

# From Ethanol to Salsolinol: Role of Ethanol Metabolites in the Effects of Ethanol

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**ABSTRACT:** In spite of the global reputation of ethanol as the psychopharmacologically active ingredient of alcoholic drinks, the neurobiological basis of the central effects of ethanol still presents some dark sides due to a number of unanswered questions related to both its precise mechanism of action and its metabolism. Accordingly, ethanol represents the interesting example of a compound whose actions cannot be explained as simply due to the involvement of a single receptor/neurotransmitter, a scenario further complicated by the robust evidence that two main metabolites, acetaldehyde and salsolinol, exert many effects similar to those of their parent compound. The present review recapitulates, in a perspective manner, the major and most recent advances that in the last decades boosted a significant growth in the understanding on the role of ethanol metabolism, in particular, in the neurobiological basis of its central effects.

**KEYWORDS:** ethanol, acetaldehyde, salsolinol

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## Introduction

Ethanol is the main psychopharmacologically active ingredient of alcoholic drinks and, accordingly, is recognized as the compound potentially responsible for alcohol use disorders (AUDs), as defined by the *Diagnostic and Statistical Manual of Mental Disorders* (fifth edition),<sup>1</sup> as well as for a number of alcohol-related chronic diseases that heavily affect individuals and society. In Europe, AUDs and AUD-related pathologies (mostly liver, cardiological, and neurological disorders) affect over 4 million people, costing roughly 740 million euros in terms of direct and indirect costs,<sup>2</sup> whereas in the USA, these numbers roughly approximate to 16.3 million people<sup>3</sup> and 223.5 billion USD.<sup>4–6</sup> Consequently, understanding the neurobiological basis of AUDs still represents a main challenge for the scientific community devoted to disclose the mechanisms at the basis of the ability of this simple compound to alter behavior. The disclosure of its mechanism(s) of action could allow the progress toward the availability of new medications useful to prevent and treat AUDs.

Interestingly, although the molecular target(s) of ethanol have not yet been precisely determined, a large body of literature refers to its central effects as primarily due to an action onto GABA<sub>A</sub> receptors, whereby the acute action of ethanol results in a facilitation of inhibitory conductance mediated by this receptor channel.<sup>7</sup> Moreover, it also acts

onto N-Methyl-D-Aspartate (NMDA) glutamate receptors, whereby ethanol acutely inhibits excitatory currents mediated by NMDA–Ca<sup>2+</sup> channels.<sup>8</sup> In addition to these mechanisms, the indirect involvement of the opioid,<sup>9,10</sup> adenosine,<sup>11</sup> and mesolimbic dopaminergic<sup>12</sup> systems has become acquainted with some of the neurochemical,<sup>13–15</sup> behavioral,<sup>9</sup> and motivational<sup>16</sup> acute effects of ethanol.

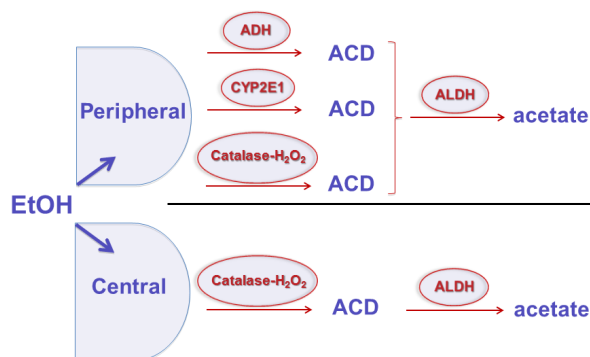
This review focuses on the role of peripheral and central metabolism of ethanol and on the role of its main biologically active metabolites, acetaldehyde and salsolinol, in the acute actions of their parent compound. A particular emphasis will be placed on the effects that acetaldehyde and salsolinol exert on the mesolimbic dopaminergic system as they appear rich in critical implications in the motivational properties of ethanol itself and represent a still promising pathway of investigation toward the understanding of the neurobiological basis of AUDs and the discovery of suitable targets for its pharmacotherapy.

## Peripheral Metabolism of Ethanol

The metabolic disposal of ethanol in the body, which yields acetaldehyde and acetate, may take place through different oxidative pathways depending on the differential expression of the involved enzymes in different organs. The main metabolic pathway responsible for ethanol metabolism is represented by type II alcohol dehydrogenase (ADH), the other isoforms of ADH

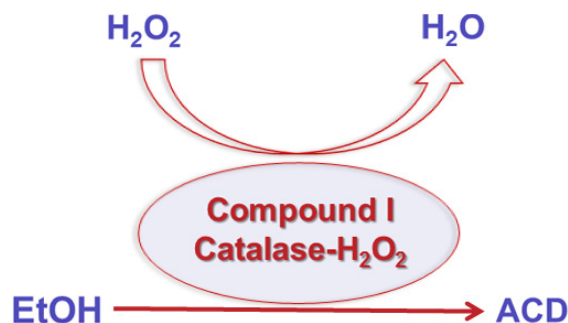
(I, III, and IV) being mostly active on alcoholic substrates with a different structure and/or longer aliphatic chains.<sup>17</sup> ADH is a nicotinamide adenine dinucleotide-dependent cytosolic enzyme that may be distinguished in different isoforms as a function of their subunit composition<sup>17</sup> and whose efficiencies may differ significantly. Accordingly, although after oral ethanol ingestion ADH represents the first significant exposure of ethanol to the enzymes responsible for its metabolism, the low efficiency of gastric enzymes and the high expression of ADH in the liver make hepatic metabolism the main oxidative pathway responsible for its disposal. Some exceptions, however, do exist. In fact, gastric ADH in most cases is a poorly efficient isoform (ie, works under a first-order kinetic until the substrate reaches a saturating concentration at which the enzyme switches to a zero-order kinetic) unless factors such as gender (females express more efficient gastric ADH isoforms than males) and fasting (stomach emptiness elicits enzymatic induction) increase their metabolic rate.<sup>17</sup> Moreover, genetic differences are critical in understanding the consequences of ethanol metabolism on its effects. Thus, type 2 ADH (ADH2\*2) is a highly efficient variant of ADH abundantly expressed in individuals of the Asian population that accounts for their high susceptibility to the aversive effects of acetaldehyde. In addition, in these subjects, an increase in acetaldehyde in plasma following ethanol ingestion is sustained and prolonged by the simultaneous expression of a poorly efficient isoform of aldehyde dehydrogenase (ALDH), the enzyme responsible for the oxidation of acetaldehyde into acetate. Therefore, Asians are generally more sensitive to the toxic effects of acetaldehyde as a consequence of the simultaneous presence of a more efficient enzymatic conversion of ethanol into acetaldehyde and of a less efficient conversion of acetaldehyde into acetate (Fig. 1).<sup>17</sup>

However, although ethanol metabolism has been conventionally viewed as carried out by liver ADH1 (class I), it has to be taken into account that other pathways, in particular in the presence of high blood ethanol levels or high drinking, may



**Figure 1.** Schematic representation of the main, peripheral and central, metabolic pathways by which ethanol is oxidized into acetaldehyde and acetate.

**Abbreviations:** ACD, acetaldehyde; EtOH, ethanol; ADH, alcohol dehydrogenase; ALDH, aldehyde dehydrogenase; CYP2E1, cytochrome P<sub>450</sub>, isoform 2E1.



**Figure 2.** Schematic representation of the catalase–H<sub>2</sub>O<sub>2</sub> metabolic pathway by which ethanol is oxidized into acetaldehyde.

**Abbreviations:** ACD, acetaldehyde; EtOH, ethanol.

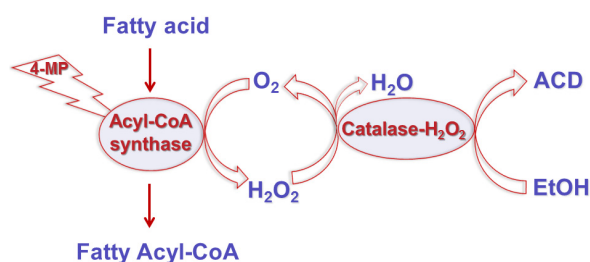
also play an important role in ethanol peripheral metabolism. Thus, besides ADH1, other components of the enzymatic machinery responsible for ethanol oxidation such as the Nicotinamide Adenine Dinucleotide Phosphate Hydrogen (NADPH)-dependent microsomal ethanol oxidizing<sup>18</sup> and the catalase–H<sub>2</sub>O<sub>2</sub> systems should be taken into account, although their contributions have yet to be fully clarified. In particular, in the microsomal ethanol oxidizing pathway, resident in the smooth endoplasmic reticulum and involving the 2E1 isoform of cytochrome P450, ethanol is converted into acetaldehyde by the redox reaction in which O<sub>2</sub> is reduced to form H<sub>2</sub>O, whereas in the catalase–H<sub>2</sub>O<sub>2</sub> system, ethanol is oxidized into acetaldehyde within the peroxisomes<sup>19</sup> by taking H<sub>2</sub>O<sub>2</sub> as a co-substrate to form compound I (Fig. 2).<sup>20</sup>

Moreover, Haseba and Ohno<sup>21</sup> reported that ADH3 (class III) may contribute to peripheral ethanol metabolism, thereby diminishing the consequences of acute ethanol intoxication and eventually supporting the ADH1-mediated ethanol metabolic disposal.<sup>21</sup>

### Peripheral Ethanol Metabolism: Beyond ADH

Inhibition of (peripheral) ADH has been described to decrease brain acetaldehyde concentration, and this is contrary to what would be predicted since, not being disposed in the periphery, ethanol would be expected to reach the brain in greater amounts. As a consequence, also acetaldehyde concentrations, following ethanol's cerebral metabolism, should be expected to be increased. In this regard, Bradford et al in 1993 hypothesized that the contribution of ADH to ethanol metabolism may have been overestimated.<sup>22,23</sup> In fact, administration to rats of 4-methylpyrazole (4-MP),<sup>24</sup> a potent inhibitor of ADH, resulted in a very low rate of ethanol elimination, suggesting that ADH could not be the main or the only pathway responsible.<sup>25</sup> This conclusion, however, was partly in contrast with the clinical observation that 4-MP inhibits the oxidation of ethanol in humans.<sup>26</sup>

Remarkably, in this regard, 4-MP has also been shown to inhibit Acyl-CoA synthase,<sup>22,23</sup> an enzyme essential to initiate the process of fatty acid oxidation (Fig. 3). Thus, by blocking



**Figure 3.** Schematic representation of the mechanism by which 4-methyl pyrazole may interfere with catalase–H<sub>2</sub>O<sub>2</sub>-mediated ethanol metabolism. 4-Methyl pyrazole may inhibit the acylation of fatty acids by inhibiting Acyl-CoA synthase and reducing H<sub>2</sub>O<sub>2</sub> availability for catalase–H<sub>2</sub>O<sub>2</sub>-mediated ethanol metabolism.

**Abbreviations:** 4-MP, 4-methyl pyrazole; ACD, acetaldehyde; EtOH, ethanol.

fatty acid oxidation, the generation of H<sub>2</sub>O<sub>2</sub> in the peroxisomes is prevented and liver catalase is indirectly inhibited.<sup>22,23,27</sup>

In agreement with this possibility, Handler et al<sup>28</sup> suggested that liver catalase plays a critical role in the conversion of ethanol into acetaldehyde. This observation indicates that hepatic ethanol metabolism may be mediated predominantly by catalase–H<sub>2</sub>O<sub>2</sub><sup>22,23,27</sup> and also suggests that simultaneous inhibition of peripheral ADH and peripheral catalase–H<sub>2</sub>O<sub>2</sub> could result in the almost quantitative prevention of ethanol metabolism.

Interestingly, the possibility that peripherally produced acetaldehyde may have a significant role in the central effects of ethanol has long been questioned also on the basis of the observation that it may poorly cross the blood–brain barrier (BBB).<sup>29–31</sup> However, this issue has been settled by the observation that ALDH highly expressed in the endothelial cells of the BBB may allow the distribution of acetaldehyde into the brain only in its presence at saturating concentrations (transforming the BBB into the so-called *enzymatic barrier* for acetaldehyde). In addition, it should be kept in mind that the experiments with the use of peripheral inhibitors of ethanol metabolism suffer of the following intrinsic limitations: first, the blockade of ADH or interference with liver catalase–H<sub>2</sub>O<sub>2</sub> system may determine a reduction in blood ethanol-derived acetaldehyde,<sup>32</sup> therefore, not available to eventually exert its peripheral and/or central effects, and second, the blockade of ADH or interference with liver catalase–H<sub>2</sub>O<sub>2</sub> system may also (and simultaneously) determine an increase in ethanol availability for its central actions.

### Central Metabolism of Ethanol

**Brain catalase.** In the central nervous system also, the enzyme catalase takes H<sub>2</sub>O<sub>2</sub> as a co-substrate to form compound I (Fig. 2),<sup>20</sup> which is the main metabolic intermediate for the metabolism of ethanol.<sup>33,34</sup> The distribution of brain catalase expression has been investigated by the immunohistochemical approach disclosing that catalase is localized mainly in the body of catecholaminergic neurons of the midbrain and

of the brain stem.<sup>35,36</sup> Brain catalase staining appears weaker than that in the liver and its distribution appears overall limited as compared to ALDH staining which, in contrast, is widely expressed in a number of brain structures.<sup>33,37</sup> However, acetaldehyde is still generated locally in pharmacologically significant amounts.<sup>33</sup> Accordingly, when brain catalase activity is impaired either pharmacologically<sup>38,39</sup> and genetically,<sup>40</sup> acetaldehyde production from brain homogenates incubated with ethanol is significantly reduced. This confirms that compound I (Fig. 2) is the intermediate which is also critically implicated in the central production of acetaldehyde; hence, for this reason, it is also realistic to admit that both the catalase–H<sub>2</sub>O<sub>2</sub> system and the cellular bioavailability of H<sub>2</sub>O<sub>2</sub> (Fig. 3) may determine the rate of acetaldehyde formation.<sup>41–43</sup>

**Detection of acetaldehyde.** Over the years, a number of experimental issues (methodological and related to limits of detection) have made difficult to gain the present critical understanding on the neurophysiological and neurochemical properties of acetaldehyde and on its role in the central effects of ethanol. The first of these critical issues was related to the quantitative measurements of acetaldehyde in the blood and, especially, in the brain. Jamal et al<sup>44</sup> provided a procedure to detect acetaldehyde in living animals by in vivo brain microdialysis.<sup>44</sup> However, the detection of acetaldehyde was limited to the condition in which its metabolism was inhibited by the ALDH inhibitor, cyanamide. The second critical issue was represented by acetaldehyde's redox transformations. In fact, besides its prompt liver and brain oxidation by ALDH2 into acetate, acetaldehyde can also be removed as a consequence of its reduction into ethanol by liver ADH.<sup>33</sup> Moreover, another critical issue, representing a limiting factor for the detection of acetaldehyde in the periphery and in the brain, is related to the fact that this chemical retains a very short (minutes) plasmatic elimination half-life<sup>45–47</sup> due, at least partly, to its high electrophilic reactivity and, therefore, to its ability to bind to nucleophilic structures and give condensation products or adducts.<sup>48–52</sup>

### Role of Acetaldehyde in the Motivational Effects of Ethanol and Involvement of the Dopaminergic System

Converging evidence attributes to either peripheral or central metabolism of ethanol into acetaldehyde a critical role in many of ethanol's central effects, including the ability to affect motivation.<sup>50,53</sup> Notably, while the peripheral metabolism of ethanol into acetaldehyde may result in central effects depending on the conditional capability of acetaldehyde to cross the BBB,<sup>37</sup> its central metabolism mostly depends on the activity of catalase–H<sub>2</sub>O<sub>2</sub> system.<sup>38</sup>

Both ethanol-derived acetaldehyde and acetaldehyde on its own have been shown to involve the mesolimbic dopaminergic system in their central effects. Evidence, in this regard, originated from the observation that, similarly to ethanol,<sup>54</sup> orally administered acetaldehyde can also stimulate

spontaneous firing of dopaminergic neurons in the ventral tegmental area (VTA),<sup>55</sup> a finding also in agreement with the observation that acetaldehyde stimulates dopamine transmission in the nucleus accumbens as determined by in vivo brain microdialysis.<sup>56,57</sup> Furthermore, indirect evidence of the involvement of the mesolimbic dopaminergic system in the effects of either acetaldehyde or ethanol-derived acetaldehyde (as demonstrated by the combined administration of ethanol and 4-MP or D-penicillamine, a compound able to sequester acetaldehyde) arose from the studies reporting their ability to elicit the activation of the extracellular signal-regulated kinase pathway<sup>58,59</sup> in the nucleus accumbens.<sup>60</sup>

Until now, at least the following four distinct pieces of evidence support the role of ethanol-derived acetaldehyde in these effects of ethanol:<sup>50,53</sup>

- (i) the demonstration that the effects of peripherally administered ethanol may be prevented by drugs able to contrast peripheral ethanol metabolism (ADH inhibitors);<sup>32,55,56,60</sup>
- (ii) the demonstration of the ability to prevent the effects of systemic administration of ethanol by drugs that inhibit and/or interfere with catalase-H<sub>2</sub>O<sub>2</sub> system;<sup>41-43,61</sup>
- (iii) the demonstration that a reduction in acetaldehyde availability by sequestering drugs could produce a reduction in the effects of ethanol either after its peripheral administration or after its central administration;<sup>32,61-67</sup>
- (iv) the demonstration that acetaldehyde exerts effects similar to those of ethanol after its peripheral<sup>32,60,65,68-71</sup> or local, intracerebral,<sup>72-75</sup> administration.

Thus, based on these premises, a number of studies on the role of acetaldehyde in the motivational effects of ethanol support the suggestion of a critical role played by acetaldehyde, both on its own and as *ethanol-derived*. These studies were mainly performed, in rats, after ethanol or acetaldehyde non-contingent (intra-gastric) administration by conditioned place preference (CPP)<sup>32,56,71</sup> and after oral operant ethanol and/or acetaldehyde self-administration<sup>43,61,65,66,68,76</sup> experiments. Remarkably, in CPP experiments, the doses of acetaldehyde and ethanol were of 20 mg/kg and 1 g/kg, respectively, indicating that acetaldehyde could be 50-fold more potent than ethanol. Similarly, acetaldehyde was reported to determine CPP after intraperitoneal<sup>69</sup> and intra-gastric administrations<sup>32,56</sup> as well as after ICV infusion.<sup>70</sup> Remarkably, the inhibition of ADH and the reduction of acetaldehyde bioavailability were reported to similarly reduce the acquisition of ethanol-induced CPP.<sup>32</sup>

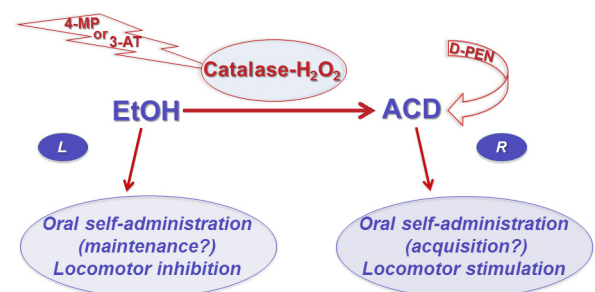
Other studies further clarified the role of ethanol-derived acetaldehyde in the motivational effects of ethanol, as determined by CPP experiments in rats of two lines selectively bred for their high (UChB) or low (UChA) voluntary ethanol intake.<sup>77</sup> In these experiments, in ethanol-naive animals of both lines, ethanol was reported to elicit a conditioned place aversion that was reverted to CPP in UChB rats by a

period of voluntary, free-choice intake of ethanol, whereas in UChA rats, in order to facilitate the development of ethanol-elicited CPP, both a period of forced ethanol drinking and the administration of 4-MP were necessary. This suggested that in UChA rats, the possibility to acquire ethanol-elicited CPP was dependent upon prior ethanol exposure and upon reduction in high blood ethanol-derived acetaldehyde.<sup>77</sup>

In operant experiments, on the other hand, acetaldehyde was reported to be self-administered orally<sup>65,78</sup> or intracerebroventricularly by ethanol-naive rats<sup>72-74</sup> into the VTA by alcohol-preferring (P) rats,<sup>79</sup> as well as intravenously.<sup>75</sup> In particular, our recent studies have pharmacologically characterized acetaldehyde self-administration by showing that it could be prevented by the blockade of  $\mu$  opioid receptors, with both naltrexone and naloxonazine,<sup>68</sup> and by the sequestering agent L-cysteine.<sup>80</sup>

### Acetaldehyde in the Maintenance Phase and the *First Hit* Hypothesis

A more recent and refined research on the role played by acetaldehyde in the ability of ethanol to elicit and maintain its self-administration disclosed that in ethanol-naive rats, acetaldehyde plays a critical role as *first hit* in the acquisition of self-administration.<sup>61,81</sup> In fact, the initial development of reinforcement (first hit) during the acquisition of voluntary ethanol intake and of the operant behavior could be prevented by reducing the generation of brain acetaldehyde,<sup>40,61,82-84</sup> by increasing its degradation,<sup>85</sup> or also by sequestering it with D-penicillamine or L-cysteine.<sup>61,64,66,67,76</sup> This research, focused on the role of acetaldehyde in the *initiation* (eg, early acquisition) phase, strikingly contrasts with the studies aimed at characterizing the role of acetaldehyde in other late/chronic phases, such as long-term maintenance, but not reinstatement upon deprivation, which can be considered as a short-term reacquisition phase of the self-administration behavior (Fig. 4, path R).



**Figure 4.** Schematic representation of the behavioral consequences of the pharmacological manipulation of the catalase-H<sub>2</sub>O<sub>2</sub>-mediated ethanol metabolism (R) or of acetaldehyde bioavailability (R). Path L indicates the effects of (non-metabolized) ethanol; path R indicates the effects of both interference (4-MP or 3-AT) with catalase-H<sub>2</sub>O<sub>2</sub>-mediated acetaldehyde production and reduction (D-PEN) of ACD bioavailability.

**Abbreviations:** 4-MP, 4-methyl pyrazole; 3-AT, 3-amino-1,2,4-aminotriazole; ACD, acetaldehyde; D-PEN, D-penicillamine; EtOH, ethanol.

Accordingly, in the maintenance phase, when a stable chronic ethanol intake, eg, operant ethanol self-administration, has already been reached, the reduction in acetaldehyde generation,<sup>61,86–88</sup> the increase in acetaldehyde degradation,<sup>85</sup> and the administration of acetaldehyde-sequestering agents<sup>61</sup> were not able to decrease the persistent ethanol intake. Thus, chronic operant ethanol intake (maintenance upon operant behavior) seems to become independent from the critical presence of acetaldehyde (Fig. 4, path R) as in the initial reinforcing mechanism(s) responsible for the *first hit* (Fig. 4, path L).<sup>61,81,87</sup> Notably, maintenance is characterized by on-going oral ethanol self-administration in which rats have reached a stable baseline rate of ethanol intake. During this phase, however, acetaldehyde might also, although indirectly, contribute by a combined mechanism: on one hand, its lack might result in further ethanol seeking and taking<sup>61</sup> and, on the other hand, the inhibition of catalase-mediated ethanol metabolism by 3-amino-1,2,4-aminotriazole (3-AT) might release the action of a non-metabolized fraction of ethanol onto GABA<sub>A</sub> receptors, as suggested for other central effects of ethanol by Marti-Prats et al<sup>89,90</sup> that might result in further perpetuating ethanol intake (maintenance)<sup>61</sup> (Fig. 4, path L).

Interestingly, in this regard, recent fascinating studies have shown that ethanol-derived acetaldehyde or acetaldehyde on its own may possess opposite effects, with respect to ethanol, after its intraperitoneal administration<sup>91</sup> or intra-VTA.<sup>89,90</sup> In particular, brain-generated acetaldehyde, after intra-VTA ethanol administration, may be involved in the stimulant effects of ethanol (ethanol-induced locomotion) via a  $\mu$  opioid receptor-mediated mechanism<sup>89</sup> (Fig. 4, path R), whereas, in contrast, the nonbiotransformed fraction of ethanol, acting through the GABA<sub>A</sub> receptors, might account for the locomotor depressant effects<sup>89,90</sup> (Fig. 4, path L). Similarly, peripheral administration or accumulation of acetaldehyde produces anxiogenic effects and induces endocrine stress responses.<sup>91</sup>

Consequently, when we state that ethanol-derived acetaldehyde could be involved in the motivational properties of ethanol, we should not exclude the possibility that a non-metabolized fraction of ethanol could act, for instance, onto GABA<sub>A</sub> receptors to exert these effects. It is worth noting, in this regard, that the administration of L-cysteine blocks the acquisition and also maintenance as well as reinstatement of drinking and seeking behaviors and the progressive ratio of oral ethanol self-administration.<sup>66,76</sup> Consistently, the pro-drug of L-cysteine, N-acetyl cysteine, has been reported to not influence the acquisition of chronic ethanol intake but to greatly inhibit ethanol intake when it is administered to animals that are consuming ethanol chronically.<sup>87</sup> Perhaps the ability of L-cysteine to bind to acetaldehyde is responsible for just the inhibition of acquisition and the reinstatement of ethanol self-administration. In fact, D-penicillamine, a synthetic amino acid that strongly binds acetaldehyde, inhibits the deprivation effect of voluntary ethanol intake<sup>67</sup> and

ethanol relapse-like drinking in operant self-administration paradigm.<sup>92</sup> This interpretation is supported by the observation that L-cysteine (but not N-acetyl cysteine) may act by different mechanisms on the acquisition and maintenance of ethanol self-administration. In this regard, we have recently shown a marked increase in the expression of the cystine/glutamate exchanger in the nucleus accumbens of ethanol dependent rats that, under chronic ethanol taking, reach on average 6–14 g/kg/day of ethanol intake.<sup>93</sup> Such an increase in the cystine/glutamate exchanger has been interpreted to represent a compensatory mechanism, although not efficient under normal or low cystine levels, to reduce extracellular glutamate levels to normal. In fact, rats that consume high ethanol levels show marked increases in extracellular glutamate.<sup>94,95</sup> However, the peripheral administration of L-cysteine or N-acetyl cysteine during maintenance could potentiate the cystine/glutamate exchanger level, which might restore extracellular glutamate levels to normal.<sup>87,93</sup>

However, the idea that acetaldehyde might be responsible for the *first hit* is in contrast with other studies revealing that a negative interference with catalase–H<sub>2</sub>O<sub>2</sub> metabolic pathway has been shown to impair the maintenance and further phases of oral ethanol self-administration<sup>43</sup> as well as of voluntary ethanol intake.<sup>83</sup> It is possible that the interference with catalase–H<sub>2</sub>O<sub>2</sub> operated by  $\alpha$ -lipoic acid ( $\alpha$ -LA), in this study, could be a stronger mechanism, more capable to reduce central (and peripheral) acetaldehyde production. Certainly, the interpretation of these outcomes is still unknown and necessitates further investigations.

Finally, in the context of the present review, it is critical to at least remember that there is a possibility of the formation of several other metabolites of ethanol such as fatty acid ethyl esters<sup>96</sup> and phosphatidylethanol.<sup>97</sup> Moreover, the production of fatty acid ethyl esters could be associated with an increased availability of H<sub>2</sub>O<sub>2</sub>, and this might support the explanation by which drugs such as  $\alpha$ -LA and L-cysteine that are able to reduce H<sub>2</sub>O<sub>2</sub> levels could decrease oral operant ethanol self-administration behavior by interfering with the neuroinflammatory process induced by ethanol or with the oxidative stress caused by acetaldehyde.<sup>98</sup> In fact, a recent work shows that the toll-like receptor 4 is involved in the induction of cytokines and chemokines responsible for promoting neuroinflammation, brain damage, behavioral and cognitive dysfunctions, and addiction. It is important to note that selective inhibition of toll-like receptor 4, although in the presence of dose-dependent non-specific effects, such as reduced animal locomotor activity, saccharine intake, and lower body core temperature, decreased ethanol drinking in both ethanol-dependent and non-dependent mice.<sup>99</sup>

### From Ethanol to Salsolinol: is Ethanol a Pro-drug of Salsolinol?

As previously anticipated, acetaldehyde, whose structure provides this molecule a high electrophilic reactivity and,



consequently, a very short half-life, is not the only pharmacologically active metabolite of ethanol. In fact, the physical-chemical properties of acetaldehyde confer this molecule a high reactivity with nucleophilic compounds such as monoamines to produce different tetrahydroisoquinolines.<sup>48,49,52</sup> In particular, acetaldehyde may react with dopamine either spontaneously<sup>48,49,52</sup> or enzymatically,<sup>100</sup> although the latter possibility still represents an issue of controversy, to yield salsolinol, 1-methyl-6,7-dihydroxy-1,2,3,4-tetrahydroisoquinoline.

The involvement of salsolinol in alcoholism has been controversial over several decades since the reported levels of salsolinol intake and their changes after ethanol exposure were not consistent, possibly due to inadequate analytical procedures and confounding factors such as diet and genetic predisposition. Accordingly, although to the best of our knowledge no studies have been provided to demonstrate a direct, quantitative, correlation between ethanol administration, blood ethanol levels, and salsolinol concentrations (in the blood and/or in the brain), it was reported that salsolinol is found in the brain following ethanol administration.<sup>44</sup> In contrast, Lee et al<sup>101</sup> observed that neither ethanol self-administration nor ethanol administration resulted in the changes of plasma or nucleus accumbens salsolinol levels in men and P rats, respectively.<sup>101</sup>

All this notwithstanding, and based on the observation that salsolinol exerts a number of behavioral and neurochemical effects similar to those of ethanol,<sup>50,51</sup> it was suggested that at least some of the effects of ethanol could be mediated by salsolinol, a possibility put forward long time earlier<sup>49</sup> but never conclusively established.<sup>51,102</sup> This demonstration, restricted to the actions of ethanol on dopamine neurons, was provided recently in a study conducted recording the changes of spontaneous firing activity of dopaminergic neurons from the posterior VTA in mesencephalic slices.<sup>103</sup> In particular, using slices from mice administered  $\alpha$ -methyl-*p*-tyrosine, an agent that could inhibit newly synthesized dopamine and its synaptic release,<sup>104</sup> this study challenged the hypothesis that ethanol may be a pro-drug of salsolinol by demonstrating that the formation of salsolinol and, hence, the ability of ethanol to increase the spontaneous firing rate of dopamine neurons in the posterior VTA could take place under the following two strictly controlled sequential events: the conversion of ethanol into acetaldehyde and the formation of salsolinol following condensation, of the newly-produced acetaldehyde, with dopamine. In particular, in the first experiment, it was confirmed the metabolic conversion of ethanol into acetaldehyde by the action of catalase-H<sub>2</sub>O<sub>2</sub>,<sup>56</sup> and in the second experiment, it was demonstrated the critical role of condensation of such ethanol-derived acetaldehyde with extracellular dopamine to form salsolinol and to stimulate spontaneous firing of dopamine neurons.<sup>103</sup> These sequential steps were controlled, respectively, by the use of the catalase-H<sub>2</sub>O<sub>2</sub> inhibitor, 3-AT, or the H<sub>2</sub>O<sub>2</sub> scavenger,  $\alpha$ -LA,<sup>103</sup> and by the use of  $\alpha$ -methyl-*p*-tyrosine. In particular, in agreement with previous studies, it was shown that ethanol (100 mM),<sup>56,105</sup> acetaldehyde

(10 nM),<sup>56</sup> and salsolinol (10 nM)<sup>106,107</sup> significantly increase the spontaneous firing rate of dopaminergic neurons in slices from control animals (eg, not administered with  $\alpha$ -methyl-*p*-tyrosine); however, it was also shown that only salsolinol (10 nM) could do so in slices from  $\alpha$ -methyl-*p*-tyrosine-administered mice.<sup>103</sup> Furthermore, this study showed that the failure of ethanol (100 mM) to stimulate the spontaneous firing rate of dopamine neurons from  $\alpha$ -methyl-*p*-tyrosine-administered mice could be reverted by the addition of exogenous dopamine.<sup>103</sup> Thus, the first experiment confirmed that, without its catalase-H<sub>2</sub>O<sub>2</sub>-mediated conversion into acetaldehyde, ethanol fails to stimulate the firing activity of dopamine neurons, whereas the second experiment disclosed that the presence of extracellular dopamine is necessary in order to form salsolinol and stimulate the spontaneous firing of dopamine neurons. A further proof that salsolinol is indeed the chemical responsible of ethanol-mediated stimulation of the dopaminergic firing was provided by the observation that salsolinol could be found only in the media containing slices from  $\alpha$ -methyl-*p*-tyrosine-administered mice in which exogenous dopamine was added and the stimulation of firing rate could be recorded.<sup>103</sup>

### Effects of Salsolinol After Local or Systemic Administration

Long before the demonstration that, at least in vitro and at least for its effects on dopamine neurons in the posterior VTA, ethanol acts as the pro-drug of salsolinol<sup>103</sup> and the effects of this compound have been studied after both its systemic and local (non-contingent and contingent) administration. The consequences of the intracerebral administration of salsolinol were addressed in an early study in long-sleep and short-sleep mice following the systemic administration of ethanol.<sup>108</sup> In this study, the effects of salsolinol on spontaneous activity in the open field and on ethanol-induced sleep time were evaluated in both lines of mice selectively bred for differential sleep time and it was found that at low doses, salsolinol could stimulate locomotor activity and decrease the sleep time of short-sleep mice, whereas at higher doses (40  $\mu$ g), it could decrease locomotor activity and increase sleep time of both lines of mice,<sup>108</sup> suggesting that at appropriate concentrations, the effect of salsolinol could be additive to those of ethanol.

The ability of salsolinol to exert reinforcing effects upon non-contingent systemic administration was reported by Matsuzawa et al.<sup>109</sup> In this study, salsolinol was reported to dose-dependently elicit CPP both in normal rats and in rats subjected to stress. Furthermore, this study was the first to provide in vivo support to the early suggestion that the effects of salsolinol could be mediated by  $\mu$  opioid receptors with the use of  $\beta$ -funaltrexamine.<sup>109</sup> Notably, the reinforcing properties of salsolinol were demonstrated in a study in which salsolinol was also shown to sustain acquisition and maintenance of its intra-posterior VTA administration in Wistar rats<sup>110</sup> in a concentration-dependent manner. Moreover, the

consequences of the local application of salsolinol in discrete brain regions (nucleus accumbens or posterior VTA) and the role of blockade of  $\mu$  opioid receptors in these effects have recently also been thoroughly investigated. In particular, Hipólito, Polache, Granero and Colleagues reported in three different studies that the local application of salsolinol in the shell and core of the nucleus accumbens differentially affects dopamine transmission in these accumbal subregions<sup>111</sup> and that the application of salsolinol in the posterior VTA, concentration dependently, stimulates locomotor activity and dopamine transmission in the shell of the nucleus accumbens.<sup>112,113</sup> Interestingly, although these studies do not provide direct evidence of the relationship between ethanol and salsolinol, given the possibility that salsolinol may be metabolically generated following ethanol administration<sup>103</sup> and given the similarities of the effects of ethanol and salsolinol,<sup>111–113</sup> they appear highly suggestive of the fact that salsolinol may play a critical role in the effects of ethanol.<sup>51</sup> Furthermore, although to our knowledge no studies have characterized fully the binding affinity of salsolinol for  $\mu$  opioid receptors,<sup>114</sup> the results of the studies by Hipólito et al<sup>111–113</sup> strongly suggest the involvement of  $\mu$  opioid receptors in these effects of salsolinol. An interesting and robust support to this interpretation has been provided by electrophysiological studies in which dopaminergic neurons from posterior VTA of mesencephalic slices were recorded following the application of salsolinol. In this study, Xie et al<sup>107</sup> demonstrated that salsolinol excites dopaminergic neurons by activating  $\mu$  opioid receptors and inhibiting GABA neurons in the posterior VTA.

### Salsolinol: the Issue of Stereoselectivity

More recently, Quintanilla et al<sup>115</sup> reported the results of their studies on the effects of salsolinol, injected intraperitoneally or intracerebrally to ethanol-naïve rats, bred as alcohol drinkers to study its motivational effects and its role on voluntary ethanol intake. In these experiments, salsolinol produced CPP and increased the locomotor activity, whether injected intraperitoneally or intra-posterior VTA. Furthermore, following systemic administration, this molecule was detected in vivo by microdialysis in the neostriatum, reaching detectable (100 nM) concentrations in the dialysates<sup>116</sup> and providing further evidence of its ability to cross the BBB.<sup>116</sup> Moreover, repeated administration of salsolinol sensitized rats to the locomotor stimulant effects and led to significant increases in voluntary ethanol consumption, which was prevented by intra-posterior VTA pre-treatment with naltrexone.<sup>115</sup>

Finally, in this context, it is critical to mention that all the above-reviewed experimental evidence refers to salsolinol (1-methyl-6,7-dihydroxy-1,2,3,4-tetrahydroisoquinoline) without any further specification, in particular, for its isomer (iso-salsolinol) and for its stereoselectivity. Indeed, in two well-designed studies, the group of Yedi Israel specified that these two critical caveats, related to the chemical nature of this

molecule, should be clearly kept in mind in future research on this compound. The first caveat refers to the presence (roughly 10–15%, w/w), in the commercially available salsolinol, of iso-salsolinol, a pharmacologically distinguishable isomer,<sup>115</sup> while the second refers to the fact that all the above-referenced evidence was originated with the use of the racemate, *R,S*-(±)-salsolinol.<sup>116</sup> As for the first issue, these authors reported that a number of effects, including drug-elicited CPP after either systemic administration or intra-posterior VTA of the commercially available salsolinol, were indeed attributable to iso-salsolinol-free salsolinol. Quintanilla et al<sup>116</sup> also elegantly demonstrated that the effects of racemate salsolinol could indeed be attributed only to (*R*)-salsolinol since the administration of (*R*)-salsolinol, but not of (*S*)-salsolinol, leads to CPP and locomotor sensitization and markedly increases voluntary ethanol consumption.<sup>116</sup>

### Conclusions

The reviewed literature supports the tenet of a key role of acetaldehyde in the central behavioral and neurochemical effects of ethanol and robustly highlights the role of peripherally and centrally produced acetaldehyde in central effects at the basis of ethanol-mediated reinforcement. Furthermore, recent studies from independent groups brought to the demonstration that ethanol could act as the pro-drug of the condensation product between acetaldehyde and dopamine<sup>103</sup> and that this condensation product, salsolinol, does indeed exert its actions as iso-salsolinol-free<sup>115</sup> as well as (*R*)-salsolinol enantiomer.<sup>116</sup> Finally, although still in the absence of clear-cut demonstration of the binding affinity of this compound for  $\mu$  opioid receptors, evidence has accumulated to suggest that this might indeed be the case.<sup>107,109,111–113</sup> All this notwithstanding, some aspects of the pharmacological profile of acetaldehyde still remain unknown, mostly due to its poor detectability that would greatly help the understanding of the mechanism(s), the kinetic(s), as well as the site(s) of its peripheral and central actions; in contrast, iso-salsolinol-free (*R*)-salsolinol seems to be mainly involved in the central effects of ethanol in particular with respect to its actions mediated through the dopaminergic mesolimbic system.

Although much progress has been made in identifying pre-clinically the role of acetaldehyde and salsolinol in the central effects of ethanol, the translation of this knowledge into the clinical setting, including their role, following an acute, pharmacologically significant, ethanol exposure in the development of AUDs, is still far to be fully disclosed. Moreover, it is important to note that in addition to acetaldehyde and salsolinol, other metabolites may play a role. Notably, many of them are used in forensic medicine as markers of ethanol intake in drinkers.<sup>96,97</sup> Finally, while it has not even been established whether ethanol-derived acetaldehyde is quantitatively transformed into salsolinol, it remains that acetaldehyde and salsolinol involve different systems and receptors implicated in ethanol effects.



In conclusion, it appears reasonable to suggest that interfering with peripheral or central ethanol metabolism as well as decreasing acetaldehyde bioavailability could be the main targets of future discoveries of pharmacological tools able to reduce and/or prevent the development and the persistence of AUDs. More importantly, from now on, it should be kept in mind that both the brain and liver peroxidative statuses are involved in the control of the acute and chronic pharmacological effects of ethanol. Thus, changes in the physiological situations, that is, in the levels of  $H_2O_2$  present in the liver and in the brain may represent a particular circumstance within the (same) organism responsible to regulate some effects induced by ethanol. Furthermore, all data obtained with drugs able to reduce  $H_2O_2$  (4-MP, 3-AT, and  $\alpha$ -LA) and acetaldehyde production/availability could be particularly important to help understanding the mechanism(s) underlying the deepest roots of ethanol binge drinking and the relationship between ethanol and its metabolites.

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### Author Contributions

ATP, MR, SP and EA contributed the writing of the manuscript, made critical revisions and approved final version. All Authors reviewed and approved of the final version of the manuscript.

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