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ORIGINAL ARTICLE

Pentylenetetrazol-kindling in mice overexpressing heat shock protein 70

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Abstract Kindling induced by the convulsant pentylenetetrazol (PTZ) is an accepted model of primary generalized epilepsy. Because seizures represent a strong distressing stimulus, stress-induced proteins such as heat shock proteins might counteract the pathology of increased neuronal excitation. Therefore, the aim of the present study was to determine whether PTZ kindling outcome parameters are influenced by heat shock protein 70 (Hsp70) overexpression in Hsp70 transgenic mice as compared to the respective wild-type mice. Kindling was performed by nine intraperitoneal injections of PTZ (ED_{16} for induction of clonic–tonic seizures, every 48 h); control animals received saline instead of PTZ. Seven days after the final injection, all mice received a PTZ challenge dose. Outcome parameters included evaluation of seizure stages and overall survival rates. In addition, histopathological findings such as cell number in hippocampal subfields CA1 and CA3 were determined. The onset of the highest convulsion stage was delayed in Hsp70 transgenic mice as compared to wild-type mice, and overall survival during kindling was improved in Hsp70 transgenic mice as compared to wild-type mice. In addition, a challenge dose after termination of kindling produced less severe seizures in Hsp70 transgenic mice than in wild-type mice. PTZ kindling did not result in significant subsequent neuronal cell loss in CA1 or CA3 neither in wild-type mice nor in the Hsp70 transgenic mice. The results of the present experiments clearly demonstrate that overexpression of Hsp70 exerts protective effects regarding seizure severity and overall survival during PTZ kindling. In addition, the decreased seizure severity in Hsp70 transgenic mice after a challenge dose suggests an interference of Hsp70 with the developmental component of kindling.

Keywords Pentylenetetrazol · Kindling · Epilepsy · Heat shock protein 70 · Mouse · Protection

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Introduction

Kindling is a widely accepted model of human epilepsy, characterized by progressive intensification of electroencephalographic and behavioral seizures evoked by initially subeffective chemical or electrical stimuli. Whereas electrical kindling is regarded as a model of complex partial epilepsy (McNamara 1984), the chemical kindling induced by the convulsant pentylenetetrazol (PTZ) represents a model of primary generalized epilepsy (Ono et al. 1990; Rossi 1996). PTZ-induced seizures are initiated by blockade of brain gamma-aminobutyric acid receptors (Olsen 1981; Ramanjaneyulu and Ticku 1984). However, the neural mechanisms involved in kindling-induced behavioral and biochemical changes remain to be determined as well as the induction of compensatory endogenous protective mechanisms. Because seizures represent a strong distressing stimulus, stress-induced proteins such as heat shock

proteins might be assumed to counteract the pathology of increased neuronal excitation.

Heat shock proteins are induced in a variety of pathological states, including cerebral ischemia, neurodegenerative diseases, or trauma, and are thought to assist the maintenance of cellular integrity; the multifactoring mechanisms underlying cell protection include prevention of protein aggregation as well as interference with apoptosis and inflammation (reviewed by Yenari et al. 2005). Regarding epilepsy, the convulsant kainic acid has been shown to increase hippocampal expression of heat shock protein 70 (Hsp70) in rats (Krueger et al. 1999), and the expression appears to correlate with seizure severity (Zhang et al. 1997). Therefore, it could be likely that Hsp70 might be involved in endogenous cellular protection during seizures.

Hsp70 overexpression has been shown to exert protective effects using in vitro stress models including heat shock or metabolic stress (Beaucamp et al. 1998; Fink et al. 1997) as well as in in vivo models of ischemia (Plumier et al. 1997; Hoehn et al. 2001; Rajdev et al. 2000).

However, there is no knowledge whether overexpression of Hsp70 exerts protective effects in epilepsy using an animal kindling model. Therefore, the aim of the present study was to determine whether PTZ kindling outcome parameters such as seizure stage and survival, as well as histopathological findings such as hippocampal cell number, are influenced by Hsp70 overexpression in Hsp70 transgenic mice.

Materials and methods

Animals

For all experiments, ethical approval was sought according to the requirements of the National Act on the use of experimental animals (Germany) and ethics committee guidelines.

Transgenic mice overexpressing the human Hsp70 under the control of the beta-actin promoter (Angelidis et al. 1991, 1996; Plumier et al. 1995) and their respective wild-type mice (CBA x C57Bl/6 hybrid mice F1) were kindly provided by Prof. C. Angelidis, Ioannina Medical School, Greece.

Male mice (25–35 g) aging 8 weeks were used for the experiments. Animals were housed under controlled laboratory conditions in a light (12 h on/12 h off), temperature (20°C±2°C), and relative air humidity (55–60%) controlled environment with free access to food and water.

PTZ kindling

For kindling, initially subeffective doses of PTZ were injected intraperitoneally (i.p.) once every 48 h for a total of nine injections. Kindling control animals received the same number of injections of saline i.p. The initially subeffective doses of PTZ corresponded to ED_{16} for induction of clonic–tonic seizures and were determined previously in separate groups of animals (for wild-type mice, 40 mg/kg; for Hsp70 transgenic mice, 35 mg/kg). The respective dose–response curves for wild-type (n=22–29 per dose) and Hsp70 transgenic mice (n=25–34 per dose) are displayed in Fig. 1. After each injection, the convulsive behavior was observed for 20 min. Seizure stages were classified as described previously (Grecksch et al. 1999; Becker et al. 1999):

Seizure stage 0: No response
Seizure stage 1: Ear and facial twitching
Seizure stage 2: Convulsive waves through the body, without rearing
Seizure stage 3: Myoclonic jerks, upright position
Seizure stage 4: Clonic–tonic convulsions, turn over into side position
Seizure stage 5: Generalized clonic–tonic convulsions, loss of postural control

PTZ challenge test

All mice from the different treatment groups received a challenge dose 7 days after the final kindling injection. This dose is, in general, subeffective in naive animals (10 mg/kg below previously determined ED_{16} dose used for kindling) and was 30 mg/kg in wild-type mice and 25 mg/kg in Hsp70 transgenic mice.

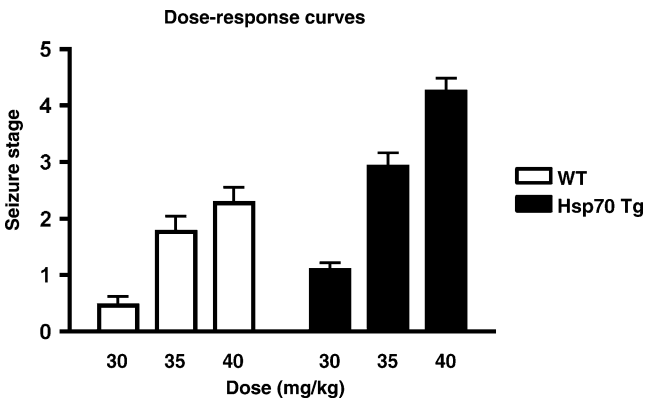

Fig. 1 Dose–response curves: mean seizure stage (mean ± SEM) following 30 mg/kg, 35 mg/kg, or 40 mg/kg PTZ in wild-type mice and Hsp70 transgenic mice (*WT* wild-type mice [*white bars*] 30 mg/kg, n=26; 35 mg/kg, n=35; 40 mg/kg, n=29; *Hsp70 Tg* Hsp70 overexpressing mice [*black bars*] 30 mg/kg, n=34; 35 mg/kg, n=25; 40 mg/kg, n=28). Results of two-way ANOVA: interaction: p=0.0085; genetic status: p<0.0001; dose: p<0.0001; Bonferroni post tests: wild-type 35 mg/kg vs Hsp70 Tg 35 mg/kg: p<0.01; wild-type 40 mg/kg vs Hsp70 Tg 40 mg/kg: p<0.001

Histopathology

Two hours after challenge injection, animals were killed, and brains were removed and stored at −70°C until further use. Cryosections were cut with a sliding microtome, and 20-μm coronal brain sections of the hippocampal region were stained with Kresylviolett for cell counting. Cell number in a 500× 500 μm field of CA1 and CA3 was counted (from the left and right sides of three sections per animal) by a person blinded for animals and experimental design.

Statistical analysis

The following parameters were compared in Hsp70 transgenic mice vs wild-type mice.

Seizure stage "Mean seizure stage" within the dose–response curves was analyzed by two-way analysis of variance (ANOVA) with the two factors "genetic status" and "dose" as sources of variation.

"Cumulative incidence of any stage 5 during the PTZ kindling procedure" was analyzed by two-way ANOVA with the two factors "genetic status" and "injections" as sources of variation. "Mean seizure stage" during the PTZ kindling procedure was analyzed by two-way repeated measures ANOVA with the two factors "genetic status" and "injections" as sources of variation.

Death/Survival "Death at first stage 5" as well as "death at any stage 5" during the PTZ kindling procedure was analyzed by chi-square test and by calculation of the relative risk with 95% confidence interval (95%CI). Survival proportions were analyzed using the log-rank test and the hazard ratio with 95%CI.

Seizure stage during challenge test Differences in seizure stage were analyzed by two-way ANOVA with the two factors "genetic status" and "treatment" as sources of variation.

Histopathology Differences in cell numbers were analyzed by two-way ANOVA with the two factors "genetic status" and "treatment" as sources of variation.

For all comparisons, a p value ≤ 0.05 was considered as statistically significant.

Calculations and statistical analysis were performed using PRISM 4 (GraphPad Software).

Results

Influence of Hsp70 overexpression on the development of kindling

As stated in "Materials and methods," the previously determined doses used for PTZ kindling were different for

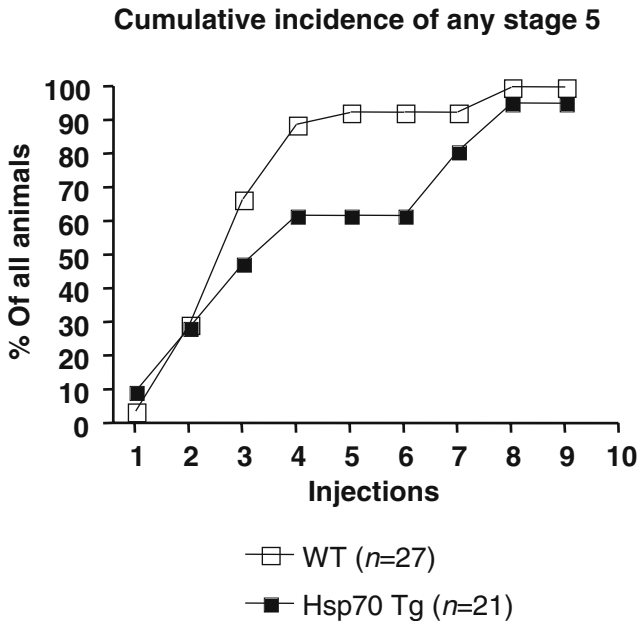

Fig. 2 Cumulative incidence of the highest seizure stage (stage 5) displayed as a percentage of all animals during the kindling procedure consisting of nine PTZ injections (inclusion of animals who survived as well as animals who died during kindling (*WT* wild-type mice [*white bars*], $n=27$; *Hsp70 Tg* Hsp70 overexpressing mice [*black bars*], $n=21$). Results of two-way ANOVA: genetic status: $p=0.0164$; injections: $p<0.0001$

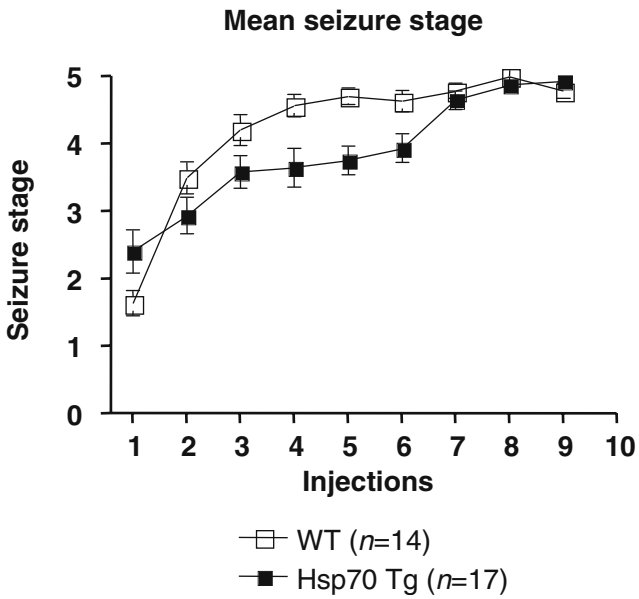

Fig. 3 Mean seizure stage (mean ± SEM) in animals who survived until the end of the kindling procedure consisting of nine PTZ injections (*WT* wild-type mice [*white bars*], $n=14$; *Hsp70 Tg* Hsp70 overexpressing mice [*black bars*], $n=17$). Results of repeated measures two-way ANOVA: interaction: $p<0.0001$; genetic status: $p=0.045$; injections: $p<0.0001$

wild-type mice (40 mg/kg) and for Hsp70 transgenic mice (35 mg/kg). Regarding the effects of the respective first kindling injection in all animals undergoing the kindling procedure, (wild-type mice n=27, Hsp70 transgenic mice n=21) the similar mean seizure stages (2.0±0.2 in wild-type mice; 2.6±0.3 in Hsp70 transgenic mice) also justified the different PTZ doses used in wild-type and Hsp70 transgenic mice.

Figure 2 displays the cumulative incidence of the highest seizure stage (stage 5) in all animals during the kindling procedure. Stage 5 was reached more rapidly in wild-type mice than in Hsp70 transgenic mice (two-way ANOVA: p=0.0164 for genetic status).

Regarding only animals who survived until the end of the kindling procedure, mean seizure stage increased more rapidly in wild-type mice than in Hsp70 transgenic mice (two-way repeated measures ANOVA: p=0.045 for genetic status). However, almost all of these animals were fully kindled after injections 8 and 9 (Fig. 3).

Influence of Hsp70 overexpression on survival during kindling

In general, only few animals died when stage 5 was reached for the first time, regardless of genetic background (Fig. 4a); in contrast, regarding death occurring at *any* stage 5 during kindling, wild-type mice died much more frequently than Hsp70 transgenic mice (chi-square: p=0.0471, relative risk 0.4154 (95%CI 0.1590–1.085; Fig. 4b).

During the whole kindling procedure, overall survival rate was decreased in wild-type mice compared to Hsp70 transgenic mice (log-rank test: p=0.0242; hazard ratio 3.129 (95%CI 1.165–8.928; Fig. 5).

How does Hsp70 overexpression affect seizure stage after a challenge dose?

As stated in "Materials and methods," the PTZ challenge dose is, in general, subeffective in naive animals (10 mg/kg below previously determined ED_{16} dose used for kindling) and was 30 mg/kg in wild-type mice and 25 mg/kg in

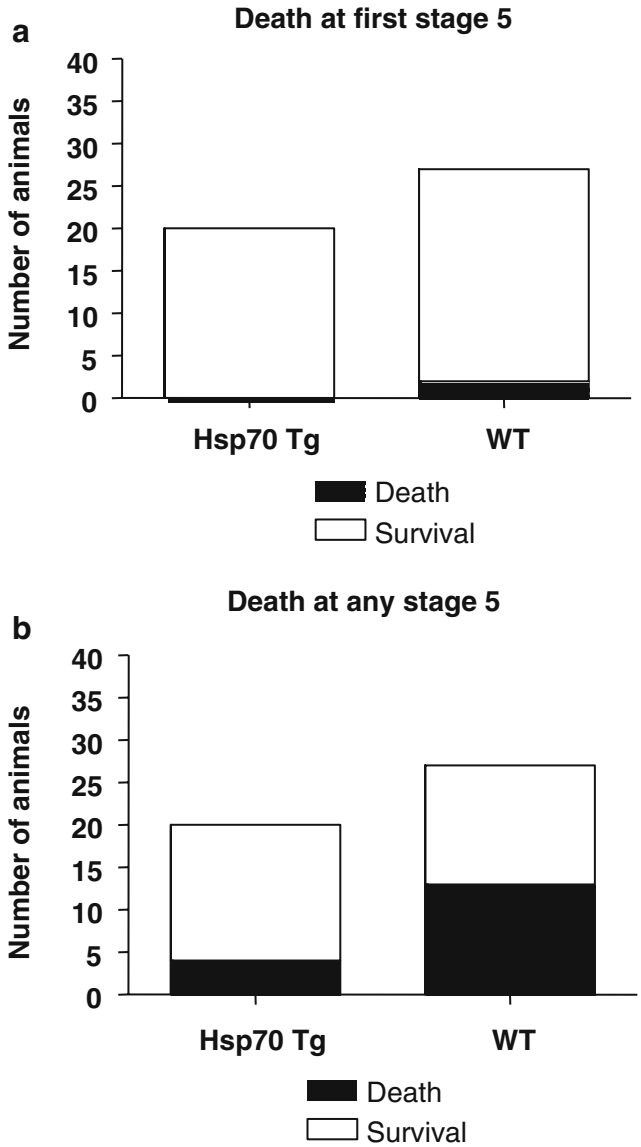

Fig. 4 **a** Death occurring when reaching stage 5 for the first time during kindling (*WT* wild-type mice, n=27; *Hsp70 Tg* Hsp70 overexpressing mice, n=21). Results of chi-square test: p n.s., relative risk 0.00. **b** Death occurring when reaching stage 5 at any time during kindling (*WT* wild-type mice, n=27; *Hsp70 Tg* Hsp70 overexpressing mice, n=21). Results of chi-square test: p=0.0471, relative risk 0.415, 95%CI 0.159–1.085)

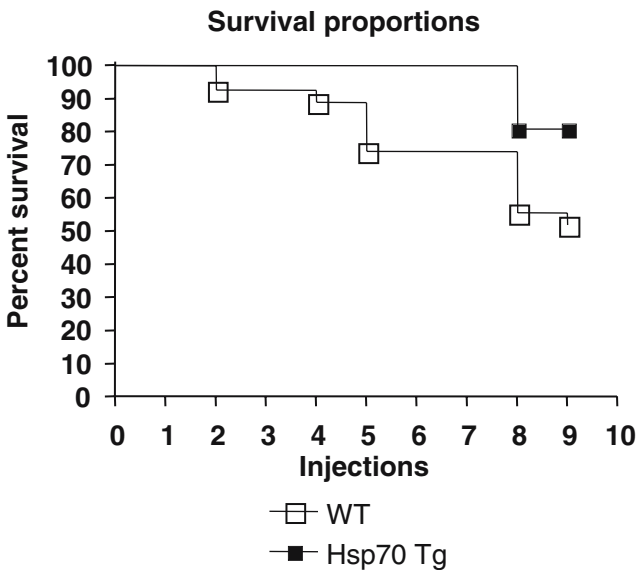

Fig. 5 Kaplan–Meier survival curves during kindling consisting of nine PTZ injections (*WT* wild-type mice [*white bars*], n=27; *Hsp70 Tg* Hsp70 overexpressing mice [*black bars*], n=21). Results of log-rank test: p=0.0242, hazard ratio 3.129 (95%CI 1.165–8.928)

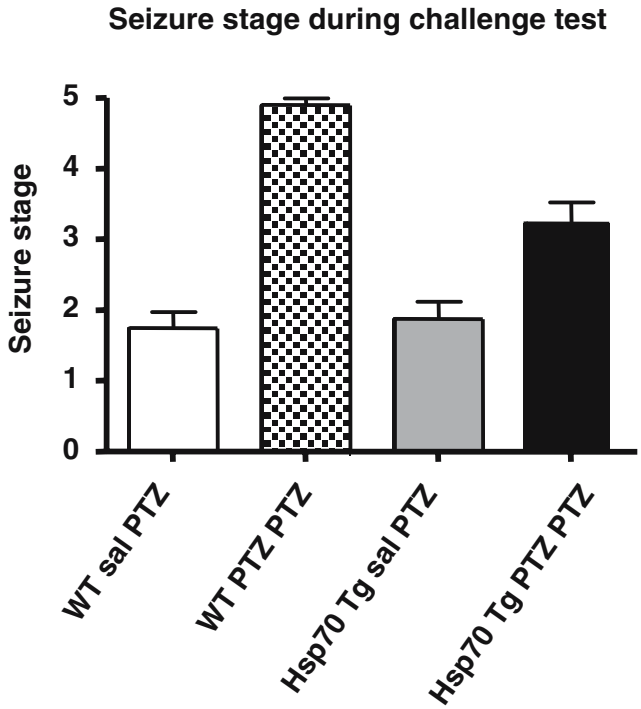

Fig. 6 Mean seizure stage (mean ± SEM) in unkindled (saline pretreated) and PTZ kindled animals after a single PTZ challenge dose. *WT sal PTZ* wild-type, saline pretreatment, PTZ challenge, n=16; *WT PTZ PTZ* wild-type, PTZ kindling, PTZ challenge, n=11; *Hsp70 Tg sal PTZ* Hsp70 transgenic, saline pretreatment, PTZ challenge, n=18; *Hsp70 Tg PTZ PTZ* Hsp70 transgenic, PTZ kindling, PTZ challenge, n=17. Statistical analysis: results of two-way ANOVA: interaction: p=0.0006; genetic status: p=0.0037; treatment: p<0.0001

Hsp70 transgenic mice. These PTZ challenge doses resulted in a similar low mean seizure stage in previously not kindled (saline pretreated) wild-type mice and Hsp70 transgenic mice (1.8±0.9 and 1.9±1.0, respectively).

In contrast, previously kindled wild-type mice responded with a mean seizure stage of 4.9±0.3, corresponding to the highest seizure stage, whereas the mean seizure stage was lower in Hsp70 transgenic mice (3.2±1.2; two-way ANOVA: p=0.0037 for genetic status, p<0.0001 for treatment; Fig. 6).

Influence of Hsp70 overexpression on histopathology

Figure 7a displays Kresylviolett staining of frontal sections of the hippocampus. Cells were counted in representative areas of CA1 and CA3 in a 500-μm square region (Fig. 7b). No significant differences were observed concerning the cell number in the CA1 region between the different treatment groups, nor between the genetic status. In the CA3 region, more cells were counted in Hsp70 transgenic mice than in wild-type mice, regardless of kindling history (two-way ANOVA: p=0.0001 for genetic status).

Discussion

The results of the present experiments clearly demonstrate that overexpression of Hsp70 exerts protective effects during PTZ kindling. The onset of the highest convulsion

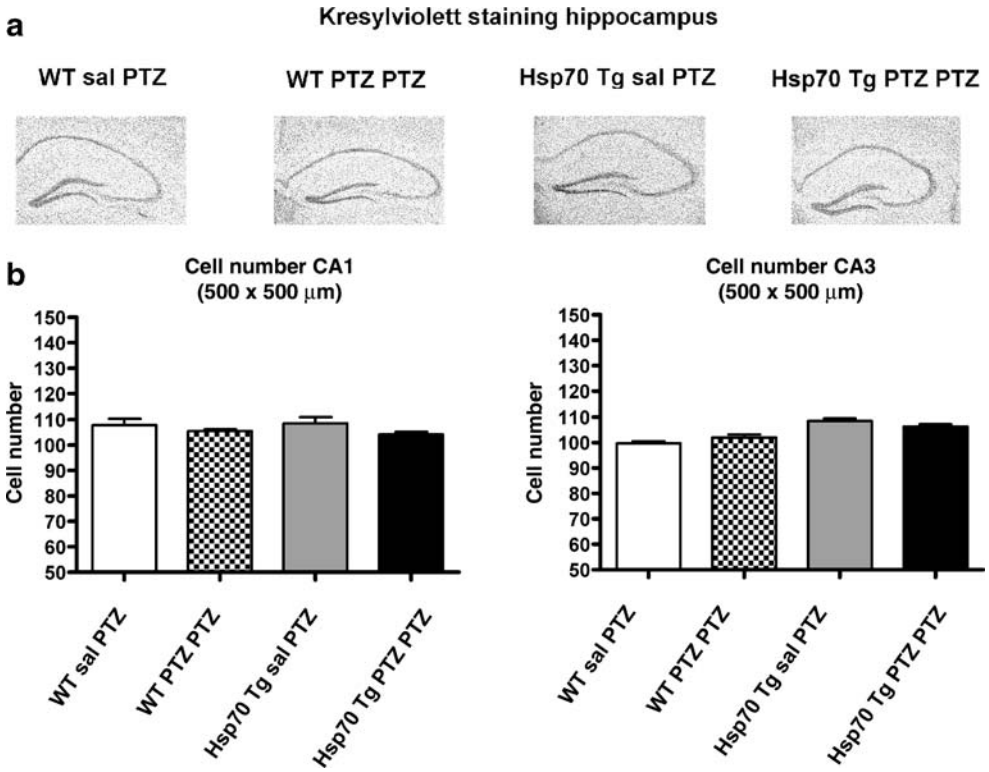

Fig. 7 **a** Representative frontal sections of the hippocampus after Kresylviolett staining. **b** Cell number counted in the hippocampal regions CA1 and CA3 (500×500 μm) in unkindled (saline pretreated) and PTZ kindled animals after a single PTZ challenge dose (n=5 per group, mean ± SEM). *WT sal PTZ* wild-type, saline pretreatment, PTZ challenge; *WT PTZ PTZ* wild-type, PTZ kindling, PTZ challenge; *Hsp 70 Tg sal PTZ* Hsp70 transgenic, saline pretreatment, PTZ challenge; *Hsp 70 Tg PTZ PTZ* Hsp70 transgenic, PTZ kindling, PTZ challenge. Statistical analysis: results of two-way ANOVA: CA1: interaction: p n.s.; genetic status: p n.s.; treatment: p: n.s.; CA3: interaction: p n.s.; genetic status: p<0.0001; treatment: p n.s.

stage was delayed in Hsp70 transgenic mice as compared to wild-type mice, and survival during kindling was improved in Hsp70 transgenic mice.

In addition, a challenge dose after termination of kindling produced less severe seizures in Hsp70 transgenic mice than in wild-type mice. Regarding the fact that kindling reflects a procedure, a process as well as a stage, overexpression of Hsp70 also seems to interfere with the developmental component of kindling.

The complex pathophysiology of PTZ kindling is still poorly understood. Several studies examining behavioral and/or histological outcome of PTZ kindling have been performed in rats. Protective effects of antagonists of δ and κ opioid receptors imply a role of the endogenous opioid system in kindling (Grecksch et al. 1999; Becker et al. 1999). Increased glutamate binding and glutamate concentrations in the hippocampus after PTZ kindling have been described (Schroeder et al. 1998). Furthermore, antagonists of the group I metabotropic glutamate receptors have been shown to positively influence seizure severity and kindling associated impairment in shuttle box learning, pointing out the impact of the glutamatergic system (Nagaraja et al. 2004). In mice, beneficial effects of the cyclooxygenase-2 inhibitor rofecoxib on seizure severity suggest the involvement of this pro-inflammatory enzyme in the pathophysiology of kindling (Dhir et al. 2006); the involvement of intraneuronal pH modulation and the potential of sodium–hydrogen exchanger system have been addressed by Ali et al. (2005) who demonstrated that amiloride, an inhibitor of the sodium–hydrogen exchange prolonged the onset of kindling and reduced the severity of seizures.

The heat shock or stress protein response is a highly conserved defense mechanism. Activation of heat shock proteins by mild hyperthermia or by pharmacological agents allows cells to withstand a subsequent metabolic insult that otherwise would be lethal, a phenomenon referred to as preconditioning. Several studies provide evidence that overexpression of Hsp70 produces protective effects similar to preconditioning. Overexpression of Hsp70 has been shown to reduce neuronal injury after transient focal ischemia, transient global ischemia, or kainic acid-induced seizures (Tsuchiya et al. 2003). In addition, neuroprotective effects of Hsp70 overexpression have been reported in a model of permanent cerebral ischemia. According to that recent MRI-based study, overall lesion size as well as tissue damage within the lesion was reduced in Hsp70 transgenic mice (van der Weerd et al. 2005).

In contrast to other epilepsy models, morphological findings after PTZ kindling have been reported to be relatively rare. It has been demonstrated that PTZ kindling in rats resulted in neuronal cell loss in distinct hippocampal regions such as CA3, which was prevented by the nootropic agent piracetam (Pohle et al. 1997). In addition, neuronal cell loss in the CA1 region has been reported to be counteracted by the κ opioid receptor antagonist enadoline (Becker et al. 1999). However, data about histological alterations after PTZ kindling in mice are scarce. Regarding kainic acid kindling in several mouse strains (kainic acid is known to induce seizures by activation of glutamate receptors), duration or severity of seizure activity was not necessarily predictive of subsequent hippocampal pyramidal cell death and/or synaptic reorganization; for example, C57BL/6J strains were resistant to cell death and synaptic reorganization despite severe behavioral seizures (McKhann et al. 2003). Therefore, it is not surprising that in our experiments PTZ kindling did not result in subsequent neuronal cell loss in CA1 or CA3 neither in wild-type mice nor in the Hsp70 transgenic mice.

In summary, the results of the present experiments clearly demonstrate that overexpression of Hsp70 exerts protective effects on seizure severity and overall survival during PTZ kindling and decreases the developmental component of kindling.

References

Ali A, Ahmad FJ, Pillai KK, Vohora D (2005) Amiloride protects against pentylenetetrazole-induced kindling in mice. Br J Pharmacol 145:880–884

Angelidis CE, Lazaridis I, Pagoulatos GN (1991) Constitutive expression of heat-shock protein 70 in mammalian cells confers thermoresistance. Eur J Biochem 199:35–39

Angelidis CE, Nova C, Lazaridis I, Kontoyiannis D, Kollias G, Pagoulator GM (1996) Overexpression of Hsp70 in transgenic mice results in increased cell thermotolerance. Transgenics 2:111–117

Beaucamp N, Harding TC, Geddes BJ, Williams J, Uney JB (1998) Overexpression of hsp70i facilitates reactivation of intracellular proteins in neurones and protects them from denaturing stress. FEBS Lett 441:215–219

Becker A, Braun H, Schröder H, Grecksch G, Höllt V (1999) Effects of enadoline on the development of pentylenetetrazol kindling, learning performance, and hippocampal morphology. Brain Res 823:191–197

Dhir A, Naidu PS, Kulkarni SK (2006) Effect of rofecoxib, a cyclooxygenase-2 inhibitor, on various biochemical parameters of brain associated with pentylenetetrazol-induced chemical kindling in mice. Fundam Clin Pharmacol 20:255–261

Fink SL, Chang LK, Ho DY (1997) Defective herpes simplex virus vectors expressing the rat brain stress-inducible heat shock protein 72 protect cultured neurons from severe heat shock. J Neurochem 68:961–969

Grecksch G, Becker A, Schroeder H, Höllt V (1999) Involvement of delta-opioid receptors in pentylenetetrazol kindling development and kindling-related processes in rats. Naunyn Schmiedebergs Arch Pharmacol 360:151–156

Hoehn B, Ringer TM, Xu L, Giffard RG, Sapolsky RM, Steinberg GK, Yenari MA (2001) Overexpression of HSP72 after induction of experimental stroke protects neurons from ischemic damage. J Cereb Blood Flow Metab 21:1303–1309

Krueger AM, Armstrong JN, Plumier J, Robertson HA, Currie RW (1999) Cell specific expression of Hsp70 in neurons and glia of

the rat hippocampus after hyperthermia and kainic acid-induced seizure activity. Brain Res Mol Brain Res 71:265–278

McKhann GM, Wenzel HJ, Robbins CA, Sosunov AA, Schwarzkroin PA (2003) Mouse strain differences in kainic acid sensitivity, seizure behavior, mortality, and hippocampal pathology. Neuroscience 122:551–561

McNamara JO (1984) Kindling: an animal model of complex partial epilepsy. Ann Neurol 16(suppl):S72–S76

Nagaraja RY, Grecksch G, Reymann KG, Schroeder H, Becker A (2004) Group I metabotropic glutamate receptors interfere in different ways with pentylenetetrazole seizures, kindling, and kindling-related learning deficits. Naunyn Schmiedebergs Arch Pharmacol 370:26–34

Olsen RW (1981) The GABA postsynaptic membrane receptor–ionophore complex. Site of action of convulsant and anticonvulsant drugs. Mol Cell Biochem 39:261–279

Ono J, Vieth RF, Walson PD (1990) Electrocorticographical observation of seizures induced by pentylenetetrazol (PTZ) injection in rats. Funct Neurology 5:345–352

Plumier JC, Ross BM, Currie RW, Angelidis CE, Kazlaris H, Kollias G, Pagoulatos GN (1995) Transgenic mice expressing the human heat shock protein 70 have improved post-ischemic myocardial recovery. J Clin Invest 95:1854–1860

Plumier JC, Krueger AM, Currie RW, Kontoyiannis D, Kollias G, Pagoulatos GN (1997) Transgenic mice expressing the human inducible Hsp70 have hippocampal neurons resistant to ischemic injury. Cell Stress Chaperones 2:162–167

Pohle W, Becker A, Grecksch G, Juhre A, Willenberg A (1997) Piracetam prevents pentylenetetrazol kindling-induced neuronal loss and learning deficits. Seizure 6:467–474

Rajdev S, Hara K, Kokubo Y, Mestril R, Dillmann W, Weinstein PR, Sharp FR (2000) Mice overexpressing rat heat shock protein 70 are protected against cerebral infarction. Ann Neurol 47:782–791

Ramanjaneyulu R, Ticku MK (1984) Interactions of pentamethylenetetrazole and tetrazole analogues with the picrotoxinin site of the benzodiazepine–GABA receptor–ionophore complex. Eur J Pharmacol 98:337–345

Rossi J 3rd (1996) Sensitization induced by kindling and kindling-related phenomena as a model for multiple chemical sensitivity. Toxicology 111:87–100

Schroeder H, Becker A, Hoellt V (1998) Sensitivity and density of glutamate receptor subtypes in the hippocampal formation are altered in pentylenetetrazole-kindled rats. Exp Brain Res 120:527–530

Tsuchiya D, Hong S, Matsumori Y, Kayama T, Swanson RA, Dillman WH, Liu J, Panter SS, Weinstein PR (2003) Overexpression of rat heat shock protein 70 reduces neuronal injury after transient focal ischemia, transient global ischemia, or kainic acid-induced seizures. Neurosurgery 53:1179–1187, discussion 1187–1188

van der Weerd L, Lythgoe MF, Badin RA, Valentim LM, Akbar MT, de Belleroche JS, Latchman DS, Gadian DG (2005). Neuroprotective effects of HSP70 overexpression after cerebral ischaemia—an MRI study. Exp Neurol 195:257–266

Yenari MA, Liu J, Zheng Z, Vexler ZS, Lee JE, Giffard RG (2005) Antiapoptotic and anti-inflammatory mechanisms of heat-shock protein protection. Ann N Y Acad Sci 1053:74–83

Zhang X, Gelowitz DL, Lai CT, Boulton AA, Yu PH (1997) Gradation of kainic acid-induced rat limbic seizures and expression of hippocampal heat shock protein-70. Eur J Neurosci 9:760–769