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Cognitive performance and convulsion risk after experimentally-induced febrile-seizures in rat

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ABSTRACT

Many reports indicated that small percentage of children with febrile seizures develop epilepsy and cognitive disorders later in adulthood. In addition, the neuronal network of the hippocampus was reported to be deranged in adult animals after being exposed to hyperthermia-induced seizures in their neonatal life. The aims of this study were to investigate (1) latency and probability of seizures, (2) spatial learning and memory, in adult rats after neonatal hyperthermia-induced febrile seizures (FS). Prolonged FS were elicited in 10-day old, male Sprague Dawleys ($n = 11$/group) by exposure to heated air (48–52 °C) for 30 min; control rats were exposed to 30 °C air. After 1.5 months the animal's cognitive performance was assessed by 5 day trial in the Morris water maze. In another experiment the latency and probability of seizures were measured in response to pentylenetetrazole (PTZ) injections (increased doses ranged from 7 to 140 mg/kg; i.p.). In water maze, both groups showed improvements in escape latency and distance swam to reach the platform; effects were significantly greater in control versus hyperthermia-treated animals on days 3 and 4. Latency and probability of PTZ-induced seizures were shorter and higher respectively, in hyperthermia-treated animals compared to controls. We concluded that FS in neonatal rats leads to enhanced susceptibility for seizures, as well as cognitive deficits in adults.

1. Introduction

Febrile seizures (FS; seizures induced by fever) are the most common form of childhood seizures affecting 2–5% of children aged six months to five years (American Academy of Paediatrics Steering, 2008). They can be classified into simple (duration of <15 min, generalized, occurs once in 24 h, and no history of neurological problems) and complex (duration of >15 min, focal and recurs within 24 h) types (Wairuru, 2004).

Whether or not FS are a risk factor for later development of epilepsy and neurocognitive sequalae remains controversial (Hesdorffer and Hauser, 2002; Hirtz, 2002). However, the likelihood is increased if the FS are complex (Millar, 2006) and the duration of fever prior to the seizures prolonged, with a family history of epilepsy, cerebral palsy, and Apgar score <7 at five minutes (Shinnar and Glauser, 2002). The risk for later development of epilepsy in children has also been reported to rise with the number of features of complex FS present (one feature has a risk of 6–8%; two features has a risk of 17–22% if two features are present, and 49% if three features are present (Annegers et al., 1987)). Controversy also exists as to whether FS are a risk factor for poor cognitive outcome (Hirtz, 2002).

Animal models of FS have been developed to study the pathogenesis and their sequalae. Studies of rats have shown that prolonged, hyperthermia (HT)-induced seizures in early life (Baram et al., 1997; Avishai-Eliner et al., 2002) have a long-lasting effect on the hippocampus that increases the risk of future seizures and of later-onset temporal lobe epilepsy (Dube et al., 2000; Dubé et al., 2006, 2007). In this seizure model, hyperthermia induces transient neuronal injury to the hippocampus without significant neuronal cell death (Toth et al., 1998; Bender et al., 2003), followed by highly specific alterations in the excitability of the hippocampus (Chen et al., 1999, 2001; Kamal et al., 2006; Notenboom et al., 2010; Kim and Connors, 2012). Such changes in neuronal excitability are known to be an important determinant of hippocampal synaptic plasticity as well as learning and memory (Giese et al., 2001; Disterhoft and Oh, 2006). To date, however, studies have failed to resolve the issue of whether or not febrile seizure-induced changes in hippocampal network excitability actually lead to subsequent

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changes in cognitive development and overt cognitive performance (Notenboom et al., 2010; Mesquita et al., 2006; Lemmens et al., 2009; Dubé et al., 2009).

Against this background, we have utilised a rat model of early-life experimentally-induced FS, to investigate the long-term susceptibility for (1) impaired cognitive performance in the Morris Water Maze (Biessels et al., 1996); (2) increased susceptibility for convulsions following administration of the epileptogenic, pentylenetetrazole (PTZ) (Hansen et al., 2012).

2. Methods

2.1. Animals

Newborn male Sprague-Dawley rats were kept in a standard animal facility (12 h light/dark cycle; lights on at 07:00am) with food and water ad libitum. Pups were from culled litters and weaned on P21. All procedures were ethically approved by the Research & Research Ethics Committee, Arabian Gulf University, Bahrain.

2.2. Hyperthermia-induced seizure

Prolonged FS were elicited in 10-day old, male Sprague-Dawley rats ($n = 11$/group) by exposure to a regulated stream of heated air (48–52 °C) as previously described (Baram et al., 1997; Dube et al., 2000; Toth et al., 1998; Kamal et al., 2006; Notenboom et al., 2010). Briefly, one to two pups were placed inside a vertically mounted Perspex tube and exposed to a controlled stream of heated air (48–52 °C) generated by an adjustable hair dryer (HL1605S/3461; Steinel, Germany), placed ~46 cm above the rats. Core temperature was measured every 2.5 min during heat treatment. Hyperthermia (HT) was defined as core temperature >39.5 °C and this was typically achieved within 5 min. Provocation of behavioural seizures was achieved by maintaining core temperature between 41 and 42 °C, and these seizures were monitored accordingly. All HT-treated rats developed seizures. As previously described, these induced behavioural seizures have been shown to correlate with electroencephalogram rhythmic epileptiform discharges from the hippocampus and amygdala (Baram et al., 1997; Dube et al., 2000). Characteristics of these seizures include arrest of heat-induced kinesis, tonic freeze postures and occasional automatisms and clonus (Notenboom et al., 2010). After 30 min of HT, the pups were rapidly cooled down in a water bath at room temperature (21–22 °C) and returned to the dam. As previously reported, our in-house data (not shown), reveal that the seizure activity is confirmed by epidural encephalogram and can be blocked by administration of pentobarbital (Notenboom et al., 2010). Weight-matched normothermic controls ($n = 11$) underwent the same protocol except that the regulated stream of air was maintained at 30 °C for the duration of exposure. Control and HT-treated rats were from the same litters.

2.3. Water maze

Assessment of learning and memory was conducted using the Morris water maze. At 1.5 months after hyperthermia ($n = 5$) or normothermia ($n = 5$) exposure, animals were assessed daily for a total of 5 days in the Morris water maze, as previously described (Kamal et al., 2006; Biessels et al., 1996). The maze consisted of a circular swimming pool (140 cm diameter and 50 cm height, filled to a depth of 30 cm) with the water maintained at room temperature. The maze was housed in a darkened room, rich in extra-maze visual cues, and illuminated by sparse red light. It was divided into 4 equal quadrants by two imaginary diagonal lines.

Each rat was given five acquisition trials/day for five consecutive days to learn the position of a hidden 'escape' platform, submerged 2 cm below the water surface, at a fixed location inside the pool. On each trial, the rats were released from one of four predetermined positions on the perimeter of the pool. The starting position was varied on each trial in a quasi-random sequence. Animals were given a maximum of two minutes to find the platform, and were allowed to remain on the platform for 30 s. Rats that failed to locate the disc were put onto it by the experimenter.

The position and movement of the animals, in the pool, was captured and analysed every 0.2 s, using a video-camera computer system, and ANY-maze video-tracking system (Stoelting Co., U.S.A.). Outcome measures were latency time and distance swum to reach the platform. These measures were considered most relevant for spatial learning and memory (Gallagher et al., 1993). Performance in the five daily trials was averaged to yield one data point per rat per day. Speed of swimming (which is a measure of motor function (Lindner, 1997)) was measured as control between the groups. To measure any bias, a trial was conducted following the last acquisition training on day-5. On the sixth day of water-maze testing the platform was removed and each animal was allowed to swim for 60 s. In this probe trial, the selective search strategy was indicated if animals performed significantly above chance (25%).

2.4. Pentylenetetrazol injections

Animals were administered with doses of the epileptogenic PTZ (Sigma P6500), 32 days following hyperthermic or normothermic exposure (at P10), in order to determine susceptibility for convulsions. Following PTZ administered (7–140 mg/kg i.p., dissolved in saline) latency and probability of seizures were compared within (i.e. across days) and between treatment groups.

2.5. Data analysis

Data was expressed as mean ± SEM. Statistical significance was set at a P value of less than 0.05. All statistical analyses were performed with Microsoft EXCEL (version 2010) and a Addinsoft™ XLSTAT (Version 2012.6.06). For the Morris maze data, daily averages for all the acquisition trials for each animal were calculated. Comparisons within- and between-treatment groups were conducted using Analysis of Variance for repeated measures ANOVA and post hoc Tukey test. For convulsion latency, between group comparisons were made by student's unpaired t-test. Probe trial differences in water-maze were tested by one sample t-test. Seizure incidence or probability was calculated using the Chi-square test.

2.6. Ethical approval

The experimental procedures were approved by the Research and Research Ethics Committee, Arabian Gulf University, Bahrain.

3. Results

3.1. Long-term effects of hyperthermia-induced seizures on probability and latency of PTZ-induced convulsions

Comparison of probability and latency of convulsions between the control and HT-treated animals was investigated by administration of PTZ. The convulsive behaviour was evaluated as described by Racine (1972). Animals reaching scale 4 or 5 were considered as positively reacting to PTZ, and the latency between PTZ administration and appearance of convulsions was measured. Convulsions occurred at a lower threshold dose of PTZ in HT-treated animals compared to controls (Fig. 1). Although all animals in both groups demonstrated convulsion in response to high doses of PTZ, but

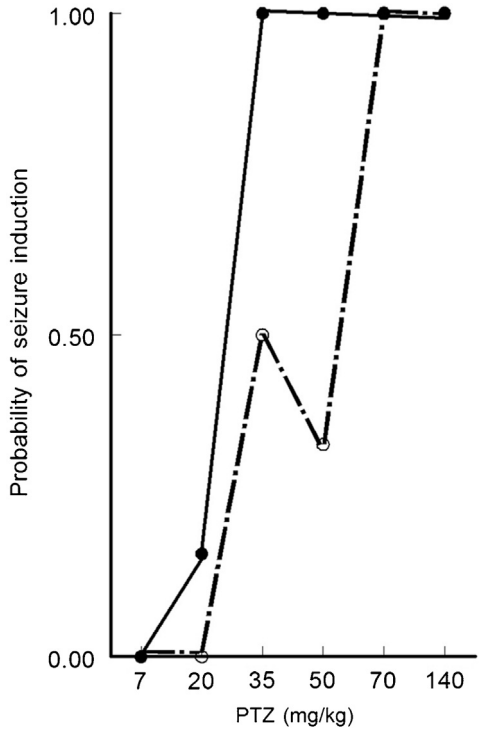

Fig. 1. A cumulative plot showing the difference in seizure susceptibility towards different doses of PTZ injection between the control (white circle) and hyperthermia rats (black circles).