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Brain & Development 31 (2009) 378–382

BRAIN & DEVELOPMENT
Official Journal of the Japanese Society of Child Neurology

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Review article

Neurobiological and physiological mechanisms of fever-related epileptiform syndromes

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Received 24 July 2008; received in revised form 30 October 2008; accepted 4 November 2008

Abstract

Febrile seizures (FS) are the most common type of convulsive events in children. FS have been extensively studied using animal models, where rat and mice pups are placed in a hyperthermic environment. Such work has largely focused on the consequences rather than on the mechanisms of experimental febrile seizures (eFS). We have recently shown that eFS are preceded by a dramatic rise in the rate of respiration. The consequent respiratory alkalosis affecting the brain and increasing neuronal excitability is a direct cause of the eFS [1]. If a similar mechanism contributes to human FS and other fever-related epileptiform syndromes, a number of factors operating at the molecular, cellular and systems level that have not been previously thought to be involved in their etiology must be considered. These include physiological and pathophysiological factors affecting CO_2 chemosensitivity as well as cellular and systemic mechanisms of acid-base regulation. Furthermore, a critical role for brain pH in FS points to novel types of susceptibility genes, which include genes coding pH-sensitive target proteins (e.g. neuronal ion channels) and pH-regulatory proteins. We will discuss these novel ideas and putative therapies based on them.
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Keywords: Febrile seizure; Hyperventilation; Respiratory alkalosis; pH; Carbon dioxide; Human; Rat; Hyperthermia; Interleukins

1. Introduction

Febrile seizures (FS), or febrile convulsions, are defined by the ILAE (International League against Epilepsy, http://www.ilae.org/) as an epileptic seizure "occurring in childhood after age 1 month, associated with a febrile illness not caused by an infection of the CNS, without previous neonatal seizures or a previous unprovoked seizure, and not meeting criteria for other acute symptomatic seizures" [2]. The majority of FS take place between 6 months and 5 years of age, peaking at 16–18 months [3–5]. The prevalence of FS varies between 2 and 8% depending on geographical and cultural factors, and also on differences in ascertainment definitions and methods [6–8]. FS are mainly generalised tonic–clonic seizures and only 4–16% of the cases show focal characteristics [9,10]. In more than two-thirds of the affected children, FS last less than 10 min, and in 9% the duration is prolonged (>15 min) and a febrile status epilepticus (usually defined on the basis of duration >30 min) occurs in 5% of children [11]. FS are classified as simple FS, which consist of a generalised convulsive seizure without focal neurological features, lasting less than 15 min and not recurring within 24 h, and complex FS which are characterized by a prolonged

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doi:10.1016/j.braindev.2008.11.011

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duration (lasting 15 min or more), focal neurological features, or seizure recurrence within a 24 h period or during the same febrile illness. FS result from a combination of genetic and environmental factors [5,12,13]. Children with FS have a positive family history in 24% for FS and in 4% for epilepsy [14]. However, several fundamental aspects in the genesis of human FS are unclear.

The mechanisms and especially the consequences of FS have been extensively studied in animal models, where rat pups are exposed to an elevated ambient temperature which leads to generalized seizures that can be monitored behaviorally [15,16]. The total duration of the eFS in this model is set to about 22–24 min to mimic the duration of complex FS in human infants. Similar models have also been used in work on mice because a number of transgenic strains of potential relevance are available but, as recognized by numerous teams studying epileptiform phenomena, EEG recordings are needed in experiments on mice to detect and confirm the occurrence of seizure activity. The present paper[1] focuses on our recent work on a rat-pup model of experimental FS (eFS) and on its implications for human FS mechanisms and potential therapeutic approaches [1,17,18].

2. A modified rat-pup hyperthermia model of eFS

In a widely-used model of eFS [16,19], rat pups at about 10–11 postnatal (P) days are heated by a hair dryer for 30 min. This leads to an extremely fast increase in body temperature from its control level (rectal temperature ~33 °C) to eFS threshold (~41 °C) within about 2–4 min [19,20]. Under the above conditions, activation of thermal nociceptors is bound to occur [21] and, in addition, inflammatory responses are regularly seen in the ears and paws of the animals. Both pain and inflammation are associated with the actions of interleukins [22] and interleukins have been implicated in the mechanisms underlying both eFS and FS (see below). In order to minimize the possible effects of pain and inflammation as confounding factors, we have used a modification of the model originally developed by Holtzman and coworkers [15], where the pups are placed in a heated chamber with an ambient temperature of about 46–48 °C [1]. The rise in body temperature is much slower than in the hair-dryer model, and eFS are seen with a delay of about 30 min [1,17]. In this context, it is important to note that the likelihood of human FS is not affected by the rate of rise of fever [23]. Further advantages of the heated-chamber model in studying eFS are described in Schuchmann et al. [17].

[1] This work was presented by one of us (K.K.) at the 11th Annual Meeting of the Infantile Seizure Society in Japan (2008).

3. Are eFS caused by hyperventilation?

While there is surprisingly little data on the effects of fever on respiratory patterns in young children, the results that are available indicate that fever induces an increase in the rate of breathing [24–26]. These considerations made us hypothesize that perhaps in the rat pups and human infants with FS, the increase in the rate of breathing is not compensated by a reduction of tidal volume, which would therefore lead to hyperventilation (defined as a loss of CO_2) and respiratory alkalosis. In the present context, this is of outmost importance because a wealth of data indicates that a rise in pH leads not only to an increase in neuronal excitability but often also to epileptiform activity [27–29].

Despite unpublished data claiming otherwise [20], it has been clearly demonstrated that both a slow and a fast rise in body temperature lead to thermal tachypnea, a pronounced increase in the rate of respiration of rat pups [17]. Indeed, respiratory responses to changes in ambient temperature are known to play a general role in the regulation of mammalian body temperature [30–32].

In our model, all P8–P11 pups showed eFS, while no seizures occurred in older ones (P22–P23). The eFS were invariably associated with an alkalosis of the brain as seen in direct measurements using an intracortical pH-sensitive electrode [1]. The seizure threshold during the respiratory alkalosis was an increase in pH of about 0.25 units from its control level. The age-dependence of the eFS probably reflects the fact that the CO_2 chemosensitivity of the P10 pups is too low [33] to enable a tight control of systemic CO_2 and pH during the hyperthermia-induced tachypnea, which therefore leads to a net loss of CO_2.

Notably, the eFS were completely abolished by exposing the pups to 5% CO_2 in air. Regarding the blocking effect of CO_2 on eFS, the following points are worth emphasizing:

(i) The effect of ambient 5% CO_2 was remarkably fast: when monitored with intrahippocampal and intracortical EEG electrodes, the electrographic seizure activity was abolished *within 20 s* (Fig. 1).
(ii) The effect of CO_2 was caused by a *partial suppression of the brain alkalosis and not by an acidosis* (cf. [34]). The slight acid shift caused by 5% CO_2 in brain pH started with a delay of several minutes and had its peak of ~0.1 units at 15–20 min.
(iii) In addition to its acute effects on brain pH and eFS, ambient CO_2 blocked two well-established long-term effects of hyperthermia: the up-regulation of the I_h current in hippocampal pyramidal neurons [35], and the up-regulation of the expression of cannabinoid CB1 receptors [36].

In summary, our work on the rat-pup model [1] provides a mechanistic basis for eFS. If hyperventilation

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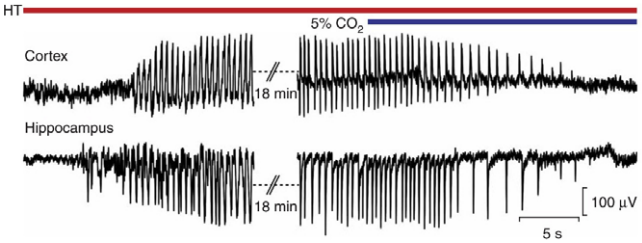

Fig. 1. Five percent ambient CO_2 blocks the hyperthermia-induced electrographic ictal activity in about 20 s. The figure shows simultaneous recordings from the cortex and hippocampus of a P9 rat pup (HT: hyperthermia). Reproduced by permission from [1].

will turn out to be a key player in human FS, the search for susceptibility genes of fever-related epileptiform dysfunctions [12,13] should include those that are involved in the control of respiration and those that play a role in the regulation of brain pH at the systemic and cellular levels. Most importantly, our results also raise the obvious question whether increasing the level of CO_2 could be used in the treatment of FS and other fever-related epileptiform dysfunctions such as FS+ and GEFS+.

4. Novel therapeutic strategies for the treatment of FS

Rectal application of diazepam, as well as nasal or buccal application of midazolam are effective in the acute management of FS [14,37,38]. Obviously, benzodiazepines do not act at the level of the triggering mechanisms of FS and furthermore, the available evidence indicates that benzodiazepines do not protect against FS or recurrent FS when applied at a dose of 0.2 mg/kg [39]. A small reduction in recurrence risk was seen at 0.3 mg/kg, but this dose had significant side effects in about one-third of the children, including somnolence or ataxia [40,41]. Finally, benzodiazepines have a broad spectrum of additional side effects, which limits their clinical use especially in young children. Therefore, novel therapies are needed for the treatment of FS especially in children with an increased risk.

We have preliminary evidence supporting the idea that a fever-related respiratory alkalosis plays a role in the generation of FS [18]. There are two obvious techniques that can be used to suppress alkaline pH shifts in humans: (1) application of CO_2-enriched air (or oxygen) in a manner analogous to that in our rat-pup model, or (2) using re-breathing from a plastic or paper bag, a standard technique also employed for instance in the treatment of hyperventilation syndrome and panic disorders [42]. Technique (1) offers the advantage of working with a well-defined level of inspiratory O_2 and CO_2. However, the application of CO_2-enriched air for medical treatment requires special permissions in several countries/states, especially within the EU and the US. A way to circumvent this problem is to use medical carbogen (5% CO_2 plus 95% O_2), which is a standard gas mixture with multiple applications in the clinic. On the other hand, the re-breathing manoeuvre (technique 2) is easy to perform practically at any place. However, using this approach with young infants requires special care because prolonged re-breathing (which is not needed) will reduce the O_2 levels to 10–12% and induce a hypoxic state. Thus, this technique should be combined with the possibility to measure end-tidal O_2 and CO_2 [18]. If one or both of the above approaches turns out to be successful in the clinic, the next step is to make them compatible for use at home.

5. Multiple mechanisms underlying eFS and FS

It is likely that there are differences in the molecular and cellular mechanisms that contribute to seizure generation in various models of eFS [1,20,43–45] and also in human FS. Notably, there is a steep rise in the levels of interleukin-1β in human FS [46], and several recent studies have shown that interleukins play a role in the modulation of eFS in animal models [47,48]. In this context, it is of much interest that "pro-seizure" interleukins have excitatory actions on neurons that mimic those of a brain alkalosis (see Section 1) in that they enhance NMDA receptor actions [49] and suppress those mediated by $GABA_A$ receptors [50]. The possible synergisms of brain alkalosis and pro-seizure interleukins in the generation of eFS and FS deserve much attention in future studies.

6. Concluding remarks

Animal models of FS cannot, of course, reproduce all characteristics of human FS. An important question here is how to calibrate developmental time across species, from rodents (rats and mice) to humans. Is a P10 rat equivalent to a human infant at 16–18 months of age when the incidence of FS is highest [3–5]? This problem does not have a clear-cut yes/no answer because different brain structures and network functions have a different developmental time course in various species [51,52]. In terms of cortical development, it is obvious that a P10 rat is much less developed than a 16–18 month-old human being: the cortex of the P10 rat produces immature-type, discontinuous EEG activity [53–55], which is strikingly similar to what is seen in preterm infants [56].

However, with regard to the above cross-species age-calibration problems, the situation may be completely different when one compares the developmental patterns of those central and peripheral systems that are responsible for the control of breathing [33]. In terms of cortical development, the P0 (newborn) rat pup is generally thought to be comparable to the human fetus at the onset of the last trimester of pregnancy. Considering that breathing of air in rat pups starts at this develop-

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mental state, the P10 pup may well be comparable with regard to respiratory functions to the 16–18 months human infant. If a lack of tight control of systemic pH/CO_2 during fever turns out to contribute to the generation of human FS, it is obvious that much more work needs to be carried out on the development of human respiratory control as well as on the effects of fever on respiratory functions in infants. This may turn out to be an exciting and clinically highly relevant area of research, which calls for multidisciplinary studies on physiological and pathophysiological mechanisms in the control of (and interactions among) neuronal excitability, body temperature, breathing and pH/CO_2 regulation in the developing brain.

In conclusion, the evidence reviewed in this paper provides strong support for the idea that a hyperthermia-induced respiratory alkalosis is a key factor in the triggering of eFS in the rat-pup model using the heated chamber [1]. With regard to human FS, the data obtained so far are consistent with this view, suggesting that a slight elevation of CO_2 can be used as a novel therapy to block FS [18].

Acknowledgements

The original research work by the authors was supported by grants from the Academy of Finland (K.K., S.V.), from the Sigrid Jusélius Foundation (S.S., K.K.), and from the European Integrated Project "EPICURE" EFP6-037315 (K.K.).

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