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Running Title Page.

The ES1/BChE KO mouse a model for BChE deficient humans

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Abstract.
Death and toxicity following cocaine use do not correlate with cocaine blood levels. One explanation for this observation is that cocaine abusers may possess one or more of the 58 possible known mutations in the butyrylcholinesterase gene (BCHE).
Butyrylcholinesterase serves as the primary cocaine hydrolase producing a non-toxic product ecgonine methyl ester. A reduction in endogenous levels of BChE may result in increased metabolism by hepatic carboxylesterase to produce norcocaine, a toxic product. Humans have carboxylesterase in tissues but not in plasma, while wild type mice have significant amounts of carboxylesterase in tissues and plasma. Knock-out mice with no plasma carboxylesterase were created to eliminate the contribution of plasma carboxylesterase in cocaine hydrolysis, thereby simulating human enzyme levels. This study tested the hypothesis that reductions in BChE such as those in humans with BChE mutations contribute to increased toxicity following cocaine use. Carboxylesterase and BChE double knock-out mice, models for humans with BChE deficiency, were challenged with a non-lethal dose of 100 mg/kg (-)-cocaine. Carboxylesterase/BChE double knock-out mice demonstrated toxic signs significantly longer than did wild type and carboxylesterase knock-out mice. The carboxylesterase/BChE deficient mice took approximately 2.5 times as long to recover from cocaine toxicities including: hypothermia, hyperactivity, stereotypical behavior, ocular effects, and dorsiflexion of the tail. The carboxylesterase/BChE double knock-out mouse model demonstrates the importance of endogenous BChE for protection against cocaine toxicity and provides an in vivo system for studying drug sensitivity of humans who carry a BChE mutation.
**Introduction.**

Butyrylcholinesterase (BChE) plays a significant role in cocaine metabolism and detoxication serving as the principal cocaine hydrolase in human serum. Exogenously delivered BChE prevents cocaine seizures in rats (Brimijoin et al., 2008) and has been proposed as a treatment for cocaine overdose and addiction in humans (Gao et al., 2008). Reduction in the level of endogenous butyrylcholinesterase exacerbates cocaine toxicity in humans (Hoffman et al., 1992). Most of the 58 known mutations in the *BCHE* gene reduce BChE activity. The atypical variant (Asp 70 Gly) has severely reduced activity toward cocaine due to its 10-fold reduced affinity for cocaine (Xie et al., 1999). People with silent BChE have 0-10% of normal BChE activity. It is hypothesized that genetic mutations that result in reduced endogenous levels of BChE may result in increased toxicity following cocaine usage. Results from a cocaine challenge study using the BChE knock-out mouse model (Li et al., 2008) suggested that people with atypical or silent BChE may be susceptible to the pathophysiological effects of cocaine at doses not harmful to the average person (Duysen et al., 2008).

Carboxylesterase has also been shown to play a role in the hydrolysis of cocaine (Redinbo et al., 2003). Carboxylesterase is found in all human tissues but not in human plasma while mice have an abundance of carboxylesterase in tissues and plasma (Li et al., 2005). To provide an in vivo model that would simulate human levels of carboxylesterase a mouse lacking plasma carboxylesterase (ES1/-) was produced. Like humans, the ES1/- mouse is deficient in plasma carboxylesterase while having normal levels of carboxylesterase in all other tissues.
Genetically defined animal models provide an essential tool that can be used to explain individual differences in sensitivity and susceptibility to central nervous system active substances such as cocaine (Morse et al., 1995). To produce a mouse model that would simulate human genetic BChE deficiencies BChE knock-out and ES1 knock-out mice were bred to produce a double knock-out model that has no BChE in any tissue and has no plasma carboxylesterase. This investigation uses this mouse as a model for humans with genetic BChE mutations to investigate possible increased toxic effects following a cocaine challenge.
Methods.

Animals. Animal work was conducted in accordance with the Guide for the Care and Use of Laboratory Animals as adopted by the National Institutes of Health. Formal approval to conduct the experiments was obtained from the Institutional Animal Care and Use Committee of the University of Nebraska Medical Center (UNMC). ES1-/-BChE-/- double knock-out mice were produced at UNMC from breeding single knock-out ES1-/-mice (Jackson Laboratories, JAX Stock No. 014096) with single knock-out BChE mice (Jackson Laboratories, JAX Stock No. 008087). All mice were in a C57BL/6 background.

Cocaine. (-)-Cocaine hydrochloride (Sigma-Aldrich St. Louis, MO) was dissolved in water to make a stock containing 34.4 mg/ml. Aliquots prepared fresh daily were maintained on ice and were discarded within 2 hours of dilution.

Challenge with 100 mg/kg cocaine. Pre-dose body weights, and surface body temperatures were recorded for adult male wild type (ES1+/+BChE+/+), ES1 knock-out (ES1-/-BChE+/+) and ES1/BChE knock-out (ES1-/-BChE-/-) mice (n=4/genotype). Prior to dosing with cocaine individual mice were observed and an inventory of baseline behaviors and physical attributes were recorded on an ethogram. General observations, behaviors and physical scores were recorded for posture, tremor, vocalization, palpebral closure, gait, mobility, arousal, stereotypical behaviors, straub tail, reflexes, and response to stimuli. Stereotypical behaviors included: circling- defined as movement in a circular pattern past a single point 10 or more times in a 30 second period, biting at the cage- defined as 5 or more bites in 30 seconds, excessive cleaning-defined as continuous cleaning behavior lasting more than 20 seconds and pivoting-
movement of the front paws from one side of the cage to the next for more than 20 seconds at a time. Hyperactivity was defined as either stereotypical or non-stereotypical movement past a single point 10 times or more in 30 seconds.

Mice were challenged with a single intraperitoneal (IP) dose of 100 mg/kg (-)-cocaine. Animals were observed continuously through 1 hour, behavioral and physical scores were recorded every 15 minutes through one hour and every 30 minutes through 5 hours. Surface body temperatures were measured and recorded every 15 minutes through 5 hours using a thermocouple skin probe (Physitemp, Clifton, NJ).

Statistics. Comparison of body temperature and time to survival data between each genotype was by Analysis of Variance (ANOVA) using a 95% confidence interval (± standard deviation). Analysis was conducted using SPSS software (IBM Corporation Chicago, IL).
Results.

Prior to treatment all mice were determined to be healthy with no untoward signs or behaviors. All animals survived and recovered to baseline levels by 5 hours post-challenge. The challenge resulted in a drop of body temperature in all genotypes through 30 minutes post dosing. (Fig. 1) From 1.0 to 4.5 hours post dosing ES1-/-BChE-/- mice demonstrated significantly lower body temperatures compared to ES1+/+BChE+/+ and ES1-/-BChE+/+ mice (p<0.01). Baseline body temperatures were recovered in ES1+/+BChE+/+ and ES1-/-BChE+/+ mice by 2.0 hours post dosing while time to temperature recovery in the ES1-/-BChE-/- mice lagged by 3.0 hours with a return to baseline at 5.0 hours. Ethogram observations (Fig. 2) revealed that ES1-/-BChE/- mice experienced a statistically significant (p<0.05) longer time to recovery to baseline for: tail position, gait, activity levels and cessation of stereotypical behaviors compared to ES1+/+BChE+/+ and ES1-/-BChE+/+ mice. ES1-/-BChE/- mice took a significantly longer time to recover normal palpebral closure compared to ES1-/-BChE+/+ mice. Two ES1-/-BChE/- mice experienced whole body tremors 10-20 minutes post dosing, while no tremors were observed in the ES1+/+BChE+/+ or ES1-/-BChE+/+ mice. No other unique signs were observed in the ES1-/-BChE-/- mice. Plasma carboxylesterase did not contribute to reduced toxicity as evidenced by the ES1-/BChE+/+ mice demonstrating similar toxic signs and time to recovery when compared to the ES1+/+BChE+/+ wild type mice.
Discussion.

Deaths due to cocaine are not dose related and blood levels do not accurately predict toxicity (Karch et al., 1998). One explanation for this variability may be differences in the levels of esteratic metabolism of cocaine as a result of genetic mutations found in the BCHE gene. The K variant is the most common variant with one person out of four being heterozygous for this mutation and 1 out of 25 being homozygous resulting in 33% lower plasma BChE activity (Bartels et al., 1992). The silent variant, with 0-10% BChE activity, is present in 1 out of 100,000 Americans (Lockridge, 1990).

Om et al. found that plasma cholinesterase levels in humans may be predictive of complications from cocaine use (Om et al., 1993). BChE hydrolyzes cocaine to ecgonine methyl ester, a pharmacologically inert substance. We hypothesized that reduced levels of plasma BChE will increase the plasma half-life of cocaine resulting in increased levels of cocaine transported to the CNS potentiating dopaminergic transmission. Cocaine that is not hydrolyzed by BChE would remain available to undergo hepatic N-demethylation into the metabolically active and vasoconstrictive norcocaine (Pellinen et al., 2000). High levels of norcocaine were found in a study of cocaine abusers that presented to the emergency room for treatment (Blaho et al., 2000) indicating a possible association between this metabolite and cocaine toxicity.

Mice deficient in both BChE and plasma carboxylesterase challenged with a non-lethal dose of cocaine experienced behavioral and physiological effects for a significantly longer period of time than did mice lacking only plasma carboxylesterase or wild type
mice. It could be predicted that at higher cocaine doses the ES1-/-BChE-/- mice would have experienced higher levels of mortality and morbidity.

The ES1 knock out mouse lacking plasma carboxylesterase was developed as a model for testing human response to chemical agents that are metabolized by plasma carboxylesterase. Humans have no carboxylesterase in their plasma while mice have high levels. The observation that following challenge with cocaine ES1-/- mice with normal levels of BChE show similar behavioral and neuro-toxic effects compared to wild type mice was surprising. The role of carboxylesterases in metabolizing cocaine is well established therefore it was anticipated that mice lacking plasma carboxylesterase would demonstrate increased toxic signs following cocaine challenge. Future studies may elucidate the role that mouse plasma carboxylesterase (ES1) plays in detoxification of cocaine.

The ES1/BChE double knock-out mouse model demonstrates the importance of endogenous BChE for protection against cocaine toxicity and provides an in vivo system for studying drug sensitivity of humans who carry a BChE mutation.
References.


Fig. 1. Surface body temperature of ES1-/−BChE-/− (white triangles), ES1-/−BChE+/+ (grey squares), and ES1+/+BChE+/+ (black circles) mice challenged with 100 mg/kg (-)-cocaine. a is significantly different than b (p<0.01) through 4.5 hours post-dosing by ANOVA analysis.

Fig. 2. Time to recovery of baseline behavior and physiology following challenge with 100 mg/kg (-)-cocaine in ES1-/−BChE-/− mice (grey bars), ES1-/−BChE+/+ (diagonal lined bars), and ES1+/+BChE+/+ (black bars). a is significantly different than b (p<0.05) by ANOVA analysis.
Figure 1

The graph shows the temperature (°C) measured at different hours post dosing. The temperature is plotted on the y-axis, ranging from 31°C to 39°C, and the hours post dosing are shown on the x-axis, ranging from 0 to 5 hours.

Different symbols and colors are used to represent different groups or conditions. The symbols 'a' and 'b' are annotated on the graph to indicate specific groups or categories for comparison.