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Rapid communication

Brain biomarkers for identifying excited delirium as a cause of sudden death

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ABSTRACT

Excited delirium (ED) syndrome is a serious medical condition associated with acute onset of agitated violent behavior that often culminates in a sudden unexplained death. While the contribution of restraint, struggle and the use of conductive energy devices (CED) to the cause and manner of death raise controversy, a CNS dysfunction of dopamine signaling may underlie the delirium and fatal autonomic dysfunction. We conducted a mortality review for a case series of ninety excited delirium deaths and present results on the association of a 2-protein biomarker signature. We conducted quantitative analyses of the dopamine transporter and heat shock protein 70 validated for specificity and degree of interindividual variation. Incident circumstances, force measures, autopsy and toxicology results were determined for all subjects. A majority of the victims in this case series tested positive for cocaine in blood and brain, although four had no licit or illicit drugs or alcohol measured at autopsy. Mean core body temperature where recorded was 40.7 °C. The expression of the heat shock protein HSPA1B transcript was elevated 1.8–4-fold in postmortem brain. The elevation of Hsp70 in autopsy brain specimens confirms that hyperthermia is an associated symptom and often a harbinger of death in these cases. Dopamine transporter levels were below the range of values measured in age-matched controls, providing pathologic evidence for increased risk of chaotic dopamine signaling in excited delirium. When combined with descriptions of the decedents' behavior prior to death, a 2-protein biomarker signature can serve as a reliable forensic tool for identifying the excited delirium syndrome at autopsy.

1. Introduction

Victims of excited delirium syndrome who present in a highly agitated state often die in police custody, while being restrained or incapacitated by deployment of conductive energy devices (CED) [1–3]. Excited delirium is one of several terms that describe a syndrome characterized by delirium and agitation, combativeness, unexpected strength and elevated body temperature. Although there is no anatomic cause of death in excited delirium, catecholamine-induced cardiac arrhythmias, restraint or positional asphyxia, or adverse cardiorespiratory effects of CED (e.g. TASER®) are often cited [3–5]. However, case reviews demonstrate that the individual is medically unstable and in a rapidly declining state that has a high risk of mortality even with medical intervention or in the absence of restraint stress or CED deployment [5,6].

Delirium is a syndrome, or group of symptoms, caused by a disturbance of consciousness in the normal functioning of the brain [7]. In hyperactive delirium, there is a change in awareness and response to the environment, which manifests as agitation, hallucinations, delusions, and psychosis. Over 150 years ago, Dr. Luther Bell described a disease in institutionalized psychiatric patients resembling some advanced stage of mania and fever as an overlooked and often unrecorded malady [8]. Fishbain and Wetli [9] recognized the same syndrome in a cocaine body packer, and their report was followed by a series of case reports of excited delirium most often associated with chronic stimulant abuse [10–16]. Although there was no single causal factor cited, long-term psychostimulant abuse appears to increase risk for excited delirium.

We present a retrospective analysis and mortality review of factors associated with sudden unexpected deaths of individuals in states of excited delirium. Scene investigation, autopsy and toxicology results were reviewed for a case series of 90 excited

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delirium deaths. The results demonstrate that a 2-protein biological marker (dopamine transporter and heat shock protein) analysis when combined with descriptions of the decedent's behavior prior to death is reliably associated with this syndrome. We conclude that the identification of postmortem biological markers (biomarkers) serve as an objective-testing method for assisting medical examiners in identifying excited delirium at autopsy.

2. Methods

2.1. Medical examiner investigations, data analysis and procedures

The deaths in this case series were routine medical examiner investigation of sudden and unexpected deaths, most of which occurred while the victims were in police custody. Each investigation of an acute, unexpected psychiatric death included a description of the scene and circumstances surrounding the death, review of records from emergency departments, and a complete autopsy with toxicologic analysis of cocaine and other licit or illicit drugs in blood. Cases of fatal excited delirium (ED) were submitted for analytical consultation from different locales (Florida, California, Georgia, Kansas, Maryland, Missouri, Pennsylvania, Tennessee, New York, Canada, UK, Sweden) if the individual exhibited an episode of delirium, bizarre and violent behavior, followed by sudden death [9,12,15,16]. Frozen unfixed neuropathological specimens were used for analytical measures of dopamine transporter and Hsp70 transcript and protein measures. Brain specimens were selected for biomarker and supplemental toxicologic analyses of cocaine and metabolites sampled from coronal blocks that had been stored at −80 °C prior to assay. Autopsy records were reviewed from an available cohort of banked specimens where medical examiners certified that death was due to cocaine intoxication and the manner of death was accidental [15,16].

Demographic data, autopsy, pathology and the circumstances of death were abstracted to create a relational database system (SPSS v. 15.0.0). General toxicological information on drugs of abuse present at death was obtained from the medical examiner's office and supplemented with additional toxicological testing in brain. Gas chromatography–mass spectrometry (GC–MS) was employed to examine occipital cortex from all excited delirium and cocaine intoxication cases for the presence of cocaine and metabolites [17,18]. Age-matched drug-free control specimens from individuals who died from natural causes or trauma ($N = 30$) were analyzed in order to obtain a range of reference values for comparison to cocaine intoxication deaths ($N = 100$) and cases of excited delirium ($N = 90$). All cases and controls selected for analytical measures were matched for age and postmortem interval.

2.2. Biomarker analyses

Saturation analysis of [^{3}H]WIN35,428 binding to the dopamine transporter was conducted in unfixed cryopreserved specimens as described previously [19]. Briefly, a fixed concentration of [^{3}H]WIN35,428 (0.5 nM) was incubated in a total volume of 2.0 mL with human striatal membranes (2.5 mg/mL) in the presence of increasing concentrations of unlabeled WIN35,428 (0.1 nM–10 μM) for 2 h at 4 °C. Cases were excluded from radioligand binding site analysis if the individual suffered a prolonged agonal state, including respiratory arrest and multi-organ failure. Samples of human putamen were dissected and the tissue was homogenized in 20 volumes of 10 mM sodium-phosphate buffer (pH 7.4) containing 0.32 M sucrose using a Brinkman polytron at setting 3 for 15 s. The membranes were pelleted by centrifugation at 32,000 × g for 15 min. The membrane pellet was washed 1 time and resuspended in ice-cold sucrose-phosphate buffer at a final dilution of 1:20 (w/v).

Gene expression of HSPA1B gene was measured in frozen brain specimens (Brodmann area 22) by real-time PCR using TaqMan Universal PCR Master Mix and the Applied Biosystems 7900HT thermocycler (ABI, Foster City, CA). TaqMan probes and proprietary primers designed based on previously reported sequences were purchased from Applied Biosystems Inc. (Foster City, CA). cDNA was amplified using TaqMan Fast Universal PCR master mix reagent at the following conditions: 20 s at 95 °C, 40 cycles: 1 s at 95 °C and 20 s at 60 °C. Target cDNA for HSPA1B, Cyclophilin (PPIA), ribosomal protein L19 (RPL19) and tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein, zeta polypeptide (YWHAZ) was amplified using TaqMan ABI MGB probe and primer set assay; Hs00359147_s1, Hs99999904_m1, Hs02338565_gh, Hs00237047_m1, respectively. PPIA, RPL19 and YWHAZ were used as housekeeping genes for normalization.

The size of the PCR fragment was determined using Go Taq Green Master Mix from Promega (Madison, WI) in a Bio-Rad iCycler (Hercules, CA). RT-PCR reactions consisted of a 2-min denaturation step at 95 °C, and 30 cycles of denaturation at 95 °C for 30 s, followed by annealing for 30 s at 62 °C, and extension for 2 min at 72 °C followed by 5 min at 72 °C. Cyclophilin was used as an internal standard. The primer pairs were as follows: HSPA1B (30 cycles): 5′-CCGAGAAGGACGAGTTTGAG-3′, and 5′-GCAGCAAA GTC CTTGAGTCC-3′, PPIA (30 cycles): 5′-TTCATCTGCACTGCCAAGAC-3′, and 5′-TCGAGTTGTCCACAGTCAGC-3′. The PCR products were visualized by 2% agarose gel electrophoresis on a Molecular Image Gel Doc XR System (Bio-Rad, Hercules, CA).

For Western blot analysis of HSPA1B protein (heat shock 70 kDa protein 1B), frozen brain specimens were processed using the Qproteome Mammalian Protein Prep Kit according to manufacturer's instructions (Qiagen, Valencia, CA). Supernatants were run on a 10% sodium dodecyl sulfate gel and transferred to Immobilon-P nitrocellulose (Millipore, Bedford, MA). After transfer, the membranes were blocked and then incubated overnight at 4 °C with mouse monoclonal anti-HSPA1B antibody (Novus Biologicals, Littleton, CO). Blots were incubated with HRP labeled goat anti-mouse IgG secondary antibody (Pierce Biotechnology, Rockford, IL). Labeled proteins were detected and quantified using SuperSignal West Pico Chemiluminescent Substrate (Pierce Biotechnology, Rockford, IL). Blots were stripped and reprobed with a monoclonal anti-alpha-tubulin antibody (Sigma, St. Louis, MO) to confirm that equal amounts of protein were loaded from each case.

2.3. Statistical analysis

Estimation of the maximal number of binding sites for [^{3}H]WIN35,428 was done using non-linear curve fitting software LIGAND (Biosoft, KELL v.6.0, Cambridge, GB-UK) and Prism (v.3.0, GraphPad, San Diego, CA). Group differences were assessed by one-way ANOVA and post-hoc analyses were conducted using the Dunnett's test and results were considered statistically significant when $p < 0.05$.

Normalized cycle threshold values (calculated automatically within the Applied Biosystems software RQ manager 1.2) were used to estimate expression ratios between the excited delirium or cocaine and control groups. Geometric averaging of multiple internal control genes was done for normalization of qPCR data [20]. Expression ratios were subjected to a log2 transform to produce fold change data. Student's t-test was used to test for significant differences between groups. One-way analysis of variance (ANOVA) was used to compare gene expression with a Tukey's post-hoc comparison (SPSS, Inc.).

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Table 1
Demographic characteristics of 90 excited delirium subjects.

Characteristic	*n*	%
Men	82	91.1
White	27	32.9
Black	36	43.9
Hispanic	19	23.2
Women	8	8.9
White	4	50.0
Black	3	37.5
Hispanic	1	12.5
Mean age (year ± S.D.)		
Men	34.2 ± 7.2	
Women	32.8 ± 6.7	
Mean height (in. ± S.D.)		
Men	69.1 ± 3.0	
Women	61.5 ± 2.1	
Mean body weight (lb ± S.D.)		
Men	200.9 ± 42.0	
Women	140.4 ± 19.6	
Body mass index (mean + S.D.)		
Men	29.6 ± 5.4	
Women	26.0 ± 2.9	
Mean body temp. (°C ± S.D)[a]	40.7 ± 2.6	
Seizures		
Present	12	13.3
Absent	78	86.7
Survival time		
<1 h	42	46.8
1–6 h	30	33.3
7–12 h	4	4.4
>12 h < 2 days	12	13.3
>2 days	2	2.2

[a] Body temperatures where available ($N = 53$, range 37.7–42.2 °C).

3. Results

Table 1 summarizes the demographic information for 90 autopsy cases of excited delirium. Excited delirium victims are young (mean age 34.2 ± 7.2) males (91.1%), with a high body mass index (29.6 ± 5.4). For decedents with recorded body temperatures, mean body temperature was 40.7 °C (Table 1). Seizures or uncontrolled shaking was observed in 13% of the cases. Many of the deaths occurred within 1 h after initial contact by the police during the period of paranoia and delirious agitation. The survival time data were analyzed from the approximate time the police first encountered the individual and do not reflect onset of delirium signs and symptoms to pronouncement of death. For most excited delirium victims, cardiorespiratory arrest occurred shortly after the use of restraints, chemical agents or deployment of a CED. In cases where resuscitation was successful, death due to rhabdomyolysis and multisystem failure occurred within 2 days. In three cases, death occurred at the victims' residence without police intervention. The death scene investigation showed elevated rectal temperatures and lack of clothing consistent with an elevated body temperature. The ransacked appearance of the dwellings, abrasions on the extremities, and positive toxicology were taken as evidence that the cause of death was drug-induced excited delirium.

Arrest circumstances and police force measures are shown in Table 2. Disturbance call encounters evidenced agitated behaviors, including destruction of property, disorderly conduct, inappropriate disrobing, running wildly in and out of traffic on streets, and kicking residents' doors, or the compulsion to break or bang on glass. Aggravated assaults on friends or family members and attempted breaking and entering were also reported. One subject in detention experienced hallucinations and flooded his cell after confinement. Attempts by correctional personnel to restrain the prisoner resulted in him violently resisting their attempts to control him followed by sudden death.

Table 2
Incident circumstance, force measures used by police, and location of death ($n = 90$).

Factor	*n*	%
Incident circumstance		
Disturbance call	45	50.0
Aggravated assault	14	15.6
Running in/out of traffic	10	11.1
Attempted breaking and entering	8	8.9
Emergency medical service (EMS) call	7	7.8
Found unconscious/dead	3	3.3
Operating vehicle erratically	2	2.2
Violent agitation while in jail detention	1	1.1
Force measures used by police		
Mechanical restraints	75	83.3
Conductive energy device (TASER®)	16	17.8
Chemical agents (pepper spray)	7	7.8
Hogtied (maximum hobble restraint)	6	6.7
Stun gun	4	4.4
Impact weapon strikes	2	2.2
Location of death		
Emergency room/hospital	35	38.9
At scene	28	31.1
EMS transport	14	15.6
Police transport	13	14.4

Force measures used by police included empty hand control for combative subjects and application of mechanical restraints (Table 2). Maximal hobble restraint or hogtying victims (placed prone with wrists tied behind the back together with the ankles) was used for only 7% of the in-custody deaths reported here. A total of seven out of the 90 cases in the series involved the use of chemical agents. Electrical incapacitation (CED) to override the CNS or pain compliance (dry stun) was used on 18% of the cases with a variable number of deployments and strikes (data not shown).

Table 3 shows the results of blood toxicology. Psychostimulants (cocaine and methamphetamine) were detected in 94% of the cases. Brain concentrations of cocaine and the cocaine metabolite benzoylecgonine in excited delirium cases were in the same range as measured in a reference cohort of accidental cocaine intoxication deaths (Table 4). The levels of cocaine measured in peripheral blood were lower in cocaine-related excited delirium deaths, in keeping with longer survival times as has been reported previously [13]. The most commonly reported route of administration was smoke inhalation ($N = 47$; 58%), followed by nasal insufflation ($N = 15$, 18.6%) intravenous drug use ($N = 1$; 1.2%) and unknown ($N = 18$; 22.2%).

One of the victims had a confirmed psychiatric diagnosis with measurable blood levels for two prescription medications

Table 3
Toxicology results for excited delirium victims.

Drug type	*n*	%
Cocaine	53	58.9
Cocaine + alcohol	24	26.8
Cocaine + amphetamines	2	2.2
Cocaine + amphetamines + alcohol	1	1.1
Cocaine + ephedrine/pseudoephedrine	1	1.1
Amphetamine/methamphetamine	1	1.1
Amphetamine/methamphetamine + alcohol	1	1.1
MDMA/MDA + alcohol	1	1.1
Ephedrine/pseudoephedrine	1	1.1
Psychiatric medication (Risperidone Citalopram)	1	1.1
None detected	4	4.4

Postmortem blood, $N = 84$; premortem blood, $N = 6$.

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Table 4
Brain and blood concentrations of cocaine and benzoylecgonine in excited delirium deaths.

	COC		ED	
	Blood (mg/L)	Brain (mg/kg)	Blood (mg/L)	Brain (mg/kg)
Cocaine	3.30 ± 0.74	4.50 ± 0.74	0.81 ± 0.11[a]	2.73 ± 0.66
Benzoylecgonine	4.00 ± 0.47	1.21 ± 0.14	3.29 ± 0.34	1.53 ± 0.15
Cocaethylene	0.39 ± 0.18	0.15 ± 0.04	0.08 ± 0.01	0.11 ± 0.02

Postmortem blood samples from cocaine intoxication deaths (COC, $N = 100$) and cocaine-related excited delirium (ED, $N = 68$). Postmortem brain samples (occipital cortex) from COC ($N = 100$) and ED ($N = 81$) subjects. Lower concentrations in blood reflect variable timeframes of survival and drug metabolism or degradation. Brain concentrations of cocaine or metabolites were not significantly different between the two groups.

[a] A significant difference was demonstrated for blood cocaine concentrations between COC and ED ($p < 0.0001$, students t-test).

(risperidone and citalopram). There were 4 cases of acute mania in the absence of any illicit or licit drugs (Table 3). One of these victims became deliriously agitated and died suddenly after recently experiencing the operational stress of extreme sleep deprivation during Swedish military maneuvers and simulated battle at sea. These case reviews underscore that not all excited delirium deaths are due to stimulant drugs albeit the majority are.

The listed cause of death from review of the death certification is shown in Table 5. Death in relation to drugs was reported in 88% of the excited delirium cases examined. Traumatic injury was cited for 5 of the incidents. Acute exhaustive mania was determined to be the cause of death for 4 subjects. In some cases, excited delirium was termed in the cause of death statement as a contributory condition secondary to cardiopulmonary arrest or myocardial infarction. Death due to gunshot wounds was reported in a psychotically agitated or delirious person shot by other citizens or police. Overall, the assessment of the death certificates demonstrates that there was no anatomic correlate for a majority of these cases.

We applied independent analytical strategies to test the reliability of association for a 2-protein biomarker signature with the occurrence of excited delirium. Since the majority of the victims of excited delirium were chronic, long-term cocaine abusers, we compared the results in excited delirium victims to drug-related deaths due to the toxic effects of cocaine alone or cocaine in combination with alcohol [15,16]. Presynaptic dopamine transporters were assayed in postmortem brain with the cocaine analog [^{3}H]WIN35,428 (Fig. 1). Saturation analysis of the total amount of radioactivity bound confirmed a significantly lower number of dopamine transporter sites (115.4 ± 2.7 pmol/g) in the anteroventral striatum of cocaine-related excited delirium victims ($N = 65$, $p < 0.001$) compared to chronic cocaine abusers (256.7 ± 7.9 pmol/g).

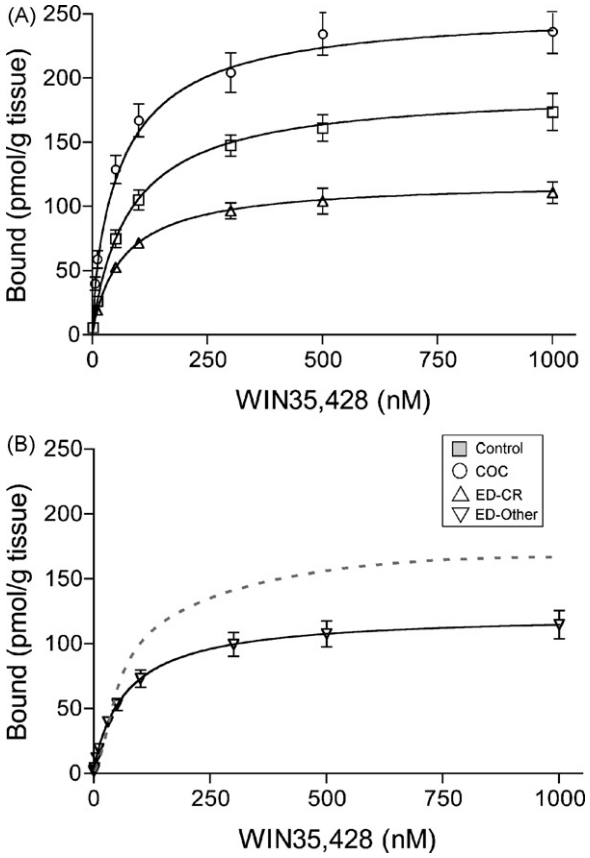

Fig. 1. Saturation binding of [^{3}H]WIN35,428 to the dopamine transporter in excited delirium. (A) Dopamine transporter number was measured by varying the concentration of WIN35,428 in ventral striatum from age-matched drug-free control subjects (□) age-range 33.7 ± 4.4, $N = 35$), cocaine intoxication deaths (○) age-range 35.2 ± 3.9, $N = 38$), and cocaine-related excited delirium victims (△) age-range 34.1 ± 2.2, $N = 65$). (B) Saturation plots illustrate maximum number of dopamine transporters in cases of acute exhaustive mania (▽) age-range 35.8 ± 6.4, $N = 4$) and compared to the average of the individual control values (dashed line). Data shown are the integrated sample estimates of the means ± S.E.M.

Panel B in Fig. 1 illustrates the average density of dopamine transporters in four subjects with no known drug abuse history who died suddenly in a state of excited delirium. Saturation analysis of WIN35,428 binding showed significantly lower dopamine transporter numbers in comparison to values measured in age-matched drug-free controls (95.6 ± 9.6 vs. 186.2 ± 3.8 pmol/g; $p < 0.001$) [21]. The average density of dopamine transporter sites was within the range of values for cocaine-related excited delirium cases illustrated in panel A. When all available data from the cohort were included in the analysis ($N = 74$), the overall maximum binding site density estimates were in excellent agreement with values determined in cocaine-related cases of excited delirium (114.2 ± 2.4 vs. 115.4 ± 2.7 pmol/g, respectively). There was no change in the estimated affinity constants (K_D values) for WIN35,428 across case and control groups, in agreement with previously published values (data not shown) [19] These results demonstrate low interindividual variability of the dopamine transporter assay and when compared to age-matched drug-free controls validate dysregulated dopamine transporter numbers as a postmortem biomarker of excited delirium.

Hyperthermia, a condition of extremely high core body temperature, is associated with induction of heat shock proteins. We examined a proprietary genome-wide database of transcriptional microarray data (Affymetrix GeneChip Plus 2 arrays,

Table 5
Cause of death for excited delirium victims.

	n	%
Acute cocaine toxicity	29	32.4
Acute exhaustive mania	4	4.4
Blunt trauma	2	2.2
Cardiopulmonary arrest (excited delirium nos.)	1	1.1
Cocaine psychosis	10	11.1
Cocaine excited delirium	32	35.6
Excited delirium (MDMA)	1	1.1
Excited delirium (methamphetamine intoxication)	1	1.1
Gunshot wounds	3	3.3
Hyperthermia (phentermine, ephedrine/pseudoephedrine)	1	1.1
Multi-organ failure (cocaine intoxication)	3	3.3
Myocardial infarction		
Excited delirium due to cocaine	1	1.1
Excited delirium nos	1	1.1
Ventricular fibrillation	1	1.1

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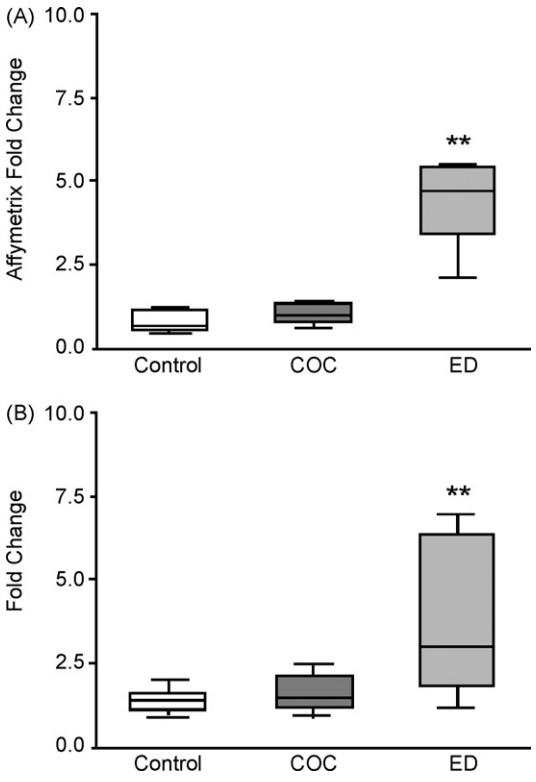

Fig. 2. Heat shock protein 70 gene expression in excited delirium deaths. (A) Raw Affymetrix data (Microarray Analysis Suite version 5.0) illustrate the differential expression of HSPA1B gene. Box plots illustrate range and median values. Cocaine-related excited delirium; $N = 12$; $^{**}p < 0.001$ (B) Quantitative real-time RT-PCR of HSPA1B expression in brain. Excited delirium cases were included in qPCR analysis if RNA integrity numbers (RIN values) were >6.0. Excited delirium, $N = 80$; $^{**}p < 0.001$.

courtesy of GeneLogic, Inc., Gaithersburg, MD) from cocaine-related cases of excited delirium for regulation of the heat shock family of genes. In comparison to cocaine intoxication deaths, we demonstrated a significant upregulation in the HSPA1B transcript (Fig. 2A, $p < 0.001$). This gene codes for heat shock protein 70 (Hsp70), which is elevated in brain whenever core body temperature reaches or exceeds 39 °C [22]. We validated this observation in a larger sample of cases using real-time PCR on brain samples that had intact RNA (RNA integrity number > 6.0; $N = 80$, Fig. 2B). The range of HSPA1B fold change values determined by qPCR are in excellent agreement with the microarray results, providing evidence for induction of Hsp70 transcript in brain samples from victims of excited delirium.

HSPA1B transcript size and relative abundance was measured by RT-PCR and semiquantitative results determined by densitometry (Fig. 3A). The HSPA1B transcript in human brain gave a single band with increased expression in excited delirium cases as compared either to cocaine intoxication deaths or controls (Fig. 3A, $N = 20$, $p < 0.001$). We observed a significant increase in the relative expression of the HSPA1B transcript, in keeping with the quantitative results shown in Fig. 2.

We tested for transient increases in Hsp70 protein on the same cases selected for RT-PCR as an additional test for induction of this biomarker of hyperthermia (Fig. 3C and D). Quantitation of immunopositive bands from Western blots demonstrated higher HSPA1B protein expression in cocaine-related excited delirium victims compared to cocaine intoxication deaths and age-matched drug-free control subjects (Fig. 3C). Solubilized proteins from the temporal cortex (Brodmann area 22) were compared to a dilutional protein pool prepared from age-matched drug-free controls. Protein extracts from individual excited delirium and cocaine intoxication deaths and matched control cases were probed with an anti-Hsp70 antibody. A single band was observed at the expected molecular mass of 70 kDa in agreement with the results seen for the recombinant protein (Fig. 3C). Denser Hsp70-positive bands were consistently observed in excited delirium victims as compared to control subjects (Fig. 3C; $p < 0.01$). Densitometric analysis of the immunoblots revealed no change in the level of alpha-tubulin (50 kDA) across experimental sample runs, which ensured evenness of protein loading. Although we cannot estimate a precise time course for induction of Hsp70 protein in human brain, the results suggest that inducible Hsp70 as a biomarker of thermal stress is a frequent condition in excited delirium.

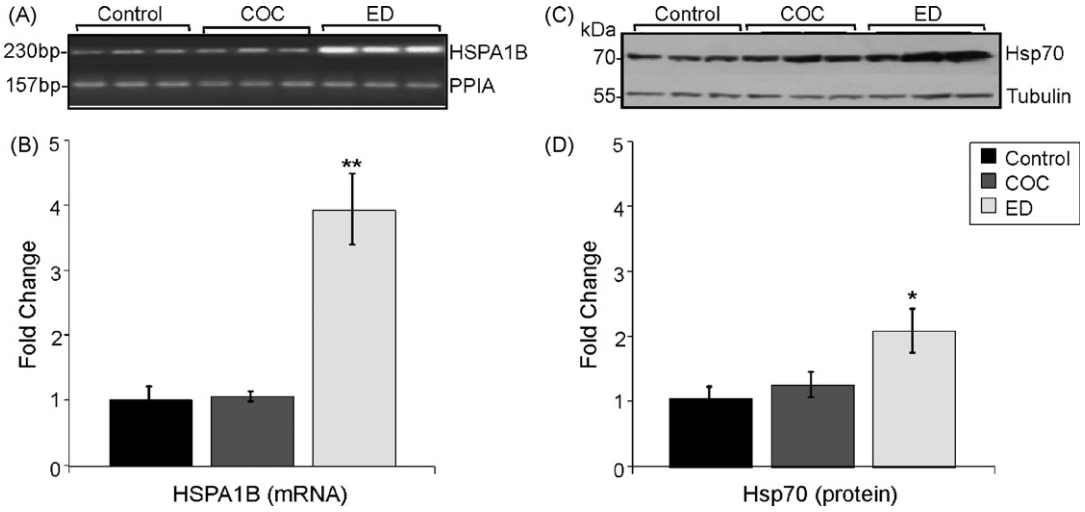

Fig. 3. Comparative analyses of heat shock protein 70 transcript and protein in cases of excited delirium. (A) RT-PCR assay of HSPA1B transcript in human brain. Representative cases are shown. HSPA1B was normalized to an endogenous control gene cyclophilin. (B) Graphical representation of the relative mRNA levels in excited delirium, control and cocaine groups ($N = 20$, respectively). (C) Western blot analysis of Hsp70 protein expression. Representative immunoblots show Hsp70 immunopositive bands in cocaine-related excited delirium and cocaine intoxication deaths as compared with age-matched drug-free control subjects ($N = 20$, each group as shown in panels A and B. (D) Quantitation of protein expression normalized to alpha tubulin. Optical density measurements are shown as fold-change values. Data are means ± S.E. $^{*}p < 0.01$, $^{**}p < 0.001$ (one-tailed t-test).

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4. Discussion

We report here the first association of a 2-protein biomarker study of excited delirium deaths. Although pathological findings in excited delirium exclude an obvious cause of death, Hsp70 induction in cases of excited delirium indicates that death likely occurs soon after the person becomes hyperthermic. Dopamine transporter assays showed sensitivity and a low degree of interindividual variability in excited delirium deaths. The association of dopamine transporter dysregulation with fatal excited delirium implicates chaotic dopamine signaling in the pathogenesis of this syndrome and offers the potential for developing more objective testing methods, including the identification of dopaminergic candidate genes that may confer risk.

Victims of excited delirium display sudden onset of paranoia and alternate between calm behavior and extreme agitation. When confronted by police, who are invariably called to the scene, the victim intensifies the violence and paranoia. An intense struggle ensues, when the victim exhibits incredible "superhuman" strength and is impervious to the usual police techniques of pain control, including pepper spray, peroneal baton strikes and multiple CED deployments. The intense struggle requires the efforts of many police officers, who are finally able to restrain the victim and apply physical restraints. Usually, within minutes of being restrained, the victim loses all vital signs. Core body temperatures average 40.5 °C [2,3,13]. Resuscitation of excited delirium victims often results in a failed course of hospital treatment, characterized by a fatal sequence of rhabdomyolysis and renal failure [23,24]. While many factors are associated with sudden death in individuals requiring restraint for excited delirium [24], these individuals develop a disturbance in thought, behavior and mood, and become agitated and violent consistent with an underlying CNS mechanism or brain disorder. We demonstrate a marked induction of the HSPA1B transcript in brain specimens from cases of excited delirium. The heat-shock response is an immediate transient response to heat with the level of the 70-kDa protein most closely related to the magnitude of the thermal stress [25,26]. Hyperthermia is considered strong supportive evidence for the diagnosis of excited delirium, but is not considered an absolute requirement.

The syndrome of excited delirium in cocaine abusers is perhaps best characterized, because toxicology testing demonstrates that cocaine is the most frequently reported illicit drug. Psychological autopsy reports demonstrate that most are chronic freebase ("crack") cocaine users, usually engaged in a recent binge of drug use. Inhibition of dopamine transporter function is thought to be the primary mechanism underlying cocaine's addictive effects [27]. The dopamine transporter is critical in determining the concentration of extracellular dopamine and overall dopaminergic tone [28,29]. By blocking the transporter protein, cocaine allows released dopamine to persist in the extracellular space and prolongs dopamine receptor stimulation, which leads to behavioral activation. One of the neuroadaptive changes to chronic cocaine abuse is a compensatory upregulation of dopamine transporter numbers and function [12,19,30–32]. We have demonstrated previously that there is a failure to upregulate dopamine transporter function in cases of cocaine-related excited delirium. All psychostimulants (cocaine, methamphetamine and MDMA) cause increases in synaptic levels of DA [33], which may explain the association of chronic psychostimulant abuse with excited delirium. A central role of dopamine is to mediate the "salience" of environmental events and internal representations in a dynamic process characterized by time and stimulus-dependent regulation [34]. The failure of dopaminergic regulation leads to a functional hyperdopaminergia, which triggers unexpected onset of delirium and extreme agitation in these victims [35].

Kosten and Kleber proposed that cocaine-induced excited delirium is a variant of the neuroleptic malignant syndrome, a rare adverse reaction to psychotropic drugs in psychiatric patients that is characterized by hyperthermia, extrapyramidal signs, altered consciousness, and autonomic dysfunction [36]. Acute exhaustive mania, as originally described by Luther Bell in psychiatric patients [8], is indistinguishable from excited delirium related to chronic abuse of psychostimulants (cocaine, methamphetamine, MDMA). Excited delirium also may occur in some psychiatric patients who are medication noncompliant [3]. We have demonstrated that dopamine transporter levels fall below the normal range for all victims of excited delirium, including those with no known history of drug abuse and a negative toxicology screen at autopsy. Our results show that the unabated conditions, which favor the development of excited delirium, are psychostimulant abuse, extreme mental stress or an underlying psychiatric condition. Neuroanatomic connections between the brain and the heart provide links that allow cardiac arrhythmias to occur in response to abnormal brain activation [37,38]. We suggest that a final common pathway for excited delirium related to chronic stimulant drug abuse, extreme environmental stress or other psychopathologies may be a failure of the dopamine transporter to dynamically regulate synaptic dopamine. This failure of regulation leads to a hyperdopaminergic state, which causes the violent behavior, delirium, agitation, and sudden death when the neurocardiac axis is activated. A dopamine transporter murine model of hyperdopaminergic state displays a distinctive cardiorespiratory and thermal phenotype, providing further support for the altered dopamine regulation hypothesis of excited delirium [39].

Police when suddenly confronted with psychotic, violent persons, set into motion an escalation of the use of force continuum, and death may occur despite the appropriate application of sublethal control techniques. The violent nature of the conflict between police and excited delirium victims, often witnessed by citizens and sometimes the news media, may lead to accusations of excessive use of force and community outrage. If death occurs while police officers are trying to restrain the victims, the police are assumed to be responsible with subsequent civil litigation against the municipality, the police department, and the individual police officers to be expected. The tendency to confuse proximity with causality, become greater when the necropsy fails to disclose an anatomic cause of death [11]. Because these cases come to legal review, measures should be taken to ensure that events and findings are clearly documented. We have demonstrated that dopamine transporter and Hsp70 proteins are indicators of abnormal biological processes that afford an objective measure to assess excited delirium at autopsy. The high sensitivity and low degree of interindividual variability provide proof-of-concept that when combined with descriptions of the decedents' behavior prior to death, a 2-protein biomarker analysis has validity for use in assigning excited delirium as a cause of death.

Conflict of interest statement

We have no conflict of interest.

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