 1998; Napoli, Blusztajn, & Mellott, 2008); increase hippocampal responsiveness to carbachol (Montoya et al., 2000); and increase basal dendritic arborization in hippocampal CA1 pyramidal neurons (Li et al., 2004). Animal studies have shown that prenatal ethanol exposure decreases phosphatidylcholine levels in the hippocampus (Wen & Kim, 2004). In humans, a magnetic resonance spectroscopy study has found decreased levels of choline in some brain regions of 12-year-old children diagnosed with FAS (Astley et al., 2009). Using the same approach, it has been shown that choline administration to humans increases the level of choline and its metabolites (e.g. phosphocholine) in the brain (Babb et al., 2004).

Conclusion

Developmental exposure to ethanol alters cholinergic muscarinic receptor signaling, particularly in the hippocampus, a brain region which is believed to mediate many of the effects seen after ethanol exposure (Berman & Hannigan, 2000). While abstaining from alcohol consumption is obviously the best preventive measure for FASD and FAS, it is recognized that therapeutic approaches would be valuable. In this regard, studies

showing the beneficial effects of choline appear the most promising (Jones, 2011; Thomas et al., 2010). The observations that choline, which is easily available and safe, can ameliorate some developmental neurotoxic effects of ethanol, in particular hippocampus-mediated behaviors, make this compound an ideal candidate for such endeavors. The mechanisms underlying the beneficial action of choline in animal models have not been determined; however, several studies have shown that a primary target for choline is the cholinergic system in the hippocampus, which is also targeted by ethanol. The main biological substrates of choline's action in the nervous system are related to the cholinergic system (e.g. acetylcholine, phosphatidylcholine, PLD). The hypothesis that choline supplementation would antagonize ethanol's developmental neurotoxicity by "potentiating" cholinergic actions in the developing nervous system appears to warrant further investigation. Also of interest, from a practical clinical viewpoint, is the finding that choline appears to conserve its efficacy even if given after ethanol administration (Thomas et al., 2000), which would be of much relevance in the continuous quest for therapeutic interventions for FAS/FASD.

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