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REVIEW

Sudden and unexpected death in early life: proceedings of a symposium in honor of Dr. Henry F. Krous

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Abstract Reported here are the proceedings of a symposium given in honor of Dr. Henry F. Krous upon his retirement as Clinical Professor of Pathology and Pediatrics at the University of California Schools of Medicine, and as Director of the San Diego SIDS/SUDC Research Project. Dr. Krous' distinguished 37-year-career was dedicated to research into sudden unexpected death in infancy and childhood, notably the sudden infant death syndrome (SIDS) and sudden unexplained death in childhood (SUDC). The presentations were given at the International Conference on Stillbirth, SIDS, and infant survival on October 5, 2012, in Baltimore, MD, USA. Eight colleagues of Dr. Krous whose own professional careers were touched by his efforts discussed forensic issues related to SIDS, tissue banking, animal models in SIDS, brainstem studies in SIDS, genetic studies in SIDS, establishment of a SUDC registry, neuropathologic research in SUDC, and potential shared mechanisms underlying sudden and unexpected death in early life. The wide scope of the presentations crossed the disciplines of forensic pathology, pediatric pathology, neuropathology, neuroscience, physiology, genetics, and bereavement, and attest to Dr. Krous' far-reaching influence upon SIDS and SUDC research.

Keywords Autopsy protocol · Brainstem · Death scene investigation · Hypoxia · Serotonin · Sudden infant death syndrome (SIDS) · Sudden unexplained death in childhood (SUDC)

Introduction

This article is a composite of presentations given in honor of Dr. Henry F. Krous upon his retirement as Clinical Professor of Pathology and Pediatrics at the University of California Schools of Medicine, and as Director of the San

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Diego SIDS/SUDC Research Project. Dr. Krous' distinguished 37-year-career was dedicated to research into sudden unexpected death in infancy and childhood, notably the sudden infant death syndrome (SIDS) and sudden unexplained death in childhood (SUDC). Dr. Krous relates that it was the sudden unexpected death of the infant daughter of one of his fellow interns that led to his introduction to Dr. J. Bruce Beckwith, the world renowned pediatric pathologist with seminal publications in the pathology of SIDS [1–4], who inspired Dr. Krous' research into SIDS.

To highlight but a few of Dr. Krous' many career achievements, in 1991 he assisted in the development of California legislation aimed at the improvement of the investigation of sudden unexpected infant death, and he served as chair of a state-wide multidisciplinary committee that developed and implemented standardized death scene investigation and autopsy protocols. At this time, he also formed the San Diego SIDS Research Project that focused upon refinements in the pathology of SIDS. Recognizing the unique opportunities afforded by the California SIDS legislation, Dr. Krous welcomed the collaboration of investigators worldwide who were pursuing SIDS research into this project that had a formal liaison with the San Diego County medical examiner's office and involved the sharing of precious tissues of infants dying suddenly and unexpectedly for research. In 1999, Dr. Krous broadened his research to include cases of sudden unexpected death in childhood, and he renamed the San Diego SIDS Research Project as the San Diego SIDS/SUDC Research Project to include childhood cases over 1 year of age (the operational cut-off of the SIDS definition), thereby initiating the first comprehensive research into SUDC. In his career, Dr. Krous put forward both a widely utilized SIDS definition (i.e. the sudden unexpected death of an infant <1 year of age, with onset of the fatal episode apparently occurring during sleep, that remains unexplained after a thorough investigation, including performance of a complete autopsy and review of the circumstances of death and clinical history) [5]; and a SUDC definition (i.e. the sudden death of a child older than 1 year of age that remains unexplained after a thorough investigation, including review of the clinical history and circumstances of death, and the performance of a complete autopsy with appropriate ancillary testing) [6]. Among numerous awards, Dr. Krous was presented with the Senator Daniel E. Boatwright Award for "extraordinary public service on behalf of Californians touched by SIDS."

The following presentations were given at the symposium in honor of Dr. Krous at the International Conference on Stillbirth, SIDS, and Infant Survival on October 5, 2012, in Baltimore, MD, USA. They were given by eight colleagues of Dr. Krous whose own professional careers were touched in significant ways by Dr. Krous' efforts and/or through direct collaborations with him in SIDS and/or SUDC research. The three medical examiners, Drs. Rognum, Byard, and Hyma (in collaboration with Dr. McEntire), highlighted forensic issues that were of major importance to Dr. Krous, i.e. the definition of SIDS, the standardization of autopsy and death scene protocols, and the collection and banking of tissues for research. The three basic researchers, Drs. Paterson, Nattie, and Haddad, spoke to potential physiological and genetic mechanisms of sudden death in SIDS, particularly as they relate to the altered homeostatic responses to asphyxia, hypercarbia, and hypoxia in vulnerable SIDS infants. The work of Dr. Paterson in the laboratory of Dr. Kinney depends directly upon collaboration with the SIDS/SUDC Research Project and the San Diego County medical examiner's system for tissue accrual and phenotyping. It also illustrates the critical importance of Dr. Krous' endeavors in the facilitation of basic research in SIDS through such collaborations including in the career development of young SIDS investigators such as Dr. Paterson. Dr. Nattie's work builds to a significant degree upon the brainstem findings of Dr. Paterson and others in the laboratory of Dr. Kinney, and illustrates the influence of Dr. Krous' efforts in the development of animal modeling of SIDS and the search for therapeutic interventions based upon physiological mechanisms. Dr. Haddad is a long time colleague of Dr. Krous at Rady Children's Hospital in San Diego and he, too, has been influenced by the work of Dr. Krous in the development of his highly regarded research program into the mechanisms of cell death following hypoxia–ischemia, including as it relates to SIDS. The presentations by Ms. Crandall and Dr. Kinney highlight Dr. Krous' contributions to SUDC including the development of; (1) an international registry of SUDC cases for research; and (2) potential neural mechanisms related to sudden death in toddlers based upon cases in this registry. The establishment of the SUDC Program, founded by Dr. Krous and Ms. Crandall, also speak to Dr. Krous' efforts to increase public awareness of sudden and unexpected death in childhood, and to assist in the grieving process of affected families through medical counsel.

Forensic-related issues in SIDS

The definition of SIDS: Dr. Torleiv O. Rognum

The SIDS definition (1969) [1] and the SIDS ICD code (1971) were important steps forward for research regarding sudden unexpected deaths in infants and small children. They also provided support to grieving families and diminished the guilt and blame characteristic of these deaths [2]. This SIDS definition "per exclusion," however,

resulted in debates about the relationship of particularly findings to the cause of death. Moreover, there have been local "epidemics" due to non-evidence based diagnostic trends [7], the "special" (presumably biased) ideas of pathologists or researchers, or the need of a "diagnostic dustbin" [8]. The back-to-sleep campaigns in the early 1990s resulted in a dramatic drop in the number of SIDS deaths [9, 10] and consequently a reduction in total post-neonatal infant mortality [11, 12]. Thus, pathologists realized that the 1969 SIDS definition needed revision [3, 13]. In 2003, Drs. Beckwith and Krous suggested the need for a new definition, and that sudden death in small infants, older infants, and small children has different distinctive features. Dr. Beckwith repeated his call for a re-examination of the definition of SIDS with the possibility of including positive diagnostic criteria with stratification to delineate particular subsets [2], so, in 2003, Dr. Krous gathered a panel of pediatric and forensic pathologists and pediatricians to develop a new definition. This important initiative resulted in the San Diego definition [5]: the category IA Classic SIDS now requires an age more than 21 days and <9 months, a normal clinical history, growth and development, and no similar deaths in close genetic relatives. Death should occur during sleep. Death scene investigation should not disclose an explanation for death, and the autopsy should exclude fatal pathological findings, evidence of trauma, thymic stress effect, and toxicological, microbiologic, radiologic, vitreous chemical or metabolic explanations for death. Category IB SIDS includes incompletely documented cases. Category II SIDS includes cases with a wider age range and other discrepancies. Finally, the category unclassified sudden infant death includes deaths that do not meet the category II SIDS, including cases in which autopsies are not performed.

After the risk reduction campaign the observation was made that the reduced SIDS rates were mainly due to a reduction in the number of *small* SIDS infants, between 2 and 4 months of age, and sleeping prone with slight clinical symptoms of infection prior to death [14]. This observation should generate new hypotheses in SIDS research. Research on sudden unexpected death in children over 1 year of age (SUDC) needs other hypotheses than SIDS. The determined work of Dr. Krous through several decades, including development of the international standardized autopsy protocol for sudden unexpected infant death [15, 21] and the San Diego definition [5], has had a clear-cut result and will contribute to improved future research.

Standardization of protocols in the investigation of sudden infant death syndrome: Dr. Roger W. Byard

A major problem with the investigation and diagnosis of all categories of sudden infant and early childhood death, not only in the area of SIDS, has been the lack of standardized approaches to these cases. Death scenes were sometimes reviewed in a very perfunctory fashion, with parental interviews occurring days after the death over the telephone. Autopsy examinations were equally variable, with minimal special dissections being undertaken and very patchy use of ancillary studies. This was despite studies that clearly demonstrated the value of every stage of the autopsy assessment [16]. Not only have death scene and autopsy investigations been suboptimal, but also these investigations have even involved the use of definitional terms, despite clearly promulgated definitions in the international literature [17–20]. For example, in 2006 an analysis of peer-reviewed publications on SIDS found that 58 % of reports either failed to specify the definition of SIDS that was used or cited a non-standard version [18]. A follow up study in 2012 documented an improvement, with this number falling to 32 % [19]; however, the other side of the coin is that nearly one-third of studies are still not providing details of their diagnostic criteria for SIDS.

The problem persisted in the early 1990s with many studies relying on data that derived from cases where full evaluations had not been undertaken. In an effort to correct this serious defect in the SIDS diagnostic and research fields, the Pathology Working Group under the chair of Dr. Henry Krous (as part of the Global Strategy Task Force initiated by the National Institute of [NICHD] and SIDS International) was set the task of establishing a standardized autopsy protocol for infant deaths. The aims of the protocol were to standardize autopsy practices and improve diagnostic accuracy, provide additional information to supplement information obtained from the clinical history review and death scene examination, enhance opportunities to further reduce infant death rates and enable more meaningful comparisons of infant death rates to be made between populations, and to improve the quality of research into unexpected infant death. At the same time, the Centers for Disease Control (CDC) began to develop a standardized death scene examination. This resulted in the initial sudden unexplained infant death investigation report form (SUIDIRF) which is available on the CDC website [20].

The work of the pathology group culminated in the International Standardized Autopsy Protocol which was first published in 1995 [15, 21]. This template was endorsed by a variety of professional groups including the National Association of Medical Examiners (NAME) and the Society for Pediatric Pathology (SPP) in the United States. Although there has been some resistance to the adoption of what are sometimes viewed as too rigid guidelines, the point about such protocols is that they provide a gold standard that can then be adapted to local conditions.

It has become quite clear over the latter part of the last century, and in the decade or so of this century, that the more carefully investigated infant and early childhood deaths are, the more likely it is that occult conditions and situations will be identified. For example, the increased identification of infant deaths attributable to accidental asphyxia was only made possible after detailed death scene evaluations were conducted [22]. Similarly, the possibility of serotonergic deficiencies in SIDS infants was not considered until standardized autopsies had been performed with adequate provision of tissues for research purposes [23].

Protocols and uniform definitions have provided us all with guidelines to follow when we are tackling the difficult issue of an unexpected infant death. They ensure that we are reminded of necessary steps and processes, as well as ensuring that cases from a variety of jurisdictions have all been approached in a similar peer-reviewed manner. One of the most important developments in pediatric forensic pathology in recent years has been the development and implementation of these protocols under the guidance of Dr. Krous. Their use has been validated by the identification of a wide range of rare conditions that previously would have been included under the umbrella of SIDS, John Emery's unfortunate "convenient diagnostic dustbin [8]."

Tissue consortiums in sudden unexpected infant death: Drs. Bruce Hyma and Betty McEntire

The sudden unexpected death of an infant continues to challenge the forensic science community while bringing untold emotional trauma to parents and extended family members. Forensic pathologists, medical examiners, and coroners have investigated these deaths with the assistance of law enforcement. However, other than in San Diego, there is no US comprehensive infrastructure for providing the tissue and medical-legal information to researchers. To that end, the SUID tissue consortium was created, following the San Diego SUID program model, for three purposes: to provide much needed tissue and body fluids for researchers from infants who die suddenly, to develop age-matched tissue and body fluid controls, and to help understand the medical abnormalities that may increase the risk of sudden death in infants. The consortium provides various tissue samples for research use along with the complete autopsy findings and medical-legal report including the scene investigation.

Four medical examiner districts in south Florida (Miami-Dade, Collier, Broward and Palm Beach) are currently participating in the consortium with consent assistance from the University of Miami Tissue Bank's Donor Services (UMTB). With timely, informed consent from the designated legally authorized person in each case, body fluids (serum and cerebrospinal fluid [CSF]), tissue samples (heart, lung, kidney, liver and thymus) and the brain of infants (1 year of age or less regardless of cause of death) are collected during the autopsy process. Organ and tissue transplantation, as well as the evidentiary needs of the medical examiner, take precedence over research tissue, so not all samples may be collected in every case. Serum and CSF are preserved at −80 °F after centrifugation. Duplicate tissue samples are preserved at −80 °F and in 10 % buffered formalin. The medulla, brainstem, left cerebrum and left cerebellum are preserved at −80 °F and the right cerebrum in 10 % buffered formalin. These body fluid and tissue samples are banked at the National Institute for Child Health and Development Brain and Tissue Bank at the University of Maryland (NICHD BTB). De-identified, medical examiner investigative data is collected by the American SIDS Institute and attached to each body fluid/tissue set. The American SIDS Institute provides laboratory equipment and freezers; the NICHD BTB provides specimen kits, shipping materials and shipping labels. Webinar training is conducted. Additionally, the NICHD BTB provides the submitting medical examiner department with a neuropathology report on each brain received.

The SUID tissue consortium has been collecting and preserving tissue and organ samples from this population of infants since August 2011. Our current consent rate is 25 %, with a target of 50 %. The American SIDS Institute holds monthly phone conferences with participating district medical examiners, UMTB and the NICHD BTB to monitor the progress of consented cases, exchange investigative information and address system deficiencies. Efforts are underway to expand this project to other districts in Florida, as well as to other states. The process is best structured within a medicolegal death investigative system, which has the support and cooperation of tissue donor service professionals and a conducive, local and state, legal framework. With time, this tissue consortium will be a valuable, national resource for current and next generations of SUID research.

Research into the mechanisms of sudden infant death

The role of genetic factors in the pathogenesis of brainstem abnormalities in SIDS: Dr. David S. Paterson

The basic premise of the brainstem hypothesis of SIDS is that an important subset of SIDS cases has an underlying abnormality in the homeostatic network in the medulla oblongata that results in a failure of protective responses to life-threatening stressors during sleep in a critical

developmental period [24, 25]. Over the last two decades, using tissue specimens that have been provided to us almost exclusively through our collaboration with Dr. Krous, the SIDS/SUDC Research Project, and the San Diego County medical examiner's system our laboratory has reported multiple neurotransmitter and related abnormalities, including reductions in the binding density of serotonin (5-HT) [26–29] and γ-amino butyric acid (GABA) [30] receptors, reduced brainstem levels of 5-HT and tryptophan hydroxylase (TPH2) (the rate limiting enzyme regulating 5-HT synthesis) [29], and reductions in the level of the 14-3-3 signal transduction family of proteins [31] in regions of the medulla oblongata that are critically involved in regulation of homeostatic function. We propose that the death of an infant from SIDS involves the dysfunctional interaction of these (and potentially other) signaling systems in the medullary homeostatic network during sleep.

Importantly, the pathogenesis of these abnormalities remains unknown but is proposed to be multifactorial, involving a combination of environmental and genetic risk factors. Epidemiological studies have been very successful in identifying environmental risk factors involved including prone sleep position and maternal cigarette and alcohol use during pregnancy [32, 33]. A genetic component to SIDS is indicated by the increased risk of SIDS in subsequent siblings [34] ethnic and racial disparities in SIDS rates that are not entirely attributable to socio-economic factors and by the observation that while some infants exposed to environmental risk factors and stressors die of SIDS other infants with identical exposures do not [32], suggesting that some infants are genetically susceptible or pre-disposed to SIDS. Multiple studies have attempted to elucidate the genetic component to SIDS and a large number of genes and systems have been associated with increased SIDS risk including cardiac ion channels, 5-HT signaling, autonomic nervous system, immune system, and metabolism and energy pathway-related genes [35]. Of specific interest to our research are the genetic variations that potentially alter 5-HT neurotransmission as these may cause or contribute to the brainstem 5-HT abnormalities reported by us in SIDS cases. Genetic variations affecting 5-HT neurotransmission that have been associated with SIDS include, the 5-HT transporter (5-HTT) promoter polymorphism (5-HTTLPR) [36–39], a variation in the fifth Ewing variant gene (FEV) [40], and a polymorphism in the promoter region of the monoamine oxidase A (MAO) gene [41]. The 5-HTT plays a key role in regulating 5-HT neurotransmission via its transport of synaptic 5-HT back into the neuron terminal. The LL genotype of the 5-HTTLPR is associated with increased gene transcription and 5-HT transport in vitro and is postulated to increase the risk of SIDS by reducing the availability of synaptic 5-HT thereby exacerbating the existing deficit in brainstem 5-HT. The FEV gene is the human homologue of the murine transcription factor Pet-1 that is critical in the development of 5-HT neurons [42, 43]. Rand et al. observed that a rare insertion mutation in the FEV gene (IVS2-191_190insA) was more frequently observed in SIDS cases compared to controls and more frequently in African Americans compared to Caucasians, raising the possibility that this mutation my cause or contribute to the medullary 5-HT abnormalities observed in SIDS cases and putatively also the increased incidence of SIDS observed in African Americans [49]. MAO is the main catabolic enzyme responsible for terminating the action of 5-HT. A polymorphism in the promoter region of this gene that results in reduced gene expression and putatively reduced 5-HT breakdown has been observed in increased frequency in Caucasian male but not female SIDS cases compared to controls and thus may contribute to abnormal 5-HT neurotransmission, and may also in part play a role in the increased incidence of SIDS observed in male compared to female infants. In our laboratory, we have initiated, therefore, a program of studies to determine if these gene variations play a role in the pathogenesis of the medullary 5-HT abnormalities reported by us in SIDS brainstems. Following genotyping of 179 SIDS cases and 139 controls in the San Diego SIDS dataset, however, we did not observe an association between the LL genotype or L allele of the 5-HTTLPR and SIDS [44]. Similarly, in 71 SIDS cases and 53 controls we were unable to replicate the findings of Rand et al. [40], and instead observed that the mutation was present in both SIDS and control populations and that it appeared to be a common variant in the African American population [45]. The results from these studies do not, therefore, support the idea that these polymorphisms play a major role in the pathogenesis of the medullary 5-HT abnormalities in SIDS. Genetic studies currently underway in our laboratory include analysis of the MAOA promoter polymorphism, and single nucleotide polymorphism (SNP) microarray analysis, the results of which should be available in the near future. Taken as a whole, the observations from the studies of genetic variation in SIDS support the idea that SIDS is oligogenic in nature, i.e. no single gene mutation is responsible, but that multiple gene polymorphisms that are individually responsible for a small increase in SIDS risk, likely occur in different combinations simultaneously in an infant and act synergistically to significantly increase the risk of SIDS. Alternatively, a rare variation or mutation with significant functional impact upon a system, e.g. a copy number variation (CNV) potentially constitutes the genetic component to SIDS risk. Indeed, Toruner et al. [46] identified CNVs in a major cluster of histone genes in 3 of 27 SIDS cases. We are currently pursuing analysis of CNV in

SIDS in our database using a comparative genome wide hybridization approach.

Insights into SIDS pathogenesis and possible "treatment" from animal models: Dr. Eugene E. Nattie

Studies of brainstems from SIDS cases, collected largely due to the industry and dedication of Dr. Krous and his team in conjunction with the committed efforts of the medical examiners of the San Diego County medical examiner's office and Dr. Kinney's laboratory, have uncovered a cluster of abnormalities as described by Drs. Kinney and Paterson, and colleagues [24, 28–31]. Within our NICHD-funded program project on SIDS, the responsibility of the Dartmouth Physiology group, which I represent, has been to define in animal models possible mechanisms by which these brainstem abnormalities might cause sudden death during a specific postnatal age period, i.e. a window of development. In rodent pups during early postnatal development, we study three aspects of physiology relevant to SIDS: (1) arousal from sleep [47]; (2) the laryngeal chemoreflex, which powerfully inhibits breathing [48–51]; and (3) responses to various forms of intermittent exposure to hypoxia [52, 53]. Recently, we have focused upon the role of the neurotransmitter serotonin (5-HT) in brainstem-mediated physiologic responses due to the mounting evidence from the laboratory of Dr. Kinney and other investigative groups that 5-HT parameters are reproducibly abnormal in SIDS brainstems. We have developed or utilized a variety of models of 5-HT dysfunction including rat pups with dietary induced tryptophan deficiency [55] or toxin induced chemical lesions [52] and transgenic mouse pups with knock-out of the key 5-HT transcription factor, Pet1 [53, 54], or of the 5-HT transport protein [56], or acute reversible "silencing" of 5HT neurons by injection of a ligand specific for activation of a receptor expressed solely in 5-HT neurons that hyperpolarizes ("silences") them [57].

In normal rat pups, we have found a postnatal age-dependency of the potency of the apnea producing laryngeal chemoreflex (LCR) [50], and a potentiation of this reflex by exposure to smoke or nicotine [48, 49] and by increased temperature [49, 50], the latter an effect localized to TRPV1 receptors in the nucleus of the solitary tract (NTS) [51]. We have also demonstrated a 5-HT induced shortening of the time of apnea induced by stimulation of the LCR by microinjection of 5-HT into the NTS (Donnelly, Leiter, Bartlett, unpublished observations).

In rat pups from dams fed a diet deficient in tryptophan, which causes moderate brainstem 5-HT depletion [55], and in mice with knock-out of the 5-HTT gene [56], we have found a reduced ventilatory response to carbon dioxide (CO_2) at P15 and P25. In the tryptophan deficient rat pups and in transgenic mice with altered 5-HT function (Pet1 nulls with 90 % loss of medullary 5-HT), we have found: (1) a delay in the time it takes to arouse from sleep with hypoxic stimulation (Darnall et al., unpublished observations); and (2) a decreased ability to recover from brief, induced periods of anoxia (autoresuscitation) [52]. In this last case, we have observed age-specific mortality in pups 90 % deficient in brainstem 5-HT (the Pet1 null mouse) [53]. In rat pups with chemical lesions of 5HT neurons induced at P2, we have also observed age-specific autoresuscitation failure [52]. Further, we are able to "rescue" the Pet1 null mice by "treatment" with caffeine (Cummings et al., unpublished observations). In both our Pet1 null 5-HT deficiency model [54] and our acute silencing of 5-HT neuron model [57], we have observed dysfunction in body temperature regulation with mild cold exposure.

These findings point to roles for 5-HT in important physiological regulatory processes relevant to the pathogenesis of SIDS including: ventilatory responses to increased CO_2; body temperature regulation; responses to severe repeated hypoxia (autoresuscitation); arousal from sleep; and the potency of the LCR and its interaction with nicotine exposure and warm temperatures. They support the general hypothesis that brainstem abnormalities of the neurotransmitter 5-HT can cause sudden death during an age-specific window of postnatal development, and suggest that treatment of infants identified as at risk may be possible.

Mechanisms of cell death in neurons and glia following hypoxia/ischemia: Dr. Gabriel G. Haddad

Over the past half century, SIDS has not suffered from a scarcity of hypotheses, aimed at explaining its pathogenesis and determining the factors that lead to the demise of the infant in the first several months of life. More recently, with the help of scientists and clinicians alike, theories have surfaced favoring the idea of abnormalities in brainstem development resulting in physiologic catastrophes, possibly triggered by environmental events. Dr. Krous was one of the major leaders in SIDS that shaped our current thinking [5, 6, 33, 58, 59].

Our laboratory has been interested in the response of nerve and glial cells to alterations in oxygen (O_2) and CO_2 and the mechanisms that lead to cell injury (rodents) or cell survival (*Drosophila*) during asphyxia. We focus on brain hypoxia in mice particularly on cells in the penumbral region. The main idea is clinically driven in the sense that, generally, the outset of a stroke or infarct in sensitive tissues such as brain or heart is difficult to prevent or contain, but that the penumbral (surrounding) region is potentially rescuable if we enhance our understanding of the pathobiology of cell death in this region.

To mimic penumbral conditions, we employ a slice culture system and use an ischemic solution (IS), a solution

that resembles the milieu of the infarct rim [60]. Our data show significant cell injury following IS exposure for hours after the exposure of this solution. We also studied the effect of each component in IS (e.g. acidosis, hypoxia, glucose) and we isolated the contribution of each of these. For example, using IS or ion replacements in IS revealed that cell death in the neuropil was modest when potassium (K)(+) was increased or pH lowered. High K (+) is most effective in reducing cell death when bicarbonate HCO(3)(−) was normal or high. Gene expression studies indicated that among 46,000 transcripts tested, chemokine receptor-like 2 (CCRL2) was one of the most significantly up-regulated. In order to test the importance of CCRL2 in vivo (and not only in vitro), brain lesions were assessed after middle carotid artery occlusion. There was a smaller infarct volume and reduced neurological deficits in CCRL2 knockout mice than in control. We conclude that activation of neuroinflammatory pathways play a critical role in hypoxic/ischemic cell death in brain.

Utilizing this novel in vitro brain slice model of the penumbra, we also examined the free radical profile of ischemic murine hippocampal neurons using electron paramagnetic resonance spectroscopy, and also the role of Nox in this generation and in cell fate [61]. We found that free radical production increased ~75 % at 2 h of IS, and this increase was abolished by: (1) scavenging of extracellular free radicals with superoxide dismutase (SOD); (2) a general anion channel antagonist; or (3) the Nox inhibitor apocynin. Similarly, at 24 h of ischemia, [ATP] decreased >95 % and vital dye uptake increased sixfold relative to controls; whereas apocynin, the chloride channel antagonist 5-nitro-2-(3-phenylpropylamino)-benzoate (NPPB), or the free radical scavenger *N*-acetyl cysteine (NAC) each provided moderate neuroprotection, ameliorating 13–32 % of [ATP]-depletion and 19–56 % of vital dye uptake at 24 h. Our results support a cytotoxic role for Nox-mediated free radical production from penumbral neurons during the ischemic period.

Understanding SUDC

Establishment of theSUDC program and the San Diego SUDC research project: Ms. Laura Crandall

In 1999, the first formal recognition of "post infancy SIDS," now known as sudden and unexplained death in childhood (SUDC), was presented in Atlanta, Georgia by Dr. Krous at the SIDS Alliance annual meeting. His presentation highlighted the gaps in knowledge, support, and research for these tragedies. Seven affected families were in the audience who asked many difficult and unanswerable questions about sudden death beyond the first year of life, and brought out potential differences between these deaths and those <1 year of age. As a result of this unique interaction in Atlanta, a rich collaboration was born to address the critically underreported problem of SUDC through research, support and advocacy. In coordination and partnership, the first database and retrospective review of SUDC cases was accomplished through the formation of the San Diego SUDC Research Project under the direction of Dr. Krous. The SUDC Program (a program of the CJ Foundation for SIDS) was created to address bereavement services, advocacy, awareness, and fundraising under the direction of Ms. Crandall.

The San Diego SUDC Research project to date has evaluated more than 160 children who have succumbed to sudden unexpected deaths as well as SUDC [6]. It is very rare, and so far, an unpredictable and unpreventable tragedy. It occurs in 1 out of every 100,000 toddlers [62]. With the brave participation of bereaved parents, as well as the Medical Examiners and Coroners who investigated these deaths, vital information has been gathered and analyzed and helped create multiple peer reviewed journal articles to improve our understanding of these rare tragedies [e.g. 6, 63–70].

The SUDC Program, working simultaneously in partnership with Dr. Krous and the San Diego SUDC Research Project, addresses the many needs of the growing SUDC community and has created a worldwide network of support, information, advocacy and research. It continues to alter the SUDC landscape today by: providing no cost bereavement services to more than 500 families affected by sudden unexpected deaths in children, including SUDC, throughout the US and in 14 additional countries. It works with medical examiners and coroners to ensure comprehensive investigations in order to meet the sensitive needs of families, promote legislative advocacy for comprehensive and standardized investigations, and fundraises to support critically needed research. The role of Dr. Krous in SUDC will be forever acknowledged and gratefully appreciated for the voice he gave to so many children lost to SUDC. In great admiration, the SUDC Program honored Dr. Krous with the *2012 SUDC Star of the Decade Award*.

Neuropathologic research in SUDC—implications for shared neural mechanisms of sudden death in early life: Dr. Hannah C. Kinney

The SUDC Research Project under the direction of Dr. Krous developed an international registry of SUDC cases, which are rare and difficult for a single investigator to accrue for analysis of common profiles. In a series of cases of sudden and unexpected death in 64 toddlers (1–5 years old) from the initial cases in the registry [63], we found that 77 % (49/64) of the deaths were SUDC, i.e. unexplained

after a complete autopsy and death scene investigation; the remaining deaths were explained by various miscellaneous causes. In addition, 24 % (12/49) of the SUDC cases had a personal history of febrile seizures—an incidence approximately fivefold higher than that of 2–5 % in the general pediatric population [63]. Also, 67 % (8/12) of the 12 SUDC cases with a personal history of febrile seizures also had a family history of febrile seizures—an incidence 2.8-fold higher than the incidence of familial febrile seizures in individuals with febrile seizures in the general pediatric population [63]. Of the SUDC cases with an individual and/or family history of febrile seizures, 82 % (9/11 with available hippocampal sections) demonstrated gross asymmetry and/or microscopic anomalies (microdysgenesis) of the hippocampus/temporal lobe [63, 64]. Of note, 47 % (7/15) of SUDC cases *without* a personal and/or family history of febrile seizures also had hippocampal anomalies [63]. A three-generation pedigree was obtained in an initial series of 6 families enrolled in the San Diego SUDC Research Project with a toddler with SUDC, febrile seizures, *and* family history of febrile seizures [63]. Autosomal dominant inheritance of febrile seizures was observed in three families; a fourth demonstrated autosomal dominant inheritance with incomplete penetrance [65]. Thus, a genetic susceptibility to febrile seizures may be important in defining this SUDC subset.

The hippocampal/temporal lobe anomalies in the SUDC cases with and without febrile seizure histories were similar to those observed in chronic temporal lobe epilepsy or sudden unexpected death in epilepsy (SUDEP) [71–74]. SUDEP is defined as the sudden and unexplained death in patients with a known history of epilepsy that is not related to trauma, accidents, or status epilepticus [75]. By analogy to SUDEP associated with temporal lobe pathology, sudden death in SUDC cases with temporal lobe anomalies may result from a cardiac arrhythmia or respiratory arrest that originates from an epileptogenic discharge in an abnormal temporal lobe. The hippocampus helps modulates cardiorespiratory control vial mono- and poly-synaptic connections to relevant brainstem regions [76, 77]; propagation of asynchronous discharges from an epileptogenic focus in the hippocampus to these brainstem regions may result in cardiorespiratory dysfunction and sudden death, including during sleep. The association of SUDC with febrile seizures suggests that an unwitnessed febrile seizure during the fatal sleep period contributes to sudden death. The lack of febrile seizures in some SUDC cases with temporal lobe anomalies, on the other hand, suggests that such seizures are not essential in the events leading to sudden death. It now appears that hippocampal asymmetry similar to that reported in toddlers is also associated with sudden death in *infants,* a not surprising finding, given that the age cut-off of 1 year between SIDS and SUDC is operational. As proof-of-principal, we recently reported the case of a 10-month-old boy whose death was classified as SIDS by the coroner, yet, in whom there was mild asymmetry of the hippocampus with microdysgenetic features [78].

Is there a neural mechanistic link among SIDS, SUDC, and SUDEP in at least a subset of cases? Are some SIDS and SUDC cases variants of SUDEP presenting in early life? There are several key similarities in between SIDS and SUDC: (1) the individuals seem healthy prior to death; (2) the death is sudden and unexpected; (3) the death is unexplained by the conventional criteria of an autopsy and death scene investigation; (4) death is typically related to a sleep period; and (5) the position discovered is usually prone. The death in SUDEP is likewise sudden and unexpected and characteristically related to a sleep period and the prone sleep position; it remains unexplained, as even brain autopsy findings do not explain the death. The key differences between SIDS, SUDC, and SUDEP are: (1) age at presentation; and (2) the occurrence of epilepsy in SUDEP by definition. Yet, young children with febrile seizures are at risk for the development of temporal lobe epilepsy in adolescence and adulthood [79], and those with SUDC may die prior to the temporal evolution of the febrile seizure disorder to a non-febrile one. Moreover, it is possible that the single or recurrent episodes of apnea reported in certain infants who subsequently die of SIDS [80] may be the sole manifestation of seizures, as such isolated apneic seizures have been reported in newborns and infants with temporal lobe pathology [81]. Febrile seizures have not been reported in SIDS infants [82], but febrile seizures do not typically present until 6 months of age, the time-point by which approximately 90 % of SIDS deaths have already occurred.

We suggest that an underlying disorder related to 5-HT, a so-called serotonopathy, may link SIDS, SUDC, and SUDEP together. Serotonin is produced exclusively in the brainstem in populations located in caudal and rostral domains of 5-HT neurons. The caudal 5-HT domain (raphe, extra-raphe, and ventral 5-HT neurons in the medulla and caudal pons) primarily projects to spinal, cerebellar, and other brainstem sites, and is critical to the modulation of homeostasis [83]. The rostral 5-HT domain (5-HT neurons predominately in the rostral raphe in the rostral pons and midbrain), on the other hand, projects upward to forebrain sites, and is involved in cognition, mood, and arousal [83]. The potential vulnerability of the hippocampus among forebrain sites to 5-HT pathology reflects, at least in part, its extensive innervation by the rostral raphe with heavy concentrations of 5-HT_{1A} receptors and 5-HT terminals [84]. Abnormalities of 5-HT parameters in the caudal and/or rostral 5-HT domains in the brainstem may lead to developmental abnormalities in their projection sites, e.g.

the hippocampus, as 5-HT acts early in development as a trophic factor to influence neuronal proliferation, migration, and/or differentiation [85]. There is increasing evidence that 5-HT plays a major role in seizures [75], and that 5-HT_{1A} receptors, including in the hippocampus, are involved in epileptogenesis and/or propagation [75]. Moreover, reductions in 5-HT_{1A} receptor binding have been reported in the hippocampus/temporal lobe in patients with temporal lobe epilepsy in multiple neuroimaging studies [86]. We hypothesize that a subset of SIDS, SUDC, and SUDEP cases represent a spectrum of a serotonopathy that affects the caudal and rostral 5-HT domains in the brainstem and their preferential targets, including the hippocampus, and that this serotonopathy presents at different ages due to different developmental, environmental, and genetic factors. Given the triple risk model for SIDS that considers sudden death the result of the simultaneous coalescence of the vulnerable infant, critical developmental period, and exogenous stressor [87], we suggest that certain vulnerable infants escape the critical period of the first year of life because they do not meet an exogenous stressor, e.g. prone sleep—yet they die suddenly beyond infancy due to the persistent vulnerability (e.g. serotonopathy) and precipitating factors in a different developmental period. This idea of the delay of sudden death to beyond infancy is supported by the recent report of an increasing rate of SUDC parallel to a decreasing rate of SIDS in Ireland [88]. We speculate that as vulnerable infants pass beyond the 6-month mark, they may be at risk for the onset of febrile and/or non-febrile seizures, as well as the risk for sudden and unexpected death in childhood or possibly beyond. To prove the serotonopathy hypothesis, shared 5-HT abnormalities must be determined in the caudal and rostral 5-HT domains and their projection sites, including the hippocampus, in SIDS, SUDC, and SUDEP cases. To date, 5-HT abnormalities have been sought in the brainstem in SIDS infants but not in association with 5-HT abnormalities in the hippocampus; 5-HT abnormalities have been sought in the hippocampus in SUDEP, but not in the brainstem; and 5-HT abnormalities have not been sought in either the brainstem or hippocampus in SUDC. Efforts to fill in these gaps in knowledge are currently underway in our laboratory as we search of potential neural links between SIDS, SUDC, and SUDEP.

Conclusion

The breadth and depth of the presentations at this symposium in honor of Dr. Krous crossed the disciplines of forensic pathology, pediatric pathology, neuropathology, neuroscience, physiology, genetics, and bereavement, and attest to the far-reaching influence of Dr. Krous upon SIDS and SUDC research. We end with a heart-felt note of appreciation to Dr. Krous for his foresight, drive, and compassion in the search to understand sudden and unexpected death in early life, and to eradicate it forever.

Key points

1. Reported here are the proceedings of a symposium given in honor of Dr. Henry F. Krous upon his retirement as Clinical Professor of Pathology and Pediatrics at the University of California Schools of Medicine, and as Director of the San Diego SIDS/SUDC Research Project.
2. Dr. Krous' distinguished 37-year-career was dedicated to research into sudden unexpected death in infancy and childhood, notably the SIDS and SUDC.
3. Presentations were given that were related to forensic issues in SIDS, tissue banking, animal models in SIDS, brainstem studies in SIDS, genetic studies in SIDS, establishment of a SUDC registry, neuropathologic research in SUDC, and potential shared mechanisms underlying sudden and unexpected death in early life.
4. The wide scope of the presentations crossed the disciplines of forensic pathology, pediatric pathology, neuropathology, neuroscience, physiology, genetics, and bereavement, and attest to Dr. Krous' far-reaching influence upon SIDS and SUDC research.

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