Mackenzie Pavlik
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Developing a Method for the N-Methylation of Amphetamine in Human S9

Dr. Robert Powers
Department of Forensic Science

The relatively rapid N-demethylation of methamphetamine, producing amphetamine, is well recognized as the basis for the appearance of amphetamine in biological fluid and hair samples of individuals using methamphetamine as a recreational or therapeutic drug. In contrast, there is no expectation that individuals either abusing, or receiving amphetamine for therapeutic purposes (e.g. as treatment for ADHD) will generate any significant levels of methamphetamine. Thus, the appearance of that species in drug abuse monitoring samples is routinely presumed to reflect methamphetamine abuse, often with significant medical or legal consequences.

The validity of this assumption has recently come into question in a number of cases in various legal forums. Amphetamine is primarily eliminated as the unchanged parent drug, or via deamination through phenylacetone, benzoic acid, and subsequent conjugation products. However, the methylation of a small proportion of amphetamine could theoretically yield small concentrations, and potentially detectable levels of methamphetamine, and lead to an erroneous conclusion of illicit methamphetamine use. While not a preferred metabolic pathway, the methylation of amphetamine to form methamphetamine as described by the action of the enzyme norephedrine N-methyl transferase was demonstrated in the 1950s by the Nobel prize winning biochemist Julius Axelrod during his extensive research on the biotransformation of sympathomimetic amines (Axelrod 1954).

As an initial step in exploring the relationship between the N-methyl and -demethylation reactions, we have developed a method for the n-methylation of amphetamine analogs in human S9 based on Axelrod's 1962 study on Serotonin. We chose to utilize norephedrine, the C1-hydroxy analogue of amphetamine, as the basis our experiments, as amphetamine is a schedule 2N drug.

Enzyme assays were completed in 1mL aliquots with a 200mM Trizma buffer at pH 7.6 and consisted of 200µL S9 (Sigma), 15µL 20mg/mL norephedrine, and 350µL 55mM s-adenosylmethionine (SAM; Sigma). Enzyme incubations were performed at 37°C and were stopped at 0 and 60 minutes via the addition of 300 µL 1M pH 9.0 Trizma buffer and 100 µL 0.5g/mL KF. The solution was then extracted 3 x with 0.5mL EtOAc. The mixture was centrifuged, and the supernatant organic phases were combined and evaporated to ~ 100 µL under N_2 and analyzed by GC/MS for ephedrine. Our result indicated that N-methyl transferase from human S9, normally functional in neurotransmitter synthetic pathways, is capable of generating methamphetamine from amphetamine in this incubation system

Further research is needed to determine the extent that amphetamine N-methyltransferase is able to generate methamphetamine. Evaluation of the kinetic properties of the enzyme will allow a determination if methamphetamine can reasonably be expected to be present in cases of chronic amphetamine administration. This requires a greater understanding of the equilibrium achieved between methamphetamine and amphetamine as a function of the actions of the N-demethylase and N-methyltransferase, in cases of amphetamine chronic use or exposure, or the impact of product inhibition by methamphetamine or its structural analogues on that equilibrium.

References

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