K56 Effect of Ethanol on Succinyl Semialdehyde Dehydrogenase—Implications for Exacerbation of GHB Toxicity

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After attending this presentation, attendees will gain a greater understanding of the central nervous system, its endogenous inhibitory neurotransmitters and their metabolism, and the effects of ethanol on part of that metabolic process.

This presentation will impact the forensic science community by offering a mechanistic explanation for one aspect of the combined effects of ethanol/GHB co-ingestion. This knowledge will help forensic pathologists and toxicologists evaluate and interpret drug results in DFSA cases where both ethanol and GHB are determined to have been present.

Drug Facilitated Sexual Assault (DFSA) cases routinely involve the use of central nervous system (CNS) depressant agents such as benzodiazepines, barbiturates, and more recently, γ-hydroxybutyrate (GHB). A common mechanistic basis for the actions of these agents is an effect on GABA-nergic inhibitory neurotransmission. GABA (γ-amino butyric acid) is the primary inhibitory neurotransmitter in the CNS, functioning as a post-synaptic ligand-gated chloride channel agonist (GABA receptor agonist). Activation of the GABA receptor by either endogenous GABA, or by xenobiotics, results in the influx of chloride ion into the post-synaptic neuron, resulting in a hyperpolarization (inhibition) of that neuronal membrane. Removal of GABA from the synaptic space following the neurotransmission event is a function of uptake and catabolism of GABA by astrocytic cells in proximity to the synapse rather than re-uptake directly into the pre-synaptic neuron. Succinyl Semialdehyde Dehydrogenase (SSADH) is a central enzyme in the oxidative degradation of GABA and GHB, converting their common oxidative metabolite, succinyl semialdehyde (SSA), to succinate as an end product. SSA is produced directly from GABA by an enzyme-catalysed transamination (with alpha-keto glutarate, (αKG)), and from GHB by a GHB-dehydrogenase (GHBDH)-catalysed oxidation. GHB dehydrogenase is a cytosolic enzyme reducing NAD^+ as a cofactor, while SSADH and the transaminase are mitochondrial enzymes, with SSADH reducing $NADP^+$ as a cofactor.

Ethanol is commonly found in DFSA cases, either alone or in combination with other CNS depressants, including GHB, and its presence may have an impact on the interpretation of drug findings in such cases. Ethanol has been shown to exacerbate the effects of GHB; however, a mechanistic basis for that effect has not been demonstrated. It has been hypothesized that one consequence of alcohol ingestion in the body is an inhibition of SSADH by both ethanol and its oxidative metabolite, acetaldehyde, because of the structural homology between ethanol, acetaldehyde, and carbons 3 and 4 of SSA. Inhibition of SSADH would be expected to increase the effective half-life of GABA in the body, with the consequential increase in background GABA concentration, and GABA-mediated CNS depressant activity. Initial experiments with a combined enzyme system consisting of GABA-αKG transaminase/SSADH indicated that ethanol inhibited enzyme activity at a concentration equivalent to 0.4 g/dL, but did not do so appreciably at a concentration equivalent to 0.1g/dL, suggesting that any such effect of ethanol on SSADH would only be a factor in significant alcohol ingestions. Kinetic evaluation of initial reaction rates by UV spectrophotometry (monitoring generation of NADPH) indicated that ethanol affected SSADH rather than the GABA-αKG transaminase. Substrate-velocity experiments indicated that SSADH in the preparation had a Michaelis constant (K_m) for SSA of 49 μM in the absence of ethanol, and 61 μM in the presence of 0.4g/dL ethanol, as determined by Lineweaver-Burke plot. Maximal velocity (V_{max}) of the enzyme was unaffected by the inclusion of ethanol, a pattern consistent with competitive inhibition.

Based on the effect of ethanol on SSADH, it is suggested that the ingestion of alcohol in the body would, in a concentration-dependent manner, inhibit SSADH, thereby decreasing the rate of GABA- and/or GHB-derived SSA oxidation, and potentially increasing both the half-life of endogenous GABA and exogenous GHB. This effect may play a contributory role to the CNS depressant consequences of significant ethanol ingestions and combined ethanol-GHB exposures, such as could be seen in some DFSA cases.

Ethanol, GHB, GABA

 ** Presenting Author*