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Bilateral, vascular and perivascular glial upregulation of heat shock protein-27 after repeated epileptic seizures

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Abstract

Heat shock protein-27 (HSP-27) is an inducible stress response protein. It inhibits apoptotic cell death and is a reliable marker for oxidative stress. We studied the induction of HSP-27 in rat brains on days 1, 4 and 14 after repeated, pentylenetetrazole (PTZ)-induced seizures using immunohistochemisty. Saline treated control rats showed no induction of HSP-27. HSP-27 reactive astrocytes were rarely seen 1 or 4 days after PTZ injection. When present, single astrocytes were located in the cortex and/or the hippocampus. After 14 days PTZ treatment, a bilateral distribution of HSP-27 immunoreactive glia was present in piriform and entorhinal cortices and in the dentate gyrus of most brains. Rats with most intense HSP-27 upregulation showed HSP-27 in amygdala and thalamic nuclei. Astrocytes associated with blood vessels presented strongest HSP-27 staining, but did not show upregulation of gial fibrillary acidic protein and none responded with HSP-47 expression. Additionally, HSP-27 immunoreactivity increased in the endothelial cells of blood vessels in the affected brain regions, although no neuronal induction occurred. Contrastingly, a subconvulsive dose of the glutamine synthetase inhibitor L-methionine sulfoxime, which acts directly on astrocytes, resulted in a rapid, homogeneous astrocyte-specific HSP-27 upregulation within 24 h. Thus, repeated PTZ-induced seizure activity elicits a focal ''heat shock'' response in endothelial cells and astrocytes of selected cerebral regions indicating that expression of HSP-27 occurred in a seizure-dependent manner within the affected cerebral circuitries. Therefore, this PTZ-model of repeated seizure activity exhibited a cortical pattern of HSP-27 expression which is most comparable to that known from patients with epilepsy.

Keywords: Epilepsy; Astrocytes; Endothelial cells; Blood–brain-barrier; Oxidative stress; Pentylentetrazole

1. Introduction

Heat shock proteins (HSPs) belong to a highly conserved family of proteins, some of which are inducible by various noxious stimuli and are part of the cellular defence system. HSPs represent chaperones acting as specific carriers and/or participate in protein-folding (Jakob and Buchner, 1994; Kiang and Tsokos, 1998; Hartl and Hayer-Hartl, 2002). HSP-70 and HSP-27 serve as binding proteins for Vitamin D and estradiol, respectively (Gacad and Adams, 1998; Lu et al., 2002; Chen et al., 2003). Both HSP-70 and HSP-27 are induced in the CNS during heat shock, ischemia, hypoxia and kainate-induced seizures contributing to the phenomenon of preconditioning (Currie et al., 2000; He and Lemasters, 2003). For HSP-27 and the mouse homolog HSP-25, it is known that they inhibit apoptotic neuronal death (Wagstaff et al., 1999; Akbar et al., 2003) by acting at the permeability transition pore of mitochondria (He and Lemasters, 2003), thus inhibiting FAS/APO-1 signalling and preserving the endogenous antioxidant glutathione (Mehlen et al., 1996a,b). In a more general view, HSPs are also considered to represent a reliable marker for tissues affected by oxidative stress (Kregel, 2002). More recently free

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radicals and oxidative stress were identified to play a major role in epilepsy. They contribute mainly to cell loss (Kovacs et al., 2002; Bidmon et al., 2002; Patel, 2002; Patel and Li, 2003; Chung and Han, 2003; Savaskan et al., 2003; Bashkatova et al., 2003).

Several animal models have been developed specifically addressing certain aspects of epileptic activity or pathological consequences found in human patients (White, 2002). Between the kainate model and the pentylenetetrazole (PTZ) models certain differences exist, the most important one which is the fact that PTZ does not cause direct neurotoxicity (Valente et al., 2004). These differences seem to result in model specific heat shock responses, since HSP-70 is upregulated after seizures induced by kainate but not after seizures elicited by PTZ, NMDA, or lindane (Planas et al., 1994). In addition, after treatment with kainate most authors reported an induction of HSP-27 (Akbar et al., 2001; Kato et al., 1999; Plumier et al., 1996), which is especially linked to apoptotic cell death in this model and which affects glial cells and neurons (Akbar et al., 2003). In comparison, for the PTZ model only a strong induction of HSP-72 has been established (Nehlig and Pereira de Vasconcelos, 1996; Motte et al., 1997; Arzimanoglou et al., 2002).

In the temporal cortex and hippocampus of human patients suffering from intractable epilepsy HSP-27 is homogenously or focally induced and its expression remained confined to astrocytes and blood vessels (Erdamar et al., 2000; Bidmon et al., 2004). Therefore, we were searching for an animal model showing a seizure-related HSP-27 induction comparable to that reported for temporal cortex of patients with epilepsy.

Since generalized seizure activity elicited by PTZ i.p. (40 mg/kg) results in a sequence of bioelectrical events which are indistinguishable from those seen in the EEG of human epileptic patients (Caspers and Speckmann, 1972), we used this model to study seizure induced HSP-27 induction.

2. Materials and methods

2.1. Pentylenetetrazole treatment

Male Wistar rats (220–250 g bodyweight) were placed in cages mounted onto a recording stage in order to register seizure activity and duration. One group of rats ($n = 8$) was injected i.p. with a single dose of PTZ at a concentration of 40 mg/kg dissolved in physiological saline (10 mg/0.5 ml) to induce acute seizures. Control rats ($n = 4$) received saline only. These animals were sacrificed 24 h after injection. Additional groups of rats were injected every 48 h (except on weekends, 72 h), with 40 mg PTZ/kg for a total of 4 days ($n = 4$) or 14 days ($n = 12$), respectively. According to previously described experimental protocols (Caspers and Speckmann, 1972; Rauca et al., 2004), the intervals of PTZ applications at the mentioned concentrations of the drug guaranteed continuous epileptic activity with lowest side effects. Control rats ($n = 8$) received saline injections only. Rats were anesthetized with sodium pentobarbital and fixed by cardiac perfusion using ice-cold physiological saline followed by Zamboni's fixative 24 h after their last injection with PTZ.

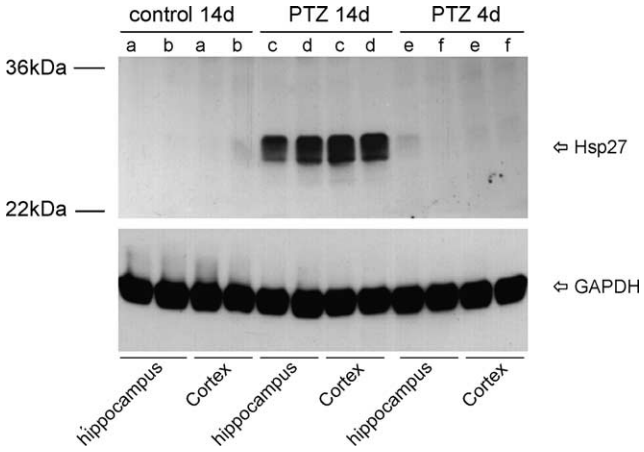

Fig. 1. Western blot analysis showing examples for the presence of a increased HSP-27 immunoreactive bands in protein extracts prepared from cerebral cortex and hippocampus of rats injected repeatedly with 40 mg PTZ/kg bodyweight for 14 days (c, d) in comparison to saline treated control rats (a, b) and rats treated with PTZ for 4 days. Note that only two consecutive injections with PTZ up to day 4 were not enough to induce HSP-27 expression up to the level of detectable amounts of immunoreactive protein.

Some treated and some control rats (Fig. 1) were perfused with saline only, the brains were removed, and cortical as well as hippocampal tissue probes of one hemisphere were used for western blot analysis whereas the other hemisphere was immersion-fixed in Zamboni fixative for immunohistochemistry. Following fixation, all brains were cryoprotected in PBS containing 25% sucrose, frozen and sectioned as described (Bidmon et al., 2001).

In order to determine whether PTZ affects astrocytes directly, we compared the induction of HSP-27 seen in PTZ-treated animals with rats in which the astrocyte-specific glutamine synthetase had been inhibited with L-methionine sulfoxime (MSO; Sigma, Deisenhofen). For this, eight additional rats were injected i.p. with a subconvulsive dose of MSO 100 mg/kg dissolved in physiological saline (Paulsen et al., 1988). After 8 h ($n = 3$) and 24 h these rats were anesthetized with sodium pentobarbital and perfusion-fixed as described above.

2.2. Western blot analysis

Tissue probes were lysed at 4 °C with 10 mmol/L Tris–HCl buffer (pH 7.4) containing 1% Triton X-100, 150 mmol/L NaCl, 1 mmol/L EDTA, 1 mmol/L EGTA, 20 mmol/L NaF, 0.2 mmol/L phenylmethylsulfonyl fluoride (PMSF), and 0.5% Nonidet P-40 (NP-40). Following homogenization, the lysates were centrifuged at 20,000 × g at 4 °C.