Published in final edited form as:

Curr Top Behav Neurosci. 2013 ; 13: 163–184. doi:10.1007/7854_2011_198.

What's in that Drink: The Biological Actions of Ethanol, Acetaldehyde, and Salsolinol

Gerald A. Deehan Jr.¹, Mark S. Brodie², and Zachary A. Rodd¹

¹ Institute of Psychiatric Research, Department of Psychiatry, Indiana University: School of Medicine, Indianapolis, IN USA

² Department of Physiology and Biophysics, University of Illinois at Chicago, Chicago, IL USA

Abstract

Alcohol abuse and alcoholism represent substantial problems that affect a large portion of individuals throughout the world. Extensive research continues to be conducted in an effort to identify the biological basis of the reinforcing properties of alcohol in order to develop effective pharmacotherapeutic and behavioral interventions. One theory that has developed within the alcohol field over the past 4 decades postulates that the reinforcing properties of alcohol are due to the action of the metabolites/products of alcohol within the central nervous system (CNS). The most extreme version of this theory suggests that the biologically active metabolites/products of alcohol, created from the breakdown from alcohol, are the ultimate source of the reinforcing properties of alcohol. The contrary theory proposes that the reinforcing properties of alcohol are mediated completely through the interaction of the ethanol molecule with several neurochemical systems within the CNS. While there are scientific findings that offer support for both of these stances, the reinforcing properties of alcohol are most likely generated through a complex series of peripheral and central effects of both alcohol and its metabolites. Nonetheless, the development of a greater understanding for how the metabolites/products of alcohol contribute to the reinforcing properties of alcohol is an important factor in the development of efficacious pharmacotherapies for alcohol abuse and alcoholism. This chapter is intended to provide a historical perspective of the role of acetaldehyde (the first metabolite of alcohol) in alcohol reinforcement as well as review the basic research literature on the effects of acetaldehyde (and acetaldehyde metabolites/products) within the CNS and how these function with regard to alcohol reward.

1. Introduction

The vast majority of North Americans and Europeans will consume alcoholic beverages during their lives. The imbuelement of alcoholic beverages is associated with the goal of obtaining a positive emotional state. The biological basis of the reinforcing properties of alcohol has not been completely established. A recurring theory in the alcohol field is that the reinforcing properties of alcohol are not produced by the ethanol (EtOH) molecule itself, but are dependent upon the action of EtOH metabolites/products. This stance asserts that

EtOH is a pro-drug; EtOH is the base compound for biologically active metabolites/products that are the ultimate source of the reinforcing properties of EtOH. Therefore to understand the biological basis of alcoholism, there is a need to study the metabolites/products of EtOH. Figure 1 is a general schematic representing the main aspects of the central and peripheral metabolism of alcohol, and the pharmacological interactions of each metabolite/condensation product, elucidated by research over the past century.

The principles underlying the 'EtOH is a pro-drug' theory are; 1) the concentrations required to observe EtOH effects in the CNS are too high for conventional pharmacological processes, 2) various behavioral/physiological consequences of alcohol consumption are observed for durations which surpass the bioavailability of EtOH within the system, and 3) metabolism of EtOH to acetaldehyde (ACD) within the CNS mediates most, if not all, of the CNS effects of EtOH. The contrary hypothesis holds that EtOH directly interacts with a number of neurotransmitter systems, and it is through these interactions that alcohol exerts its effects. The 'EtOH Alone' hypothesis asserts that ACD is aversive in the periphery and exists for such a short time period that it could not possibly mediate the persistent effects observed following alcohol intoxication.

Regardless of these opposing stances, genetic studies have clearly linked ACD metabolism with a risk for alcoholism. Specifically, genetic polymorphisms in alcohol dehydrogenase (ADH) and aldehyde dehydrogenase (ALDH) reduce the likelihood of the development of alcoholism (Edenberg, 2011). Such polymorphisms are in genes that encode proteins that regulate the production and metabolism of alcohol and ACD, with both types of polymorphisms ultimately affecting ACD levels in the body. Peripheral increases in ACD levels produce an aversive state (e.g. flush, nausea, and sweating) which reduces the likelihood of continued EtOH consumption (Peng and Yin, 2009). Recent research has established a polygenic contribution of the ADH gene cluster outlining a likely role for several ADH genes in the development of alcoholism (Frank et al., 2011). Alterations in the ALDH2 gene, and the subsequent increase in ACD following EtOH consumption, have been thoroughly documented as protective in nature, with regard to the development of alcoholism amongst Asian populations (Ball, 2008). Emerging evidence suggests that such protection may occur through two mechanisms: 1) an increase in the aversive effects of EtOH through increased ACD levels in the periphery and 2) a decrease in EtOH reward through a functional alteration in dopamine (DA) metabolism (Lu et al., 2010). Inhibition of the ALDH2 gene acted to increase brain levels of tetrahydropapaveroline (THP; a tetrahydroisoquinoline) effectively decreasing cocaine induced DA signaling within the reward pathway of the brain (Yao et al., 2011) and it has been documented that higher levels of THP decrease EtOH consumption and preference (Duncan & Deitrich, 1980). Thus, genetic polymorphisms may exhibit protective properties through a number of peripheral and central mechanisms working in tandem.

Currently, there are three pharmacotherapeutic agents for the treatment of alcoholism that are approved by the US FDA. These compounds have limited efficacy in preventing relapse to alcohol consumption or reducing on-going alcohol consumption (Bergmark, 2008). Despite the decades of research, the biological basis of the effects of alcohol is not unequivocally known. The failure to develop efficacious pharmaceuticals for the treatment

of alcoholism may be predicated upon the failure to fully determine the complex biological consequences of EtOH consumption, and that increasing the understanding of the role of ACD and ACD products in the reinforcing properties of EtOH may assist in the development of potential future pharmacotherapies for the treatment of alcoholism. The intent of this review is to provide a historical perspective of ACD and review the basic research literature of the effects of ACD (and ACD metabolites) within the CNS.

1.1 Relevance and History of Acetaldehyde and Alcoholism

Alcoholism represents a substantial health issue in the United States and throughout the world. Currently, there are over 18 million Americans that abuse alcohol or are classified as alcohol dependent (NIAAA, 2008). The World Health Organization has estimated that 140 million individuals throughout the world could be currently diagnosed with alcohol dependency (WHO, 2003). For perspective, 140 million is approximately the population of Russia, the 9th largest country in the world by population. In the United States and Eastern Europe, between 10 and 20% of males, and 5 to 10% of females, could be diagnosed with alcohol dependency (WHO, 2003).

Like the majority of drugs of abuse, the reinforcing action of alcohol primarily occurs within the dopamine (DA) reward pathway, which originates in the ventral midbrain (ventral tegmental area; VTA) and projects to forebrain regions that include the nucleus accumbens (Acb) and the medial prefrontal cortex (mPFC; Koob et al., 1998; McBride et al., 1999). Furthermore, manipulation of the DA, serotonin (5-HT), γ -aminobutyric acid (GABA), opioid, and glutamate (GLU) systems within the DA reward pathway, or within structures or areas that project to the DA reward pathway, has been found to effectively alter alcohol-related behaviors in preclinical research (Vengeliene et al., 2008; Koob et al., 1998).

Over the past century, researchers have described the aversive effects of alcohol following the ingestion of a number of compounds which, in turn, significantly affected alcohol related behaviors in both preclinical as well as clinical populations. Koelsch (1914) described a number of transitory symptoms following alcohol consumption in factory workers manufacturing calcium cyanamide, an organic compound originally synthesized for the production of metals. Following even a minimal intake of alcohol, individuals exposed to calcium cyanamide would become “hypersensitive to alcohol” and experience flushing (redness) of the face, headaches, shortness of breath, and increased heart rate eventually followed by fatigue (Koelsch, 1914). Chiffot (1916) reported that consumption of an ink cap mushroom (*coprinus atramentarius*) prior to consuming alcohol displayed a similar ‘aversive’ reaction to EtOH consumption. Coprine, an amino acid in the ink cap mushroom, was determined to produce the aversive physical responses to alcohol consumption (Reynolds & Lowe, 1965). Another naturalist observation reported that workers at a rubber parts plant exposed to tetramethylthiuram experienced the similar redness in the face, increased heart rate, and palpitations when consuming alcohol (Williams, 1937). This led Williams to postulate: “If the chemical compound is not harmful to man, one wonders whether one had discovered the cure for alcoholism.” During research attempting to treat intestinal worms, Erik Jacobsen and Jens Hald self-treated themselves with tetraethylthiuramdisulphide, and both men reported similar aversive symptoms (redness of

the face, increased heart rate, sleepiness, etc.) following alcohol consumption. Additionally, Hald and Jacobsen (1948) subsequently indicated that blocking ALDH resulted in a sharp increase in blood levels of ACD which in turn resulted in an increase in the aversive side effects of drinking (i.e. flushing of the skin, headaches, nausea, and shortness of breath). Tetraethylthiuramdisulphide has since been given the name disulfuram (marketed as antabuse) and was the first compound approved for the treatment of alcoholism by the US FDA.

While the exact physiological actions of the aforementioned compounds were not immediately clear, further research identified that they had a common mechanism of action in that they blocked the action of the enzyme ALDH which acts to break down ACD, the first metabolite of alcohol. Early research identified a direct relationship between alcohol consumption and blood ACD levels as Stotz (1943) reported that binge drinking episode (BEC 100-180 mg%) produced blood ACD levels 35 times higher than baseline levels. Similarly, blood ACD levels can be greatly augmented by antabuse treatment. In socially drinking individuals, treatment with antabuse increased blood ACD levels 5-10 fold compared to controls (Hald & Jacobsen, 1948; Larsen, 1948). Additionally, antabuse treatment rendered ACD detectable in the breath of human participants following alcohol exposure (Hald & Jacobsen, 1948). Animal research soon elucidated the metabolic pathway of EtOH and replicated the observation that ACD could be detected in the breath of rabbits receiving antabuse and alcohol (Hald et al., 1949a,b).

Given the clinical implications of the early antabuse studies, several theories emerged associating alcoholism and ACD (Carpenter & MacLeod, 1952; Davis & Walsh, 1970; Griffiths et al., 1974; Myers & Veale 1969; Truitt & Walsh, 1971). The most strident theories suggested that ACD was responsible for all of the effects associated with alcohol and that alcoholism would be more appropriately termed acetaldehydism (Raskin, 1975; Truitt and Walsh, 1971). Contrary studies suggested that ACD did not significantly mediate the effects of alcohol, and only trace amounts of ACD could be found in the cerebrospinal fluid or brain following alcohol consumption (Eriksson et al., 1980; Pikkariainen et al., 1979; Kiianmaa & Virtanen, 1978; Sippel, 1974). These findings coupled with the observation that ACD could only cross the blood brain barrier at high concentrations (Eriksson, 1977; Petersen & Tabakoff, 1979; Sippel, 1974; Tabakoff et al., 1976) suggested that ACD could not and did not contribute to the behavioral or pharmacological effects of alcohol. Subsequent evidence of the local formation of ACD within the brain research rejuvenated the theory that ACD could mediate the biological effects of EtOH consumption (Cohen et al., 1980).

1.2 Acetaldehyde Production in CNS

In the periphery, ACD is formed from EtOH through the action of alcohol dehydrogenase (ADH) primarily in the liver. In the brain, ADH is inactive (Zimatkin et al., 1998), and formation of acetaldehyde from EtOH in the brain is achieved primarily through the action of another enzyme, catalase (Smith et al., 1997; Sippel, 1974; Zimatkin, 1991). Originally, it was postulated that peripheral ACD was unlikely to enter the CNS due to the prevalence of ALDH and the blood-brain barrier (Hunt, 1996). Further research indicated that high levels of peripherally administered ACD results in detection of ACD in the brain within minutes

(Ward et al., 1997) Therefore, peripheral ACD may overwhelm the peripheral ALDH, allowing some percentage of ACD to enter the brain (Quertemont et al., 2005). Additional local metabolic pathways (e.g. mitochondrial cytochrome P450) can also result in the formation of ACD from EtOH in the brain (Zakhari, 2006). It is these non-ALDH metabolic pathways that produce the majority of ACD within the brain following EtOH consumption (Zimatkin et al., 1998).

2. Acetaldehyde Reactivity

ACD is a highly reactive compound that reacts with several endogenous catecholamines to form biologically active compounds (Cohen, 1976; Cohen & Collins, 1970; Davis & Walsh, 1970; Walsh et al., 1970). With regards to research addressing alcohol use disorders, the compounds that have been of interest fall into two main classes: 1) the tetrahydroisoquinoline alkaloids (THIQs), which are formed through the direct and indirect interaction of ACD with monoamines (DA, epinephrine, and norepinephrine; Cohen, 1976) and 2) the tetrahydro- β -carboline (TBCs), which are formed through the condensation of ACD with the indoleamines (tryptophan and tryptamine; Buckholtz, 1980). The most commonly investigated THIQs, tetrahydropapaveroline (THP) and salsolinol (SAL), have been detected in the brain following the administration of alcohol (Weiner, 1980). The TBCs have also received attention as to their underlying role in alcohol-associated behaviors and the physiological effects of alcohol; contradictory data on the actions of TBCs has been reported.

2.1 Alcohol, Acetaldehyde, and Acetaldehyde Products

Research studies have attempted to establish that ACD is a necessary component for the manifestation of the neurobiological and behavioral aspects of alcohol use disorders. Such studies have made use of compounds that inhibit the formation of ACD or sequester ACD into a stable non-reactive adduct. Early studies utilized the compound 3-amino-1,2,4-triazole (triazole), which inhibits brain catalase activity, halting the primary pathway for the breakdown of alcohol in the brain. Triazole administration decreased alcohol consumption in rats and mice (Aragon & Amit, 1992; Koechling & Amit, 1994), reduced EtOH-related motor depression in rats (Aragon et al., 1985), and EtOH-stimulated motor activity in mice (Escarabajal et al., 2000). Triazole also decreased the intake of saccharin-quinine solution (Rotzinger et al., 1994) and food (Tampier et al., 1995) suggesting the compound could be causing non-specific effects unrelated to ACD formation as a result of alcohol exposure. Similar research has been completed using the compound D-penicillamine, which acts to sequester ACD into a non-reactive stable adduct but does not alter EtOH metabolism. Studies using D-penicillamine have established that sequestering ACD results in a reduction of alcohol intake and a decrease in alcohol conditioned place preference in rats (Diana et al., 2008; Font et al., 2006b; Peana et al., 2008). D-penicillamine also reduces alcohol conditioned place preference and alcohol induced motor depression in mice (Font et al., 2005; Font et al., 2006a).

Research has focused on the contribution of the THIQs to physiological effects of alcohol and ACD. Early studies indicated that ACD acted to inhibit the metabolism of dopaldehyde

alkaloid, which is formed from the condensation of dopamine and acetaldehyde, resulting in greater levels of THP (Davis & Walsh, 1970). Central administration of low concentrations of THP into the lateral ventricles produced an increased preference and consumption of EtOH in rats and primates (McCoy et al., 2003; Melchior & Myers, 1977; Myers & Melchior, 1977). Higher doses of THP resulted in decreased alcohol preference and consumption (Duncan & Deitrich, 1980). Microinjections of lower doses of THP into regions in the mesolimbic reward pathway, including the VTA or the Acb, acted to increase EtOH alcohol preference in rats (Myers & Privette, 1989; Duncan & Fernando, 1991). Research has attempted to elucidate the neurochemical systems mediating the effects of THP on EtOH consumption (Myers & Privette, 1989; Privette & Myers, 1989). Buspirone (a 5-HT_{1A} receptor agonist) decreased the THP-stimulated augmentation of EtOH consumption (Privette et al., 1988). Further research with THP has not been conducted.

Systemic administration of SAL increased EtOH consumption and preference (Myers & Melchior, 1977). The ability of SAL to augment EtOH consumption is centrally mediated since microinjections of SAL into the lateral ventricle increased EtOH consumption and preference (Purvis et al., 1980). Similar to ACD, research has indicated that SAL possesses reinforcing/rewarding properties. Peripherally administered SAL will condition a place preference (Matsuzawa et al., 2000).

Compared to other ACD products, the neurochemical basis of SAL effects has been studied more intensively. SAL inhibits catecholamine reuptake (Alpers et al., 1975; Heikkila et al., 1971; Tuomisto & Tuomisto, 1973) and/or metabolizing enzymes such as catecholmethyltransferase and monoamine oxidase (Alpers et al., 1975; Collins et al., 1973) resulting in increases in catecholamine levels. SAL has been shown to significantly decrease striatal levels of 5-HT metabolic enzymes, subsequently increasing 5-HT to a levels 20 fold higher than DA (Nakahara et al., 1994). Additionally, SAL has been shown to possess a high affinity for the μ (MOR) opioid receptor (Airaksinen et al., 1984). SAL-induced locomotor activity, conditioned place preference, and stimulated DA release in the AcbSh can be reduced by microinjections of MOR antagonists into the posterior VTA (Hipolito et al, 2010, 2011).

There has been less attention on the role of TBCs in alcohol use disorders compared to ACD or the THIQs. Initial studies indicated that acute peripheral administration of TBC derivatives acted to decrease the preference for alcohol over water in rats (Geller & Purdy, 1975; Geller et al., 1973; Messiha & Geller, 1976). Chronic intra-cerebral ventricular (ICV) microinjections of the TBC tryptoline significantly increased alcohol preference and consumption (Adell & Myers, 1994; Airaksinen et al., 1983; Huttunen & Myers, 1987; Myers & Melchior, 1977; Tuomisto et al., 1982). Co-infusion of tryptoline and THP augmented alcohol preference and consumption in a synergistic nature (Myers & Oblinger, 1977). Infusion of TBC into the hippocampus of low alcohol drinking (LAD) rats increased alcohol preference and consumption (Adell & Myers, 1995; Huttunen & Myers, 1987) through increases in both 5-HT and norepinephrine levels (Adell & Myers, 1995). TBCs exhibit an affinity for the δ opioid receptor (DOR) (Airaksinen et al., 1984), but the pharmacological properties of TBCs have not been fully examined.

3. Acetaldehyde is Pharmacologically Active in the CNS

ICV microinjections of ACD increased preference for and consumption of alcohol in rodents (Brown et al., 1979; 1980). It was also discovered that ACD possessed reinforcing properties as rats would readily self-administer ACD through both ICV (Amit et al., 1977; Brown et al., 1979; Brown et al., 1980) and intra-venous (IV; Myers et al., 1984) routes. Animals receiving ICV infusions of ACD exhibited a conditioned place preference (CPP) associated with the drug (Smith et al., 1984) while peripheral injections of ACD induced a conditioned taste aversion similar to alcohol (Aragon et al., 1986).

Central and peripheral administration of ACD produces a CPP in a variety of rat lines (Quintanilla & Tampier, 2003; Spina et al., 2010). Quertemont and De Witte (2001) reported that rats showed a dose dependent stimulus preference when ACD was administered peripherally. Much like EtOH, ICV-administered ACD at lower doses, produced an elevation in locomotor activity in rats (Correa et al., 2003), while peripheral and central administration of high doses of ACD produced motor depression in both rats and mice (Durlach et al., 1988; Holtzman & Schneider, 1974; Myers et al., 1987; Quertemont et al., 2004; Tambour et al., 2006). Similar biphasic effects on locomotor activity have been observed following vapor exposure to ACD (Ortiz et al, 1974).

While there is still dispute over the extent to which ACD contributes to the neurobiological and behavioral actions of alcohol, emerging evidence indicates that ACD is biologically active and may mediate, in part, alterations in behavior produced by EtOH exposure/consumption. Research has shown that both central and peripheral administration of ACD cause an increase in alcohol consumption in rats (Brown et al., 1979; 1980). Rats will exhibit ACD induced CPP and stimulus preference suggesting that ACD is rewarding (Quertemont & De Witte, 2001; Quintanilla & Tampier, 2003; Smith et al., 1984; Spina et al., 2010); blocking or sequestering the formation of ACD resulting from alcohol exposure produces alterations in the neurobiological and behavioral effects of alcohol (Aragon & Amit, 1992; Diana et al., 2008; Font et al., 2005; Font et al., 2006a; Font et al., 2006b; Kaharanian et al., 2011; Koechling & Amit, 1994; Peana et al., 2008). While it is currently difficult to assert that ACD is absolutely necessary for the neurobiological and behavioral actions of alcohol, data show that ACD is likely to be involved to some extent.

3.1. Acetaldehyde in the VTA – In vivo electrophysiology

ACD has excitatory actions on neurons of the VTA as clearly demonstrated by the effects on dopamine release and on the firing frequency of individual VTA neurons. In experiments using in vivo recording methods, ACD was injected intravenously at doses of from 5 to 40 mg/kg, and a dose-dependent increase in firing of dopaminergic VTA neurons was reported (Foddai et al., 2004). Thus, ACD parallel the effects observed with EtOH, but at 50 times lower concentrations. The effects of EtOH on VTA neuronal activity was blocked by systemic pretreatment with the ADH inhibitor 4-methylpyrazole, but this drug had no effect on ACD-induced excitation (Foddai et al., 2004), suggesting that the excitatory effects of EtOH on the VTA are mediated by ACD. The data also implicated that peripheral ACD formation (mediated by ADH) rather than central ACD formation (which would be mediated by catalase) was the basis of the finding. Sequestration of ACD by in vivo administration of

D-penicillamine is sufficient to block the excitatory effects of intragastrically administered EtOH or intragastrically administered ACD (Enrico et al., 2009).

The clear effects of manipulation of acetaldehyde by a variety of methods (e.g., enzyme antagonism or acetaldehyde sequestration) indicate that this is a robust *in vivo* phenomenon, and the effect of EtOH on dopaminergic VTA neurons *in vivo* is dependent on acetaldehyde.

3.2. Acetaldehyde in the VTA – *In vitro* studies

Significant studies have been performed using brain slice preparations showing that acutely applied ACD can increase the firing frequency of dopaminergic VTA neurons. The key results of that study indicate that ACD-induced activation of dopaminergic VTA neurons mimics EtOH-induced excitation (Diana et al., 2008), and is produced at much lower concentrations (10-100 nM) compared to EtOH (typical excitatory concentrations of 20-120 mM; Brodie and Appel, 1998; Brodie et al., 1990). Furthermore, EtOH applied in the presence of a catalase inhibitor, 3-aminotriazole (1 mM), failed to produce its characteristic excitation of the VTA neurons in this study. In exploring the mechanism of ACD excitation of VTA neurons, Melis et al. (2007) examined the effect of ACD on two ion currents, A-current and h-current. An A-current represents a rapidly-inactivating potassium current that can contribute to spike after hyperpolarization and is involved in the regulation of firing frequency of dopaminergic VTA neurons (Koyama and Appel, 2006a). On the other hand, h-current is a characteristic current of dopaminergic VTA neurons that is activated at membrane potentials about 20 mV negative to the resting membrane potential; it may contribute to the spontaneous firing rate of dopaminergic VTA neurons from mice (Okamoto et al. 2006; McDaid et al. 2008) but not those from rats (McDaid et al. 2008; Appel et al. 2003), but its major role is likely to be in the regulation of excitability and synaptic signal integration in dopaminergic VTA neurons (Inyushin et al. 2010). The authors noted a rightward voltage shift produced by ACD on I_A (Melis et al., 2007). Also noted was a significant increase in h-current produced by acutely applied ACD; this is consistent with an effect of EtOH, which has been shown to acutely increase I_h of VTA neurons in brain slices (Brodie and Appel, 1998; Okamoto et al., 2006). In addition, the authors blocked ACD-induced excitation with two specific ion channel blockers: 4-aminopyridine (10 mM) which blocks A-current and ZD7288, which blocks h-current. Both agents apparently blocked the ACD-induced excitation of the VTA neurons (Melis et al., 2007). The firing rate was increased by 4-aminopyridine alone, and no further increase in firing was observed with the addition of ACD. In contrast, ZD7288 (30 μ M) alone reduced the firing rate of dopaminergic VTA neurons (as has been seen by some (Okamoto et al., 2006) but not others (Appel et al., 2003), and no ACD-induced excitation was observed in the presence of ZD7288.

The most parsimonious model suggests that EtOH is metabolized to ACD by catalase locally in the VTA, and the authors of these studies suggest that, in general, EtOH actions on the VTA are mediated by ACD.

3.3. Differences in the action of acetaldehyde: Methodological considerations

It is an inherently unsatisfying argument to invoke methodological differences to explain contrary experimental results, but in the case of brain slice electrophysiology, often this is

the underlying source of controversy. Variables such as ionic concentrations in external or internal media, purity of reagents, species, strain or supplier of subjects, and even plane of section, can alter the results and can lead to different conclusions among investigators. Controversies in the literature among investigators may not be amenable to resolution simply due to the multiplicity of unknown variables. Each laboratory achieves consistency of results by controlling many of these variables, but each may control these variables differently. With acetaldehyde, the differences among laboratories may become more extreme, as the reactivity and labile chemical nature of acetaldehyde may yield a null result, even when other variables are controlled. Parasagittal section of the midbrain may yield slices with relatively more intact glutamatergic fibers than coronal sections; ACD has been shown to interact with glutamatergic systems (Padilla-de la Torre et al., 2008). There is ample evidence that ZD7288 reduces glutamate release as well as blocking h-current; if a portion of ACD effects were produced by actions on presynaptic glutamatergic endings in the VTA, differences in the viability of the glutamate terminals could explain differences in the effects of ACD and other agents.

3.4 Differences between acetaldehyde and alcohol: electrophysiological studies

While the results of these studies, especially those of the enzyme antagonist experiments, indicate that EtOH actions on the firing of mesolimbic dopaminergic neurons are mediated by ACD formed in the VTA, there are some clear differences in the mechanisms of action of the two agents. ACD-induced excitation of dopaminergic VTA neurons appears to be mediated by effects on h-current and A-current (Melis et al., 2007). EtOH excitation in dopaminergic neurons of the rat has been shown to be blocked by quinidine, but not by blockers of h-current (Appel et al., 2003). Furthermore, EtOH excites individual VTA neurons dissociated from brain slices (Brodie et al., 1999; Ye et al., 2001), a preparation that would be expected to reduce the ability of synthesized ACD to act on these independent VTA neurons, and the concentration-response for EtOH excitation of dissociated dopaminergic VTA neurons (Brodie et al., 1999) is similar to that observed in brain slices (Brodie et al., 1990).

Clearly, the results of Melis, et al indicate that ACD affects both A-current and h-current, but these effects are not consistent with an EtOH-like action. Acute (Brodie and Appel, 1998) and chronic (Okamoto et al., 2006) EtOH has clear effects on h-current, and it suggests that some of the effects of EtOH on firing frequency should be mediated by h-current. Despite the finding in one study that suggested that EtOH excitation of dopaminergic VTA neurons of mice could be blocked with ZD7288 (Okamoto et al., 2006), additional studies of this phenomenon indicate that apparent reduction of EtOH excitation was more likely due to effects of ZD7288 that are not related to its action on h-channels (McDaid et al., 2008). The role of h-current in modulating the firing frequency of dopaminergic neurons of the VTA may differ in different preparations and in different rodents, as ZD7288 alone produces a decrease in firing rate in some studies (Melis et al., 2007; Okamoto et al., 2006), but not in others (Appel et al., 2003) except at high concentrations (Seutin et al., 2001). Under conditions and in species in which ZD7288 does not affect the firing rate, it also does not affect EtOH action. The action of ZD7288 and other blockers of ion channels may depend on specific experimental conditions (see above).

One agent that has been shown to decrease EtOH excitation in rat dopaminergic VTA neurons is quinidine (Appel et al., 2003), which was not tested against ACD-induced excitation. EtOH also decreases M-current (Koyama et al., 2007), and the effect of ACD on M-current is unknown. M-current is a voltage dependent, sustained potassium current that affects the firing frequency of dopaminergic VTA neurons (Koyama and Appel, 2006b). A study of the effects of EtOH on the ion channel responsible for M-current is one example of the difficulty in cataloging the effects of agents on ionic currents and then postulating a functional role for those currents on cell activity. EtOH reduces M-current of dopaminergic VTA neurons in a concentration-dependent manner (Koyama et al., 2007), but the selective M-current blocker XE-991 did not significantly reduce EtOH-induced excitation (Koyama et al., 2007). The effects of EtOH on M-current may be physiologically important in some processes, (for example, adaptation to chronic EtOH exposure), modulation of M-current does not alter the acute excitatory effect of EtOH.

The data indicate the EtOH and ACD are not producing the same electrophysiological effects on VTA DA neurons, but the results of studies indicating the lack of an EtOH effect on dopaminergic neurons in the presence of antagonists of ADH (Foddai et al., 2004) or catalase (Melis et al., 2007; Diana et al., 2008) are compelling, and suggest that the metabolism of EtOH to ACD mediate EtOH-induced excitation in dopaminergic VTA neurons. It seems most likely that ACD is a crucial component of the overall effects of EtOH on dopaminergic neurons of the VTA; the essential action of ACD could be parallel to EtOH, or it could enhance EtOH-induced changes. Blockade of the formation of ACD can reduce the response of dopaminergic VTA neurons to EtOH, and could serve as a platform for development of agents that reduce the rewarding and reinforcing actions of EtOH.

3.5 Behavioral Pharmacology of Acetaldehyde within the Mesolimbic Dopamine System

Despite the inconsistent electrophysiological studies, the results of behavioral pharmacological research examining the affects of acetaldehyde within the mesolimbic dopamine (VTA and nucleus accumbens) have been relatively consistent. EtOH, ACD, and SAL are directly self-administered into the posterior, but not anterior, VTA (Rodd et al., 2008, 2005; Rodd-Henricks et al., 2002). Self-administration of all 3 compounds into the posterior VTA can be extinguished by co-administrations of quinpirole, a D₂ receptor agonist (Rodd et al., 2005, 2008). Thus, the reinforcing actions of all 3 compounds in the posterior VTA is dependent upon VTA DA neuronal activity, since quinpirole would act to stimulate D₂ autoreceptors (reduction in DA activity). SAL microinjected into the VTA will produce a conditioned place preference (Hipolito et al., 2010, 2011). Reverse microdialysis of SAL has been shown to modulate DA levels within the Acb (Hipolito et al., 2009). EtOH and SAL self-administration, but not ACD, directly into the posterior VTA is extinguished by co-administration of 5HT receptor antagonists (Rodd et al., 2005, 2008). EtOH has direct actions on 5HT₃ receptors (Lovinger and White, 1991) and SAL can increase the release of 5HT (Maruyama et al., 1992, 1993), but ACD does not express an affinity for 5HT₃ receptors (Li, 2000).

Examining the concentration of SAL, ACD, and EtOH required to support self-administration directly into the posterior VTA provides an indication of the efficacy between

the compounds. EtOH is typically self-administered between the ranges of 20-80 mM directly into the posterior VTA (Rodd et al., 2003, 2005). ACD is reinforcing between 6-90 μ M, while SAL is self-administered between 0.03-0.3 μ M directly into the posterior VTA (Rodd et al., 2005, 2008). Thus, the concentration required for SAL to support self-administration into the posterior VTA is 200-fold less than that required for ACD and 300×10^3 lower than EtOH. All of these concentrations are within the range observed in the brain following oral consumption of EtOH (Haber et al., 1997, Zimatkin et al., 1998), and are thus pharmacologically relevant.

In addition, the dose response curves of the 3 compounds would support the stance that EtOH is possibly a pro-drug. However, EtOH self-administration directly into the posterior VTA is not altered by co-administration of a catalase antagonist (3-amino-1,2,4-triazole; triazole; Rodd et al., 2005). Yet, ACD could have been produced within the posterior VTA following self-infusion of EtOH through a catalase-independent pathway. A more recent study has examined the possible role for ACD in the etiology of alcohol use/abuse through the use of lentiviral vectors that decrease catalase activity or increase ADH activity in the VTA (Kaharanian et al., 2011). Administration of either lentiviral vector into the VTA acted to decrease voluntary consumption of EtOH as well as EtOH stimulated DA release in the AcbSh suggesting that the breakdown of EtOH into ACD is a component of EtOH reward (Kaharanian et al., 2011). A later study replicated such findings in that an anti-catalase viral vector was once again successful in decreasing EtOH intake in naïve rats (Quintanilla et al., 2011). However, the anti-catalase viral vector was only successful at decreasing EtOH consumption, in animals that had previous access to EtOH over the course of 60 days, following a period of imposed abstinence (i.e. during relapse; Quintanilla et al., 2011).

The ability of a compound to stimulate VTA DA neurons can also be measured by determining the effects of compounds directly applied to the VTA on DA release in downstream projection areas. The effects of DA release in the Acb following microinjections of drugs into the VTA have been examined for EtOH, ACD, and ACD products. Myers & Robinson (1999) were first to report that THP microinjected into the anterior VTA had a direct effect on DA release in the Acb. Further, THP was detected *in vivo* in the striatum (Haber et al., 1997) and Acb (Baum et al., 1999) showing that THP could be formed in the brain within structures involved in drug reward.

Recent studies have utilized the same equipment employed for intracranial self-administration studies to determine the effects of microinjection of a compound into the posterior VTA on DA levels in the AcbSh. Microinjections of 200 mg% EtOH (optimal concentration) into the posterior VTA increased DA levels in the AcbSh (Ding et al., 2009, 2011). Similar to the intracranial self-administration data, lower concentrations of ACD (23 μ M) and SAL (0.3 μ M) microinjected into the posterior VTA were able to evoke dopamine release in the AcbSh (Deehan et al., in press). The data sets also reveal that SAL was able to increase DA levels in the AcbSh at a greater amount than EtOH (Fig. 2). Therefore, there were parallel findings between the concentration required to produce reinforcement and that required to stimulate VTA DA neurons and evoke DA release in the AcbSh.

The research examining the effects of SAL and ACD in other brain regions has lagged behind that conducted in the VTA. Both EtOH and SAL are self-administered into the AcbSh, but not AcbC (Engleman et al., 2009; Rodd et al., 2003). Similar to the VTA data set, SAL was self-administered into the AcbSh at significantly (400×10^3) lower concentrations than EtOH. Both EtOH and SAL self-administration into the AcbSh could be extinguished by co-administration of a $D_{2/3}$ antagonist (sulpiride). Therefore, the reinforcing properties of both SAL and EtOH within the AcbSh are dependent upon activation of post-synaptic DA receptors. The reinforcing properties or neurochemical effects of EtOH, SAL and ACD in other brain regions (e.g., medial prefrontal cortex or central amygdala) have not been extensively studied.

4. General Summary

The current literature indicates that the reinforcing properties, and the behavioral consequences, of EtOH are mediated, in part, by ACD and ACD products (SAL). In general, the data reflect the complexity of EtOH within the CNS. There is evidence that EtOH can directly act at receptors and to stimulate VTA DA neurons (Brodie et al., 1999; Ye et al., 2001; Lovinger and White, 1991). Convergent evidence that both ACD and SAL have distinct actions within the mesolimbic dopamine system has recently been reported. Additional studies have indicated that EtOH consumption and reinforcement may be mediated by the conversion of EtOH into ACD. The following statements are supported by current research; 1) EtOH can have direct actions to produce neurochemical, electrophysiological, and behavioral consequences, 2) some of EtOH's actions may be mediated by, in part, the conversion of EtOH into ACD, 3) ACD in the CNS has reinforcing properties which are mediated by the mesolimbic dopamine system, 4) products derived from ACD can also produce reinforcement within the mesolimbic dopamine system, 5) the actions of EtOH, ACD, and SAL within the mesolimbic dopamine system can occur at physiologically relevant levels, and 6) whole areas of research about the EtOH-ACD-SAL system has not been elucidated. Ultimately, the sequelae of alcoholism may be based upon a complex series of interwoven peripheral and central effects of EtOH and its metabolites. It is perhaps our lack of understanding of this complex system that has prevented the development of successful pharmacotherapeutics for the treatment of alcoholism.

List of abbreviations

Triazole	3-amino-1,2,4-triazole
ACD	Acetaldehyde
ADH	Alcohol Dehydrogenase
I_A	A-type Potassium Current
BEC	Blood Ethanol Concentration
CNS	Central Nervous System
CPP	Conditioned Place Preference

DOR	Delta Opioid Receptor
D₂	Dopamine 2
DA	Dopamine
EtOH	Ethanol
GABA	Gamma-aminobutyric Acid
GLU	Glutamate
I_H	Hyperpolarization-activated Inward Current
ICV	Intra-Cerebral Ventricular
IV	Intra-venous
mPFC	Medial Prefrontal Cortex
μM	Micro-molar
MOR	Mu Opioid Receptor
NIAAA	National Institute for Alcohol Abuse and Alcoholism
AcbSh	Nucleus Accumbens Shell
Acb	Nucleus Accumbens
5HT₃	Serotonin 3
5-HT	Serotonin
TBCs	Tetrahydrobetacarbolines
SAL	Salsolinol
THIQs	Tetrahydroisoquinoline alkaloids
THP	Tetrahydropapaveraoline
ALDH	Aldehyde Dehydrogenase
US FDA	United States Food and Drug Administration
VTa	Ventral Tegmental Area
WHO	World Health Organization

5. References

- Adell A, Myers RD. 5-HT, dopamine, norepinephrine, and related metabolites in brain of low alcohol drinking (LAD) rats shift after chronic intra-hippocampal infusion of harman. *Neurochem Res.* 1995; 20:209–215. [PubMed: 7540261]

- Adell A, Myers RD. Increased alcohol intake in low alcohol drinking rats after chronic infusion of the beta-carboline harman into the hippocampus. *Pharmacol Biochem Behav.* 1994; 49:949–953. [PubMed: 7886112]
- Airaksinen MM, Mahonen M, Tuomisto L, Peura P, Eriksson CJ. Tetrahydro-beta-carbolines: effect on alcohol intake in rats. *Pharmacol Biochem Behav.* 1983; 18(Suppl 1):525–529. [PubMed: 6634862]
- Airaksinen MM, Saano V, Steidel E, Juvonen H, Huhtikangas A, Gynther J. Binding of beta-carbolines and tetrahydroisoquinolines by opiate receptors of the delta-type. *Acta Pharmacol Toxicol (Copenh).* 1984; 55:380–385. [PubMed: 6099027]
- Alpers HS, McLaughlin, Nix WM, Davis VE. Inhibition of catecholamine uptake and retention in synaptosomal preparations by tetrahydroisoquinoline and tetrahydroprotoberberine alkaloids. *Biochem Pharmacol.* 1975; 24:1391–1396.
- Amit Z, Brown ZW, Rockman GE. Possible involvement of acetaldehyde, norepinephrine and their tetrahydroisoquinoline derivatives in the regulation of ethanol self-administration. *Drug Alcohol Depend.* 1977; 2:495–500. [PubMed: 913244]
- Appel SB, Liu Z, McElvain MA, Brodie MS. Ethanol excitation of dopaminergic ventral tegmental area neurons is blocked by quinidine. *J Pharmacol Exp Ther.* 2003; 306:437–446. [PubMed: 12721326]
- Aragon CM, Abitbol M, Amit Z. Acetaldehyde may mediate reinforcement and aversion produced by ethanol. An examination using a conditioned taste-aversion paradigm. *Neuropharmacology.* 1986; 25:79–83. [PubMed: 3951679]
- Aragon CM, Amit Z. The effect of 3-amino-1,2,4-triazole on voluntary ethanol consumption: evidence for brain catalase involvement in the mechanism of action. *Neuropharmacology.* 1992; 31:709–712. [PubMed: 1407407]
- Aragon CM, Spivak K, Amit Z. Blockade of ethanol induced conditioned taste aversion by 3-amino-1,2,4-triazole: evidence for catalase mediated synthesis of acetaldehyde in rat brain. *Life Sci.* 1985; 37:2077–2084. [PubMed: 2999539]
- Ball D. Addiction science and its genetics. *Addiction.* 2008; 103:360–367. [PubMed: 18042191]
- Baum SS, Hill R, Kiianmaa K, Rommelspacher H. Effect of ethanol on (R)- and (S)-Salsolinol, salsoline, and THP in the nucleus accumbens of AA and ANA rats. *Alcohol.* 1999; 18:165–169. [PubMed: 10456568]
- Bergmark A. On treatment mechanisms-what can we learn from the COMBINE study. *Addiction.* 2008; 103:703–705. [PubMed: 18190670]
- Brodie MS, Appel SB. The effects of ethanol on dopaminergic neurons of the ventral tegmental area studied with intracellular recording in brain slices. *Alcohol Clin Exp Res.* 1998; 22:236–244. [PubMed: 9514313]
- Brodie MS, Pesold C, Appel SB. Ethanol directly excites dopaminergic ventral tegmental area reward neurons. *Alcohol Clin Exp Res.* 1999; 23:1848–1852. [PubMed: 10591603]
- Brodie MS, Shefner SA, Dunwiddie TV. Ethanol increases the firing rate of dopamine neurons of the rat ventral tegmental area in vitro. *Brain Res.* 1990; 508:65–69. [PubMed: 2337793]
- Brown ZW, Amit Z, Rockman GE. Intraventricular self-administration of acetaldehyde, but not ethanol, in naive laboratory rats. *Psychopharmacology (Berl).* 1979; 64:271–276. [PubMed: 41277]
- Brown ZW, Amit Z, Smith B. Intraventricular self-administration of acetaldehyde and voluntary consumption of ethanol in rats. *Behav Neural Biol.* 1980; 28:150–155. [PubMed: 7362581]
- Buckholtz NS. Neurobiology of tetrahydro-beta-carbolines. *Life Sci.* 1980; 27:893–903. [PubMed: 6107810]
- Carpenter RK, Macleod LD. The effects of ethyl alcohol and acetaldehyde on maze behaviour and motor co-ordination in rats. *J Ment Sci.* 1952; 98:167–173. [PubMed: 14898215]
- Chiffot MJ. Sur un cas de rubefaction de la face, tendant a se generalizer a la suite de l'ngestion du *Coprinus atramentarius*. *Fr. Bull. Soc. Mycol. France.* 1916; 32:63.
- Cohen G. Alkaloid products in the metabolism of alcohol and biogenic amines. *Biochem Pharmacol.* 1976; 25:1123–1128. [PubMed: 7258]
- Cohen G, Collins M. Alkaloids from catecholamines in adrenal tissue: possible role in alcoholism. *Science.* 1970; 167:1749–1751. [PubMed: 5461272]

- Cohen G, Sinet PM, Heikkila R. Ethanol oxidation by rat brain in vivo. *Alcohol Clin Exp Res.* 1980; 4:366–370. [PubMed: 7004234]
- Collins AC, Cashaw JL, Davis VE. Dopamine-derived tetrahydroisoquinoline alkaloids--inhibitors of neuroamine metabolism. *Biochem Pharmacol.* 1973; 22:2337–2348. [PubMed: 4733684]
- Correa M, Arizzi MN, Betz A, Mingote S, Salamone JD. Open field locomotor effects in rats after intraventricular injections of ethanol and the ethanol metabolites acetaldehyde and acetate. *Brain Res Bull.* 2003; 62:197–202. [PubMed: 14698353]
- Davis VE, Walsh MJ. Alcohol, amines, and alkaloids: a possible biochemical basis for alcohol addiction. *Science.* 1970; 167:1005–1007. [PubMed: 5460776]
- Diana M, Peana AT, Sirca D, Lintas A, Melis M, Enrico P. Crucial role of acetaldehyde in alcohol activation of the mesolimbic dopamine system. *Ann N Y Acad Sci.* 2008; 1139:307–317. [PubMed: 18991876]
- Ding ZM, Oster SM, Hall SR, Engleman EA, Hauser SR, McBride WJ, Rodd ZA. The stimulating effects of ethanol on ventral tegmental area dopamine neurons projecting to the ventral pallidum and medial prefrontal cortex in female Wistar rats: regional difference and involvement of serotonin-3 receptors. *Psychopharmacology (Berl).* 2011; 216:245–255. [PubMed: 21340473]
- Ding ZM, Rodd ZA, Engleman EA, McBride WJ. Sensitization of ventral tegmental area dopamine neurons to the stimulating effects of ethanol. *Alcohol Clin Exp Res.* 2009; 33:1571–1581. [PubMed: 19485970]
- Duncan C, Deitrich RA. A critical evaluation of tetrahydroisoquinoline induced ethanol preference in rats. *Pharmacol Biochem Behav.* 1980; 13:265–281. [PubMed: 7413698]
- Duncan CC, Fernando PW. Effects of tetrahydropapaveroline in the nucleus accumbens and the ventral tegmental area on ethanol preference in the rat. *Alcohol.* 1991; 8:87–90. [PubMed: 2064757]
- Durlach J, Rinjard P, Sprince H, Smith GG. Similar antagonistic effects of Ca N-acetylhomotaurinate on depression of motor activity and lethality induced by acetaldehyde or ethanol. *Methods Find Exp Clin Pharmacol.* 1988; 10:437–447. [PubMed: 3419248]
- Edenberg HJ. Common and rare variants in alcohol dependence. *Biol Psychiatry.* 2011; 70:498–499. [PubMed: 21864733]
- Engleman EA, Ding ZM, Oster SM, Toalston JE, Bell RL, Murphy JM, McBride WJ, Rodd ZA. Ethanol is self-administered into the nucleus accumbens shell, but not the core: evidence of genetic sensitivity. *Alcohol Clin Exp Res.* 2009; 33:2162–2171. [PubMed: 19764930]
- Enrico P, Sirca D, Mereu M, Peana AT, Lintas A, Golosio A, Diana M. Acetaldehyde sequestering prevents ethanol-induced stimulation of mesolimbic dopamine transmission. *Drug Alcohol Depend.* 2009; 100:265–271. [PubMed: 19070441]
- Eriksson CJ. Acetaldehyde metabolism in vivo during ethanol oxidation. *Adv Exp Med Biol.* 1977; 85A:319–341. [PubMed: 335831]
- Eriksson CJ, Hillbom ME, Sovijarvi AR. Difficulties in measuring human blood acetaldehyde concentrations during ethanol intoxication. *Adv Exp Med Biol.* 1980; 126:439–451. [PubMed: 7405691]
- Escarabajal D, Miquel M, Aragon CM. A psychopharmacological study of the relationship between brain catalase activity and ethanol-induced locomotor activity in mice. *J Stud Alcohol.* 2000; 61:493–498. [PubMed: 10928718]
- Foddai M, Dosia G, Spiga S, Diana M. Acetaldehyde increases dopaminergic neuronal activity in the VTA. *Neuropsychopharmacology.* 2004; 29:530–536. [PubMed: 14973432]
- Font L, Aragon CM, Miquel M. Ethanol-induced conditioned place preference, but not aversion, is blocked by treatment with D -penicillamine, an inactivation agent for acetaldehyde. *Psychopharmacology (Berl).* 2006a; 184:56–64. [PubMed: 16344987]
- Font L, Aragon CM, Miquel M. Voluntary ethanol consumption decreases after the inactivation of central acetaldehyde by d-penicillamine. *Behav Brain Res.* 2006b; 171:78–86. [PubMed: 16621047]
- Font L, Miquel M, Aragon CM. Prevention of ethanol-induced behavioral stimulation by D-penicillamine: a sequestration agent for acetaldehyde. *Alcohol Clin Exp Res.* 2005; 29:1156–1164. [PubMed: 16046870]

- Frank J, Cichon S, Treutlein J, Ridinger M, Mattheisen M, Hoffmann P, Herms S, Wodarz N, Soyka M, Zill P, Maier W, Mossner R, Gaebel W, Dahmen N, Scherbaum N, Schmal C, Steffens M, Lucae S, Ising M, Muller-Myhsok B, Nothen MM, Mann K, Kiefer F, Rietschel M. Genome-wide significant association between alcohol dependence and a variant in the ADH gene cluster. *Addict Biol.* 2011 Epub ahead of print.
- Geller I, Purdy R. Alteration of ethanol preference in rats; effects of beta-carbolines. *Adv Exp Med Biol.* 1975; 59:295–301. [PubMed: 1237221]
- Geller I, Purdy R, Merritt JH. Alterations in ethanol preference in the rat: the role of brain biogenic amines. *Ann N Y Acad Sci.* 1973; 215:54–59. [PubMed: 4541162]
- Griffiths PJ, Littleton JM, Ortiz A. Proceedings: Chronic administration of acetaldehyde to mice: behavioural and biochemical similarities with chronic ethanol administration. *Br J Pharmacol.* 1974; 50:441P.
- Haber H, Roske I, Rottmann M, Georgi M, Melzig MF. Alcohol induces formation of morphine precursors in the striatum of rats. *Life Sci.* 1997; 60:79–89. [PubMed: 9000113]
- Hald J, Jacobsen E. A drug sensitizing the organism to ethyl alcohol. *Lancet.* 1948; 2:1001–1004. [PubMed: 18103475]
- Hald J, Jacobsen E, Larsen V. Formation of acetaldehyde in the organism in relation to dosage of antabuse (tetraethylthiuramdisulphide) and to alcohol-concentration in blood. *Acta Pharmacol Toxicol (Copenh).* 1949a; 5:179–188. [PubMed: 18143956]
- Hald J, Jacobsen E, Larsen V. The rate of acetaldehyde metabolism in isolated livers and hind limbs of rabbits treated with antabuse (tetraethylthiuramdisulphide). *Acta Pharmacol Toxicol (Copenh).* 1949b; 5:298–308. [PubMed: 15393459]
- Heikkila R, Cohen G, Dembiec D. Tetrahydroisoquinoline alkaloids: uptake by rat brain homogenates and inhibition of catecholamine uptake. *J Pharmacol Exp Ther.* 1971; 179:250–258. [PubMed: 5133601]
- Hipolito L, Marti-Prats L, Sanchez-Catalan MJ, Polache A, Granero L. Induction of conditioned place preference and dopamine release by salsolinol in posterior VTA of rats: Involvement of mu-opioid receptors. *Neurochem Int.* 2011 Epub ahead of print.
- Hipolito L, Sanchez-Catalan MJ, Granero L, Polache A. Local salsolinol modulates dopamine extracellular levels from rat nucleus accumbens: shell/core differences. *Neurochem Int.* 2009; 55:187–192. [PubMed: 19524107]
- Hipolito L, Sanchez-Catalan MJ, Zornoza T, Polache A, Granero L. Locomotor stimulant effects of acute and repeated intrategmental injections of salsolinol in rats: role of mu-opioid receptors. *Psychopharmacology (Berl).* 2010; 209:1–11. [PubMed: 20084370]
- Holtzman SG, Schneider FH. Comparison of acetaldehyde and ethanol: depression of motor activity in mice. *Life Sci.* 1974; 14:1243–1250. [PubMed: 4823637]
- Hunt WA. Role of acetaldehyde in the actions of ethanol on the brain--a review. *Alcohol.* 1996; 13:147–151. [PubMed: 8814648]
- Huttunen P, Myers RD. Anatomical localization in hippocampus of tetrahydro-beta-carboline-induced alcohol drinking in the rat. *Alcohol.* 1987; 4:181–187. [PubMed: 3036178]
- Inyushin MU, Arencibia-Albite F, Vazquez-Torres R, Velez-Hernandez ME, Jimenez-Rivera CA. Alpha-2 noradrenergic receptor activation inhibits the hyperpolarization-activated cation current (I_h) in neurons of the ventral tegmental area. *Neuroscience.* 2010; 167:287–297. [PubMed: 20122999]
- Karahanian E, Quintanilla ME, Tampier L, Rivera-Meza M, Bustamante D, Gonzalez-Lira V, Morales P, Herrera-Marschitz M, Israel Y. Ethanol as a prodrug: brain metabolism of ethanol mediates its reinforcing effects. *Alcohol Clin Exp Res.* 2011; 35:606–612. [PubMed: 21332529]
- Kiianmaa K, Virtanen P. Ethanol and acetaldehyde levels in cerebrospinal fluid during ethanol oxidation in the rat. *Neurosci Lett.* 1978; 10:181–186. [PubMed: 19605277]
- Koechling UM, Amit Z. Effects of 3-amino-1,2,4-triazole on brain catalase in the mediation of ethanol consumption in mice. *Alcohol.* 1994; 11:235–239. [PubMed: 8060524]
- Koelsch F. Uber neuartige gewerbliche Erkrankungen in Kalkstickstoffbetrieben. *Munch. Med. Wochschr.* 1914; 61:1869–1970.

- Koob GF, Roberts AJ, Schulteis G, Parsons LH, Heyser CJ, Hyytia P, Merlo-Pich E, Weiss F. Neurocircuitry targets in ethanol reward and dependence. *Alcohol Clin Exp Res.* 1998; 22:3–9. [PubMed: 9514280]
- Koyama S, Appel SB. A-type K⁺ Current of Dopamine and GABA Neurons in the Ventral Tegmental Area. *J Neurophysiol.* 2006a; 96:544–554. [PubMed: 16611837]
- Koyama S, Appel SB. Characterization of M-current in ventral tegmental area dopamine neurons. *J Neurophysiol.* 2006b; 96:535–543. (2006b). [PubMed: 16394077]
- Koyama S, Brodie MS, Appel SB. Ethanol inhibition of m-current and ethanol-induced direct excitation of ventral tegmental area dopamine neurons. *J Neurophysiol.* 2007; 97:1977–1985. [PubMed: 16956995]
- Larsen V. The effect of experimental animals of antabuse (tetraethylthiuramdisulphide) in combination with alcohol. *Acta Pharmacol.* 1948; 4:321–332.
- Li TK. Pharmacogenetics of responses to alcohol and genes that influence alcohol drinking. *J Stud Alcohol.* 2000; 61:5–12. [PubMed: 10627090]
- Lovinger DM, White G. Ethanol potentiation of 5-hydroxytryptamine₃ receptor-mediated ion current in neuroblastoma cells and isolated adult mammalian neurons. *Mol Pharmacol.* 1991; 40:263–270. [PubMed: 1715016]
- Lu RB, Lee JF, Huang SY, Lee SY, Chang YH, Kuo PH, Chen SL, Chen SH, Chu CH, Lin WW, Wu PL, Ko HC. Interaction between ALDH2*1*1 and DRD2/ANKK1 TaqI A1A1 genes may be associated with antisocial personality disorder not co-morbid with alcoholism. *Addict Biol.* 2010 Epub ahead of print.
- Maruyama W, Nakahara D, Dostert P, Hashiguchi H, Ohta S, Hirobe M, Takahashi A, Nagatsu T, Naoi M. Selective release of serotonin by endogenous alkaloids, 1-methyl-6,7-dihydroxy-1,2,3,4-tetrahydroisoquinolines, (R)- and (S)salsolinol, in the rat striatum; in vivo microdialysis study. *Neurosci Lett.* 1993; 149:115–118. [PubMed: 8474680]
- Maruyama W, Nakahara D, Ota M, Takahashi T, Takahashi A, Nagatsu T, Naoi M. N-methylation of dopamine-derived 6,7-dihydroxy-1,2,3,4-tetrahydroisoquinoline, (R)-salsolinol, in rat brains: in vivo microdialysis study. *J Neurochem.* 1992; 59:395–400. [PubMed: 1629715]
- Matsuzawa S, Suzuki T, Misawa M. Involvement of mu-opioid receptor in the salsolinol-associated place preference in rats exposed to conditioned fear stress. *Alcohol Clin Exp Res.* 2000; 24:366–372. [PubMed: 10776678]
- McBride WJ, Murphy JM, Ikemoto S. Localization of brain reinforcement mechanisms: intracranial self-administration and intracranial place-conditioning studies. *Behav Brain Res.* 1999; 101:129–152. [PubMed: 10372570]
- McCoy JG, Strawbridge C, McMurtrey KD, Kane VB, Ward CP. A re-evaluation of the role of tetrahydropapaveroline in ethanol consumption in rats. *Brain Res Bull.* 2003; 60:59–65. [PubMed: 12725893]
- McDaid J, McElvain MA, Brodie MS. Ethanol effects on dopaminergic ventral tegmental area neurons during block of I_h: involvement of barium-sensitive potassium currents. *J Neurophysiol.* 2008; 100:1202–1210. [PubMed: 18614756]
- Melchior CL, Myers RD. Preference for alcohol evoked by tetrahydropapaveroline (THP) chronically infused in the cerebral ventricle of the rat. *Pharmacol Biochem Behav.* 1977; 7:19–35. [PubMed: 561961]
- Melis M, Enrico P, Peana AT, Diana M. Acetaldehyde mediates alcohol activation of the mesolimbic dopamine system. *Eur J Neurosci.* 2007; 26:2824–2833. [PubMed: 18001279]
- Messiha FS, Geller I. Effect of noreleagnine, a beta-carboline derivative on ethanol preference in the rat. *Proc West Pharmacol Soc.* 1976; 19:336–340. [PubMed: 996002]
- Myers WD, Gibson S, Ng KT, Singer G. Sex differences in acetaldehyde on body temperature and open-field performance in the rat. *Drug Alcohol Depend.* 1987; 19:1–6. [PubMed: 3816535]
- Myers RD, Melchior CL. Alcohol drinking: abnormal intake caused by tetrahydropapaveroline in brain. *Science.* 1977; 196:554–556. [PubMed: 557839]
- Myers WD, Ng KT, Marzuki S, Myers RD, Singer G. Alteration of alcohol drinking in the rat by peripherally self-administered acetaldehyde. *Alcohol.* 1984; 1:229–236. [PubMed: 6536289]

- Myers RD, Oblinger MM. Alcohol drinking in the rat induced by acute intra-cerebral infusion of two tetrahydroisoquinolines and a beta-carboline. *Drug Alcohol Depend.* 1977; 2:469–483. [PubMed: 562253]
- Myers RD, Privette TH. A neuroanatomical substrate for alcohol drinking: identification of tetrahydropapaveroline (THP)-reactive sites in the rat brain. *Brain Res Bull.* 1989; 22:899–911. [PubMed: 2765949]
- Myers RD, Robinson DE. Tetrahydropapaveroline injected in the ventral tegmental area shifts dopamine efflux differentially in the shell and core of nucleus accumbens in high-ethanol-preferring (HEP) rats. *Alcohol.* 1999; 18:83–90. [PubMed: 10386670]
- Myers RD, Veale WL. Alterations in volitional alcohol intake produced in rats by chronic intraventricular infusions of acetaldehyde, paraldehyde or methanol. *Arch Int Pharmacodyn Ther.* 1969; 180:100–113. [PubMed: 5356997]
- Nakahara D, Maruyama W, Hashiguti H, Naoi M. Characterization of the in vivo action of (R)-salsolinol, an endogenous metabolite of alcohol, on serotonin and dopamine metabolism: a microdialysis study. *Brain Res.* 1994; 644:226–232. [PubMed: 8050034]
- National Institute on Alcohol Abuse and Alcoholism (NIAAA). President's budget request for NIAAA – Director's statement before the house subcommittee on labor-HHS appropriations. 2008. Retrieved from: http://www.niaaa.nih.gov/AboutNIAAA/CongressionalInformation/Testimony/Pages/statement_5_08.aspx
- Okamoto T, Harnett MT, Morikawa H. Hyperpolarization-activated cation current (I_h) is an ethanol target in midbrain dopamine neurons of mice. *J Neurophysiol.* 2006; 95:619–626. [PubMed: 16148268]
- Ortiz A, Griffiths PJ, Littleton JM. A comparison of the effects of chronic administration of ethanol and acetaldehyde to mice: evidence for a role of acetaldehyde in ethanol dependence. *J Pharm Pharmacol.* 1974; 26:249–260. [PubMed: 4151380]
- Padilla-de la Torre M, Franco-Perez J, Santamaria A, Galvan S, Gonzalez E, Paz C. Effect of acetaldehyde on behavioral and neurochemical changes induced by MK-801 in rats. *Ann N Y Acad Sci.* 2008; 1139:259–267. [PubMed: 18991871]
- Peana AT, Enrico P, Assaretti AR, Pulighe E, Muggironi G, Nieddu M, Piga A, Lintas A, Diana M. Key role of ethanol-derived acetaldehyde in the motivational properties induced by intragastric ethanol: a conditioned place preference study in the rat. *Alcohol Clin Exp Res.* 2008; 32:249–258. [PubMed: 18162073]
- Peng GS, Yin SJ. Effect of the allelic variants of Aldehyde dehydrogenase ALDH2*2 and alcohol dehydrogenase ADH1B*2 on blood acetaldehyde concentrations. *Hum Genomics.* 2009; 3:121–127. [PubMed: 19164089]
- Petersen DR, Tabakoff B. Characterization of brain acetaldehyde oxidizing systems in the mouse. *Drug Alcohol Depend.* 1979; 4:137–144. [PubMed: 510163]
- Pikkarainen PH, Salaspuro MP, Lieber CS. A method for the determination of "free" acetaldehyde in plasma. *Alcohol Clin Exp Res.* 1979; 3:259–261. [PubMed: 384838]
- Privette TH, Hornsby RL, Myers RD. Buspirone alters alcohol drinking induced in rats by tetrahydropapaveroline injected into brain monoaminergic pathways. *Alcohol.* 1988; 5:147–152. [PubMed: 3395461]
- Privette TH, Myers RD. Anatomical mapping of tetrahydropapaveroline-reactive sites in brain mediating suppression of alcohol drinking in the rat. *Brain Res Bull.* 1989; 22:1039–1048. [PubMed: 2790497]
- Purvis PL, Hirst M, Baskerville JC. Voluntary ethanol consumption in the rat following peripheral administrations of 3-carboxy-salsolinol. *Subst Alcohol Actions Misuse.* 1980; 1:439–445. [PubMed: 7302792]
- Quertemont E, De Witte P. Conditioned stimulus preference after acetaldehyde but not ethanol injections. *Pharmacol Biochem Behav.* 2001; 68:449–454. [PubMed: 11325398]
- Quertemont E, Grant KA, Correa M, Arizzi MN, Salamone JD, Tambour S, Aragon CM, McBride WJ, Rodd ZA, Goldstein A, Zaffaroni A, Li TK, Pisano M, Diana M. The role of acetaldehyde in the central effects of ethanol. *Alcohol Clin Exp Res.* 2005; 29:221–234. [PubMed: 15714045]

- Quertemont E, Tambour S, Bernaerts P, Zimatkin SM, Tirelli E. Behavioral characterization of acetaldehyde in C57BL/6J mice: locomotor, hypnotic, anxiolytic and amnesic effects. *Psychopharmacology (Berl)*. 2004; 177:84–92. [PubMed: 15160264]
- Quintanilla ME, Tampier L. Acetaldehyde-reinforcing effects: differences in low-alcohol-drinking (UChA) and high-alcohol-drinking (UChB) rats. *Alcohol*. 2003; 31:63–69. [PubMed: 14615012]
- Quintanilla ME, Tampier L, Karahanian E, Rivera-Meza M, Herrera-Marschitz M, Israel Y. Reward and Relapse: Complete Gene-Induced Dissociation in an Animal Model of Alcohol Dependence. *Alcohol Clin Exp Res*. 2011 Epub ahead of print.
- Raskin NH. Editorial: Alcoholism or acetaldehydism? *N Engl J Med*. 1975; 292:422–423. [PubMed: 1110729]
- Reynolds WA, Lowe FH. Mushrooms and a Toxic Reaction to Alcohol: Report of Four Cases. *N Engl J Med*. 1965; 272:630–631. [PubMed: 14255341]
- Rodd ZA, Bell RL, Zhang Y, Goldstein A, Zaffaroni A, McBride WJ, Li TK. Salsolinol produces reinforcing effects in the nucleus accumbens shell of alcohol-preferring (P) rats. *Alcohol Clin Exp Res*. 2003; 27:440–449. [PubMed: 12658109]
- Rodd ZA, Bell RL, Zhang Y, Murphy JM, Goldstein A, Zaffaroni A, Li TK, McBride WJ. Regional heterogeneity for the intracranial self-administration of ethanol and acetaldehyde within the ventral tegmental area of alcohol-preferring (P) rats: involvement of dopamine and serotonin. *Neuropsychopharmacology*. 2005; 30:330–338. [PubMed: 15383830]
- Rodd ZA, Oster SM, Ding ZM, Toalston JE, Deehan G, Bell RL, Li TK, McBride WJ. The reinforcing properties of salsolinol in the ventral tegmental area: evidence for regional heterogeneity and the involvement of serotonin and dopamine. *Alcohol Clin Exp Res*. 2008; 32:230–239. [PubMed: 18162075]
- Rodd-Henricks ZA, Melendez RI, Zaffaroni A, Goldstein A, McBride WJ, Li TK. The reinforcing effects of acetaldehyde in the posterior ventral tegmental area of alcohol-preferring rats. *Pharmacol Biochem Behav*. 2002; 72:55–64. [PubMed: 11900769]
- Rotzinger S, Smith BR, Amit Z. Catalase inhibition attenuates the acquisition of ethanol and saccharin-quinine consumption in laboratory rats. *Behav Pharmacol*. 1994; 5:203–209. [PubMed: 11224269]
- Seutin V, Massotte L, Renette MF, Dresse A. Evidence for a modulatory role of Ih on the firing of a subgroup of midbrain dopamine neurons. *Neuroreport*. 2001; 12:255–258. [PubMed: 11209930]
- Sippel HW. The acetaldehyde content in rat brain during ethanol metabolism. *J Neurochem*. 1974; 23:451–452. [PubMed: 4417541]
- Smith BR, Amit Z, Splawinsky J. Conditioned place preference induced by intraventricular infusions of acetaldehyde. *Alcohol*. 1984; 1:193–195. [PubMed: 6536284]
- Smith BR, Aragon CM, Amit Z. Catalase and the production of brain acetaldehyde: a possible mediator of the psychopharmacological effects of ethanol. *Addiction Biology*. 1997; 2:277–289. [PubMed: 26735784]
- Spina L, Longoni R, Vinci S, Ibba F, Peana AT, Muggironi G, Spiga S, Acquas E. Role of dopamine D1 receptors and extracellular signal regulated kinase in the motivational properties of acetaldehyde as assessed by place preference conditioning. *Alcohol Clin Exp Res*. 2010; 34:607–616. [PubMed: 20102564]
- Stotz E. A colorimetric determination of acetaldehyde in blood. *The Journal of Biological Chemistry*. 1943; 148:585–591.
- Tabakoff B, Anderson RA, Ritzmann RF. Brain acetaldehyde after ethanol administration. *Biochem Pharmacol*. 1976; 25:1305–1309. [PubMed: 938553]
- Tambour S, Didone V, Tirelli E, Quertemont E. Locomotor effects of ethanol and acetaldehyde after peripheral and intraventricular injections in Swiss and C57BL/6J mice. *Behav Brain Res*. 2006; 172:145–154. [PubMed: 16764949]
- Tampier L, Quintanilla ME, Mardones J. Effects of aminotriazole on ethanol, water, and food intake and on brain catalase in UChA and UChB rats. *Alcohol*. 1995; 12:341–344. [PubMed: 7546330]
- Truitt, EB., Jr.; Walsh, MJ. The role of acetaldehyde in the actions of ethanol. In: Kissin, B.; Begleiter, H., editors. *The Biology of Alcoholism*. Plenum Press; New York: 1971.

- Tuomisto L, Airaksinen MM, Peura P, Eriksson CJ. Alcohol drinking in the rat: increases following intracerebroventricular treatment with tetrahydro-beta-carbolines. *Pharmacol Biochem Behav.* 1982; 17:831–836. [PubMed: 7178191]
- Tuomisto L, Tuomisto J. Inhibition of monoamine uptake in synaptosomes by tetrahydroharmine and tetrahydroisoquinoline compounds. *Naunyn Schmiedebergs Arch Pharmacol.* 1973; 279:371–380. [PubMed: 4150087]
- Vengeliene V, Bilbao A, Molander A, Spanagel R. Neuropharmacology of alcohol addiction. *Br J Pharmacol.* 2008; 154:299–315. [PubMed: 18311194]
- Walsh MJ, Davis VE, Yamanaka Y. Tetrahydropapaveroline: an alkaloid metabolite of dopamine in vitro. *J Pharmacol Exp Ther.* 1970; 174:388–400. [PubMed: 4318487]
- Ward RJ, Colantuoni C, Dahchour A, Quertemont E, De Witte P. Acetaldehyde-induced changes in monoamine and amino acid extracellular microdialysate content of the nucleus accumbens. *Neuropharmacology.* 1997; 36:225–232. [PubMed: 9144660]
- Weiner H. Estimation of the in vivo concentration of salsolinol and tetrahydropapaveroline in rat brain after the administration of ethanol. *Subst Alcohol Actions Misuse.* 1980; 1:317–322. [PubMed: 7347923]
- Williams EE. Effects of alcohol on workers with carbon disulfide. *The Journal of the American Medical Association.* 1937; 109:1472–1473.
- World Health Organization (WHO). WHO to meet beverage company representatives to discuss health-related alcohol issues. 2003. Retrieved from: <http://www.who.int/mediacentre/news/releases/2003/pr6/en/>
- Yao L, Fan P, Arolfo M, Jiang Z, Olive MF, Zablocki J, Sun HL, Chu N, Lee J, Kim HY, Leung K, Shryock J, Blackburn B, Diamond I. Inhibition of aldehyde dehydrogenase-2 suppresses cocaine seeking by generating THP, a cocaine use-dependent inhibitor of dopamine synthesis. *Nat Med.* 2010; 16:1024–1028. [PubMed: 20729865]
- Ye JH, Tao L, Ren J, Schaefer R, Krnjevic K, Liu PL, Schiller DA, McArdle JJ. Ethanol potentiation of glycine-induced responses in dissociated neurons of rat ventral tegmental area. *J Pharmacol Exp Ther.* 2001; 296:77–83. [PubMed: 11123365]
- Zakhari S. Overview: how is alcohol metabolized by the body? *Alcohol Res Health.* 2006; 29:245–254. [PubMed: 17718403]
- Zimatkin SM. Histochemical study of aldehyde dehydrogenase in the rat CNS. *J Neurochem.* 1991; 56:1–11. [PubMed: 1987314]
- Zimatkin SM, Liopo AV, Deitrich RA. Distribution and kinetics of ethanol metabolism in rat brain. *Alcohol Clin Exp Res.* 1998; 22:1623–1627. [PubMed: 9835273]

Figure 1.
General schematic representation of the central and peripheral metabolic pathways that function to eliminate alcohol from the human body.

Figure 2.

Maximal dialysate levels of dopamine (DA) in the nucleus accumbens shell (AcbSh) as a result of microinjections of 43 mM ethanol (EtOH), 23 μM acetaldehyde (ACD), or 0.3 μM salsolinol (SAL) into the posterior ventral tegmental area (pVTA),