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TITLE PAGE

Cocaine Hydrolase Gene Transfer Demonstrates Cardiac Safety and Efficacy Against Cocaine-Induced QT Prolongation in Mice

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Abbreviations:

AAV: Adeno-associated viral vector

BChE : Butyrylcholinesterase

CocH: Cocaine Hydrolase

ECG: Electrocardiogram

EME: Ecgonine methyl ester

FDA: U S Food and Drug Administration

hdAD: helper dependent adenoviral vector

IND: Investigational new drug permit

DFP: Diisopropyl fluorophosphates

ANOVA: Analysis of variance

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ABSTRACT

Cocaine addiction is associated with devastating medical consequences including cardiotoxicity and risk-conferring prolongation of the QT interval. Viral gene transfer of cocaine hydrolase engineered from butyrylcholinesterase offers therapeutic promise for treatment-seeking drug users. While previous preclinical studies have demonstrated benefits of this strategy without signs of toxicity, the specific cardiac safety and efficacy of engineered butyrylcholinesterase viral delivery remains unknown. Here, telemetric recording of electrocardiograms from awake, unrestrained mice receiving a course of moderately large cocaine doses (30 mg/kg, twice daily for 3 weeks) revealed protection against a two-fold prolongation of the QT interval conferred by pre-treatment with cocaine hydrolase vector. By itself this prophylactic treatment did not affect QT interval duration or cardiac structure, demonstrating that viral delivery of cocaine hydrolase has no intrinsic cardiac toxicity and, on the contrary, actively protects against cocaine-induced QT prolongation.

INTRODUCTION

Among drugs of abuse, cocaine stands out for cardiovascular toxicity, often leading to emergency department visits (Hahn and Hoffman, 2001). A common effect of cocaine abuse is prolongation of the QT interval in the electrocardiogram (ECG), which, when extreme, leads to *torsades de pointes*, a life-threatening ventricular dysrhythmia (Kloner et al., 1992; Bauman and DiDomenico, 2002; Phillips et al., 2009). There is no available pharmacotherapy that would prevent these adverse outcomes (Mendelson et al., 1996; Wood et al., 2009).

To fill this gap we have pursued gene therapy means to enhance cocaine metabolism in an effort to block both the reward and the toxicity of this drug. Animal studies show some benefit from butyrylcholinesterase (BChE) a common plasma enzyme. Native BChE destroys cocaine slowly, but computer simulations and site-directed mutagenesis have uncovered critical active-site mutations that collectively enhance cocaine hydrolysis by ~ 1500-fold (Sun et al., 2002; Xie et al., 1999; Yang et al., 2010; Zheng et al., 2010). Such a cocaine hydrolase (CocH) cleaves cocaine into ecgonine methyl ester and benzoic acid, both of which lack reward value and toxicity (Murthy et al., 2015). This enzyme is now a central focus in efforts to develop a gene transfer therapy for cocaine abuse.

The preferred intervention strategy requires long-term CocH expression. Instead of multiple injections or slow release preparations, gene transfer by adeno-associated viral vector (AAV) or helper dependent adenoviral vector (hdAD) looks more promising. Over a range of species, this technology can drive CocH to extremely high levels, for years after a single treatment and without apparent toxicity (Murthy et al., 2014a; Murthy et al., 2014b). The result is a greatly accelerated cocaine hydrolysis and a near-complete suppression of physiological, behavioral, and toxicological responses to cocaine (Carroll et al., 2012; Gao et al., 2004; Brimijoin et al.,

2013; Gao et al., 2013; Geng et al., 2013). We consider that a successful translation of this therapy to treatment-seeking cocaine users might aid them in becoming abstinent and, later, reduce their risk of relapse into drug taking.

Translating CocH gene transfer technology into clinical application demands careful evaluation in animal models. Multiple studies have shown negligible physiological, biochemical, neurobehavioral and neuromuscular toxicity from BChE injections and gene transfer (Murthy et al., 2015; Murthy et al., 2014a; Murthy et al., 2014b; Brimijoin et al., 2008; Saxena et al., 2005; Saxena et al, 2011; Weber et al., 2011). Nonetheless, the issue of cardiovascular safety needs closer attention. The present study used telemetry to capture ECGs from conscious free-moving mice undergoing long-term cocaine treatment. There were two objectives. The first was to determine whether CocH-based gene therapy is safe to the heart's structure and function. The second was to test our hypothesis that, beyond not being harmful, CocH gene transfer protects against cocaine cardiotoxicity exemplified by prolongation of the QT interval on the electrocardiogram.

MATERIALS AND METHODS

Animals and Housing Balb/c male mice (age: 7 - 8 weeks; weight: 26.2 ± 0.5 g) purchased from Harlan (Madison, WI) were group-housed in plastic cages with access to water and food (Purina Laboratory Chow, Purina Mills, Minneapolis, MN) in rooms controlled for temperature (24 °C), humidity (40 - 50%), and illumination (lights on 6 a.m., lights off 6 pm). To monitor food and water consumption, mice were housed individually from 3 days before surgery until the end of the experiment. The protocol (A20812) was approved by the Mayo Clinic Institutional Animal Care and Use Committee, and experiments were conducted in accordance with the Principles of

Laboratory Animal Care in an Association for Assessment and Accreditation of Laboratory Animal Care International (AAALAC) accredited facility.

Surgery and Telemetry To monitor heart rate and ECG continuously in conscious mice, ETA-F10 telemetry devices (weight, 1.6 g; volume 1.1 cc; Data Sciences International, St. Paul, MN) were implanted in the peritoneal cavity, and wire leads were tunneled subcutaneously in a "lead II configuration" (O'Cochlain et al., 2004; Kane et al., 2006; Alekseev et al., 2010). Implantation surgery was conducted strictly according to AAALAC-approved aseptic conditions, one day after removal of anterior neck and abdominal hair. Anesthesia was achieved with isoflurane (5% for induction, 1.5% for maintenance in pure oxygen) and body temperature was maintained with a water-jacketed table at 37 °C. Implantation surgery was highly successful. Of the 27 mice subjected to telemetry device implantation in 27 mice, only one failed to recover fully and was euthanized after 3 days. Two other mice were euthanized because of persistent chewing on the telemetry leads. None died as a result of viral transduction or cocaine administration. All 24 of these telemetry implanted mice were randomly assigned to three experimental groups. In general, core body temperature stabilized at ~ 38 °C by 4 to 5 weeks. At this point the vector group received dexamethasone (10 and 5 mg/kg mg/kg, i.p.) 16 h and 2 h before virus injection, and a further 5 mg/kg 24 h later. These treatments suppressed acute immune responses that impaired enzyme transduction. Telemetry data were acquired weekly for 3 weeks at 2kHz following 4-5 weeks of recovery from telemetry implantation (when core temperature stabilized), and 4 weeks after vector injection, as shown in the schematic (Fig. 1).

Viral gene transfer AAV-8 vector incorporating cDNA for mouse BChE with amino acid substitutions for improved cocaine hydrolysis (A199S/S227A/S287G/A328W/Y332G), was prepared as previously (17). This CocH sequence was ligated into pAAV-VIP vector (Balazs et

al., 2012) between Not I and BamH I restriction sites. An AAV-VIP-CocH mutant plasmid was co-transfected into HEK293T cells with pHelper and pAAV2/8, using **X-treme GENE HP** DNA Transfection Reagent (Roche, Indianapolis, IN). AAV virus was purified from cell lysates by ultracentrifugation against Optiprep Density Gradient Medium-Iodixanol (Sigma-Aldrich, St Louis, MO). After recovery from telemetry implantation the 12-week-old mice identified for gene transfer treatment were given vector (200 µl, 10^{13} viral particles) via tail-vein.

Blood collection Blood (< 0.1 ml) was taken from the cheek with a 21-gauge mouse-lancet and bleeding was stopped with sterile gauze. Samples were centrifuged for 15 min (8000g) and plasma was stored at -20 °C. Cocaine hydrolase activity was assayed in a solvent-partitioning assay with ^{3}H-cocaine (50 nCi) and cold cocaine (18 µM) as described (Brimijoin et al., 2002). A related procedure determined levels of ^{3}H-cocaine and benzoic acid, with diisopropyl fluorophosphate (DFP) added to halt enzymatic breakdown.

Histology One day after the last cocaine administration, whole hearts were removed, rinsed, blotted dry, and flash-frozen in liquid nitrogen-chilled isopentane. Micrographs of 5 µm transverse sections of the heart, including left and right ventricles, stained with hematoxylin-eosin were taken with a KS-4000 Axiocam digital camera on an Axioplan 2 light microscope (Carl Zeiss MicroImaging, Thornwood, NY). Quantitative histological measurements were made under treatment-blind conditions.

Drugs Cocaine HCl was obtained from the National Institute of Drug Abuse Drug Supply Program (Research Triangle Park, NC).

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Intraperitoneal Injections For all experiments, the intended dose of cocaine was freshly dissolved in 0.9% NaCl before injection. Concentrations were adjusted to deliver ~300 µl per subject for a 30 mg/kg cocaine dose. Control mice received comparable volumes of saline.

Data Analysis Electrocardiographic analysis was performed at 3 different time points for each recording session (approximate time points: initial 3-7 min, middle – 12-17 min, and last 22 – 27 min) under normal sinus rhythm. The RR interval refers to the distance between adjacent R waves. The QT interval was defined as the time from the start of the Q wave to the end of the T-wave (return to isoelectric point) on the electrogram. The QT interval was corrected for heart rate (QTc) by using the following formula (Kane et al., 2006; Mitchell et al., 1998):

$$QTc(ms) = \frac{QT(ms)}{\sqrt{RR(ms)/100}}$$

Statistics Data were analyzed using Student's t-tests with Microsoft Excel (Redmond, WA) and two-way analysis of variance (ANOVA) with Holm-Sidak testing for post hoc analyses using GraphPad Prism Statistical Software Version 6.0 (San Diego, CA). Statistical significance was accepted if $P < 0.05$.

RESULTS

Experimental Sequence After acclimation on arrival, mice received telemetry implants. Upon recovery, mice were randomly assigned to the three groups: Vector, Saline, and Cocaine (N = 8 each). The Vector group received CocH gene transfer and, when stably high enzyme expression was achieved, were entered on the 3-week course of cocaine treatment (flow chart, Fig. 1). The other 16 mice were divided into pure negative controls (saline only) and positive controls (single saline injection in place of vector, followed by the course of cocaine).

Time course of CocH expression Initial experiments addressed the levels and duration of CocH in plasma after gene-transfer. Plasma cocaine hydrolytic activity reached an average level of 35 U/ml, 2 to 4 weeks after administration of AAV-CocH vector, and remained stable for the remainder of the experiment (data not shown). Consistent with our recent reports (Murthy et al., 2014a; Geng et al., 2013) the average rise in enzyme-driven cocaine hydrolysis capacity was ~ 100,000 fold above the pre-treatment level generated by endogenous BChE (0.0004 U/ml).

Temperature Shortly after telemetry implantation mice developed moderate fever (~ 40 °C) but returned to ~ 38 °C within 5 weeks. After confirming stable enzyme expression but before cocaine administration, telemetry showed an average body temperature of 37.9 ± 0.2 °C, with no difference between vector-treated and control animals. Weekly recordings throughout the experiment continued to reveal no significant differences between treatment groups (vector versus control) or within groups (before and after cocaine). Thus neither vector treatment nor cocaine injection had discernible effects on body temperature (Table. 1).

Heart rate As with body temperature, neither viral vector nor cocaine administration induced a change in heart rate. At baseline and during the cocaine treatment regimen, no significant fluctuations were observed in cocaine positive controls (500 ± 35 bpm at baseline, 540 ± 33 bpm at day 7, 549 ± 30 bpm at day 14 and 513 ± 36 bpm at day 21), saline controls (499 ± 46 bpm at baseline, 482 ± 47 bpm at day 7, 522.36 ± 40.32 bpm at day 14 and 543.21 ± 22 bpm at day 21) or vector treated mice (560 ± 20 bpm at baseline, 556 ± 13 bpm at day 7, 529 ± 426 bpm at day 14 and 530 ± 28 bpm at day 21) ((Fig. 2). Likewise, heart rate in vector treated mice did not change from pre-treatment levels.

Cocaine-induced prolongation of QTc interval Mice given only saline showed stable QTc intervals throughout the 3 weeks of injections and observation (Fig. 3). In contrast, mice treated with cocaine alone developed a statistically significant QTc prolongation that increased progressively over the course of drug administration: 30.2 ± 2.6 ms at baseline, 37.5 ± 3.3 ms at day 7 (P = 0.01), 35.0 ± 3.5 ms at day 14 (P = 0.44), and 47.4 ± 3.3 ms at day 21 (P = 0.02). Vector alone did not affect QT intervals: 22.6 ± 0.9 ms at baseline and 27.8 ± 3.4 ms at day 7 after virus delivery (P = 0.09). Most important, after three weeks of cocaine treatment this group of mice showed no QT prolongation: final value = 26.2 ± 1.6 ms, non-significantly lower than the pre-cocaine level.

In addition, QRS analysis revealed no cocaine- induced changes in QRS duration, either in unprotected mice (12.8 ± 1.0 ms at baseline *versus* 12.4 ± 1.0 ms at 3 wks; P = 0.71), or in mice pre-treated with CocH vector (11.2 ± 0.3 ms at baseline *versus* 13.0 ± 1.4 ms at 3 wks; P = 0.29).

Histology Fresh-frozen cryostat sections stained with hematoxylin-eosin revealed no indication of cardiac myopathy in the left ventricles of cocaine positive controls, saline controls or vector treated mice (Fig. 4a,b,d). Furthermore, the cardiomyocyte cross-sectional area was not affected by administration of cocaine alone or in the presence of vector as compared to saline (Fig. 4c; ANOVA P = 0.70). This outcome confirmed expectations that three weeks of cocaine dosing should be adequate to induce electrophysiological abnormalities but not grossly evident cardiomyopathy.

DISCUSSION

To pave the way for an eventual human trial of cocaine hydrolase gene transfer therapy, we have conducted several preclinical studies to examine neurobehavioral, neuromuscular,

metabolic and physiological parameters (Murthy et al., 2015; Murthy et al., 2014a; Murthy et al., 2014b). Despite the high levels of cocaine hydrolase achieved in mice, rats, and rhesus monkeys, no indications of toxicity or physiological dysfunction emerged, but cardiac effects remained to be tested. Because cocaine is cardiotoxic*,* it is important to eliminate the possibility that hydrolase gene transfer might disturb heart rhythms. A key metric of treatment-induced electrophysiological dysfunction is prolongation of the QT interval. Drug-induced QT prolongation can lead to fatal *torsades de pointes*, a primary cause of failure in drug discovery projects. Because FDA routinely mandates QT reports as a condition for an investigational new drug permit (IND), it is important to document the effects of CocH and cocaine, alone or combined, on QT intervals in mice.

Cocaine acts to block presynaptic reuptake of dopamine, norepinephrine and serotonin in the autonomic and central nervous systems and enhances adrenergic tone. Excessive sympathetic stimulation can lead to life-threatening cardiac stress. Thus, an agent that limits cocaine's actions at adrenergic synapses should have clinical value. Our proposed gene therapy focuses on reducing behavioral impact by destroying cocaine before it penetrates the blood-brain barrier. This strategy is highly effective in rodent models, where one treatment with vector-generated CocH can accelerate cocaine hydrolysis by 1000-fold or more and suppress lever pressing for cocaine reward for years (Zlebnik et al 2014). However, until now it had not been determined whether such treatment would be cardio-protective or, alternatively, might enhance risk in users taking larger cocaine doses to compensate for their diminished reward value.

Here, we addressed two fundamental questions: 1) Do high circulating levels of cholinesterase after viral gene transfer have any adverse cardiovascular effects? 2) Can gene transfer of cocaine hydrolase alleviate the consequences of continued cocaine use, specifically the risk-conferring prolongation of QT intervals?

As a partial approach to the first question, we examined the effect of vector and cocaine treatment on cardiac structure. Chronic cocaine abuse is known to induce cardiac hypertrophy in humans. In a mouse line especially susceptible to cardiac stressors, Reyes et al (2009) recently found that a 3-week regimen of sub-lethal cocaine doses induced left ventricular damage and impaired ejection velocity in mice lacking functional ATP-sensitive potassium (K_{ATP}) channels, which are established molecular sensors that protect the heart against conditions that impose an energetic overload. Our findings are in line with the control group in that prior study, which exhibited no cardiac histopathology at the administered cocaine dose. This was true in unprotected positive controls and also in the vector-treated mice exposed to moderately high level cocaine. Of particular importance for translational prospects, the present results provide evidence that vector treatment and expression of the BChE transgene designated as CocH do not induce cardiac histopathology by themselves or enhance the inherent cardiotoxicity of cocaine. This outcome supports our view that the viral delivery of CocH is not likely to impose a substantial risk of cardiotoxicity in human users, even if they continue taking cocaine after treatment. In fact the treatment might well provide a degree of protection.

Clinical studies clearly document QT prolongation in cocaine users (Erwin and Deliargyris 2002; Taylor et al., 2004; Magnano et al., 2006; Levin et al., 2008), but murine models of cocaine-induced QT interval prolongation have not been reported. Here, addressing question two, weekly ECG recordings during the course of cocaine treatment showed a cumulative, dose-dependent prolongation of QT, but no change in heart rate, in mice that did not receive CocH vector. That outcome is consistent with another report that 10 days of 40 mg/kg cocaine dosing caused no heart rate fluctuations (Sutliff et al., 2003). Interestingly, that treatment failed to induce QT prolongation. Furthermore, vector pretreatment to secure the presence of CocH in the circulation before exposure to cocaine prevented the drug-induced prolongation of the QT interval. Interestingly, QRS duration was not affected. This outcome suggests that the QT

interval prolongation as a result of cocaine administration was related to an increase in repolarization time, which is in line with previous reports (Guo et al., 2006; Haigney et al., 2006). It is possible that the same mechanism is responsible for the effects seen in our study, as mice have been shown to also express Kcnh2-encoded ERG channels. This implies that gene transfer of cocaine hydrolase should indeed alleviate the adverse cardiovascular action of cocaine, adding further support for future clinical applications of this gene-based therapy.

In vector treated mice, cocaine degrades rapidly into ecgonine methyl ester (EME) and benzoic acid. Some older literature has suggested that EME does affect blood vessel smooth muscle (Zakusov et al., 1978; Kurth et al., 1993) but we recently found no transient blood pressure changes after cocaine doses up to 80 mg/kg i.v. in mice pre-treated with CocH vector (Murthy et al., 2015). Because cocaine disappeared from the blood stream within seconds of administration, the equimolar mixture of cocaine metabolites generated by CocH action must be neither hypertensive nor hypotensive. Our present experiments, delivering 60 mg cocaine per kg per day, generated approximately 40 mg/kg of EME for 3 weeks without affecting body temperature, heart rate, or QT intervals. This result is strong evidence that rapid conversion of large cocaine doses into EME and benzoic acid is unlikely to pose significant risk for vector-treated human subjects.

Clinically, chronic cocaine users exhibit thermoregulatory aberrations, impaired sweating, and cutaneous vasodilation (Crandall et al., 2002). However, in our murine model of drug use there were no sustained fluctuations in temperature throughout the cocaine delivery regimen, either in the positive controls or in the vector-treated mice. Also worth noting, given the autonomic nervous system's involvement in thermal stress and thermoregulation, vector treatment and excess of transgene BChE had no long-term effect on body temperature.

We conclude that neither exposure to viral vector, nor elevated expression of modified BChE, nor cocaine metabolites enzymatically generated during a prolonged course of drug injections have detectable adverse effects on the cardiovascular system. On the contrary, CocH vector treatment in this murine model effectively prevented cocaine-induced QTc prolongation. This outcome is encouraging in regard to an eventual translation of CocH vector into human use. However, as significant differences in cardiac electrophysiology between mice and humans are well known, further testing in larger animal models is warranted before human trials should be considered. Nonetheless, the safety and efficacy documented here provide encouragement for the idea that cocaine hydrolase gene transfer might someday form the basis of a robust treatment to aid cocaine users to achieve relapse-free abstention from that destructive drug.

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Author Contributions

Participated in research design: Murthy, Reyes and Brimijoin

Conducted Experiments: Murthy, Reyes, Geng and Gao

Performed data analysis: Murthy and Reyes

Wrote or contributed to the writing of the manuscript: Murthy, Reyes and Brimijoin

Conflict of Interest

The authors declare no conflicts of interest, including biotech founders shares, sub-licenses, or patent assignments.

REFERENCES

Alekseev AE, Reyes S, Yamada S, Hodgson-Zingman DM, Sattiraju S, Zhu Z, Sierra A, Gerbin M, Coetzee WA, Goldhamer DJ, Terzic A, and Zingman LV (2010) Sarcolemmal ATP-sensitive K(+) channels control energy expenditure determining body weight. *Cell Metab* **11**: 58-69.

Balazs AB, Chen J, Hong CM, Rao DS, Yang L, and Baltimore D (2012) Antibody-based protection against HIV infection by vectored immunoprophylaxis. *Nature* **481**: 81-84.

Bauman JL, and DiDomenico RJ (2002) Cocaine-induced channelopathies: emerging evidence on the multiple mechanisms of sudden death. *J Cardiovasc Pharmacol Ther* **7**: 195-202.

Brimijoin S, Shen ML, and Sun H (2002. Radiometric solvent-partitioning assay for screening cocaine hydrolases and measuring cocaine levels in milligram tissue samples. *Anal Biochem* **309**: 200-205.

Brimijoin S, Gao Y, Anker JJ, Gliddon LA, Lafleur D, Shah R, Zhao Q, Singh M, and Caroll ME (2008) A cocaine hydrolase engineered from human butyrylcholinesterase selectively blocks cocaine toxicity and reinstatement of drug seeking in rats. *Neuropsychopharmacology* **33**: 2715-25.

Brimijoin S, Orson F, Kosten TR, Kinsey B, Shen XY, White SJ, and Gao Y (2013) Anti-cocaine antibody and butyrylcholinesterase-derived cocaine hydrolase exert cooperative effects on cocaine pharmacokinetics and cocaine-induced locomotor activity in mice. *Chemico-biological interactions* **203**: 212-216.

Carroll ME, Zlebnik NE, Anker JJ, Kosten TR, Orson FM, Shen X, Kinsey B, Parks RJ, Gao Y, and Brimijoin S (2012) Combined Cocaine Hydrolase Gene Transfer and Anti-Cocaine Vaccine Synergistically Block Cocaine-Induced Locomotion. *PLoS One* **7**: e43536.

Crandall CG, Vongpatanasin W, and Victor RG (2002) Mechanism of cocaine-induced hyperthermia in humans. *Ann Intern Med* **136**: 785-791.

Erwin MB, and Deliargyris EN (2002) Cocaine-associated chest pain. *Am J Med Sci* **324**:37-44.

Gao Y, and Brimijoin S (2004) An engineered cocaine hydrolase blunts and reverses cardiovascular responses to cocaine in rats. *J Pharmacol Exp Ther* **310**: 1046-1052.

Gao Y, Geng L, Orson F, Kinsey B, Kosten TR, Shen X, and Brimijoin S (2013) Effects of anti-cocaine vaccine and viral gene transfer of cocaine hydrolase in mice on cocaine toxicity including motor strength and liver damage. *Chemico-biological interactions* **203**: 208-211.

Geng L, Gao Y, Chen X, Hou S, Zhan CG, Radic Z, Parks RJ, Russell SJ, Pham L, and Brimijoin S (2013) Gene transfer of mutant mouse cholinesterase provides high lifetime expression and reduced cocaine responses with no evident toxicity. *PLoS One* **8**: e67446.

Guo J, Gang H, and Zhang S (2006) Molecular determinants of cocaine block of human ether-á-go-go-related gene potassium channels. *J Pharmacol Exp Ther*. **317**: 865-74.

Haigney MC, Alam S, Tebo S, Marhefka G, Elkashef A, Kahn R, Chiang CN, Vocci F, and Cantilena L (2006) Intravenous Cocaine and QT Variability. J *Cardiovasc Electrophysiol*. **17**:610-6.

Hahn IH, and Hoffman RS (2001) Cocaine use and acute myocardial infarction. *Emerg Med Clin North Am* **19**: 493-510.

Kane GC, Behfar A, Dyer RB, O'Cochlain DF, Liu XK, Hodgson DM, Reyes S, Miki T, Seino S, and Terzic A (2006) KCNJ11 gene knockout of the Kir6.2 KATP channel causes maladaptive remodeling and heart failure in hypertension. *Hum Mol Genet* **15**: 2285-2297.

Kurth CD, Monitto C, Albuquerque ML, Feuer P, Anday E, and Shaw L (1993) Cocaine and its metabolites constrict cerebral arterioles in newborn pigs. *J Pharmacol Exp Ther* **265**: 587-591.

Kloner RA, Hale S, Alker K, and Rezkalla S (1992) The effects of acute and chronic cocaine use on the heart. *Circulation.* **85**: 407-19.

Levin KH, Copersino ML, Epstein D, Boyd SJ, and Gorelick, DA (2008) Longitudinal ECG changes in cocaine users during extended abstinence. *Drug Alcohol Depend.* **95**: 160-163.

Magnano AR, Talathoti NB, Hallur R, Jurur DT, Dizon J, Holleran S, Bloomfield DM, Collins E, and Garan H (2006) Effect of acute cocaine administration on the QTc interval of habitual users. *Am J Cardiol* **97**: 1244-1246.

Mendelson JH, and Mello NK. Management of cocaine abuse and dependence (1996) *N Engl J Med* **334**: 965-972.

Mitchell GF, Jeron A, and Koren G (1998) Measurement of heart rate and Q-T interval in the conscious mouse. *Am J Physiol* **274**: H747-H751.

Murthy V, Gao Y, Geng L, LeBrasseur N, White T, and Brimijoin S (2014a) Preclinical studies on neurobehavioral and neuromuscular effects of cocaine hydrolase gene therapy in mice. *J Mol Neurosci* **53**: 409-416.

Murthy V, Gao Y, Geng L, LeBrasseur NK, White TA, Parks RJ, and Brimijoin S (2014b) Physiologic and metabolic safety of butyrylcholinesterase gene therapy in mice. *Vaccine* **32**: 4155-4162.

Murthy V, Geng L, Gao Y, Zhang B, Miller JD, Reyes S, and Brimijoin S (2015) Reward and Toxicity of Cocaine Metabolites Generated by Cocaine Hydrolase. *Cell Mol Neurobiol* **35**: 819-826.

O'Cochlain DF, Perez-Terzic C, Reyes S, Kane GC, Behfar A, Hodgson DM, Strommen JS, Liu XK, van den Broek W, Wansink DG, Wieringa B, and Terzic A (2004) Transgenic overexpression of human DMPK accumulates into hypertrophic cardiomyopathy, myotonic myopathy and hypotension traits of myotonic dystrophy. *Hum Mol Genet* **13**: 2505-2518.

Phillips K, Luk A, Soor GS, Abraham JR, Leong S, and Butany J (2009) Cocaine cardiotoxicity: a review of the pathophysiology, pathology, and treatment options. *Am J Cardiovasc Drugs* **9**: 177-196.

Reyes S, Kane GC, Zingman LV, Yamada S, and Terzic A (2009) Targeted disruption of KATP channels aggravates cardiac toxicity in cocaine abuse. *Clin Transl Sci* **2**:361-365.

Saxena A, Sun W, Luo C, and Doctor BP (2005) Human serum butyrylcholinesterase: in vitro and in vivo stability, pharmacokinetics, and safety in mice. *Chemico-biological interactions* **157**: 199-203.

Saxena A, Sun W, Fedorko JM, Koplovitz I, and Doctor BP (2011) Prophylaxis with human serum butyrylcholinesterase protects guinea pigs exposed to multiple lethal doses of soman or VX. *Biochem Pharmacol* **81**: 164-9.

Sun H, Pang YP, Lockridge O, and Brimijoin S (2002) Re-engineering butyrylcholinesterase as a cocaine hydrolase. *Mol Pharmacol* **62**: 220-224.

Sutliff RL, Haase C, Russ R, Holt BD, Morris R, Norman AB, and Lewis W (2003) Cocaine increases mortality and cardiac mass in a murine transgenic model of acquired immune deficiency syndrome. *Lab Invest* **83**: 983-989.

Taylor D, Parish D, Thompson L, and Cavaliere M (2004) Cocaine induced prolongation of the QT interval. *Emerg Med J* **21**: 252-253.

Weber A, Butterweck H, Mais-Paul U, Teschner W, Lei L, Muchitsch EM, Kolarich D, Altmann F, Ehrlich HJ, and Schwarz HP (2011) Biochemical, molecular and preclinical characterization of a double-virus-reduced human butyrylcholinesterase preparation designed for clinical use. *Vox Sang* **100**: 285-97.

Wood DM, Dargan PI, and Hoffman RS (2009) Management of cocaine-induced cardiac arrhythmias due to cardiac ion channel dysfunction. *Clin Toxicol* **47**: 14–23.

Xie W, Altamirano CV, Bartels CF, Speirs RJ, Cashman JR, and Lockridge O (1999) An improved cocaine hydrolase: the A328Y mutant of human butyrylcholinesterase is 4-fold more efficient. *Mol Pharmacol* **55**: 83-91.

Yang W, Xue L, Fang L, Chen X, and Zhan CG (2010) Characterization of a high-activity mutant of human butyrylcholinesterase against (-)-cocaine. *Chemico-biological interactions* **187**: 148-152.

Zakusov VV, Kostochka LM, and Skoldinov AP (1978) Effect of cocaine molecule fragments on the central nervous system. *Biull Eksp Biol Med* **86**: 435-438.

Zheng F, Yang W, Xue L, Hou S, Liu J, and Zhan CG (2010) Design of high-activity mutants of human butyrylcholinesterase against (-)-cocaine: structural and energetic factors affecting the catalytic efficiency. *Biochemistry* **49**: 9113-9119.

Zlebnik NE, Brimijoin S, Gao Y, Saykao AT, Parks RJ, and Carroll ME (2014) Long-term reduction of cocaine self-administration in rats treated with adenoviral vector-delivered cocaine hydrolase: evidence for enzymatic activity. *Neuropsychopharmacology* **39**: 1538-1546.

Footnotes

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Figure Legends

Figure 1. Experimental flow chart. Mice were acclimated on arrival followed by implantation of telemetry devices. After surgical recovery mice were randomly assigned for vector (AAV-CocH, 10^{13} viral particles, i.v., N = 8) or saline pre-treatment (N = 16). Four weeks later, plasma CocH expression was confirmed in the Vector group and baseline ECG was recorded in all groups. As indicated, Saline Controls received only saline i.p., twice daily ~ 300 µl per injection. Cocaine and Vector groups (N = 8 each) both received 30 mg/kg cocaine i.p., twice daily. All mice were recorded each week (30 min sessions).

Figure 2. Heart rate in Vector mice (N = 8) and age-matched Saline and Cocaine controls, (N = 7 each). Data are from 30 min of recording (mean ± SEM). Baseline heart rates ranged from 450 – 600 bpm. No significant differences were detected: *P* = N.S. for all possible comparisons by 2-way ANOVA.

Figure 3. (**a**) Representative ECG's from each treatment group: Saline controls; Positive controls given cocaine; Vector-expressing mice given cocaine. Scale - the horizontal scale bar represents 50 ms, and the vertical scale bar represents 0.1 mV. (**b**) Description of ECG component waves, highlighting P, Q, R, S and T waves, as well as QRS and QT intervals. (**c**) QT intervals measured manually and corrected for heart rate (QTc; see Methods) from Vector mice (N = 7- 8); Saline controls (N = 5 - 7) and Cocaine mice (N = 4 - 8) over the course of cocaine treatment. Data represent mean ± SEM. * $P < 0.05$ vs same group baseline.

Figure 4. (**a - c**) Representative 40x micrographs of the left ventricular posterior free wall in experimental groups as labeled (saline, N = 4; cocaine, N =3; vector, N = 3). (**d**) Cross-sectional

area is not significantly altered after repeated exposure to cocaine alone or in the presence of vector. Scale bar = 200 μm.

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Table 1. Body temperature in Vector mice (N = 8) and age-matched Saline and Cocaine mice (N = 7 each). Temperature data were averaged across 30 min intervals. Data represent mean ± SEM. At baseline, mean temperature across all groups was 37.9 ± 0.2 °C. No group differences were observed: *P* = N.S. for all possible comparisons by 2-way ANOVA.

Treatment	Temperature (°C) (Mean ± SEM)			
	Baseline	Week 1	Week 2	Week 3
Saline (N = 7)	37.7 ± 0.4	37.8 ± 0.2	37.8 ± 0.2	38.0 ± 0.2
Cocaine (N = 7)	37.8 ± 0.4	38.0 ± 0.4	38.1 ± 0.3	37.6 ± 0.4
Vector (N = 8)	38.2 ± 0.4	37.7 ± 0.2	38.0 ± 0.3	37.8 ± 0.2

Figure 1

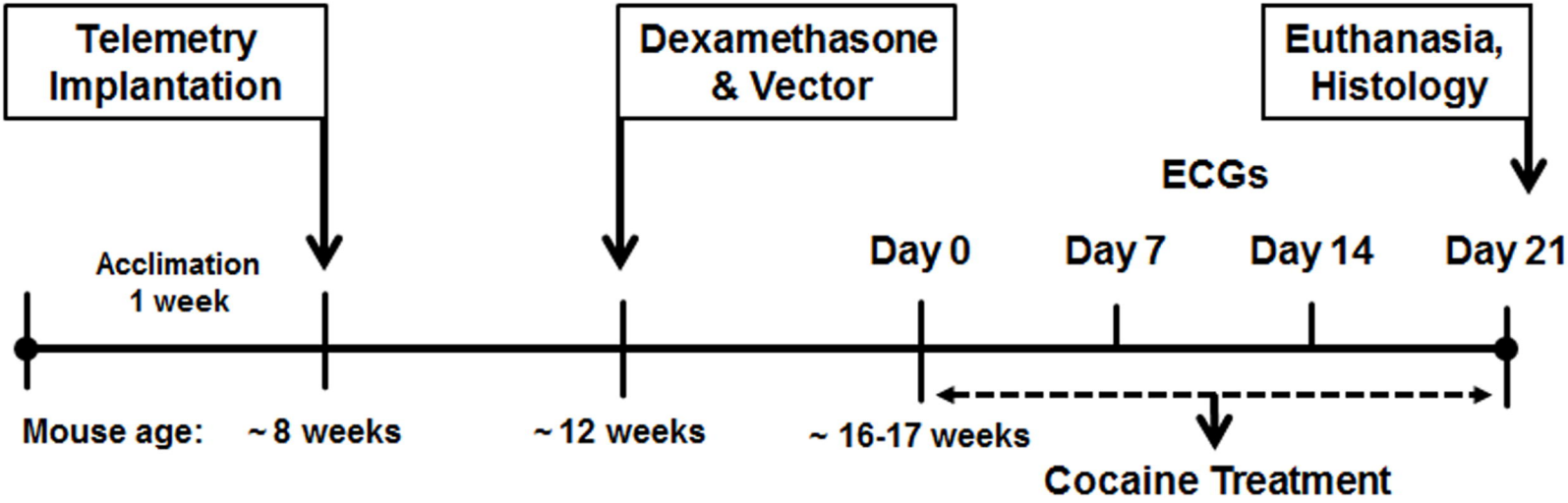
Telemetry Implantation
Dexamethasone & Vector
Euthanasia, Histology
ECGs
Acclimation 1 week
Day 0
Day 7
Day 14
Day 21
Mouse age:
~ 8 weeks
~ 12 weeks
~ 16-17 weeks
Cocaine Treatment

Figure 2

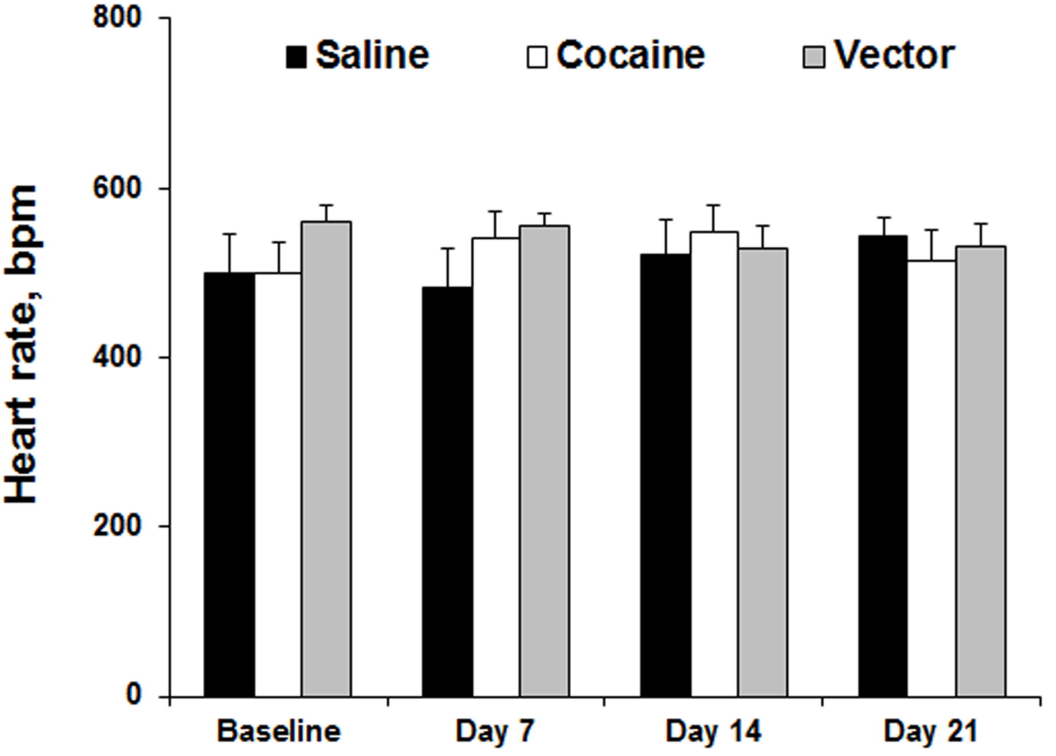
800
600
400
200
0
Heart rate, bpm
Saline
Cocaine
Vector
Baseline
Day 7
Day 14
Day 21

Figure 3

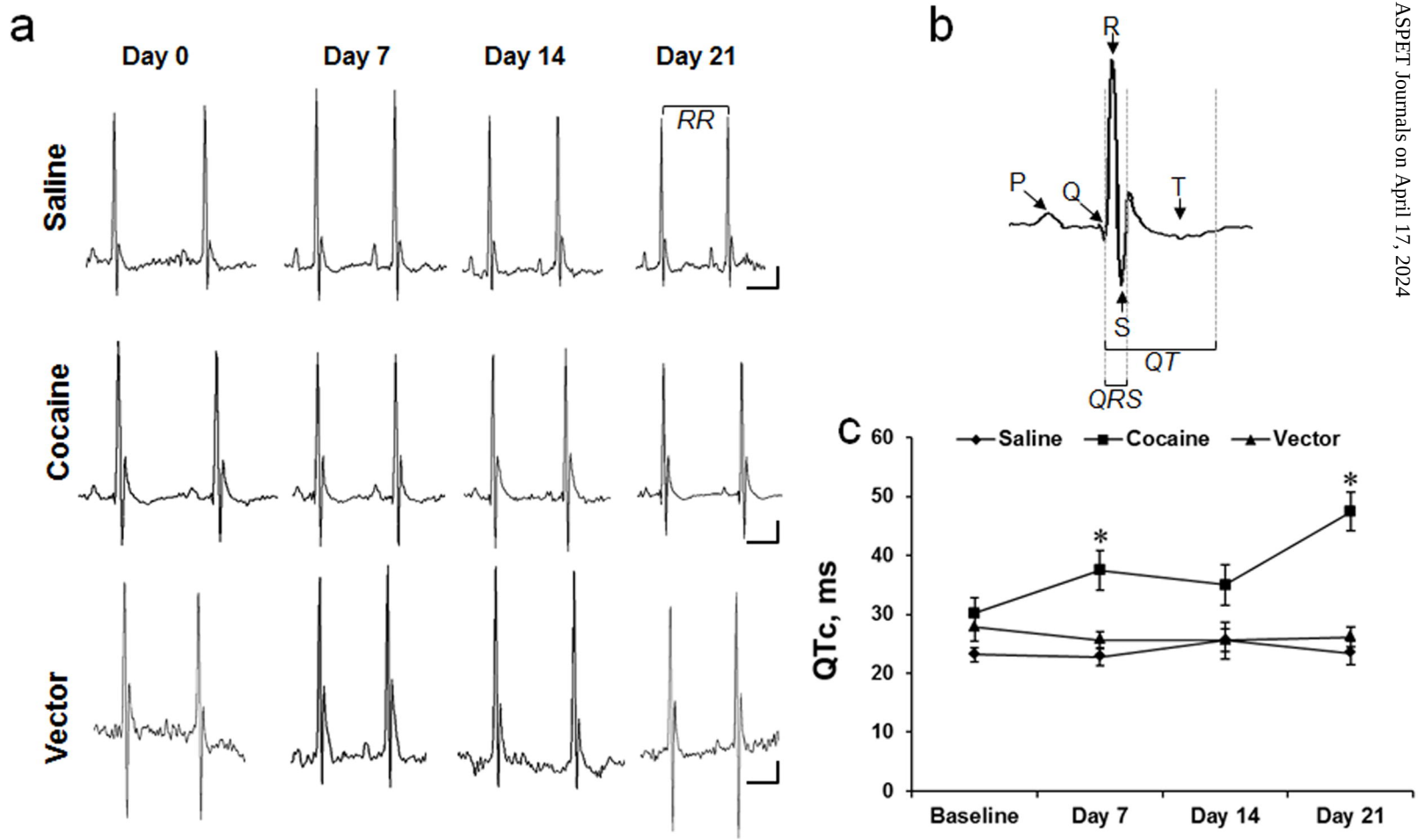

Figure 4