Areas of susceptibility of the predisposed immature rat brain to hyperthermic seizures and resultant neurodevelopment delay : an MRI and PET study

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Introduction

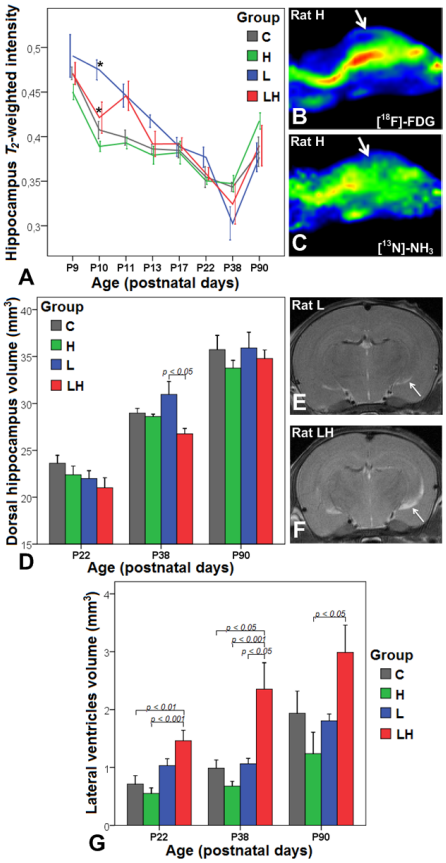
Retrospective studies have correlated temporal lobe epilepsy (TLE) and mesial temporal sclerosis (MTS) with a past history of atypical febrile seizures (FS). A causal relation between the two conditions is not clear in epilepsy literature. Recently, a study on a pediatric population has shown that cortical dysplasia (CD) and MTS were frequent findings in children with refractory TLE [1]. An animal model was developed to establish the relation between CD, FS and TLE. This model consisted of inducing a cortical cryolesion on the right fronto-parietal cortex of neonatal rats at post-natal day (P1) and then inducing hyperthermic seizures (HS) at P10. A cortical lesion was found to predispose animals to hyperthermic seizures and prolonged seizures [2]. Also, more than 85 % of adult rats developed spontaneous recurrent seizures recorded from the temporal lobe [3]. Histology has shown morphologic abnormality of the hippocampus at P22 [4]. In order to better understand the neurodevelopmental changes that occur after HS on a predisposed brain, longitudinal *in vivo* magnetic resonance imaging (MRI) volumetric measures were obtained. Since atrophy of the hippocampus is a common feature in MTS, this structure was closely examined. In addition, the topographic variations of MRI T_2-weighted signal intensity were studied to determine brain areas involved in HS. Positron emission tomography (PET) images were acquired during prolonged hyperthermic seizures (PHS) to study the metabolic and blood flow changes during seizure in order to better characterize the pathophysiology of the changes observed with MRI.

Material and Methods

Animals: Twenty Sprague-Dawley pups were equally divided into four groups: naive controls (C), hyperthermic seizures (H), cortical cryolesion (L), and finally cortical cryolesion and hyperthermic seizures (LH). **Induction of cortical lesions:** A 2 mm diameter copper cylinder cooled in liquid nitrogen was positioned for 10 s on the skull of P1 pups to induce a right fronto-parietal focal microgyrus [5]. **Hyperthermic seizure procedures:** HS: P10 pups were individually placed in a plexiglas box heated with warm dry air (47°C) until a generalized seizure started [2]. PHS: Same as HS but the animals were kept for 3 min in the hyperthermic environment after the generalized seizure. **MRI T_2 signal:** MRI experiments were performed with a 7 T animal scanner. Axial T_2-weighted images were acquired at P9, P10 (5 h post-hyperthermia), P11, P13, P17, P22, P38 and P90 with a fast spin echo (FSE) sequence (TR: 4000 ms; TE_{eff}: 48 ms; ES: 12 ms; eight echoes; acquisition matrix size: 256 × 256; FOV: 25 × 25 mm^2; averages: 16; slice thickness: 1 mm). **MRI volumetric images:** Axial T_2-weighted images were acquired at P22, P38 and P90 with a FSE sequence (TR: 6000 ms; TE_{eff}: 52 ms; ES: 13 ms; eight echoes; acquisition matrix size: 256 × 256; FOV: 30 × 30 mm^2; averages: 16; slice thickness: 500 µm). **PET images:** PET images were acquired with a dedicated small animal scanner [6]. [^{18}F]-FDG (n = 5; 26.5 ± 5.0 MBq) was injected intraperitoneally before the PHS protocol. Animals were then anesthetised and positioned for imaging. Brain vascularisation was assessed with [^{13}N]-NH_3 (n = 3; 21.0 ± 5.2 MBq) immediately after the PHS protocol. **Image analysis:** Volumetric analysis: All images were co-registered with an in-house rat brain template using SPM5 software [7]. Hemispheres, dorsal hippocampus, ventral hippocampus and lateral ventricles were then manually delineated to obtain volumetric measures with an in-house program (Matlab, The Mathworks). Analysis of MRI T_2-weighted intensity: Regions of interest were manually drawn on limbic and extra-limbic structures. MRI T_2-weighted intensity of the 4 groups was normalised with the gray matter signal intensity from the C group.

Results

At P10, 5 h following HS, hypointensity in the hippocampus was seen in the LH group as compared with the L group (**A**). This difference was not seen between the non-lesion groups C and H. Two structures were predominantly affected by the hyperthermic seizures on the predisposed brain: hippocampus (L : 0.48 ± 0.01; LH : 0.42 ± 0.02; $p < 0.05$) and the corpus callosum/external capsule (L : 0.50 ± 0.01; LH : 0.42 ± 0.03; $p < 0.05$). PET images revelead hypometabolism and lower blood flow in the dorsal hippocampus and cortical regions during HS and the post-ictal period of H pups during PHS (**B** and **C**). The volumetric follow-up revealed that both dorsal hippocampus (L: 31.0 ± 1.4 mm^3; LH: 28.6 ± 0.6 mm^3; $p < 0.01$) (**D**) and the lateral ventricles (L: 1.06 ± 0.09 mm^3; LH: 2.35 ± 0.46 mm^3; $p < 0.05$) (**E**, **F** and **G**) were statistically different at P38.



(A) T_2-weighted intensity in the hippocampus. * indicates $P < 0.05$. **(B,C)** Sagittal metabolic and perfusion PET images acquired from H pups at P10 during PHS. White arrows indicate the dorsal hippocampus. **(D)** Longitudinal dorsal hippocampus volumes. **(E,F)** White arrows show the ventricular dilatation of an L and LH rat at P22. **(G)** Longitudinal lateral ventricle volumes.

Discussion

A T_2-weighted intensity change in the hippocampus has been previously observed in HS [8]. To our knowledge, we present the first study of T_2-weighted intensity as early as 5 h after HS. PET results indicate an imbalance between metabolism and vascularisation in the hippocampus. This may lead to an increased deoxyhemoglobin level responsible for shortening T_2. Interestingly, others have observed hyperintensity in certain limbic structures 24 h after seizure [8,9]. This was not seen in this study and is believed to be caused by the difference in the HS animal protocol. In this study, the pups were subjected to hyperthermia only until generalised seizure compared with other protocols that maintained the animal in this environment for an extended period. In conjunction with the signal change, the deleterious neurodevelopmental effects of HS on a predisposed brain can be appreciated in our results. We link the signal change of the hippocampus with a developmental delay of the hippocampus (and a compensatory dilatation of the lateral ventricles) that is noticeable at P38.

Conclusion

MRI and PET were combined for the first time to study the consequences of seizures on a predisposed rat brain. Our results suggest a causal relationship between a T_2-weighted signal change resulting from metabolism/vascularisation imbalance after HS and a consequent developmental delay of the hippocampus.

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