Experimental Neurology 240 (2013) 108–111

Contents lists available at SciVerse ScienceDirect

Experimental Neurology

journal homepage: www.elsevier.com/locate/yexnr

Commentary

Fishing for the mechanisms causing febrile seizures: Employing a novel model to uncover the physiological generators of seizures with fever☆

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ARTICLE INFO

Article history:
Received 2 October 2012
Revised 9 November 2012
Accepted 13 November 2012
Available online 22 November 2012

Keywords:
Febrile seizure
Animal model
Ion channels
Gene expression

Febrile seizures are a common neurologic condition affecting children between 1 and 5 years of age. Febrile seizures (FS) are the most common type of seizures for a person to experience. Up to 5% of children will experience at least one febrile seizure before the age of five (Hauser, 1997; Piperidou et al., 2002). Even though many children will suffer a febrile seizure, fortunately the majority are considered benign. There is, however, evidence that some with febrile seizures will go on to develop epilepsy later in life. Therefore, understanding the mechanisms leading to the development of febrile seizures and developing models to determine the molecular, cellular, and network effects of febrile seizure is important if pediatricians and child neurologist will ever be able to predict and ultimately therapeutically alter, who may have a bad outcome from febrile seizures.

Febrile seizures can be either simple or complex. A "simple FS" is both brief in duration (<15 min) and has no lateralizing features. A prolonged seizure (>15 min), with focal features, or recurrence of a FS within 24 h is termed a "complex FS" (Nelson and Ellenberg, 1987). While there are a number of studies on the effect of febrile seizures in the rodent brain using a model of hyperthermia-induced seizures (Brewster et al., 2002; Dube et al., 2000; Dube et al., 2006; McClelland et al., 2011), very few studies exist on why children have seizures with fever (Dube et al.; Dube et al., 2012; Maroso et al., 2011). The work by Hunt et al. "A novel zebrafish model of hyperthermia-induced seizures reveals a role for TRPV4 channels and NMDA-type glutamate receptors" in the September issue of Experimental Neurology (Hunt et al., 2012) attempts to both develop a novel model of temperature induced seizures and address the question of the molecular etiology of seizures with fever. The study by Hunt et al. is well done, but like most interesting studies, raises as many new questions as it answers. Before, commenting on the current study, a brief overview of the existing models and literature on febrile seizures is needed to fully understand the potential importance and novel insights of the Hunt et al. manuscript.

Febrile seizures are heterogeneous in both etiology and outcome. Children with febrile seizures are more likely to have a family history of febrile seizures or epilepsy and a prior neurologic abnormality (Berg et al., 1999). This suggests an underlying genetic component to development of FS. There have been some associations within families with changes in the interleukin receptors or other immune mediators (Virta et al., 2002). Immune system changes are a rare cause of a possible genetic linkage to febrile seizures. The more common genetic linkage to febrile seizures is with changes in ion channel and neurotransmitter receptor genes (Dube et al.; Dube et al., 2012; Scheffer et al., 2005; Sijben et al., 2009). Indeed, children with mutations in the voltage gated sodium channel, *SCN1A*, often first present with febrile seizures and remain susceptible to seizures with fever their whole lives(Scheffer and Berkovic, 1997; Scheffer et al., 2007). In addition to mutations in *SCN1A*, mutations in *SCN2A* and *GABGR2* have both been linked to epilepsy with a prominent febrile seizure component (Harkin et al., 2002). Another rare ion channel gene that may predispose to febrile seizures refers to mutations in the hyperpolarization-induced cation channel, *HCN1* (Dibbens et al., 2010). Mutations in this gene have been linked to a few families with febrile seizures. Another channel class proposed, but not yet shown, to be involved in febrile seizures is the TRPV channels (Dube et al., 2012). The current study is the first to directly test this potential relationship.

Controversy exists regarding the relationship of FS and the potential development of epilepsy later on in life. As stated above, most children with febrile seizures have a benign disorder with no long-term consequences of the seizures. Some however, may go on to develop epilepsy. Both clinical and basic science studies have attempted to address this question. Knowing the answer is important if pediatricians and child neurologist are to determine if febrile seizures are of concern. Overall, there is no consensus regarding this question and various studies suggest that febrile seizures are an important factor, but are neither necessary nor

☆ Commentary on Experimental Neurology Manuscript (YEXNR-11177) entitled "A novel Zebrafish model of hyperthermia-induced seizures reveals a role for TRPV4 channels and NMDA-type glutamate receptors".

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0014-4886/$ – see front matter
http://dx.doi.org/10.1016/j.expneurol.2012.11.016

sufficient to lead to epilepsy later in life (Ahmad and Marsh, 2010; Berg et al., 1999; Camfield and Camfield, 1997; Dube et al.; Dube et al., 2012; McClelland et al., 2011; Nelson and Ellenberg, 1986; Veliskova et al., 2004). The work by Hunt et al. (Hunt et al., 2012) is a second model that could be used to clarify if febrile seizures contribute to epilepsy or learning differences later in life in an animal model of FS.

To date, there has not been a great deal of work on the mechanisms contributing to development of FS. Currently, the only experimental model of febrile seizures is a rodent model of febrile status epilepticus (a prolonged febrile seizure lasting more than 15 min) (Baram et al., 1997) that utilizes a hair dryer to rapidly warm a rat to 40–41 °C. At this temperature, the rat exhibits seizures, and then stops when the temperature is lowered. The first work on this model reported on the development of age specific seizures without the development of spontaneous seizures later on in life (Dube et al., 2000). Subsequent studies have shown that 35% of rats with induced FS on P10 (approximately equivalent to a 3–12 month old child) develop electroclinical seizures in adulthood (Dube et al., 2006). Studies using this model have reported changes in excitability (Chen et al., 2001; Chen et al., 1999), gene expression (Brewster et al., 2002), and network effects(Bender et al., 2003), but with little or no cell loss, clear mossy fiber sprouting, or neurogenesis (Bender et al., 2003; Brewster et al., 2002; Toth et al., 1998). Data from the rodent febrile status model has begun to answer some of the important questions, such as whether a prolonged FS injures the hippocampus. While the original studies on the experimental FSE model did not report hippocampal cell death, Dube et al. subsequently performed serially MRI examinations on rats' experimental FSE and found acute T2 signal abnormality without any associated neuronal loss (Dube et al., 2004). Together, all of these studies have concluded that early prolonged febrile seizures do cause a lowered seizure threshold later in the life of the rats (Dube et al., 2000), supporting the hypothesis that FSE can result in long-term consequences in a developing brain.

The current paper does not address the long-term effects of febrile seizures on the developing brain, but rather attempts to elucidate why fever leads to seizures. A few hypothesis have been put forth to explain the brain's susceptibility to seize with increased temperatures. One hypothesis asserts that fever alters ion channel kinetics and axonal conduction velocity thereby making the normal network hyperexcitable (Dube et al., 2007). Another hypothesis asserts that the mediators of fever directly alter neuronal function. A number of studies have looked at the role cytokines, specifically Interleukin 1 beta (IL-1 β) in the brain in both fever and hyperthermia (Heida and Pittman, 2005; Vezzani et al.). This particular cytokine appears to be necessary to generate FS in rats. Rats lacking the Interleukin 1 receptor (IL-1R1) gene have a much higher temperature threshold to develop FS (Dube et al., 2005). In addition, IL1 β receptors are expressed in high density in the hippocampus and activation of the receptor triggers downstream effects via both MAP kinase and NF κB signaling (Takao et al., 1990). Activation of these signaling cascades alters gene expression, which eventually modifies the normal neuronal circuitry into an epileptic circuit. More recently a few papers have implicated viruses to be potentially directly involved in seizure generation and in the long-term changes in the hippocampus after a febrile seizure. Human herpes virus 6B (HHV6B) is a common cause of infection and has been found more commonly in pathological specimens from patients with intractable epilepsy undergoing hippocampal resections compared to subjects with neocortical resections (Theodore et al., 2008). Besides HHV6B, other viruses, HHV-6 and 8 and HSV-1, were detected by polymerase chain reaction (PCR) in 6 of 33 subjects who underwent temporal lobectomy for refractory mesial temporal lobe epilepsy (MTLE) (Karatas et al., 2008).

To this background, the current study adds important information about the potential role of Transient Receptor Potential Vanilliod (TRPV) channels in the induction of seizures in the setting of fever. TRPV channels are a family of ligand gated non-selective cation channels (Kauer and Gibson, 2009). TRPV channels are unusual channels in that the ligand is not a protein or neurotransmitter but rather physical stimuli like heat, pressure or lipophillic molecules act on the extracellular side of the channel (Gavva, 2008; Kauer and Gibson, 2009). These channels were first discovered as being the channel that responds to capsaicin, the molecule responsible for the heat in hot peppers (Kauer and Gibson, 2009). These channels are highly calcium permeable suggesting that they may play an important role in vesicular release at a presynaptic nerve terminal. There are currently 6 members of the TRPV channels, with TRPV1–4 being very similar and TRPV5 and 6 having significantly different channel and binding properties (Kauer and Gibson, 2009; Matta and Ahern, 2011). TRPV1–4 are all expressed in the peripheral and central nervous system while TRPV5 and 6 are expressed in endothelial cells (Kauer and Gibson, 2009). The function of the brain expressed channels is still a matter of active research, but it is clear that they play an important role in setting the core body temperature, peripheral temperature sensation, as well as osmo-regulation (Gavva, 2008; Steenland et al., 2006). Body temperature is regulated by the supraoptic and preoptic nuclei in the hypothalamus. TRPV channels are expressed in these nuclei and likely act to sense temperature and alter the activity of these neurons, which then regulate the normal body temperature controlling mechanisms. This action of TRPV channels in body temperature regulation lead Hunt et al. to postulate that over activation of these channels might lead to the generation of a seizure with a fever (Hunt et al., 2012).

The manuscript details the characterization of a novel model for inducing febrile seizures in zebrafish larvae. The first process in developing a model for fever-induced seizures is to determine that a direct relationship exists between the temperature of an animal and seizure like activity in that animal. As the model is in larval zebrafish, the group used a procedure of embedding the larvae in agarose and perfusing the embedded fish with water of an increasing temperature (Baraban et al., 2005; Beck et al., 2004; Gilland et al., 1999). As fish are poikilothermic their body temperature rises with the water temperature. The authors were able to record field potentials from the region of the telencephalon using a glass microelectrode. When the agar temperature reached 25.5 ± 0.3 °C from 22.0 ± 0.3 °C there was a field potential change that resembles an electrographic seizure from EEG in humans, rodents, and dogs — the more common species used for epilepsy research (Hunt et al., 2012). These authors state that the discharges recorded with temperature elevation were similar to discharges recorded when the animals were perfused with pro-convulsant drugs (Baraban et al., 2007; Baraban et al., 2005). While the field potential recordings are fairly convincing of a seizure like discharge within the forebrain of the fish, there is a lack of a behavioral correlate as the animals are anesthetized and embedded in agarose. While not necessary to establish the zebrafish as a model for febrile seizures, observing a behavioral change in a separate set of experiments during an increase in water temperature would make the model more complete.

In contrast to febrile seizures in children, the seizures in the zebrafish stopped when the temperature of the water/agar was lowered back to room temperature (Hunt et al., 2012). The zebrafish model is similar to the rodent model where the seizures also stop when the body temperature returns to normal (Baram et al., 1997). The majority of febrile seizures in children last less than a few minutes even in the presence of persistently elevated body temperature. Another difference between the two experimental models and the human condition is that in children the fever is internally generated by the numerous mechanisms that elevate the body temperature to aid in fighting the infection that triggered the fever. This difference suggests that the hypothesis mentioned above regarding the role of the inflammatory mediators in febrile seizure initiation may indeed be true, at least partially.

Hunt et al. describe the maturation in the complexity and duration of the febrile seizure in the zebrafish over 4 days of larval development,