Pronounced increase in breathing rate in the “hair dryer model” of experimental febrile seizures

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SUMMARY
In a study using a heated chamber for induction of experimental febrile seizures (eFS) in rat pups, ictal activity was shown to be precipitated by a respiratory alkalosis (Schuchmann et al., 2006). In sharp contrast to this, in a recent review Dubé et al., (2007) suggest that the respiratory alkalosis is model specific, and that no increase in respiratory rate is observed in the widely used “hair dryer model” of eFS. The data in the present work, based on well-established techniques for measuring respiratory rates in rat pups, show a pronounced increase in the “hair dryer model” with values that are slightly higher than those recorded in the heated chamber model. Hence, a temperature-evoked increase in respiration is a common feature of these two models of eFS.

KEY WORDS: Neonate rat, Hyperthermia, Hyperventilation, Epilepsy.

Febrile seizures (FS) present the most common type of convulsive events in children (Stafstrom, 2002). “Simple FS” characterized by their brief duration of generalized cortical epileptiform are considered benign. However, the spectrum of fever-related epileptic syndromes includes a wide range of disease states, and some of them are associated with an increased risk of subsequent epilepsy (Berg et al., 1998; Shinnar, 1998). A major nonbenign category includes “complex FS” that have a prolonged duration or include partial (focal) seizure activity (Stafstrom, 2002). Although a number of susceptibility genes associated with fever-induced convulsions have been identified (Baulac et al., 2004), the neurophysiological mechanisms underlying these disease states have not been identified.

In order to study the mechanisms and consequences of FS, animal models have been developed where an increase in body temperature is induced by elevating the ambient temperature (e.g., Holtzman et al., 1981; Baram et al., 1997). In one of the most widely used models (Baram et al., 1997; Dubé and Baram, 2005), rat pups at postnatal (P) days of about 10–11 are exposed to a stream of hot air from a hair dryer for 30 min, which leads to a very rapid increase in body temperature and to experimental FS (eFS) that commence within about 2-4 min (Dubé and Baram, 2005; Dubé et al., 2007). The total duration of the eFS in this model is set to about 22–24 min to mimic complex FS.

In a recent study, we modified the above model in a manner in which the pups are placed in a heated chamber with an ambient temperature of about 48°C, and in which heat transfer is not heavily dependent on convection, as is the case with the hair dryer. The rise in body temperature is much slower, and eFS are seen with a delay of about 30 min from the start of the hyperthermia exposure (Schuchmann et al., 2006). A key finding in this work was that the eFS were preceded by a steady rise in the rate of respiration, up to 160% of the control level. This resulted in a respiratory alkalosis (net loss of CO2) as demonstrated by an alkaline shift that was measured using a pH-selective electrode implanted in the cortex. Notably, several lines of evidence suggested that the heat-induced respiratory alkalosis was a direct cause of the eFS. A key observation was that the eFS were completely suppressed within 20 s by exposure of the pups to a low (5%) level of ambient CO2.

In light of the data above, it was surprising that in a recent review, Baram and coworkers (Dubé et al., 2007) argue on the basis of unpublished data (see Corrigendum...
for Dubé et al., 2007) that the dramatic increase in body temperature in the "hair dryer model" is associated with little change (∼3%) in the rate of respiration just before the onset of convulsions. Obviously, this kind of a discrepancy between the two models would have profound consequences on the interpretation and possible clinical applications of the data derived from them, and hence we felt that it is pertinent to reexamine whether a thermal respiratory response is indeed virtually absent in the "hair dryer model." The data obtained using well-established methods for measuring respiratory rates clearly showed that this is not the case. The increase in respiratory rate observed just before the onset of seizures is very similar in the hair dryer and in the heated chamber model.

**MATERIALS AND METHODS**

In this study, all the data are from P10 Wistar rat pups. The experiments on eFS in the heated chamber model were carried out as described before (Schuchmann et al., 2006). The protocol involving the hair dryer model was based on detailed descriptions in original research papers and reviews (Baram et al., 1997; for methods and references, see Dubé and Baram, 2005). The breathing rate of the pups was monitored by recording abdominal breathing movements using a piezo crystal sensor (movement sensor 230; Siemens-Elema AB, Solna, Sweden) placed on the abdomen. Rectal temperature was measured using a thermocouple (K101; Volcraft, Hirschau, Germany). The onset of behavioral seizures was observed in real time, and confirmed by an independent observer on the basis of video recordings (25 frames/s). The values in the Results section and in the bar graph in Fig. 1 present mean ± s.e.m. Statistical significance was tested using Student’s paired t-test, and analysis of variance (ANOVA) followed by Bonferroni/Dunn comparison in the multiple comparison shown in Fig. 1.

**RESULTS**

In the experiments with the heated chamber, the baseline level of rectal temperature was 33.6 ± 0.5 °C (n = 9 pups). Under these experimental conditions, the seizure threshold temperature was 41.2 ± 0.6 °C and seizures occurred after 28.9 ± 4.3 min. During the minute that preceded seizure onset, the respiration rate had increased from its baseline of 162.6 ± 9.7 breaths/min to 246 ± 7.7 breaths/min (p < 0.001), indicating an increase in the mean respiration rate of 51% (Fig. 1A, C).

In the experiments with the hair dryer model for the eFS induction, the baseline level of rectal temperature was 33.0 ± 0.2 °C (n = 6 pups here and below). This is similar to the value 33.4 ± 0.16 °C reported by Dubé et al., (2007). The heat exposure was adjusted to closely mimic the time course of increase in body temperature and seizure induction as described by the above authors, provided for comparison in [brackets] below. In our experiments, the seizure threshold temperature was 40.1 ± 0.3 °C [40.74 ± 0.13 °C], and the seizures commenced within 3.2 min [2.9 min]. In sharp contrast to the unpublished data communicated by Dubé et al. (2007), a pronounced increase in respiratory rate took place during the hyperthermia. The baseline level was 179.8 ± 5.3 breaths/min, followed by 298.7 ± 10.1 breaths/min (p < 0.001) during the 60 s that preceded seizure onset (Fig. 1B, C). This implies an
average increase of 66% in the rate of respiration induced in the hair dryer model, which is slightly higher ($p < 0.05$) than the heated chamber model.

**DISCUSSION**

This study shows that with regard to the heat-induced respiratory response, the heated chamber and hair dryer models are not qualitatively different. If anything, the increase in respiratory rate just before seizure onset was slightly higher in the hair dryer model. Whether there are other differences in the two models remains to be seen in future work. Notably, two main long-term consequences of eFS, an increase in the Ih current (Chen et al., 2001) and in the expression of CB1 receptors (Chen et al., 2003) are similar in both models.

Although the present work does not demonstrate any physiological differences between the two eFS models, there are some methodological details that are worth considering. Regarding the hair dryer model, “if core temperature $>41.5^\circ$C, pups are removed to a cool metal surface for 2 min to prevent excessive heating. The cycle of warming for 2 min, temperature measure, and continued warming or ‘time-out’ is maintained for a total of 30 min, resulting in seizures of $\sim 24.1$ min” (Dubé and Baram, 2005). From an experimental point of view, the heated chamber model has the advantage that after adjusting the ambient temperature suitable for eFS induction with low mortality, there is no need to remove the pups for arbitrary periods of time from the hyperthermic environment. This makes the heated chamber model suitable for rigorously quantitative tests on the acute actions of, for example, anticonvulsant drugs, or of the influence of the genetic background and/or mutations on eFS. In addition, intracranial EEG measurements are readily done in the heated chamber model (Schuchmann et al., 2006), which is an important point especially in work on mice where reliable behavioral seizure detection and scoring is often difficult and unreliable.

When considering the clinical relevance of data obtained using the two models, it may be worthwhile to take a direct quote from a paper by Berg (1993): “Pediatricians are frequently taught that a rapid rise in temperature is responsible for causing a febrile seizure; yet there are no clinical data to support this hypothesis. The few experimental data are based on hyperthermia-induced seizures in animals and are of no clear relevance to naturally occurring fevers and accompanying seizures. Further, the experimental findings are not consistent across studies. By contrast, there is substantial evidence indicating that the height of temperature plays a role in eliciting a febrile seizure.” A similar conclusion was made by Stafstrom (2002).

To summarize, both the heated chamber and hair dryer model induce an increase in respiration in rat pups, which has been suggested to play a crucial role in the generation of eFS (Schuchmann et al., 2006). Rodent models may offer valuable insights into mechanisms responsible for the induction and long-lasting effects of eFS. However, because of their obvious limitations, systematic clinical trials are required to investigate the relevance of the suggested mechanisms for the human situation from both etiological and therapeutic points of view.

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**REFERENCES**


