Acetaldehyde and salsolinol in ethanol's two-step mechanism of action: An overview

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Abstract

In the last years, numerous studies have supported the idea that, at least in part, motivational and neuropharmacological effects of ethanol are mediated by its first brain-derived metabolite, acetaldehyde, and its bioderivate salsolinol. This review aims at gathering and shaping as a whole the evidence on their role in the mechanism of action of ethanol. Acetaldehyde and salsolinol interact with the reward brain system and are involved as *primum movens* of motivational and addictive behaviour that can be especially relevant to ethanol use disorders. Understanding the neurobiology of acetaldehyde and salsolinol holds promising potential for the development of novel pharmacological approaches for reducing ethanol abuse.

Introduction

In the last years, numerous studies have supported the idea that, at least in part, motivational and neuropharmacological effects of ethanol (EtOH) are mediated by its first brain-derived metabolite, acetaldehyde (ACD) and/or its bioderivates, salsolinol (SAL), above all.[1,2] ACD is formed in the brain mainly through a catalase-mediated reaction.[3] SAL, on the other hand, can be formed in the brain through the non-enzymatic condensation of ACD and dopamine (DA).[4] Over the past four decades, several studies have investigated the involvement of ACD and SAL in the behavioural and neurobiological effects of EtOH, and we hypothesise that both compounds play a functional and specific role in the development of EtOH abuse and alcoholism.[5-8]

The aim of this review is to gather and shape as a whole the evidence in support of this hypothesis.

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Key words: ethanol, acetaldehyde, salsolinol, reward.

Contributions: AC, GL preformed the literature search; AC, GL, MG, EC wrote the manuscript draft; AC prepared the figures. All the authors approved the final version of the manuscript.

Conflict of interest: the authors declare no potential conflict of interest.

Received for publication: 14 April 2017.
Revision received: 10 June 2017.
Accepted for publication: 15 June 2017.

Journal of Biological Research 2017; 90:6751
doi:10.4081/jbr.2017.6751

Materials and Methods

The literature search targeted evidence-based guidelines, evidence-based summaries, systematic reviews and recent experimental research on ACD and SAL central and behavioural effects. The keywords used were *ethanol*, *acetaldehyde*, *salsolinol* and *dopaminergic pathway.* Through this simple strategy, we identified more than 1000 sources using PubMed and Scopus (last accessed via PubMed and SCOPUS on April 2017).

From EtOH to ACD

ACD is produced in the human body after the consumption of EtOH in a tissue-specific fashion,[9-11] and occurs naturally in alcoholic beverages. Indeed, substantial ACD concentrations have been detected in several products, accounting for apple wines and ciders, fortified wines and spirits such as sugarcane spirits (cuxa; cachaça), agave spirits and calvados,[12-18] to which it gives a distinctive flavour.

It is widely recognised the relevance of EtOH chemosensory stimuli in eliciting craving and associated drug-seeking responses in EtOH-experienced individuals.[19,20] Indeed, EtOH and ACD gain immediate access to the central nervous system via their complex chemosensory attributes. Importantly, these sensory pathways are linked to limbic forebrain and cortical areas involved in controlling motivation and feeding.[21-23] Whatever its source, either as original substance or as EtOH bioderivate, ACD possesses stimulating effects on some areas of the reward pathway in the brain, *i.e.*, ventral tegmental area (VTA) and nucleus accumbens (NAc), leading to DA release, positive reinforcement and induction of dependence.[24-27]

In the intracranial self-administration paradigm, whereby rats receive response-contingent infusions of a compound directly into a discrete brain region, rats readily self-administer ACD into the VTA.[28,29] Specifically within the VTA, ACD is able to activate DA neurons by significantly increasing their firing rate, similarly to

EtOH.[30-33] Moreover, DA neurons within the posterior VTA (pVTA) exhibit a significantly greater sensitivity to ACD compared to EtOH so that 23μM ACD is effective at significantly increasing DA efflux within the NAc shell to levels 200% above baseline.[28]

The neurochemical feature underlying ACD availability in the VTA is paralleled by behavioural evidence of ACD's own reinforcing properties in the conditioned place preference (CPP), a behavioural paradigm widely used to explore rewarding effects of drugs.[34] A high preference for ACD-paired cues is observed when ACD is administered both intraperitoneally and orally.[35,36] Although place preference is suggestive of drug-associated reinforcement, it focuses on automatic or implicit expressions of reward, rather than on active motivated behaviour. Besides, report exists on the evaluation of acquisition and maintenance of ACD drinking behaviour in self-administration paradigms in rats. As EtOH, rats voluntarily self-administer ACD in a two-bottle choice drinking-paradigm; moreover ACD intake increases when higher concentration is provided.[37-40] The flavour and taste of ACD solution may actually serve as conditioned stimuli of post-ingestional effects.[41]

Positive reinforcing properties of ACD have been further investigated by using operant self-administration paradigms, in which animals are trained to emit a specific response (lever press or nose poke) for gaining the reinforcement.[42] ACD shows reinforcing effects at concentrations 1000 lower than EtOH.[28,43] When introduced by the natural oral route, ACD is reported to induce and maintain operant drinking behaviour according to fixed and progressive ratios of reinforcement.[6,44] In addiction research, the operant conditioning paradigm has always been considered an invaluable tool, since it allows to thoroughly explore discrete features of addictive behaviour, as reported for humans in the Diagnostic and Statistical Manual of Mental Disorders - 5th edition.[45] Indeed, ACD-drinking rats display resistance to extinction when reinforce delivery is withheld, and a powerful deprivation-effect when ACD availability is resumed after repeated cycles of deprivation.[6,7,44,46] Notably, evidence from the operant-conflict paradigm has shown that the operant response for ACD persists also in the presence of an aversive stimulus,[6,46] further highlighting the motivational effect of the compound.

Although Peana *et al.*[44] reported that brain ACD levels do not significantly differ between rats consuming oral ACD and those consuming water in their experimental conditions, recent evidence shows a significant increase in ACD brain content when ACD is introduced by a free-access paradigm. Indeed, following a 4-week two-bottle choice paradigm with ACD at 3.2% v/v, ACD concentration in the brain is increased by 29.52% with respect to control levels.[37] The discrepancy may be due to the different ACD drinking pattern and to the detection technique itself. In the ACD free-access paradigm, ACD is consumed chronically and continuously on rat's demand, producing higher blood levels than in the operant-drinking sessions, that may overcome ACD-dehydrogenase activity and cross the blood-brain barrier. Moreover, gas chromatography with headspace, although specific for aldehyde detection, might display poor sensitivity for low concentration of analytes, probably making ACD detection awkward. Dinitrophenylhydrazine-acetonitrile derivatization instead, could overcome these limits. Indeed, using this technique, extraction and purification are unnecessary, making the procedure simple, rapid and accurate, allowing to measure subtle but significant variations in ACD levels in the brain.[47-49]

ACD interaction with the reward system legitimises its involvement as *primum movens* of motivational and addictive behaviour that can be especially relevant to EtOH use disorders.

DA plays a fundamental role in the expression of operant behaviour elicited by rewards and reward-related stimuli. Importantly, ACD induces DA release in the NAc shell at the same doses used in CPP studies.[32,36,50-52] Consequently, when quinpirole is used to activate presynaptic D2 autoreceptors, thus reducing ACD-induced DA release, a profound inhibition of seeking-behaviour for ACD occurs.[7,28] In accordance with chronic EtOH-induced down-regulation of DA signalling in the limbic regions,[53] subchronic stimulation of postsynaptic D2/D3 receptors, by the administration of ropinirole during ACD deprivation, turns off rats craving and inhibits relapse when ACD is available.[7]

Along with DA transmission, the endocannabinoid system plays an important role in value attribution processing and in modulation of EtOH-seeking behaviour,[54-56] in view of its role as fine modulator of incoming inputs within the limbic brain regions.[57-61] Indeed, ACD-seeking behaviour and punishment resistance in the operant-conflict paradigm, and withdrawal symptoms following ACD intoxication, are blunted when CB1 signalling is inhibited by the administration of a CB1 specific antagonist AM281.[46,62] Overall this evidence suggests that ACD reinforcing activity involves endocannabinoids production, which in turn, modulates DA mesocorticolimbic pathway through CB1 receptors (Figure 1). Hence, the pharmacological inhibition of CB1 signalling might represent a promising strategy for counteracting the neurochemical imbalance associated with ACD- and EtOH- addictive behaviour.

From ACD to SAL

Pre-clinical studies considered so far have shown that ACD is a neuroactive molecule with its own psychopharmacological properties, that can be considered as a necessary component for the occurrence of the neurobiological and behavioural effects of EtOH.[63] Despite of its short half-life,[63,64] ACD may condensate, either spontaneously or enzymatically, with nucleophilic compounds, such as monoamines, to produce tetrahydroisoquinolines[65]. When condensation occurs with DA, ACD generates 1-methyl-6,7-dihydroxy-1,2,3,4-tetrahydroisoquinoline, SAL. In particular SAL is formed either by non-enzymatic Pictet-Spengler condensation with ACD, yielding racemic (R/S)-SAL, or through enzymatic biosynthesis by (R)-SAL synthase, which enantio-selectively synthesizes (R)-SAL from DA and ACD.[66-69]

Endogenous identification of SAL has been analytically challenging because of its very low levels in the brain.[70] However the implementation of sensitive, reliable, and versatile methods for SAL analysis in the brain,[69] has helped proving that SAL content increases in several brain regions (NAc, caudate putamen, midbrain, hypothalamus) after very different alcohol drinking procedures.[69,71-73] Interestingly, under EtOH and DA co-application higher levels of SAL are determined in slices from naïve mice.[74] Conversely, SAL itself can promote EtOH drinking: this has been proved by early findings in the rat and corroborated by evidence from primate studies showing abnormal alcohol intake produced by centrally infused SAL.[75,76] The observed reciprocal interaction supports the theory that amine-aldehyde metabolites may constitute a causal neurochemical factor in the onset of the rewarding properties of EtOH and in the development of EtOH addiction.[77,78]. Behavioural observations in rats following direct injection of only 30 pmol SAL into the VTA report behavioural sensitisation, strong motor activity and significant increases in voluntary EtOH consumption.[79] Moreover, significant place preference is induced by SAL, given either intraperitoneally or by local micro injection into the pVTA.[80,81] Notably, rats readily self-administer SAL into the NAc shell and pVTA,[29,43] suggesting that SAL itself may act as a reinforcer in the mesolimbic system. Indeed, in a recent elegant study, Melis and colleagues[74] found that, similarly to EtOH and ACD, SAL significantly stimulates the firing rate of

DA cells in the pVTA. Specifically, the onset of the effects of EtOH, ACD and SAL is similar and EtOH derivatives reveal overlapping dose-response curves.[74] This is in accordance with previous studies showing that SAL stimulates DA release in the pVTA in a inverted U-shape manner, showing a peak DA efflux (up to 300% of baseline) and a significantly low response at 3μM.[33] SAL reinforcing effects are thus mediated by activation of DA neurons and are associated with enhanced DA levels in the ipsilateral NAc shell since, similarly to ACD, co-infusion of quinpirole reduces SAL reinforcing effects.[29] Recent studies have postulated that the stimulatory action of SAL on the firing rate of DA cells might be due to activation of the opioid system.[81-84] Indeed SAL is a morphine-like alkaloid, and can generate motivational effects through its binding to μ opioid receptors (MORs).[80] In confirmation, preclinical studies report that SAL-induced CPP, consequently to its systemic administration, is blocked by naloxone, a nonspecific MORs antagonist, while SAL-induced locomotor stimulation is attenuated by the administration of b-funaltrexamine, a selective MORs antagonist.[79,80] Furthermore, CPP and EtOH intake are completely blocked by naltrexone administration into the pVTA, as reported recently by Quintanilla and colleagues.[85] Altogether these findings suggest that SAL addictive-like behavioural effects are mediated through opioidergic modulation in the reward pathway, resulting in suppression of GABAergic inhibition, and concurrent stimulation of excitatory afferents (Figure 2). Given that EtOH acute actions on spontaneous activity of DA neurons might be the net effect resulting from complex synaptic changes at both inhibitory and excitatory inputs integrated with cell membrane properties,[86,87] SAL activity at the molecular level provides new insights to look into the neurobiological basis of alcoholism and suggests exciting avenues of future research.

Conclusions

Recurring theories in the EtOH field pinpoint the attention at EtOH's active metabolites/products as main players of the its reinforcing properties. The interaction of ACD and its condensate product, SAL, with the DA system strongly supports the development of discrete features of addictive behaviour. However, several questions on this matter deserve further study. For instance, the effects of ACD exposure during the developmental period are largely unexplored. In humans, prenatal EtOH consumption may cause several neurodevelopmental defects that could be due to ACD formation. Indeed, EtOH can readily cross the placental barrier and blood-brain-barrier, and in the developing rat brain, catalase plays an important role in ACD formation. The formation of ACD in the fetal rat brain, in turn, contributes to the production of elevated levels of SAL and other alkaloids.[48] Since the developing central nervous system is extremely sensitive to pharmacological and environmental manipulations,[88-94] increasing

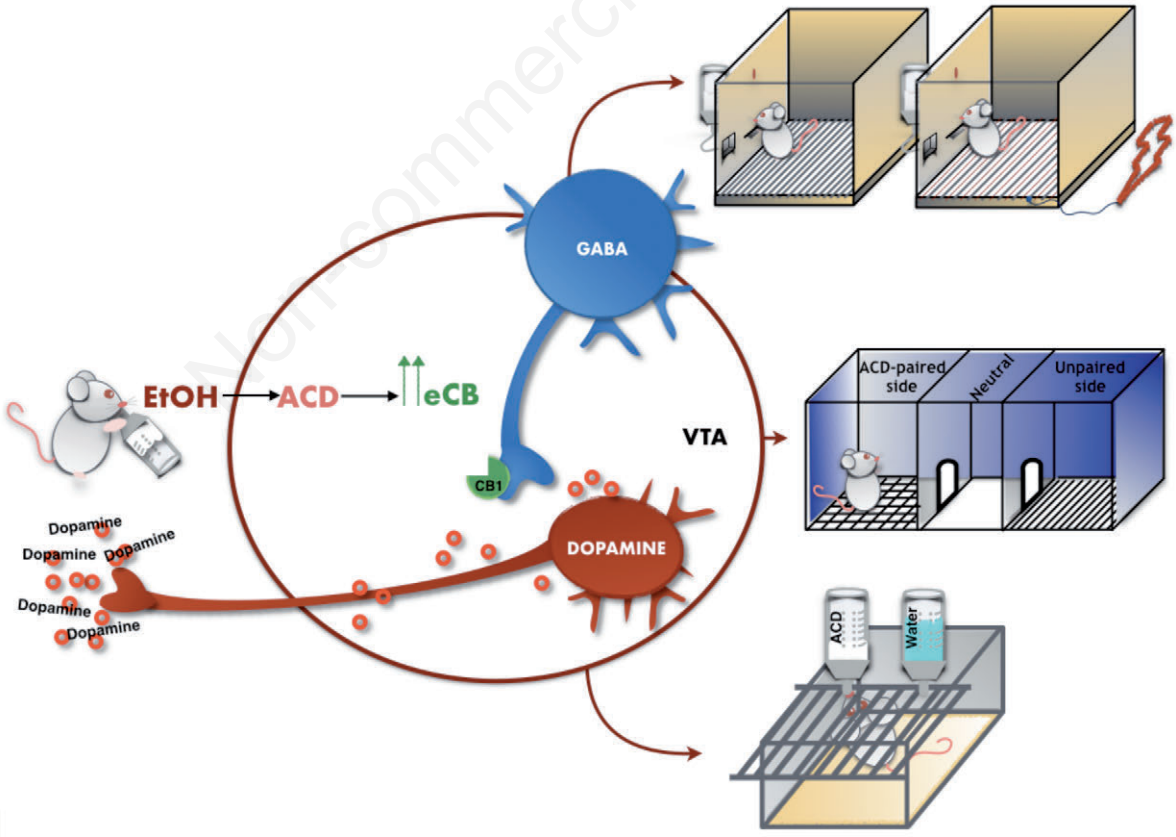

Figure 1. Schematic representation of acetaldehyde's mechanism of action and effects. Acetaldehyde potentiates the endocannabinoidergic tone, thus increasing dopamine release from the ventral tegmental area. Acetaldehyde has motivational properties and behavioural effects, measurable through operant responding, operant-conflict paradigm, conditioned place preference and free-access paradigm.

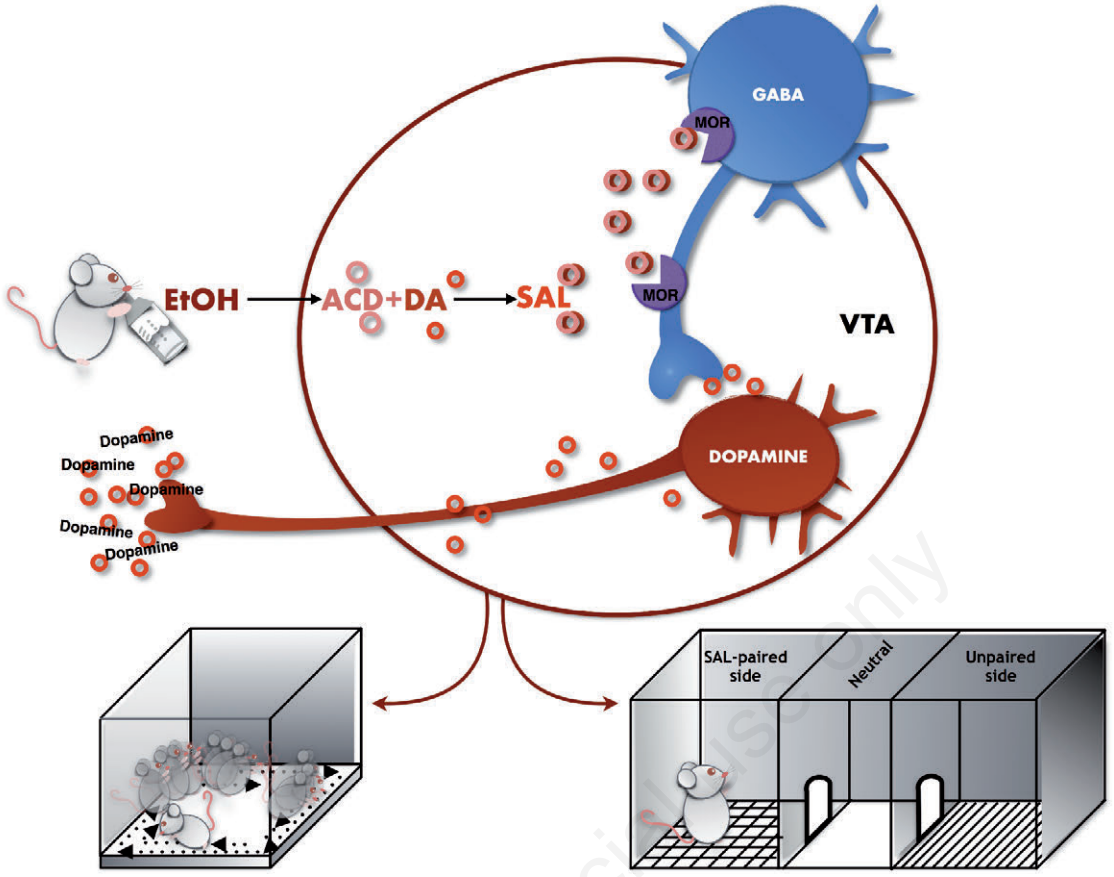

Figure 2. Schematic representation of salsolinol's mechanisms on activation of MORs on the GABAergic neurons and/or their afferents and salsolinol's behavioural effects and motivational properties in conditioned place preference.

attention must be paid to assess the consequences of perinatal ACD and SAL exposure, that could be enduring and outlast adolescence and adulthood. Given the paucity of data on this topic, this review is also to be intended as a spur to thoroughly evaluate ACD and SAL as strong contributors to EtOH two-step molecular activity in the developing brain, and the related addictive phenotypes later in life.

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