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Transient hyperthermia protects against subsequent seizures and epilepsy-induced cell damage in the rat

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Many mild preconditioning stress conditions, including physical and metabolic injuries, increase the resistance of neurons to subsequent more severe stresses of the same or different type. This "tolerance phenomenon" lasts one to several weeks, providing a unique opportunity to investigate endogenous neuroprotective mechanisms. The aim of this study was to find a physiological and easily applicable preconditioning stimulus able to confer protection against convulsant-induced neuronal damage and seizures. We found that moderate transient hyperthermic preconditioning markedly reduced kainic-acid-induced neuronal cell loss and attenuated susceptibility to bicuculline-induced seizures. Prevention of cell damage (~50%) was efficient both in vitro in organotypic hippocampal slice cultures and in vivo in adult rats. This protection lasted about 1 week and peaked 3 to 5 days after pretreatment. Unraveling the mechanisms of heat shock preconditioning-induced protection against epilepsy should lead to the development of new therapeutic strategies.
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Introduction

The central nervous system, like other organs, uses some outstanding strategies to protect itself against a variety of environmental injuries. Insults prone to put the brain at risk, such as stroke, head trauma, spinal cord injury, hypoxia–ischemia, and epilepsy, stimulate signals that trigger a cascade of events intended to avoid further neuronal damage. Preconditioning with a short subtoxic insult can confer "brain tolerance", characterized by the transient resistance of brain tissue to a subsequent deleterious insult. Tolerance has mostly been described for ischemic insults, but has also been reported for other types of insult, including brain trauma and epilepsy (Dirnagl et al., 2003; Rejdak et al., 2001).

These studies showed that any preconditioning insult able to confer tolerance triggers a defense response that protects vulnerable brain structures from injury. This delayed response, which starts about 1 day after preconditioning and lasts for 7 to 15 days, is mainly explained by genetic remodeling through de novo synthesis of stress proteins enhancing endogenous neuroprotection (Dirnagl et al., 2003; Leker and Neufeld, 2003). Elucidation of the mechanisms enabling such protection may provide new insights into endogenous neuroprotection and therefore favor the development of novel therapies for a range of disorders associated with neuronal death.

"Epileptic tolerance" has also been described in several models. The preconditioning stimuli described mostly consisted of a noninjurious epileptic episode: normal or rapid hippocampal kindling (Kelly and McIntyre, 1994; Penner et al., 2001), kainic acid (KA)-induced seizures (El Bahh et al., 1997, 2001; Lere et al., 2002; Najm et al., 1998), bicuculline-induced seizures (Sasahira et al., 1995), or electroshock (Kondratyev et al., 2001). Other studies described a cross-tolerance effect following hypoxic/ischemic insult (Emerson et al., 1999a,b, 2000; Plamondon et al., 1999; Pohle and Rauca, 1994; Rejdak et al., 2000) or pharmacological preconditioning (Blondeau et al., 2000) before an epileptic challenge. In all these studies, the initial stimulus conferred protection against the neuronal damage induced by chemoconvulsants (KA, pilocarpine, or bicuculline).

The aim of this study was to identify a new preconditioning stimulus with more advantageous properties than those previously described. Most of the preconditioning stimuli used until now were harmful or invasive, requiring the implantation of stimulating electrodes in the brain. Thus, we sought a relatively benign and easily applicable stimulus that could efficiently prevent the neuropathology induced by subsequent epileptic challenge. As it is well known that cells respond to injury stimuli in a highly conserved, archetypal fashion and as several experiments have shown that hyperthermia protects against subsequent hypoxic–ischemic insults (Joyeux-Faure et al., 2003; Kitagawa et al., 1991; Xu et al., 2002; Zhang et al., 2000), we explored the protective preconditioning effect of hyperthermia in epilepsy.

In addition to the potential protective effect of heat shock preconditioning on KA-induced cytotoxicity, we investigated the

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possibility that hyperthermia can exert an anticonvulsive effect. Thus, we studied the effect of transient elevation of the whole body temperature on bicuculline-induced seizures. Bicuculline, a GABA-A antagonist, induces paroxysmal discharges and clonic or tonic–clonic convulsions depending on the dose and conditions (Zouhar et al., 1989).

Here, we describe a new model of epileptic cross-tolerance using a simple and safe preconditioning stimulus. Prior hyperthermia was shown to protect the vulnerable fields of the hippocampus against subsequent damage resulting from KA both in vitro and in vivo. Furthermore, we found that this preconditioning stimulus reduced susceptibility to bicuculline. Deciphering the complex mechanisms of this endogenous neuroprotective effect should lead to new therapeutic strategies.

Materials and methods

In vitro studies

Hippocampal slice cultures

Cultures were prepared as previously described with some modifications (Stoppini et al., 1991). Hippocampi were removed from 12-day-old Wistar rats incubated in Hank's balanced salt solution (HBSS) supplemented with 1% glucose and cut into slices (350 to 400 μm thick) using a McIllwain tissue chopper. Slices were then laid on porous, transparent millicell-CM membranes (4 slices per membrane, Millipore). One milliliter of a culture medium consisting of Eagle's minimal essential medium (44%) (Gibco), HBSS (31%) (Gibco), heat-inactivated horse serum (22%) (Gibco), D-glucose (5 mg/ml) (Sigma), L-glutamine (1 mM) (Sigma), fungizone (3.75 μg/ml) (Sigma), and anti-mycoplasma (1%) (Sigma) was placed on each slice. Cultures were maintained in an incubator at 35.5°C in a humidified 5% CO_2 atmosphere. Fresh medium was added every 3 days thereafter. Prior to each experiment, slices were placed in medium containing propidium iodide (PI) and discarded if PI fluorescence was detected at this time.

Heat shock preconditioning

The cultures were placed in prewarmed medium at 45°C for 60 min, before being put into the incubator maintained at 35.5°C.

Kainic acid treatment

The vulnerability of hippocampal slices to direct KA treatment is specific and highly dependent on KA concentration. Thus, we first determined the concentration of KA necessary to generate selective neuronal damage in organotypic slices that mimics the effect of KA in in vivo experiments. This concentration was 5 μM, in agreement with other in vitro studies (Best et al., 1996; Nvue et al., 2004).

Slices (263) were used for experimentation after 2 weeks in culture. At various time points after preconditioning in heated medium (1, 2, 3, 5, or 10 days), KA was directly added in the culture medium. Control slices received KA without preconditioning at the same corresponding time points. Two days after KA administration, slices were fixed with 4% paraformaldehyde in 0.1 M phosphate buffer pH 7.4.

Assessment of neuronal cell loss using propidium iodide staining

KA-induced excitotoxicity was quantified in standardized conditions by measuring the amount of nontoxic, stable fluorescent dye propidium iodide (PI) incorporated. Loss of membrane integrity allows PI to enter dead or dying cells, where it binds to DNA and becomes highly fluorescent.

PI was added to cells (5 μg/ml; Molecular Probes) and incubated for 20 min. Cultures were then observed using a fluorescent inverted Nikon microscope equipped with a standard rhodamine filter and a digital camera (Nikon DMX 200). Images were captured and the densitometric analysis was done using a computer-assisted image analysis system (Lucia). The hippocampal subfields CA3/CA4 were identified on the transmission image. These predetermined areas expressing PI fluorescence above the background level were counted using the "density slice" function of the image software. For each experimental group, results are expressed as the percentage of cell death compared with the non-preconditioned KA control group. Statistical analysis was done with these data. In order to minimize possible differences according to each experiments, PI fluorescent staining measured in each KA groups was considered to represent 100% cell death. These calculations were corroborated by cresyl violet staining.

In vivo studies

In vivo experiments were done in 63 adult Wistar male rats weighing between 300 and 400 g.

Every efforts was made to minimize suffering, to avoid discomfort, and to reduce the number of animals used. All animal experiments were conducted in accordance with the European Committee Council Directive and approved by the local Animal Experiments Committee (authorization 006817).

Animals were housed in individual cages in controlled lighting (12:12 light/dark cycle) and temperature (22°C) conditions and allowed free access to food and water.

Heat shock preconditioning

Rats were placed in a 45°C warming box for 30 min. Their body temperature was taken with a rectal probe just before entering and just after leaving the box. After the heat stress, the rats were placed on a cold surface to stop hyperthermia rapidly. Apart from the period of heat stress, their body temperature was normal throughout the experiment.

Kainic acid injection

KA was injected via the intracerebroventricular (icv) route at three different times (3, 7, or 10 days) following the preconditioning heat shock ($n = 23$). KA-induced neuronal damage was also measured in rats that received KA without preconditioning stimulus ($n = 11$) and sham animals that received icv saline solution after hyperthermic preconditioning ($n = 5$). Infusions were performed as described previously (Nadler et al., 1980; Yin et al., 2002). Animals were anesthetized with Equithesin (4 ml/kg) and placed in a stereotaxic restraint apparatus. We slowly injected 0.6 μl of KA (1 μg/μl) or saline solution (NaCl 0.9%) into the right ventricle over a 25-min period (using a 1-μl Hamilton syringe) at the following coordinates: AP:−0.7 mm from bregma; ML: ±1.3 mm from the midline and DV: −3.6 mm from the dura matter surface according to the Paxinos and Watson atlas (1986). KA-injected rats were observed for signs of behavioral seizure activity, which typically consists of altered responsiveness to environmental stimuli, irregular tonic–clonic movements of the extremities, and loss of postural tone (Zhang et al., 1998). Rats were observed and returned to their cages after 4 to 6 h, when the most severe

behavioral seizures had usually ceased. All of the surviving animals exhibited one or more episodes of bilateral forelimb clonic or tonic activity and were used for further studies.

Tissue processing

Seven days after KA or saline injection, the animals were deeply anesthetized and perfused with 4% paraformaldehyde through the left ventricle. Brains were removed and postfixed for 2 h in 4% paraformaldehyde at 4°C. Coronal sections (40 μm) through the dorsal hippocampus were cut with a sliding vibratome and stored in 0.1 M phosphate-buffered saline (PBS), pH 7.4, supplemented with 1% sodium azide until processing for immunohistochemical and histological analyses.

Quantification of cell loss using propidium iodide staining

Cell survival in the CA3–CA4 field was quantified using the PI staining protocol of Van Bogaert (Van Bogaert et al., 2001). Slides were first soaked in Tris-buffered saline (TBS) (0.1 M; pH 7.4), supplemented with 0.3% Triton X-100. They were then incubated for 5 min in a solution of Tris–HCl (pH 7.6), deoxyribonuclease A (type IV) (500 μg/ml), and PI (50 μg/ml) dissolved in Tris–HCl, pH 7.6. Finally, they were rinsed with 0.1 M TBS pH 7.4 and mounted in an antifading medium (Mowiol, citifluor). PI fluorescence was quantified with a digital camera (Nikon DMX 200) and analyzed by LUCIA software.

Histological staining

To confirm the PI cell loss results, free-floating coronal microtome sections (40 μm) taken through the middle of the dentate gyrus in the dorsal hippocampal region (between 3.2 and 4.2 mm from the bregma) were stained with the nuclear antigen NeuN. Endogenous peroxidase was blocked by adding PBS pH 7.4 supplemented with 0.3% H_2O_2. Nonspecific sites were then saturated with a blocking solution of 10% NSS (normal horse serum) and 0.3% Triton X-100 in PBS, pH 7.4. Slices were incubated overnight at 4°C with the primary anti-NeuN antibody (mouse monoclonal; Chemicon) at a final dilution of 1/300 in PBS. Sections were washed several times in PBS, incubated for 2 h at room temperature (RT) with secondary biotinylated rabbit anti-mouse immunoglobulins (Dako) diluted 1/400 in blocking solution (5% NSS/0.1% Triton X-100 in PBS, pH 7.4), rinsed in PBS and incubated for 1 h at RT in avidin–biotin horseradish peroxidase complex (Dako) in PBS. The sections were rinsed in PBS. Peroxidase activity was detected by incubation for a few minutes with a mixture of DAB (diaminobenzidine) (10 mg/ml), nickel ammonium sulphate (60 mg/ml), and H_2O_2 (33 μl/ml) in Tris–HCl, pH 7.6. The reaction was stopped by washing the sections in Tris–HCl, pH 7.4. Sections were then rinsed in PBS, mounted on gelatin-coated slides. The slices were then counterstained in the cresyl violet to reveal dead cells.

EEG recordings and bicuculline-induced seizures

Rats were anesthetized with Equithesin and screws were implanted over the frontal and occipital cortex and cemented to a socket to allow them to be connected to a digitized EEG recorder (Biopack System Acknowledge). EEG recordings were taken at least 5 days after implantation. Bicuculline (Merk) was freshly prepared in acetone and administered at a concentration of 7.5 mg/kg. Twelve rats were submitted to heat stress (30 min at 45°C) and received an ip injection of bicuculline 3 days later. Twelve control animals were placed in the same box but maintained at ambient temperature and injected 3 days later. As above, EEG recordings and behavioral observations lasted 30 min.

Statistical analysis

Statistical analyses were performed with the Statview software. The Student's *t* test was used to compare the KA group and the HS + KA group in the in vitro study. A nonparametric test (Mann–Whitney *U* test) was used to compare the results from in vivo study. A χ^2 test and the nonparametric test (Mann–Whitney *U* test) were used for bicuculline experiments. Differences were considered significant if $P < 0.005$.

Results

In vitro experiments

Based on the results of our pilot studies, we used a concentration of 5 μM KA in the in vitro experiments to mimic the selective neuronal cell damage pattern observed in vivo following icv administration of KA.

Forty-eight hours after exposing organotypic hippocampal slices to this dose of KA, we observed selective neuronal cell death in the CA3 subfield (Fig. 1). PI exclusion measurements and cresyl violet staining showed that about 38 ± 13 % of cells died in CA3. For each set of experiments, this value was taken to represent 100%, i.e., the maximum of KA-induced cell death in the absence of preconditioning. The other hippocampal subfields were not affected by this dose. Virtually no cells were lost in CA1 or in the dentate gyrus. Therefore, these areas were not taken into account for this study.

Heat shock preconditioning exerted a time-dependent effect on KA-induced neurotoxicity in CA3 (Fig. 2). Protection was maximal 3 days after hyperthermic shock (60 min at 41°C). At this time point, KA-induced cell death was 55.3% lower in preconditioned slices than in control KA-treated slices. The protection was less pronounced, although still significant, 2 and 5 days after heat shock (respectively, 34.1 and 39.9 % reduction).

Thus, thermal preconditioning protected the vulnerable CA3 hippocampal subfield against KA cytotoxicity in slice cultures. This neuroprotection was transitory, lasting about 1 week and peaking at 3 days.

In vivo experiments

Hyperthermic preconditioning prevents KA-induced hippocampal cell death

Given our in vitro results and the length of neuroprotection conferred by other preconditioning stimuli in other models, we investigated the effect of heat shock preconditioning on the outcome of KA injection 3, 7, and 10 days later.

Animals maintained in normothermic conditions had a mean whole temperature of 37.8°C ± 0.5°C. In the preconditioning group, the body temperature 41.6°C ± 0.5°C at the end of the 30-min period of heat stress.

No apparent abnormal behavioral signs were noted in these animals. Cortical EEG recordings did not reveal any signs of epileptic manifestation in the 7 days after heat stress. In addition, increase in body temperature did not induce any neuronal cell death in the various subfields of the hippocampus studied, as revealed by PI quantification and histological observation. Finally,
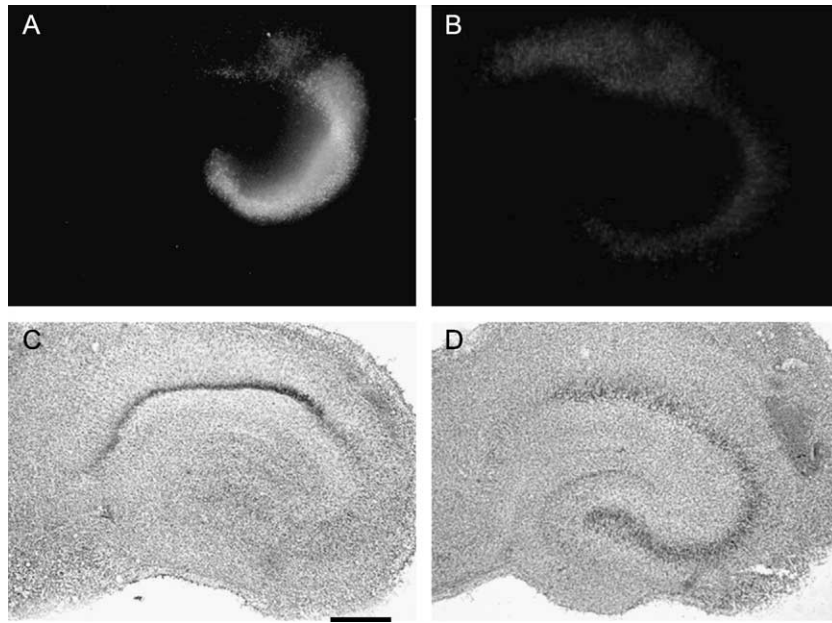
Fig. 1. In vitro experiments. Organotypic hippocampal slice cultures showing propidium iodide (PI) staining patterns (A,B) and cresyl violet staining (C,D). (A and C) Slices treated with KA alone. (B and D) Slices preconditioned at 45°C, 7 days before adding KA. Scale bar = 500 μm.

heat stress followed 1 week later by icv injection of physiological saline did not induce any cell loss in the hippocampus. Conversely, in animals injected with KA, 96% of cells in the CA3/4 subfields were lost (Fig. 3). Heat shock preconditioning protected against KA cytotoxicity in a time-dependent manner. A significant reduction in neuronal death was observed 3 and 7 days after preconditioning (41.3% and 40.1%, respectively) (Fig. 4). Ten days after preconditioning, no neuroprotective effect was observed despite a slight tendency for reduced cell death in CA3/4.

Hyperthermic preconditioning prevents bicuculline-induced seizures

EEG and behavioral manifestations induced by 7.5 mg/kg of bicuculline are variable in the rat, ranging from discrete paroxysmal discharges to severe seizures followed by death. The following events can be observed (listed in order of increasing severity). The first abnormal sign is the appearance of rhythmic

EEG episodes, consisting of isolated spikes or polyspikes of small amplitude (Fig. 5). Most of these discharges are accompanied by a sudden and brief jump reaction. Following these EEG abnormalities, clonic seizures, characterized by rhythmic head nodding and clonus of the forelimb, can be evoked. Finally, tonic–clonic seizures occur in some animals and most often result in death.

Animals exhibited very rarely full motor seizures. Only two sham-treated animals and one treated animal exhibited clonic or clonic–tonic seizures (see Table 1). Concerning the EEG analysis, the latency for the first occurrence of paroxysmal EEG manifestations was shorter in the treated animals than in the control group, but the difference was not significant. However, a significant difference between experimental and control groups was found related to the number of animals displaying manifest EEG signs. Eleven of the 12 control animals injected with bicuculline only developed epileptic signs. Half of the rat that received a heat shock 3 days before administration of bicuculline had no detectable EEG paroxysmal signs. This difference was statistically significant ($P <$
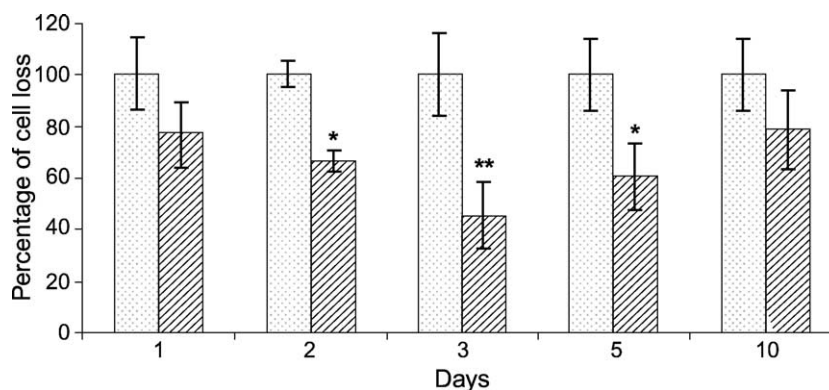

Fig. 2. Time-dependent neuroprotective effect of hyperthermic preconditioning on subsequent KA-induced cell damage in organotypic hippocampal slice cultures. For each of the five experimental groups, KA-induced cell death was considered to be 100% in not preconditioned control animals (dotted histograms). One to 10 days after preconditioning (striped histograms), cell loss was measured according to the control values. Protection was statistically significant 2, 3, and 5 days after hyperthermia. *$P < 0.05$, **$P < 0.01$. Data are expressed as mean ± SEM.
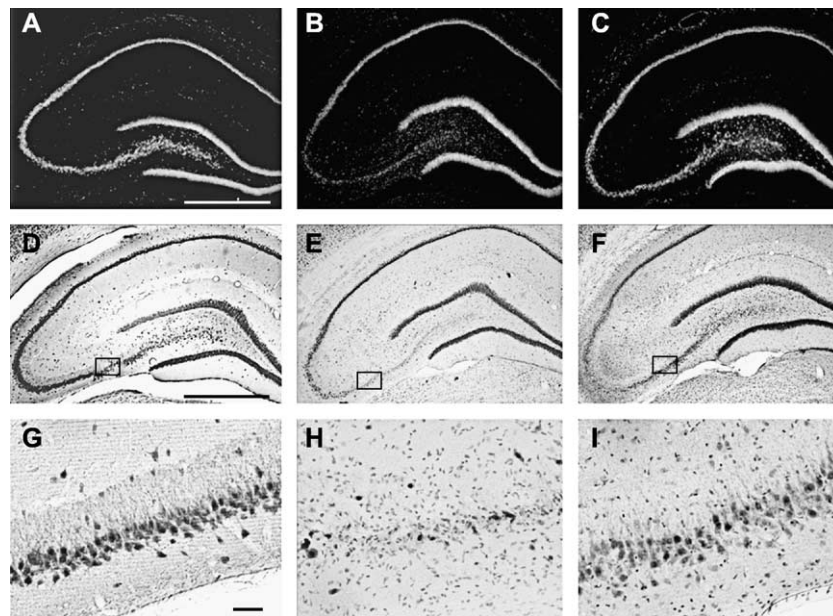
Fig. 3. In vivo experiments. Propidium iodide staining patterns (A–C) and NeuN immunolabelling of cresyl violet counterstained transverse hippocampal sections (D–I) taken from rats receiving saline injection (A, D, G), icv KA alone (B, E, H) or KA after transient heat shock stimulus (C, F, I). Scale bars in A–D = 1 mm; in E–F = 100 μm.

0.02). Thus, the reduced susceptibility to paroxysmal discharges induced by bicuculline mostly concerned the threshold for the onset of the first epileptic signs.

Discussion

The use of preconditioning stimuli to dampen the detrimental effects of ischemia has been extensively investigated. Numerous stimuli, including heat shock, have been shown to mitigate the neuropathological consequences of a subsequent ischemic insult (Chopp et al., 1989; Kitagawa et al., 1991; Xu et al., 2002). Heat shock preconditioning also protects against subsequent light-induced retinal damage (Barbe et al., 1988) and against spinal cord injury (Zhang et al., 2000). Subtoxic administration of convulsants, kindling, electroshocks, and hypoxic and ischemic insults can prevent the neuronal damage induced by KA, pilocarpine, and bicuculline (El Bahh et al., 1997, 2001; Emerson et al., 1999a,b; Kelly and McIntyre, 1994; Kondratyev et al., 2001; Lere et al., 2002; Najm et al., 1998; Penner et al., 2001; Plamondon et al., 1999; Pohle and Rauca, 1994; Rejdak et al., 2000; Sasahira et al., 1995). Until now, no groups have looked at whether prior hyperthermia can protect against epilepsy. Thus, this study provides the first evidence that transient hyperthermic pretreatment protects against status-epilepticus-induced cell death. It also appears to protect against bicuculline-induced seizures.

Our results are consistent with most of the preconditioning stress stimuli that induce an evolutionarily conserved defense system. These stimuli, including heat shock pretreatment, nearly all evoke a reactive adaptive and survival response, involving the reprogramming of gene expression intended to protect cells against heat or other insults. As seizures can develop following
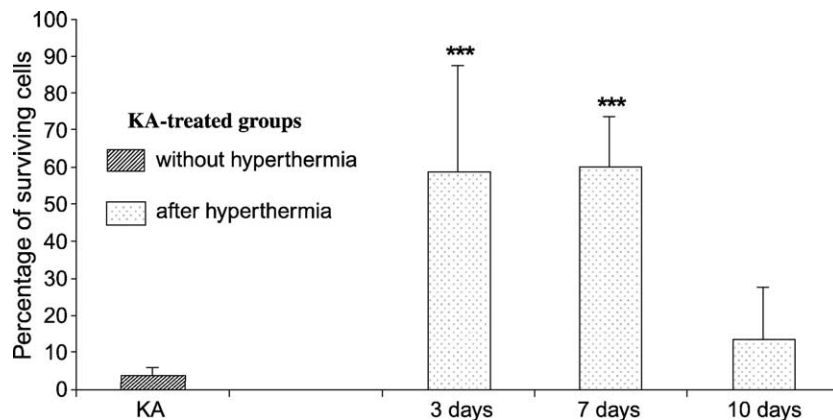

Fig. 4. Surviving cells in CA3 after heat shock preconditioning. Intraventricular microinjection of KA induced an almost complete loss of CA3 pyramidal cells (KA group). This cell loss was markedly reduced when animals were submitted to heat shock preconditioning 3, 7, or 10 days before administration of the convulsant. Differences were statistically significant between the control KA group and the hyperthermic groups at 3 and 7 days. ***$P < 0.001$. Data are expressed as mean ± SEM.

Table 1
Summarizing the paroxysmal EEG and behavioral manifestations observed following i.p. administration of bicuculline in sham-treated rats and in rats that received hyperthermic preconditioning stimulus 3 days before the convulsant drug

	Sham group, $n = 12$	Heat shock group, $n = 12$
Number of animals exhibiting epileptic EEG events	11	6*
Latency to the onset of epileptic signs (s)	84.5 ± 35.1	91.5 ± 45.6
Number of animals developing clonic or clonic–tonic seizures	2	1

* $P < 0.02$.

an excessive fever-like increase in temperature, the adaptive response following preconditioning may also be intended for preventing seizures, as observed here.

Heat shock: a physiological and safe preconditioning stimulus
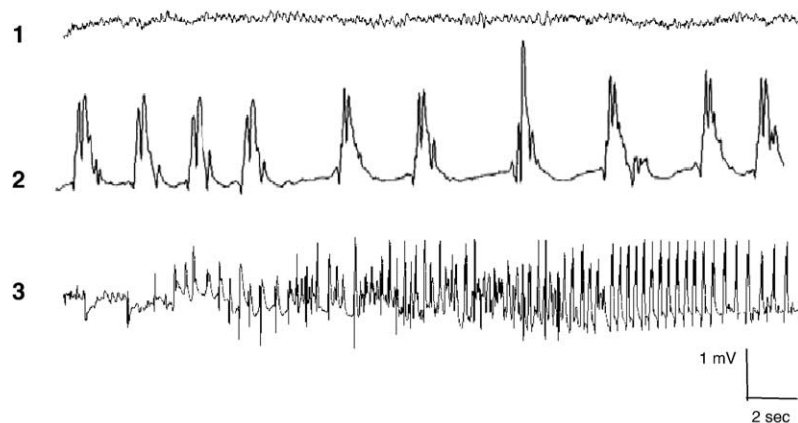
Compared with the other stresses used as preconditioning stimuli in other situations of epileptic tolerance, the model described here offers several interesting advantages. The main advantages are its physiological and safe nature as well as its suitability for investigating neuroprotection.

To avoid damaging the brain, hyperthermia must not exacerbate neuronal excitability, trigger epilepsy or induce neuronal cell loss in any vulnerable brain areas. The effects of hyperthermia generally differ according to several factors, including the degree and the duration of the temperature increase, the age of the subject, and the presence of other pathophysiological events that can occur simultaneously or delayed in time.

The likelihood of temperature changes triggering epileptic seizures differs according to brain maturity. In animals and young children, resistance to hyperthermia-induced seizures increases with maturation, with younger individuals requiring a lower degree of hyperthermia to convulse (Jiang et al., 1999; Liebregts et al., 2002). In adults, the seizure temperature thresholds are much higher. Tonic–clonic seizures can be elicited in rats by increasing the core body temperature to 42 to 44°C. We increased body temperature to 41.5°C in our in vivo experiments, thus remaining below the critical threshold for inducing detrimental side effects and within the range of physiological conditions.

Furthermore, the brain can tolerate a whole-body temperature of 42°C for 1 h without showing signs of cell death in the CNS or neurological symptoms; microscopic damage appears at higher temperatures (Khan and Brown, 2002; Leoni et al., 2000). In adults, febrile seizures have no detrimental consequences in the hippocampus and no neuropathological changes are detectable unless status epilepticus has occurred. Thus, hyperthermia appears to be a relatively safe stimulus.
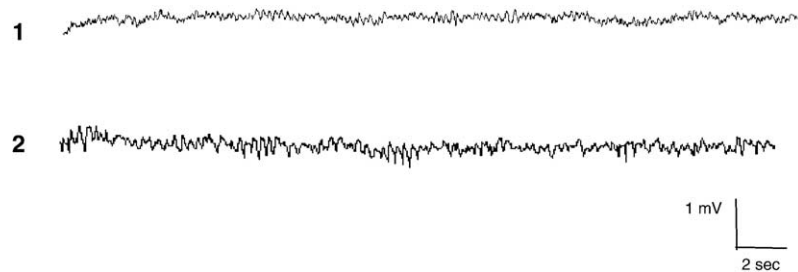

Fig. 5. Anticonvulsant effect of hyperthermic preconditioning on bicuculline-induced paroxysmal discharges. Examples of EEG recordings in control (A) and heat-treated rats (B). Normal EEG in the absence of treatment (A-1 and B-1) and after bicuculline treatment in control animals (A-2 and A-3) and in heat-treated rats (B-2).

However, when hyperthermia occurs simultaneously with pathophysiological events, such as brain trauma, inflammation, or neuropathological injuries, the secondary brain injury can be more severe. Nevertheless, as for the effect of temperature alone, elevated temperatures are required. Finally, although both clinical and experimental evidence support the hypothesis that hyperthermia increases neuronal damage when it is superimposed with different neuropathological injuries, it is still unknown whether the benefits of antipyretic therapy outweigh its risks when just temperature is elevated (Thompson et al., 2003). Consistent with our findings, the general consensus is that fever-like hyperthermia may have an adaptive value.

To make it safer, it should be possible to decrease the degree of temperature shock, while repeating the heat shock at different intervals. This may be safer and have a more sustained protective effect.

Protection against bicuculline susceptibility

To gain insight into the putative anticonvulsant effect of hyperthermic preconditioning, we analyzed the effects of applying heat shock 3 days after systemic administration of bicuculline. Bicuculline is a GABAergic inhibitor known to trigger paroxysmal activity and seizures in a dose-dependent manner (Zouhar et al., 1989). Here, we extend the previous result of Yang et al. (1996), showing that heat shock pretreatment attenuates bicuculline-induced convulsions in rats. However, only a few animals from both groups exhibited clonic or clonic–tonic seizures. Almost all animals displayed few behavioral manifestations and minor EEG signs consisting of spiking activity at different frequencies and amplitudes. In heat-treated animals, the severity of these signs was diminished and the number of animals that displayed them was significantly lower.

Our result is in line with a previous experiment showing that mice are less susceptible to exhibit PTZ-induced seizures 7 days after moderate hypoxia than when untreated (Rauca and Ruthrich, 1995).

Further studies using other experimental models of epilepsy are required to provide a more general picture of the protective effect of heat shock pretreatment against epilepsy. It would be particularly interesting to know whether this protection is effective with any type of epilepsy and whether it has an anticonvulsant effect, an antiepileptogenic effect, or both.

It would be also interesting to see whether the neuroprotective effect induced by heat shock preconditioning is related or not to the reduced bibuculline susceptibility.

Preconditioning stimuli have been repeatedly reported to generate a wide variety of metabolites and ligands that may participate in the tolerance phenomenon (Blondeau et al., 2000; Dirnagl et al., 2003; Emerson et al., 2000; Leker and Neufeld, 2003; Nvue et al., 2004; Plamondon et al., 1999; Rejdak et al., 2001; Xu et al., 2002). Although we cannot discuss each of them here, the role of GABA merits to be mentioned in the present context of delayed transient reduction of bicuculline susceptibility, because it has been reported that protection from bicuculline-induced seizures is closely correlated with a long-lasting increase in GABA content produced by prior hypoxia/ischemia preconditioning (Sieklucka et al., 1992).

A model for investigating neuroprotection

This model appears to be particularly appropriate for studies on endogenous neuroprotection and may help to unravel the under-

lying molecular mechanisms. In vitro investigations using organotypic hippocampal slice cultures can be a useful system for assessing pharmacological substances prior to or together with in vivo experiments.

It is important to highlight the time window over which the preconditioning stimulus is effective. Neuroprotective drugs and treatments are usually investigated soon after their administration. Antiepileptic compounds act in the minutes or hours after their application and their efficacy is short lived. This differs from the neuroprotection conferred by preconditioning, which is delayed with long-lived properties.

Repetitive hyperthermic pretreatment at 18-h intervals results in a stronger protective effect on ischemic insult (Kitagawa et al., 1991). Thus, it is likely that protection would be strengthened and/or lengthened by applying repeated transient heat shocks at variable intervals.

Pharmacological modulation of stress-induced brain tolerance is a promising strategy and more research is required in this field. Deciphering and exploiting the natural pathways engaged by endogenous neuroprotective systems should lead to the development of new pharmacological agents and potential clinical applications to alleviate the detrimental effects of damaging insults and to prevent epilepsy.

References

Barbe, M.F., Tytell, M., Gower, D.J., Welch, W.J., 1988. Hyperthermia protects against light damage in the rat retina. Science 241, 1817–1820.

Best, N., Sundstrom, L.E., Mitchell, J., Wheal, H.V., 1996. Pre-exposure to subtoxic levels prevents kainic acid lesiosn in organotypic hippocampal slice cultures: effects of kainic acid on parvalbumin-immunoreactive neurons and expression of heat shock protein 72 following the induction of tolerance. Eur. J. Neurosci. 8, 1209–1219.

Blondeau, N., Plamondon, H., Richelme, C., Heurteaux, C., Lazdunski, M., 2000. K(ATP) channel openers, adenosine agonists and epileptic preconditioning are stress signals inducing hippocampal neuroprotection. Neuroscience 100, 465–474.

Chopp, M., Chen, H., Ho, K.L., Dereski, M.O., Brown, E., Hetzel, F.W., Welch, K.M., 1989. Transient hyperthermia protects against subsequent forebrain ischemic cell damage in the rat. Neurology 39, 1396–1398.

Dirnagl, U., Simon, R.P., Hallenbeck, J.M., 2003. Ischemic tolerance and endogenous neuroprotection. Trends Neurosci. 26, 248–254.

El Bahh, B., Lurton, D., Sundstrom, L.E., Rougier, A., 1997. Induction of tolerance and mossy fibre neuropeptide-Y expression in the contro-lateral hippocampus following a unilateral intrahippocampal KA injection in the rat. Neurosci. Lett. 227, 135–139.

El Bahh, B., Auvergne, R., Leré, C., Brana, C., Le Gal La Salle, G., Rougier, A., 2001. Decreased epileptic susceptibility correlates with neuropeptide Y overexpression in a model of tolerance to excitotoxicity. Brain Res. 894, 209–217.

Emerson, M., Nelson, S., Samson, F., Pazdernik, T., 1999a. A global hypoxia preconditioning model: neuroprotection against seizure-induced specific gravity changes (edema) and brain damage in rats. Brain Res. Protoc. 4, 360–366.

Emerson, M.R., Nelson, S.R., Samson, F.E., Pazdernik, T.L., 1999b.

Hypoxia preconditioning attenuates brain edema associated with kainic acid-induced status epilepticus in rats. Brain Res. 825, 189–193.

Emerson, M.R., Samson, F.E., Pazdernik, T.L., 2000. Effects of hypoxia preconditioning on expression of metallothionein-1,2 and heme oxygenase-1 before and after kainic acid-induced seizures. Cell. Mol. Biol. 46, 619–626.

Jiang, W., Duong, T.M., de Lanerolle, N.C., 1999. The neuropathology of hyperthermic seizures in the rat. Epilepsia 40, 5–19.

Joyeux-Faure, M., Arnaud, C., Godin-Ribuot, D., Ribuot, C., 2003. Heat stress preconditioning and delayed myocardial protection: what is new? Cardiovasc. Res. 60, 469–477.

Kelly, M.E., McIntyre, D.C., 1994. Hippocampal kindling protects several structures from the neuronal damage resulting from kainic acid-induced status epilepticus. Brain Res. 634, 245–256.

Khan, V.R., Brown, I.R., 2002. The effect of hyperthermia on the induction of cell death in brain, testis, and thymus of the adult and developing rat. Cell Stress Chaperones 7, 73–90.

Kitagawa, K., Matsumoto, M., Tagaya, M., Kuwabara, K., Hata, R., Handa, N., Fukunaga, R., Kimura, K., Kamada, T., 1991. Hyperthermia-induced neuronal protection against ischemic injury in gerbils. J. Cereb. Blood Flow Metab. 11, 449–452.

Kondratyev, A., Sahibzada, N., Gale, K., 2001. Electroconvulsive shock exposure prevents neuronal apoptosis after kainic acid-evoked status epilepticus. Mol. Brain Res. 91, 1–13.

Leker, R.R., Neufeld, M.Y., 2003. Anti-epileptic drugs as possible neuroprotectants in cerebral ischemia. Brain Res. Rev. 42, 187–203.

Leoni, S., Brambilla, D., Risuleo, G., de Feo, G., Scarsella, G., 2000. Effect of different whole body hyperthermic sessions on the heat shock response in mice liver and brain. Mol. Cell. Biochem. 204, 41–47.

Lere, C., El Bahh, B., Le Gal La Salle, G., Rougier, A., 2002. A model of 'epileptic tolerance' for investigating neuroprotection, epileptic susceptibility and gene expression-related plastic changes. Brain Res Protoc. 9, 49–56.

Liebregts, M.T., McLachlan, R.S., Leung, L.S., 2002. Hyperthermia induces age-dependent changes in rat hippocampal excitability. Ann. Neurol. 52, 318–326.

Nadler, J.V., Perry, B.W., Gentry, C., Cotman, C.W., 1980. Degeneration of hippocampal CA3 pyramidal cells induced by intraventricular kainic acid. J. Comp. Neurol. 192, 333–359.

Najm, I.M., Hadam, J., Ckakraverty, D., Mikuni, N., Penrod, C., Sopa, C., Markarian, G., Luders, H.O., Babb, T., Baudry, M., 1998. A short episode of seizure activity protects from status epilepticus-induced neuronal damage in rat brain. Brain Res. 810, 72–75.

Nvue, R., Gorianov, V., Best, N., Sundstrom, L.E., Pringle, A.K., 2004. Time window and pharmacological characterisation of kainate-mediated preconditioning in organotypic rat hippocampal slice cultures. Neurosci. Lett. 367, 365–368.

Penner, M.R., Pinaud, R., Robertson, H.A., 2001. Rapid kindling of the hippocampus protects against neural damage resulting from status epilepticus. NeuroReport 12, 453–457.

Plamondon, H., Blondeau, N., Heurteaux, C., Lazdunski, M., 1999.

Mutually protective actions of kainic acid epileptic preconditioning and sublethal global ischemia on hippocampal neuronal death: involvement of adenosine A1 receptors and K(ATP) channels. J. Cereb. Blood Flow Met. 19, 1296–1308.

Pohle, W., Rauca, C., 1994. Hypoxia protects against the neurotoxicity of kainic acid. Brain Res. 644, 297–304.

Rauca, C., Ruthrich, H.L., 1995. Moderate hypoxia reduces pentylenetetrazol-induced seizures. Naunyn-Schmiedeberg's Arch. Pharmacol. 351, 261–267.

Rejdak, K., Rejdak, R., Stelmasiak, Z., Czuczwar, S.J., Kleinrok, Z., Sieklucka-Dziuba, M., 2000. The effect of CGP-40116 on pilocarpine evoked seizures in mice exposed to transient episode of brain ischemia. Epilepsy Res. 41, 213–222.

Rejdak, R., Rejdak, K., Sieklucka-Dziuba, M., Stelmasiak, Z., Grieb, P., 2001. Brain tolerance and preconditioning. Pol. J. Pharmacol. 53, 73–79.

Sasahira, M., Lowry, T., Simon, R.P., Greenberg, D.A., 1995. Epileptic tolerance: prior seizures protect against seizure-induced neuronal injury. Neurosci. Lett. 185, 95–98.

Sieklucka, M., Heim, C., Block, F., Sontag, K.H., 1992. Transient reduction of cerebral blood flow leads to long-lasting increase in GABA content in vulnerable structures and decreased susceptibility to bicuculline induced seizures. J. Neural Transm. 88, 87–94.

Stoppini, L., Buchs, P.A., Muller, D., 1991. A simple method for organotypic cultures of nervous tissue. J. Neurosci. Methods 37, 173–182.

Thompson, H.J., Tkacs, N.C., Saatman, K.E., Raghupathi, R., McIntosh, T.K., 2003. Hyperthermia following traumatic brain injury: a critical evaluation. Neurobiol. Dis. 12, 163–173.

Van Bogaert, P., De Tiege, X., Vanderwinden, J.M., Damhaut, P., Schiffmann, S.N., Goldman, S., 2001. Comparative study of hippocampal neuronal loss and in vivo binding of 5-HT1a receptors in the KA model of limbic epilepsy in the rat. Epilepsy Res. 47, 127–139.

Xu, H., Aibiki, M., Nagoya, J., 2002. Neuroprotective effects of hyperthermic preconditioning on infarcted volume after middle cerebral artery occlusion in rats: role of adenosine receptors. Crit. Care Med. 30, 1126–1130.

Yang, R.C., Yang, S.L., Chen, S.W., Lai, S.L., Chen, S.S., Chiang, C.S., 1996. Previous heat shock treatment attenuates bicuculline-induced convulsions in rats. Exp. Brain Res. 108, 18–22.

Yin, H., Bardgett, M.E., Csernansky, J.G., 2002. Kainic acid lesions disrupt fear-mediated memory processing. Neurobiol. Learn Mem. 77, 389–401.

Zhang, S., Chen, J., Wang, S., 1998. Spatial learning and memory induce up-regulation of nitric oxide-producing neurons in rat brain. Brain Res. 801, 101–106.

Zhang, P., Abraham, V.S., Kraft, K.R., Rabchevsky, A.G., Scheff, S.W., Swain, J.A., 2000. Hyperthermic preconditioning protects against spinal cord ischemic injury. Ann. Thorac. Surg. 70, 1490–1495.

Zouhar, A., Mares, P., Liskova-Bernaskova, K., Mudrochova, M., 1989. Motor and electrocorticographic epileptic activity induced by bicuculline in developing rats. Epilepsia 30, 501–510.