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Hypothermia Inhibits Pentylenetetrazol Kindling and Prevents Kindling-Induced Deficit in Shuttle-Box Avoidance

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RAUCA, C., W. POHLE, K. GRUNENBERG AND S. FRANZE. *Hypothermia inhibits pentylenetetrazol kindling and prevents kindling-induced deficit in shuttle-box avoidance.* PHARMACOL BIOCHEM BEHAV **65**(1) 23–30, 2000.— In this study, we evaluated the effects of hypothermic exposure on pentylenetetrazol (PTZ) kindling and the resulting deficit of shuttle-box avoidance learning in rats. Additionally, to acknowledge neuronal cell loss, we estimated the number of toluidine blue-positive cells in different brain regions after PTZ kindling and hypothermia exposure in comparison to different normothermic and hypothermic controls. To obtain hypothermic conditions over a period of up to about 3 h, 30 min after PTZ application the animals were treated with 5 mg/kg chlorpromazine (CP) and 25 min later exposed to 15°C cold water for 5 min. Under these conditions the rectal and the striatal temperature were reduced up to a maximum of 5°C. The additional injection of CP did not influence the development of PTZ kindling. Animals treated with PTZ/CP and exposed to hypothermia did not reach the criterion for kindling. Furthermore, this group of animals did not demonstrate any learning deficit. Forty-eight hours after the last kindling application the number of toluidine blue-stained cells was decreased in the investigated brain regions (hippocampal CA1 and CA3 sector, hilus, and cingular cortex) of kindled rats. Hypothermia protected from cell damage in the hippocampal CA3 sector and in the hilus. Results suggest that the inhibiting effect of hypothermia on the development of kindling and the following learning deficit possibly resulted from the suppression of cell damage in distinct brain structures on PTZ-kindled rats. © 1999 Elsevier Science Inc.

Hypothermia Cerebral protection Pentylenetetrazol (PTZ) Kindling Shuttle-box avoidance Cell damage Rats

THE kindling phenomenon is generally accepted as an experimental model of epilepsy and epileptogenesis. Kindling is characterized by an increased susceptibility to seizures after repeated application of initially subconvulsive electrical (15) or chemical (25) stimuli. Kindling has been extensively investigated as a phenomenon of epilepsy and neuronal plasticity, but its cellular mechanisms are still uncertain.

An impairment of cognitive processes was described in kindled animals (23,33) after chemically induced kindling in rats using repeated application of subconvulsive doses of pentylenetetrazol (PTZ). The performance of two-way active avoidance learning was significantly diminished (5,16). To find out the reason for the learning deficit, histopathological investigations must be performed in different brain regions of PTZ-kindled animals. Pohle et al. (1997) (31) found a neuronal loss in distinct hippocampal structures following PTZ kindling. Meldrum (1993) (26) hypothesized that there exists a positive correlation between the appearance of status epilepticus and the neuronal cell damage in the vulnerable brain structures. During acute seizures an excessive release of excitatory neurotransmitters could be observed, which plays a causative role in the development of neuronal cell death (2). A significant enhancement of glutamate and aspartate concentrations could be shown (19) after amygdala kindling. Entorhinal kindling also increased the release of glutamate in hippocampus of rats (18).

A decrease in brain temperature by at least 5°C completely inhibits the release of glutamate during ischemia (4,13,14,34).

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Hypothermia of animals also markedly attenuated neuronal damage in vulnerable brain regions such as the hippocampus (9–11). The protection is mediated by the cerebral metabolic depressant effects of decreased body temperature, which has been attributed mainly to a decrease in brain energy demands, energy failure during the ischemic insult, or both.

The beneficial effect of hypothermia has been demonstrated in neocortex and globus pallidus after flurothyl-induced epileptic brain damage (24) and in hippocampal structures following kainic acid-induced cell death (22).

Current literature supplied few references for the effects of a decrease in the body temperature on development of seizures and kindling, respectively. Therefore, the question arose as to whether hypothermia resulted in a suppression of PTZ-induced kindling, in a reduction of the kindling-induced learning impairments in a shuttle-box task, and in the protection against kindling-induced cell loss. Moreover, it is necessary to clarify whether the rectal temperature corresponds to the brain temperature under hypothermic conditions.

METHOD

Animals

The experiments were performed using 8-week-old male Wistar rats [Mol: Wist (Shoe), Moellegaard Breeding Centre GmbH, Germany]. The animals were kept under controlled laboratory conditions under a lighting regime of light:dark 12:12 (lights on at 0600 h), temperature 20 ± 2°C, and air humidity 55–60%. They had free access to commercial rat pellets (Altromin 1326) and tap water. The rats were housed in groups of five animals per cage. For all procedures followed, ethical approval was obtained prior to the experiments according to the requirements of the National Act on the use of Experimental Animals (Germany).

Experimental Groups

To study the influence of hypothermia on the development of PTZ kindling and kindling-induced consecutive processes the following groups were needed:

Normothermic rats (groups 1 to 4). The rats of group 1 were intraperitoneally (IP) injected with 5 ml physiological NaCl solution/kg body weight and received IP NaCl a second time 30 min later; the animals of group 2 received IP 37.5 mg/kg PTZ (Serva, Heidelberg, Germany) 30 min before IP injection of NaCl; group 3 was treated IP with NaCl and 5 mg/kg chlorpromazine (CP) (Arzneimittelwerk Rodleben, Germany), which were administered after 30 min; the rats of group 4 received 37.5 mg/kg PTZ and 5 mg/kg CP 30 min later.

Hypothermic rats (groups 5 to 7) The animals of group 5 were treated as described for group 1 and exposed to hypothermic conditions 25 min after the second NaCl administration. To this end, the rats were placed into 15°C cold-water bath for 5 min. This procedure was followed by drying with a rough towel. Group 6 was treated as described for group 2, but exposed to hypothermic conditions 25 min after NaCl administration; the animals of group 7 were injected with PTZ and CP as described for group 4, and exposed to hypothermia 25 min after CP application.

Measurement of Rectal and Striatal Temperature

In a separate experiment, 7-week-old rats were anesthetized by IP application of 20 mg/kg ethonidate and a hole was drilled through the individual animal's skull and the dura was pierced with a needle for the measurement of striatal temperature (coordinates: 1.25 mm posterior and 2.6 mm lateral from bregma) carried out 1 week later. On freely moving rats a special probe (Physitemp Instruments Inc., USA) was inserted into the drill hole of skull at a depth of 4.5 mm to determine the striatal temperature. Subsequently, the rectal temperature was controlled by placing a special probe 7 cm deep into the rectum. The time courses of striatal and rectal temperature were registered on 10 rats of each of the investigated experimental groups immediately before, after 30, 50, and 60 min, and then every 30 min over a period of 240 min after the first application (NaCl or PTZ).

Pentylenetetrazol Kindling

The PTZ kindling experiment was performed using the normothermic groups 2, 4, and the hypothermic groups 6 and 7. The convulsive behavior was observed immediately after PTZ application. The resultant seizures were classified according to a modified RACINE scale (32) as follows: stage 0: no response; stage 1: ear and facial twitching; stage 2: convulsive waves through the body; stage 3: myoclonic jerks and/or rearing; stage 4: clonic–tonic seizures; stage 5: generalized clonic–tonic seizures, loss of postural control.

In all groups this procedure was repeated every other day over a period of 4 weeks as described by Becker et al. (1992) (2). The animals were considered to be "kindled" after having received 13 PTZ applications and after reaching at least three consecutive stage 4 or 5 seizures.

Shuttle-Box Avoidance

Forty-eight hours after the last PTZ application to induce kindling the animals were tested for learning behavior in two-way active avoidance in the shuttle-box. The automatic shuttle-box was divided into two compartments (25 × 25 × 60 cm) separated by a 5-cm hurdle. The conditioned stimuli were light (40-W bulbs located on the central ceiling of each compartment) and a sound produced by a buzzer. The unconditioned stimulus was an electric footshock of a maximum of 1 mA delivered through stainless steel rods covering the floor. The conditioned stimuli-unconditioned stimulus interval was 4 s. One trial was limited to 20 s, and was repeated on 4 consecutive days. Sessions were always started at the same time of day (0800 h). Prior to the first session, the rats were allowed to explore the box for 5 min, and on the following day 1 min was provided.

If following conditioned stimulus the rat changed the compartment within the first 4 s, then a conditioned reaction and avoidance, respectively, was completed. The number of conditioned reactions (reaction time < 4 s) was recorded for evaluation of the learning performance.

Histological Investigations

For histological experiments seven groups of five rats were treated as described. Forty-eight hours after the last subchronic injection all rats were anesthetized with hexobarbital (100 mg/kg) and sacrified by transcardial perfusion with 50 ml phosphate-buffered saline (PBS) solution followed by 200 ml 4% paraformaldehyde diluted with PBS. The brains were removed, postfixed over night in the same fixative, dehydrated, and embedded in paraffin. Horizontal sections (10 μm) were cut in the plane of the nucleus habenulae and stained with toluidine blue. The different types of neurons were counted after coverdipping with neutral balsam.

We used 10 different sections of each animal for counting the neurons in a field of 0.06 mm^2 of each section. The number of neurons was counted by use of a counting net. Pyramidal cells of the hippocampal sectors CA1, CA3, neurons of the hilus, and the cingular cortex were studied. The means of the number of cells of the 10 investigated sections is the value for one animal. Five animals per experimental group were investigated. All countings were made in blind examination.

Statistical Evaluations

Data are presented as mean ± standard error of the mean (SEM) for each treatment group. Two-factor repeated-measures analysis of variance (ANOVA) was used for statistical evaluation of the time course of striatal and rectal temperature, of seizure development and of learning behavior in the shuttle-box, to compare all groups at each time point of the test procedure. After confirmation of significant main effects, differences among individual means were analyzed using modified LSD (Tukey) post hoc tests. The basis of statistical decision was a significance level of 0.05. The statistical analysis of histological data was performed using one-way ANOVA followed by a modified LSD (Tukey) test with a significance level of 0.05 for post hoc comparisons. The statistical calculations were carried out by means of SPSS software.

RESULTS

Striatal and Rectal Temperature

Figure 1 shows the time course of striatal temperature on the normothermic NaCl/NaCl-, pentylenetetrazol (PTZ)/NaCl-, NaCl/chlorpromazine (CP)-, and PTZ/CP-treated group and on the hypothermic animal groups administered with NaCl/NaCl, PTZ/NaCl, or PTZ/CP. In normothermic animals, the PTZ/CP treatment reduced significantly the striatal temperature by about 1°C in comparison to the other three normothermic control groups at 120 to 240 min after the PTZ injection [NaCl/NaCl: $F(1, 18) = 17.17$, $p = 0.001$; PTZ/NaCl: $F(1, 18) = 9.44$, $p = 0.007$; NaCl/CP: $F(1, 18) = 42.01$, $p = 0.000$]. Hypothermic conditions could not significantly decrease the striatal temperature in the NaCl/NaCl group [compared to the normothermic NaCl/NaCl-treated group: $F(1, 18) = 0.07$, $p = 0.796$]. But following cold-water exposure, a reduction of striatal temperature could be observed in the PTZ/NaCl-treated group only 10 min after the hypothermia exposure [compared to the normothermic PTZ/NaCl-group: $F(1, 18) = 4.43$, $p = 0.050$].

In the PTZ/CP group, hypothermic conditions considerably decreased the brain temperature compared to all normothermic groups [NaCl/NaCl: $F(1, 18) = 70.53$, $p = 0.000$; PTZ/NaCl: $F(1, 18) = 60.54$, $p = 0.000$; NaCl/CP: $F(1, 18) = 98.43$, $p = 0.000$; PTZ/CP: $F(1, 18) = 26.72$, $p = 0.000$] and to the hypothermic NaCl/NaCl, $F(1, 18) = 100.44$, $p = 0.000$, and PTZ/NaCl group, $F(1, 18) = 67.31$, $p = 0.000$, over a period of 3 h.

Figure 2 demonstrates that the rectal temperature was reduced in normothermic rats only by the combined treatment with PTZ/CP 180 and 210 min after the PTZ injection [compared to the NaCl/NaCl group: $F(1, 18) = 13.85$, $p = 0.002$; compared to the PTZ/NaCl group: $F(1, 18) = 7.99$, $p = 0.011$; compared to the NaCl/CP group: $F(1, 18) = 12.77$, $p = 0.002$]. Following hypothermia exposure, the rectal temperature of the NaCl/NaCl group was significantly reduced immediately after that, $F(1, 18) = 10.00$, $p = 0.005$. In contrast to this, a significant effect of hypothermia could be observed in the PTZ/NaCl-injected group up to 70 min after cold water exposure,

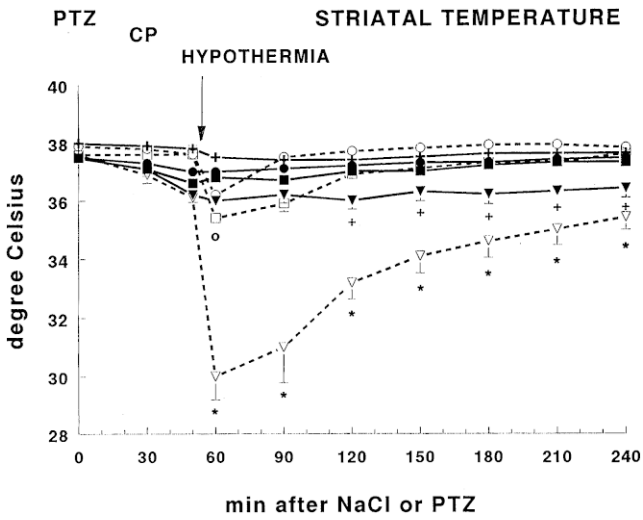

FIG. 1. Time course of striatal temperature on normothermic rats treated with NaCl/NaCl (—●—), pentylenetetrazol (PTZ, 37.5 mg/kg acutely applied)/NaCl (—■—), NaCl/chlorpromazine (CP, 5 mg/kg acutely applied) (—+—), or PTZ/CP (—▼—), and on hypothermic rats treated with NaCl/NaCl-H (—○—), PTZ/NaCl-H (—□—), or PTZ/CP-H (—▽—). X-axis, time after the first injection (NaCl or PTZ) in minutes, Y-axis, striatal temperature in degrees Celsius. Data points are means ± SEM of 10 animals per groups. Two-factor repeated-measures analysis of variance (ANOVA) was performed followed by Tukey's post hoc test: * $p < 0.05$, values of the hypothermic PTZ/CP group are significantly different from the corresponding values of the normothermic PTZ/CP group, ○ $p < 0.05$, value of the hypothermic PTZ/NaCl group is significantly different from the value of the corresponding normothermic PTZ/NaCl group at the same time point; + $p < 0.05$, values of the normothermic PTZ/CP group are significantly different from values of the normothermic NaCl/NaCl, PTZ/NaCl, and NaCl/CP groups.

$F(1, 18) = 37.18$, $p = 0.000$. The rectal temperature was significantly reduced in PTZ/CP-treated animals in comparison to all normothermic groups [compared to the NaCl/NaCl group: $F(1, 18) = 64.72$, $p = 0.000$; the PTZ/NaCl group: $F(1, 18) = 57.96$, $p = 0.000$; the NaCl/CP group: $F(1, 18) = 63.89$, $p = 0.000$; and the PTZ/CP group: $F(1, 18) = 31.06$, $p = 0.000$] and to the NaCl/NaCl- and PTZ/NaCl-treated hypothermic rats, $F(1, 18) = 73.04$, $p = 0.000$; $F(1, 18) = 33.42$, $p = 0.000$, for 3 h following hypothermia exposure. A positive correlation could be found between the striatal and the rectal temperature of all investigated rats (the Spearman correlation coefficient for all seven groups ($n = 70$) is 0.8445; $p = 0.000$). Table 1 demonstrates the Spearman correlation coefficients for all studied points in time. The correlation coefficients indicate that changes in rectal temperature run in parallel with changes in striatal temperature. The best agreement could be found 40 min after cold-water exposure and 90 min following the first injection (NaCl or PTZ), respectively.

Pentylenetetrazol Kindling

In Figure 3, the development of PTZ-induced kindling is demonstrated in normothermic groups subchronically treated with PTZ/NaCl and PTZ/CP. From the seventh up to the last PTZ-kindling injection, the rats of the PTZ/CP group responded mainly with seizure stage 4 or 5, indicating a "fully" kindled state. The group of subchronically PTZ/NaCl-treated

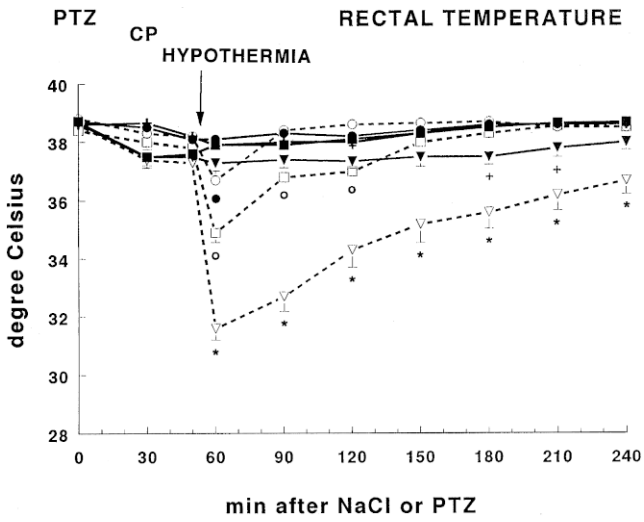

FIG. 2. Time course of rectal temperature on normothermic rats treated with NaCl/NaCl (—●—), pentylenetetrazol (PTZ, 37.5 mg/kg acutely applied)/NaCl (—■—), NaCl/chlorpromazine (CP, 5 mg/kg acutely applied) (—+—), or PTZ/CP (—▼—), and on hypothermic rats treated with NaCl/NaCl-H (—○—), PTZ/NaCl-H (—□—), or PTZ/CP-H (—▽—). X-axis, time after the first injection (NaCl or PTZ) in minutes, Y-axis, striatal temperature in degrees Celsius. Data points are means ± SEM of 10 animals per group. Two-factor repeated-measures analysis of variance (ANOVA) was performed followed by Tukey's post hoc test: * $p < 0.05$, values of the hypothermic PTZ/CP group are significantly different from the corresponding values of the normothermic PTZ/CP group; ○ $p < 0.05$, values of the hypothermic PTZ/NaCl group are significantly different from the values of the corresponding normothermic PTZ/NaCL group; ○ $p < 0.05$, value of the hypothermic NaCl/NaCl group is significantly different from the value of the corresponding normothermic NaCl/NaCl group at the same time point; + $p < 0.05$, values of the normothermic PTZ/CP group are significantly different from values of the normothermic NaCl/NaCl, PTZ/NaCl, and NaCl/CP groups.

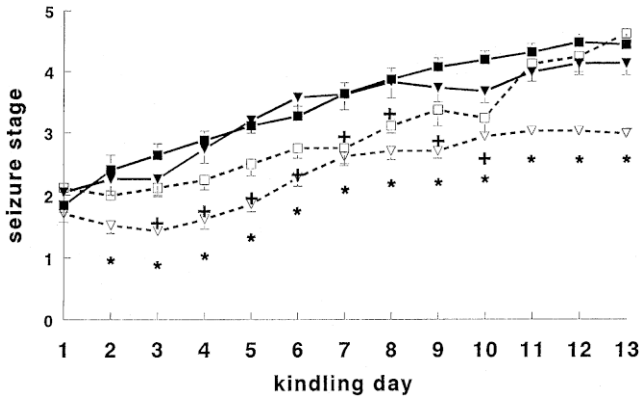

FIG. 3. Development of the susceptibility of normothermic rats to pentylenetetrazol (PTZ, 37.5 mg/kg)/NaCl (—■—) or PTZ/chlorpromazine (CP, 5 mg/kg) (—▼—), and hypothermic rats treated with PTZ/NaCl-H (—□—) or PTZ/CP-H (—▽—), in the course of kindling. Values are means ± SEM of 10 animals per group. Two-factor repeated-measures analysis of variance (ANOVA) was performed followed by Tukey's post hoc test: * $p < 0.05$, values of the hypothermic PTZ/CP group are significantly different from the corresponding values of the normothermic PTZ/CP group; + $p < 0.05$, values of the hypothermic PTZ/NaCl group are significantly different from corresponding values of the normothermic PTZ/NaCl group.

and hypothermia exposed animals attained seizure stage 3 only up to the 10th kindling day, then a fully kindling state could be observed. Up to the 10th PTZ injection significant differences could be observed between the hypothermic and normothermic treatment groups [between PTZ/NaCl groups: $F(1, 18) = 13.65$, $p = 0.002$; between PTZ/CP groups: $F(1, 18) = 17.19$, $p = 0.001$]. In contrast to this, subchronically PTZ/CP-injected and hypothermia exposed rats were not kindled [compared to normothermic PTZ/NaCl group: $F(1, 18) = 35.83$, $p = 0.000$; to normothermic PTZ/CP group: $F(1, 18) = 35.51$, $p = 0.000$].

TABLE 1

THE SPEARMAN CORRELATION COEFFICIENTS (CORR. COEF.) FOR THE DIFFERENT POINTS IN TIME ARE AS FOLLOWS

Time (min)	Corr. Coef.	*n*	*p*
0	0.6192	70	0.000
30	0.8004	70	0.000
50	0.4825	70	0.000
60	0.7993	70	0.000
90	0.8794	70	0.000
120	0.8398	70	0.000
150	0.8270	70	0.000
180	0.7081	70	0.000
210	0.6531	70	0.000
240	0.5837	70	0.000

Shuttle-Box Avoidance

In the shuttle-box experiment both the kindled groups (PTZ/NaCl- and PTZ/CP-treated animals) and the NaCl/CP-treated group differed significantly in their learning performance from the normothermic NaCl/NaCl control group, which acquired the learning task rapidly [compared to PTZ/NaCl group: $F(1, 18) = 9.14$, $p = 0.007$; to the PTZ/CP group: $F(1, 18) = 11.95$, $p = 0.003$]. Likewise, the repeated treatment with CP leads to a significant impairment of learning performance [compared to normothermic NaCl/NaCl group: $F(1, 18) = 5.17$, $p = 0.036$]. The hypothermia exposure did not significantly change the learning performance on the NaCl/NaCl group, $F(1, 18) = 1.47$, $p = 0.241$, just as in the PTZ/NaCl-administered group, $F(1, 18) = 3.80$, $p = 0.067$. In contrast, in PTZ/CP-treated rats the hypothermia and/or the fact that this group was not kindled prevented the diminished acquisition of the shuttle-box learning task [compared to normothermic PTZ/CP group: $F(1, 18) = 7.14$, $p = 0.016$]. The results are demonstrated in Figure 4.

Toluidine Blue-Positive Cells

Hypothermia exposure of rats treated subchronically with NaCl/NaCl did not influence the number of toluidine blue-positive cells in the investigated brain regions. The toluidine blue staining demonstrated a significant loss of cells in all structures studied (CA1 and CA3 sector of hippocampus, hilus, and cingular cortex) in PTZ/NaCl-kindled animals. In this PTZ/NaCl group the cold-water exposure induced a hypo-

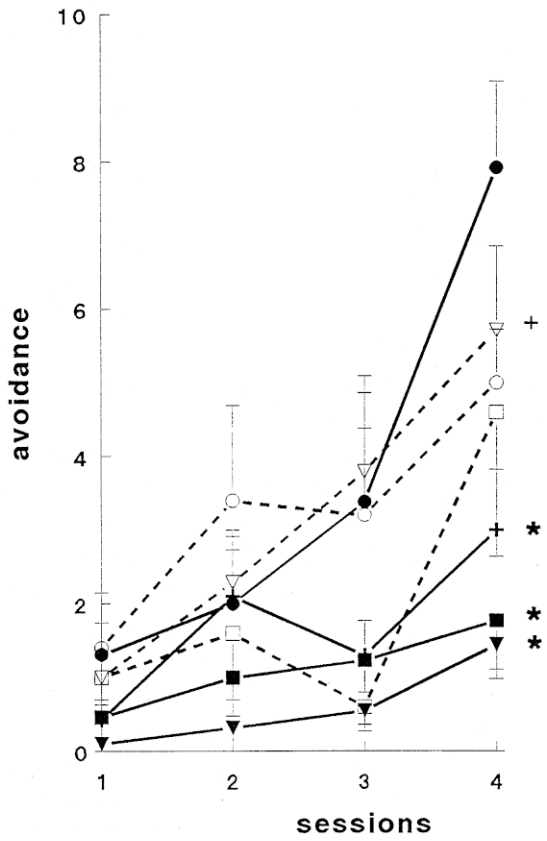

FIG. 4. Shuttle-box learning performance expressed as avoidance in normothermic NaCl/NaCl-injected controls (—●—) in comparison to normothermic pentylenetetrazol (PTZ, 37.5 mg/kg)/NaCl (—■—)-, NaCl/chlorpromazine (CP, 5 mg/kg) (—+—)-, and PTZ/CP (—▼—)-treated rats, and to the hypothermic NaCl/NaCl-H (—○—), PTZ/NaCl (—□—), and PTZ/CP (—▽—) groups. All substances were subchronically applied (13 times over a period of 4 weeks). Values are means ± SEM of 9 to 10 animals per group. Two-factor repeated-measures analysis of variance (ANOVA) was performed followed by Tukey's post hoc test: * $p < 0.05$, values of the normothermic PTZ/NaCl, NaCl/CP, or PTZ/CP groups are significantly different from the normothermic NaCl/NaCl group; + $p < 0.05$, values of the hypothermic PTZ/CP-treated rats are significantly different from the values of the normothermic PTZ/CP-injected rats.

thermia of a duration of about 1 h, which was not able to prevent the cell loss after PTZ kindling. The subchronical application of NaCl/CP- and PTZ/CP damaged the cells in the CA1 and CA3 sectors and in the hilus, but not in the cingular cortex. Following PTZ/Cp pretreatment the hypothermia continued at least for 3 h and protected against cell loss in the hippocampal CA3 sector and in the hilus, but not against cell damage in the CA1 sector. Surprisingly, CP prevented the cell damage after PTZ kindling in the cingular cortex. The histological results are showed in Figure 5.

DISCUSSION

To study the influence of hypothermia on pentylenetetrazol (PTZ) kindling and kindling-induced functional and histological changes, different groups of rats were subchronically treated with NaCl/NaCl, PTZ/NaCl, or PTZ/CP, and exposed to 15°C cold water for 5 min. Acute experiments indicate the brain temperature measured as striatal temperature was not significantly influenced in the NaCl/NaCl-treated group, but was decreased in PTZ/NaCl-injected animals 10 min after cold-water exposure. This effect disappeared 30 min later. In our experiments a reduction in striatal temperature lasting 3 h could only be induced by application of chlorpromazine (CP) switching off the central regulation of body temperature. It could be demonstrated that under hypothermic conditions the rectal temperature was decreased in the same way as the striatal temperature (see Fig. 2).

Calculating the Spearman correlation coefficient, a significant correlation between striatal and rectal temperature could be found at all investigated points in time (see Table 1). Fifty minutes after PTZ and 20 min after CP treatment, respectively, the Spearman correlation coefficient was reduced in comparison to the previous and following points in time. It may be that the preceding application of CP influenced the striatal and rectal temperature in a quantitatively different manner. The subcutaneous fat protects the body better for the loss of body heat at room temperature, whereas the brain chills through more quickly. This may be the reason for the fact that the hypothermia exposure reduced the striatal temperature to a greater extent compared with the rectal temperature. In hypothermic rats, the Spearman correlation coefficient was most enhanced at 90 min and decreased slightly up to 240 min after the beginning of the experiment. The examination of the Spearman correlation coefficient allows the conclusion that the rectal temperature is a good indicator to register differences in brain temperature.

As demonstrated in Fig. 3, at the start of the kindling experiment significant differences cannot be established between seizure scores of the different groups investigated. But over the time course of the experiment it emerged that both normothermic PTZ-treated groups were kindled. The subchronically PTZ/NaCl injected experimental group exposed to hypothermic conditions showed differences in PTZ susceptibility compared to the normothermic PTZ/NaCl-treated control up to the 10th kindling day. From 11th to 13th kindling day the animals reached seizure scores comparable with the normothermic PTZ kindling groups. Nevertheless, subchronically PTZ/CP injected animals exposed to hypothermic conditions did not attain a kindling state in comparison to normothermic PTZ/CP- or PTZ/NaCl-treated rats. From these results it may be concluded that the duration of hypothermia is connected with the protection from seizures following PTZ kindling injection.

The shuttle-box avoidance learning was tested 48 h after the end of the kindling experiment. As showed in Fig. 4, hypothermia did not influence the effect of NaCl/NaCl treatment on shuttle-box learning behavior. In the PTZ-kindled normothermic groups (PTZ/NaCl or PTZ/CP) a deficit in learning behavior could be found as described by Becker et al. (5,6,16). But the same shuttle-box avoidance learning impairment could also be observed in the group injected with NaCl/CP subchronically. Short-lasting hypothermia in the PTZ/NaCl group only delayed the development of PTZ kindling and did not significantly inhibit the learning deficit, whereas hypothermia lasting 3 h was able to prevent the kindling and to protect from impaired learning in two-way avoidance in the shuttle-box, as demonstrated following PTZ/CP treatment.

The histopathological investigations showed that the hypothermia exposure of the NaCl/NaCl-treated group has no influence regarding the number of toluidine blue-positive cells in the studied brain regions. After PTZ kindling in the normothermic PTZ/NaCl- and PTZ/CP-injected groups and after

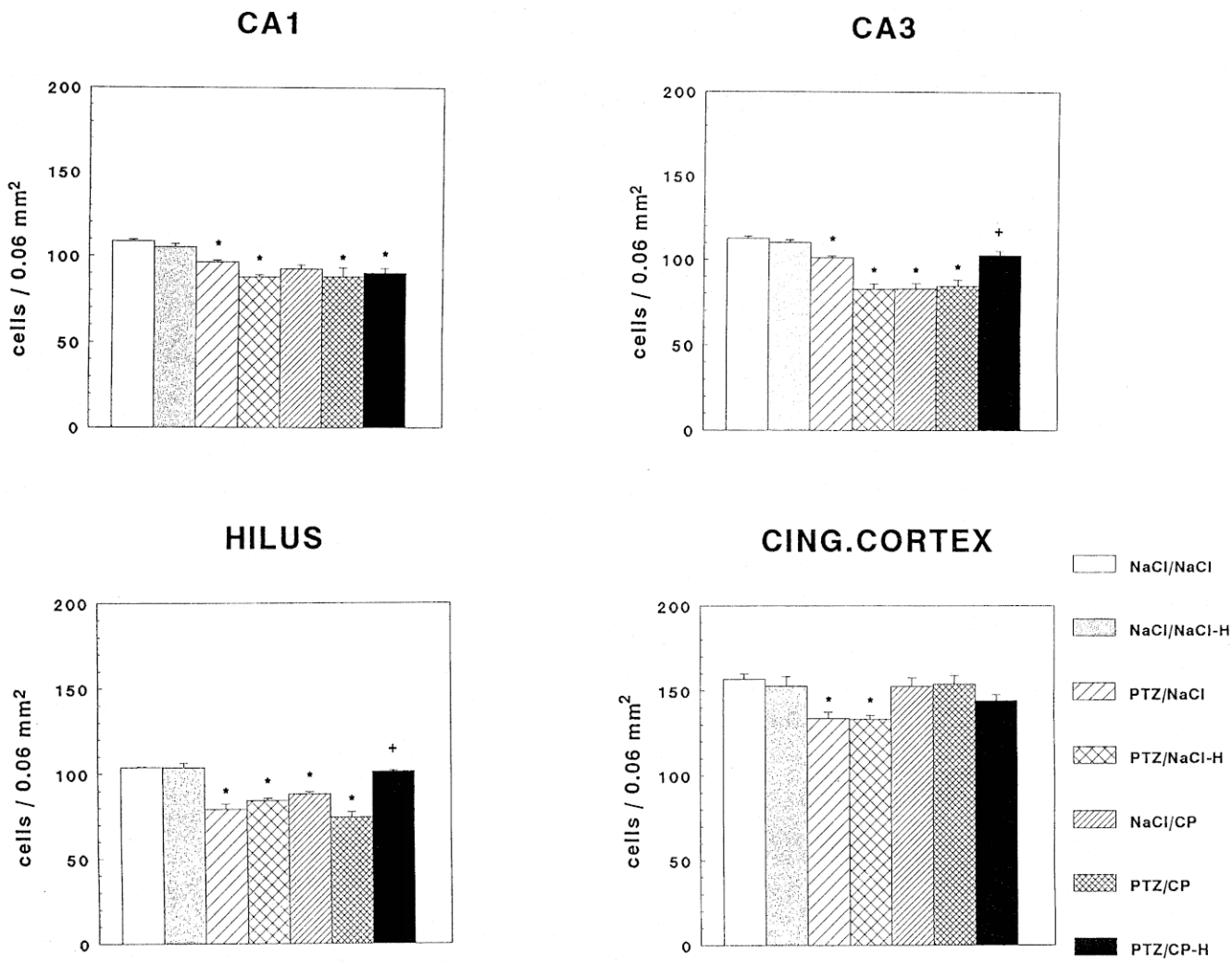

FIG. 5. Number of cells stained with toluidine blue in hippocampal CA1 (CA1) and CA3 (CA3) sector, in the hilus (HILUS) and in the cingular cortex (CING. CORTEX) 48 h after the end of subchronical NaCl/NaCl, pentylenetetrazol (PTZ, 37.5 mg/kg)/NaCl, NaCl/chlorpromazine (CP, 5 mg/kg), or PTZ/CP treatment of normothermic rats, and of NaCl/NaCl, PTZ/NaCl, or PTZ/CP treatment of hypothermic rats. Values are means ± SEM of five animals per group. One-way ANOVA was used for statistical evaluations followed Tukey's post hoc test: * $p < 0.05$, values are significantly different from the normothermic NaCl/NaCl control group at the same point; + $p < 0.05$, values of the hypothermic PTZ/CP group are significantly different from the normothermic PTZ/CP group at the same time point.

subchronical application of CP the number of toluidine stained cells was decreased in the hippocampal CA1 and CA3 sector and in the hilus. Neuronal loss could also be observed in the CA3 sector and in the hilus after subchronical application of NaCl/CP. No significant changes were detected in hippocampal CA1 sector and in the cingular cortex (CC) following subchronical NaCl/CP administration. The additional treatment of rats with CP prevented the cell loss in CC after kindling. Such biphasic effects are described for CP on cell viability in neuroblastom cells and in cardiomyocytes (1,3). CP can reduce the cytotoxocity mediated by either Ca^{2+} -dependent events or oxidative stress and reduce the cellular energy requirement (35). In contrast to this, CP causes a direct cytotoxic effect possibly mediated by a depression of mitochondrial functions (3). It may be assumed that metabolic differences between cells in the hippocampal CA3 sector and hilus on the one hand, and the CC on the other hand are the reason for the damaging or protective action of CP. Hypothermia had a protective effect on cell damage in PTZ-kindled animals as could be demonstrated in the CA3 sector and in the hilus.

It may be possible that a connection exists between the degree of neuronal cell damage in particular brain regions and the disturbance of shuttle-box learning performance after PTZ kindling.

Hypothermia of rats inhibits the development of PTZ kindling and neuronal damage in vulnerable brain structures. What happens during a moderate reduction in brain temperature lasting 120 min? It may be concluded from the protective effects of hypothermia on ischemic insults that a decrease in body temperature is able to reduce to excessive glutamate release (14,27,34,36). Therefore, the cascade of impairing events producing cell death is interrupted. An excessive glutamate release was measured in the electric focus in amygdala-kindled cats (30) and in the rat hippocampus after entorhinal kin-

dling (18). The [^{3}H]-D-aspartate release is also enhanced during the development of PTZ kindling, as could be observed after the third injection of PTZ in course of the 13 applications lasting PTZ kindling [Schröder, Pharmacol. Biochem. Behav. (in press)]. Additionally, an increased activity of the excitatory amino acid system may be caused by the inhibition of the function of the GABA ergic transmitter system (7,12) during the development of kindling. The consequence of this is, on the one hand, the progressive sensitization to the convulsant drug and, on the other hand, in analogy to the processes of ischemia/hypoxia, the damage to cells. A reduction in output of excitatory amino acids and the resulting diminution of cell death was observed after application of superoxide dismutase (SOD), an enzyme selectively scavenging free oxygen radicals, in rat forebrain neurons in culture submitted to hypoxia/reoxygenation (8). Moreover, in amygdala-kindled rats and intra-amygdaloid injection of SOD suppressed kindled seizure. This result suggests that free radicals participate in the persistence of kindled seizure susceptibility and the initiation of kindled seizures (21,28,29). Also lesions of substantia nigra in flurotyl-induced status epilepticus are ameliorated by the spin trap alpha phenyl-*N-tert*-butyl nitrone (PBN), a free radical scavenger (17). In our experiments it is possible that the activity of the excitatory amino acid system and the generation of free oxygen radicals is restricted in PTZ-kindled animals by hypothermia, as described for the inhibition of cell death after ischemia (20). In this respect it must be supposed that impairing processes are started and continued after the visible seizures by PTZ-kindled convulsions. In this period hypothermia seems to be able to stop either the enhanced glutamate release and/or the metabolic processes generating free radicals and in this way kindling is inhibited and cerebroprotective effects are achieved.

The present results are consistent with the possibility that both processes must be taken into consideration in search of new strategies for anticonvulsant drugs influencing not only epilepsy but also the resulting cognitive deficits.

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REFERENCES

1. Abe, K.; Sekizawa, T.; Kogure, K.: Biphasic effects of chlorpromazine on cell viability in a neuroblastoma cell line. Neurosci. Lett. 71:335–339; 1986.
2. Aronica, E. M.; Gortler, J. A.; Paupard, M. C.; Grooms, S. Y.; Bennett, M. V.; Zukin, R. S.: Status epilepticus-induced alterations in metabotropic glutamate receptor expression in young and adult rats. J. Neurosci. 17:8588–8595; 1997.
3. Babson, J. R.; Gravitt. N. E.; Dougherty J. M.: Chlorpromazine protection against Ca (2+)-dependent and oxidative cell injury. Limitations due to depressed mitochondrial function. Biochem. Pharmacol. 48:1509–1517; 1994.
4. Baker, C. J.; Fiore, A. J.; Frazzini, V. I.; Choudhri, T. F.; Zubay, G. P.; Solomon, R. A.: Intraischemic hypothermia decreases the release of glutamate in the cores of permanent focal cerebral infarcts. Neurosurgery 36:994–1001; 1995.
5. Becker, A.; Grecksch, G.; Rüthrich, H.-L.; Pohle, W.; Marx, B.; Matthies, H.: Kindling and its consequences on learning in rats. Behav. Neural Biol. 57:37–43; 1992.
6. Becker, A.; Grecksch, G.; Matthies, H.: The influence of diazepam on learning processes impaired by pentylenetetrazol kindling. Naunyn Schmiedebergs Arch. Pharmacol. 349:492–496; 1994.
7. Bradford, H. F.: Glutamate, GABA and epilepsy. Prog. Neurobiol. 47:477–511; 1995.
8. Cazevieille, C.; Muller, A.; Meynier, F.; Bonne, C.: Superoxide and nitric oxide cooperation in hypoxia/reoxygenation-induced neuron injury. Free Radic. Biol. Med. 14:389–395; 1993.
9. Colbourne, F.; Corbett, D.: Delayed and prolonged postichemic hypothermia is neuroprotective in the gerbil. Brain Res. 654:265–272; 1994.
10. Colbourne, F.; Sutherland, G.; Corbett, D.: Postichemic hypothermia: A critical appraisal with implications for clinical treatment. Mol. Neurobiol. 14:55–85; 1997.
11. Corbett, D.; Nurse, S.: The problem of assessing effective neuroprotection in experimental cerebral ischemia. Pro. Neurobiol. 54:531–548; 1998.
12. Corda, M. G.; Orlandi, M.; Lecca, D.; Garboni, G.; Frau, V.; Giorgi, O.: Pentylenetetrazol-induced kindling in rats: Effect of GABA function inhibitors. Pharmacol. Biochem. Behav. 40:329–333; 1991.
13. Dirig, D. M.; Hua, X. Y.; Yaksh, T. L.: Temperature dependence of basal and evoked release of amino acids and calcitonin gene-related peptide from rat dorsal spinal cord. J. Neurosci. 17:4406–4414; 1997.
14. Eilers, H.; Bickler, P. E.: Hypothermia and isoflurane similarly inhibit glutamate release evoked by chemical anoxia in rat cortical brain slices. Anesthesiology 85:600–607; 1996.
15. Goddard, G. V.: Development of epileptic seizure through brain stimulation at low intensity. Nature 214:1020–1021; 1967.
16. Grecksch, G.; Becker, A.; Rauca, C.: Effect of age on pentylenetetrazol-kindling and kindling-induced impairments of learning performance. Pharmacol. Biochem. Behav. 56:595–601; 1997.
17. He, Q. P.; Smith, M. L.; Li, P. A.; Siesjö, B. K.: Necrosis of the substantia nigra, pars reticulate, in flurothyl-induced status epilepticus is ameliorated by the spin trap alpha phenyl-*N-tert*-butyl nitrone. Free Radic. Biol. Med. 22:917–922; 1997.
18. Jarvie, P. A.; Logan, T. C.; Geula, C.; Slevin, J. T: Entorhinal kindling permanently enhances Ca^{2+}-dependent L-glutamate release in regio inferior of rat hippocampus. Brain Res. 508:188–193; 1990.
19. Kaura, S.; Bradford, H. F.; Young, A. M.; Croucher, M. J.; Hughes, P. D.: Effect of amygdaloid kindling on the content and release of amino acids from the amygdaloid complex: In vivo and in vitro studies. J. Neurochem. 65:1240–1249; 1995.
20. Kil, H. Y.; Zhang, J.; Piantadosi, C. A.: Brain temperature alters hydroxyl radical production during cerebral ischemia/reperfusion in rats. J. Cereb. Blood Flow Metab. 16:100–106; 1996.
21. Kurokouchi, A.; Mori, N.; Kumashiro, H.: Effects of superoxide dismutase on limbic status epilepticus in rats. Jpn. J. Psychiatr. Neurol. 44:394–395; 1990.
22. Liu, Z.; Gatt, A.; Mikati, M.; Holmes, G. L.: Effect of temperature on kainic acid-induced seizures. Brain Res. 631:51–58; 1993.
23. Lopez da Silva, F. H.; Gartner, J. A.; Wadman, W. J.: Kindling of the hippocampus induced spatial memory deficits in the rat. Neurosci. Lett. 63:115–120; 1986.
24. Lundgren, J.; Smith, M. L.; Blennow, G.; Siesjö, B. K.: Hyperthermia aggravates and hypothermia ameliorates epileptic brain damage. Exp. Brain Res. 99:43–55; 1994.
25. Mason, C. R.; Cooper, R. M.: A permanent change in convulsive threshold in normal and brain-damaged rats with repeated small doses of pentylenetetrazol. Epilepsia 13:663–674; 1972.
26. Meldrum, B. S.: Excitotoxicity and selective neuronal loss in epilepsy. Brain Pathol. 3:405–412; 1993.
27. Michaelis, E. K.: Molecular biology of glutamate receptors in the central nervous system and their role in excitotoxicity, oxidative stress and aging. Progr. Neurobiol. 54:369–415; 1998.
28. Mori, N.; Yokoyama, H.: Role of superoxide dismutase in a kin-

dling model of epilepsy. Comp. Biochem. Physiol. 104:373–376; 1993.
29. Mori, N.; Wada, J. A.; Watanabe, M.; Kumashiro, H.: Increased activity of superoxide dismutase in kindled seizure following intra-amygdaloid injection of superoxide dismutase in rats. Brain Res. 557:313–315; 1991.
30. Nakase, H.; Tada, T.; Hashimoto, H.; Kurakawa, S.; Hirabayashi, H.; Hoshida, T.; Sakaki, T.; Ohnishi, H.: Experimental study of the mechanism of seizure induction: Changes in the concentrations of excitatory amino acids in the epileptic focus of the cat amygdaloid kindling model. Neurol. Med. Chir. Tokyo 34:418–422; 1994.
31. Pohle, W.; Becker, A.; Grecksch, G.; Juhre, A.; Willenberg, A.: Piracetam prevents pentylenetetrazol kindling-induced neuronal loss and learning deficits. Seizure 6:467–474; 1997.
32. Racine, R. J.: Modification of seizure activity by electrical stimulation. II. Motor seizures. Electroencephalogr. Clin. Neurophysiol. 32:281–285; 1972.
33. Stone, W. S.; Gold, P. E.: Amygdala kindling effects on sleep and memory in rats. Brain Res. 449:135–140; 1988.
34. Winfree C. J.; Baker, C. J.; Connolly, E. S., Jr.; Fiore, A. J.; Solomon, R. A.: Mild hypothermia reduces penumbral glutamate levels in the rat permanent focal cerebral ischemia model. Neurosurgery 38:1216–1222; 1996.
35. Zager, E. L.; Ames, A.: Reduction of cellular energy requirements. Screening for agents that may protect against CNS ischemia. J. Neurosurg. 69:568–579; 1988.
36. Zornow, M. H.: Inhibition of glutamate release: A possible mechanism of hypothermic neuroprotection. J. Neurosurg. Anesthesiol. 7:148–151; 1995.