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Electrical but not chemical kindling increases sensitivity to some phencyclidine-like behavioral effects induced by the competitive NMDA receptor antagonist D-CPPene in rats

Piotr Wlaź [1], Ulrich Ebert, Heidrun Potschka, Wolfgang Löscher *

Department of Pharmacology, Toxicology, and Pharmacy, School of Veterinary Medicine, Bünteweg 17, 30559 Hannover, Germany

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Abstract

We have previously reported that a competitive *N*-methyl-D-aspartate (NMDA) receptor antagonist, DL-[*E*]-2-amino-4-methyl-5-phosphono-3-pentenoic acid (CGP 37849), produces stereotyped behaviors and hyperlocomotion in amygdala kindled rats at doses which do not induce such phencyclidine (PCP)-like behaviors in nonkindled rats, indicating that kindling predisposes rats to such adverse effects of competitive NMDA receptor antagonists. From these data we predicted that epileptic patients may exhibit a hypersensitivity to PCP-like adverse effects of competitive NMDA receptor antagonists, which was subsequently confirmed in a clinical trial with D-CPPene (SDZ EAA-494; 3-(2-carboxypiperazine-4-yl)propenyl-1-phosphonate). For further exploration of the functional alterations in NMDA receptor responsiveness produced by kindling, we studied whether the enhanced susceptibility of amygdala-kindled rats to PCP-like adverse effects of CGP 37849 is also observed with D-CPPene. Furthermore, we determined whether the enhanced susceptibility of kindled rats to such adverse effects occurs only after relatively short intervals following the last seizure, as used in our previous study, or is a more permanent phenomenon. For this purpose, we compared adverse effects in kindled rats not only with naive (non-implanted) controls, as done in our previous study, but used electrode-implanted nonkindled rats as an additional control to assess the possible bias of mere electrode-implantation. In addition, we studied whether the enhanced susceptibility to NMDA receptor antagonists of electrically kindled rats is also present in chemically kindled animals. In some experiments, the PCP-like uncompetitive NMDA receptor antagonist MK-801 (dizocilpine) was included for comparison. In amygdala kindled rats, D-CPPene produced significantly more stereotyped behaviors than in electrode-implanted or naive nonkindled controls. The enhanced sensitivity of electrically kindled rats to PCP-like stereotypies induced by D-CPPene was observed both 7 and 180 days after the last kindled seizure, indicating a long-lasting if not permanent hypersensitivity to these adverse effects. In addition, more intense circling was observed in amygdala kindled rats, whereas hyperlocomotion only tended to be more intense after D-CPPene in kindled rats. These alterations in D-CPPene-induced behaviors were not observed after chemical kindling with pentylenetetrazole, but D-CPPene induced significantly less hypothermia in chemically kindled rats both 7 and 70 days after the last seizure. The data demonstrate that kindling produces long-lasting alterations in some adverse effects of D-CPPene, substantiating that epileptogenesis as initiated by kindling renders the brain more susceptible to PCP-like behavioral side effects of competitive NMDA receptor antagonists.

Keywords: Epilepsy; Kindling; Pentylenetetrazole; Dizocilpine; MK-801; Phencyclidine

1. Introduction

Noncompetitive *N*-methyl-D-aspartate (NMDA) receptor antagonists, such as phencyclidine (PCP) or MK-801 (dizocilpine), induce a behavioral ('PCP-like') syndrome in rodents, consisting of amphetamine-like (e.g., hyperlocomotion, stereotypies) and barbiturate-like (e.g., motor impairment) behaviors, which is thought to reflect the psychotomimetic and motor impairing adverse effects of these drugs in humans and to limit the therapeutic usefulness of such drugs (Balster, 1987; Willetts et al., 1990; Löscher and Schmidt, 1994). It has often been proposed that competitive NMDA receptor antagonists may have advantages as potential therapeutic agents because, at least

* Corresponding author. Tel.: +49-511-856-8721; Fax: +49-511-953-8581.

[1] Present address: Department of Pharmacology, Faculty of Veterinary Medicine, Agricultural University, Lublin, Poland.

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in laboratory animals, PCP-like adverse effects are only observed at doses considerably higher than those producing therapeutically relevant effects, such as anticonvulsant or neuroprotective effects. However, in 1991, we reported that the competitive NMDA receptor antagonist DL-[*E*]-2-amino-4-methyl-5-phosphono-3-pentenoic acid (CGP 37849), while lacking anticonvulsant activity, induced PCP-like behavioral effects (hyperactivity, stereotypies) in the amygdala kindling model of temporal lobe epilepsy in rats at doses which did not induce such effects in nonkindled rats (Löscher and Hönack, 1991c), thus suggesting that epileptogenesis as initiated by kindling might render the brain more susceptible to PCP-like adverse effects of competitive NMDA receptor antagonists (Löscher and Hönack, 1991b). Because PCP-like behavioral effects in rodents are thought to reflect the psychotomimetic and reinforcing effects of PCP and related drugs in humans, we predicted that competitive NMDA receptor antagonists may bear the risk of producing such effects in epileptic patients (Löscher and Hönack, 1991b), particularly if these drugs are used in patients with complex partial seizures, because neurophysiological and neurochemical changes in NMDA-related processes in kindled brains and brain tissue from patients with temporal lobe epilepsy are comparable (Avoli, 1991; Löscher, 1993). Thus, behavioral data from kindled animals might be more predictive of behavioral adverse effects in patients with focal epilepsy than data from nonkindled animals (as generally used for behavioral studies during preclinical drug development) or models of generalized epilepsy.

More recently, data from the first clinical trial with a competitive NMDA receptor antagonist, D-CPPene (SDZ EAA-494; 3-(2-carboxypiperazine-4-yl)propenyl-1-phosphonate), in epileptic patients were published (Sveinbjornsdottir et al., 1993). During pre-clinical evaluation, this drug had been shown to be a potent and effective anticonvulsant in various seizure models, including kindling (Chapman, 1991; Dürmüller et al., 1994; Herrling et al., 1997). In healthy volunteers, D-CPPene had been well tolerated in doses up to 2000 mg/day (Sveinbjornsdottir et al., 1993). In contrast, when added to regular antiepileptic drugs in 8 patients with refractory complex partial seizures in daily doses of 500–1000 mg, D-CPPene induced severe adverse effects (confusion and disorientation, gait ataxia, sedation) in all patients, requiring hospitalisation in six patients and premature termination of the trial. Seizure control was worsened in 3 patients and unchanged in the others. Thus, in contrast to all other preclinical data on this compound, the behavioral data from studies on competitive NMDA receptor antagonists in kindled rats had predicted what happened in patients with temporal lobe epilepsy (Löscher and Schmidt, 1994). If so, animals with chronic brain dysfunction as induced by epileptogenesis should be involved in the evaluation of associated behavioral and cognitive deficits in anticonvulsant drug testing, particularly in case of novel glutamate receptor antagonists, in order to avoid underestimation of a drug's potency to induce behavioral and cognitive deficits (Wlaź and Löscher, 1998).

To further address this important topic, we undertook a series of experiments with the following aims: (1) to study whether the enhanced susceptibility of amygdala-kindled rats to PCP-like adverse effects of CGP 37849 is also observed with D-CPPene; (2) to determine whether the enhanced susceptibility of kindled rats to such adverse effects occurs only after relatively short intervals following the last seizure, as used in our first study (Löscher and Hönack, 1991c), or is a more permanent phenomenon; (3) to compare adverse effects in kindled rats not only with naive (non-implanted) controls, as done in our first study (Löscher and Hönack, 1991c), but to use electrode-implanted nonkindled rats as an additional control; (4) to study whether the enhanced susceptibility to NMDA receptor antagonists of electrically kindled rats is also present in chemically kindled animals.

2. Methods

2.1. Animals

Female Wistar rats (Harlan-Winkelmann, Borchen, F.R.G.), weighing 210–230 g, were used. The animals were purchased from the breeder at a body weight of about 200 g. Following arrival in the animal colony, the rats were kept under controlled environmental conditions (ambient temperature 24–25°C, humidity 50–60%, 12/12 h light/dark cycle, light on at 7:00 a.m.) for at least 1 week before being used in the experiments. Standard laboratory chow (Altromin 1324 standard diet) and tap water were allowed ad lib. All rats were habituated to handling and the various observational procedures and recording of rectal body temperature prior to any drug experiments.

2.2. Amygdala kindling

The rats were anesthetized with chloral hydrate (360 mg/kg i.p.) and received stereotaxic implantation (according to the surgery methods described in the atlas of Paxinos and Watson, 1986) of one bipolar electrode in the right basolateral amygdala. Coordinates for electrode implantation were AP −2.8, L −4.8, V −8.6. All coordinates were measured from bregma. Skull screws served as the indifferent reference electrode. The electrode assembly was attached to the skull by dental acrylic cement.

After a postoperative period of 2 weeks, constant current stimulations (500 μA, 1 ms, monophasic square-wave pulses, 50/s for 1 s) were delivered to the amygdala at intervals of 1 day until 10 fully kindled stage 5 seizures, i.e., focal seizures secondarily generalizing to generalized

clonic seizures and loss of balance (Racine, 1972), were elicited. Only fully kindled rats with reproducible severity and duration of seizures were used for the behavioral studies described below. The interval between the last stage 5 seizure and onset of the drug testing for behavioral alterations was either 7 or about 180 days.

Two control groups of rats were used, being age-matched with the amygdala kindled rats. One control group (sham kindled) was implanted with an electrode into the amygdala in the same way as the kindled rats but was not kindled. A second group (naive controls) did not receive stereotaxic implantation. Handling was the same in all groups. Group size in the drug experiments was 7–9 for kindled, 7–8 for sham-kindled, and 8–9 for naive control groups, group size of electrode-implanted rats varying because some rats lost their electrode assembly during the experiments. All rats were coded and the persons involved in behavioral observations were not aware which of the electrode-implanted rats were kindled, i.e., experiments were done in a 'blinded fashion'.

2.3. Chemical kindling

Rats were injected with pentylenetetrazole at an i.p. dose of 25 mg/kg every second day, except weekends, and were observed after each injection for a period of up to 45 min. Age-matched control rats were given saline at the same volume (2 ml/kg i.p.). The dose of pentylenetetrazole used for chemical kindling was based on preliminary experiments, showing that higher doses (e.g., 30 mg/kg or higher) as often used in the literature are too high for the rats used in this study and lead already to generalized tonic–clonic seizures in several rats after the first administration.

Seizures occurring during pentylenetetrazole kindling with 25 mg/kg every second day were scored by a modified Racine (1972) scale: 0, no seizure activity; 1, facial clonus; 2, sudden muscle jerks associated with behavioral rest; 3, repetitive muscle jerks associated with single episodes of rearing; 4, rearing, often accompanied by bilateral forelimb clonus; 5, rearing with loss of balance and falling accompanied by generalized clonic seizures. With 25 mg/kg every second day, rats exhibited no or only score 1/2 seizures after the first administration and reached criterion (i.e., generalized clonic seizures) after an average of 4.2 ± 0.74 injections. Rats were considered fully kindled if they exhibited 10 stage 5 seizures, i.e., generalized clonic seizures with loss of balance. In some cases, clonic seizures were followed by tonic seizures. The interval between the last stage 5 seizure and onset of the drug testing for behavioral alterations was either 7 or 70 days in average. Group size was 8–9 for kindled and 5–6 for nonkindled groups, respectively. All rats were coded and the persons involved in behavioral observations were not aware which rats were kindled, i.e., experiments were done in a 'blinded fashion'.

2.4. Experiments with D-CPPene

Two of 3 dosages, i.e., 10, 15, or 25 mg/kg i.p., of D-CPPene were examined in each model. These doses were chosen on the basis of the anticonvulsant effects of D-CPPene in the amygdala-kindling model, showing that doses of about 10 mg/kg and above exert anticonvulsant effects in fully kindled rats (Dürmüller et al., 1994). The same dosages of D-CPPene administered in kindled rats were also administered in nonkindled rats. Nonkindled rats were observed together with kindled rats to allow direct comparison of differences in behavioral effects of D-CPPene. In a typical experiment, up to 9 rats were concomitantly observed after drug administration, e.g., 3 kindled, 3 sham kindled, and 3 naive controls. All drug experiments were performed in the same groups of amygdala or pentylenetetrazole kindled groups (and respective controls); at least 7 days were interposed between the experiments in the same groups of rats. All behavioral effects of D-CPPene and MK-801 (see below) were scored by the same experienced observers, who had been extensively trained in previous experiments to assess the PCP-like behavioral effects of MK-801.

For examination of behavioral effects of D-CPPene, the animals were transferred from the vivarium to the laboratory in their home cage and placed in clear plastic cages, $590 \times 380 \times 190$ mm high. After 15 min, rectal body temperature was measured and the rats' behavior recorded (predrug control), followed by immediate i.p. drug injection. The animals were observed for alterations in behavior for up to 240 min after injection. Based on the time course of effects, behavioral scores determined at 15, 30, 60, 120, and 240 min after administration were used for comparative evaluation of drug experiments. Each individual animal was observed for about 3 min at each of these time points, including open field observation for rating of ataxia and locomotion (see below), rotarod test, and recording of body temperature. For all observations, rigorous observational protocols described elsewhere were used (Löscher and Hönack, 1991c; Löscher et al., 1992). Head weaving (swaying movements of the head and upper torso from side to side for at least one complete cycle, i.e., left–right–left), stereotyped sniffing or face washing, reciprocal forepaw treading ('piano playing'), and circling were scored using a ranked intensity scale where 0 = absent, 1 = equivocal, 2 = present and 3 = intense. For comparison between groups, scores for all stereotyped behaviors (head weaving, piano playing, stereotyped sniffing and face washing) were averaged for each animal and used for calculation of group means. Locomotion was scored in the open field as follows: −3, intense hypolocomotion; −2, hypolocomotion present; −1, hypolocomotion equivocal;

0, normal locomotion; +1, hyperlocomotion equivocal; +2, hyperlocomotion present; +3, intense hyperlocomotion. Ataxia was scored using a 6-point rating system as described previously (Löscher et al., 1992). For rating ataxia, the animals were taken out of the cage, placed in an open field for 1 min, and the intensity of ataxia was rated as follows: 1, slight ataxia in hind-legs (tottering of the hind quarters); 2, more pronounced ataxia with dragging of hind legs; 3, further increase of ataxia and more pronounced dragging of hind legs; 4, marked ataxia, animals lose balance during forward locomotion; 5, very marked ataxia with frequent loss of balance during forward locomotion; 6, permanent loss of righting reflexes, but animal still attempts to move forward. In addition to judge motor impairment by observation, rats were subjected to the rotarod test as described previously (Löscher and Hönack, 1991a).

2.5. Experiments with MK-801

For comparison with behavioral effects induced by D-CPPene, the uncompetitive NMDA receptor antagonist MK-801 (dizocilpine) was administered in kindled and nonkindled rats at a dose of 0.2 mg/kg. At this dose, MK-801 produces marked PCP-like behavioral alterations in female Wistar rats (Hönack and Löscher, 1993). Observation of behavioral effects and recording of body temperature was done as described for D-CPPene.

2.6. Drugs

D-CPPene (SDZ EAA 494) was kindly provided by NOVARTIS (Basle, Switzerland). MK-801 (as maleate salt) was purchased from RBI (Natick, MA, USA), and pentylenetetrazole from Sigma (St. Louis, MO, USA). All drugs were freshly dissolved in distilled water and injected i.p. at a volume of 2–3 ml/kg.

2.7. Statistics

For comparison between groups, the observational scores recorded at 15, 30, 60, 120, and 240 min after drug injection were summed for each animal and used for calculation of group means. In amygdala kindled rats, significance of differences in behavioral scores between kindled and the two groups of nonkindled rats was calculated by analysis of variance (ANOVA) on ranks (Kruskal–Wallis test), followed post hoc by the Mann–Whitney *U*-test. Differences in body temperature were evaluated with ANOVA followed by a post hoc *t*-test. In

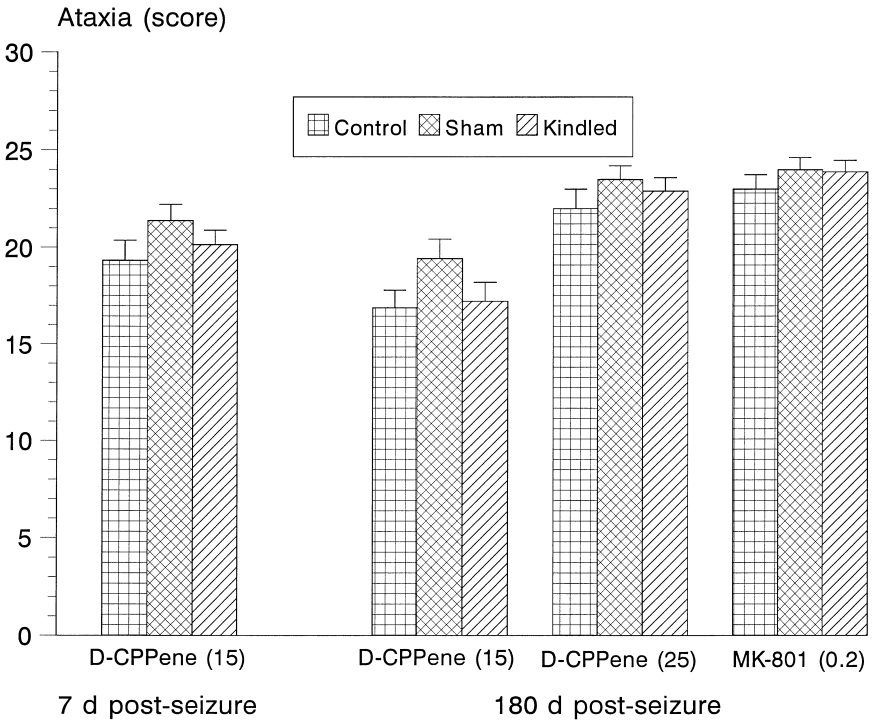

Fig. 1. Ataxia induced by D-CPPene and MK-801 in amygdala kindled and nonkindled rats. Drugs were injected in fully kindled rats after either 7 or 180 days following the last kindled seizure; doses in mg/kg are indicated in brackets. Two groups of age-matched nonkindled controls were used together with the kindled rats; one group ('sham') was electrode-implanted, the other ('control') was not electrode-implanted. The intensity of ataxia was scored at 15, 30, 60, 120 and 240 min after i.p. drug injection. Each score was summed over the five observation periods and the individual summed scores were used for calculation of group means + S.E.M. Group sizes were 7–9 (kindled), 7–9 (sham) and 9 (control), respectively. Evaluation of data by ANOVA indicated no significant differences among the group means.

pentylenetetrazole kindled rats, statistical differences between kindled and nonkindled groups were calculated by *U*-test (behavior) or *t*-test (body temperature). All tests were used two-sided and $P < 0.05$ was considered significant.

3. Results

3.1. Amygdala kindled rats

The prototype uncompetitive NMDA receptor antagonist MK-801 induced the typical PCP-like behavioral syndrome in female Wistar rats, consisting of ataxia (Fig. 1), head weaving and other stereotyped behaviors such as piano playing, sniffing and face washing (Fig. 2), circling (Fig. 3), and hyperlocomotion (Fig. 4). Body temperature was not markedly affected at the dose of MK-801 used in these experiments, i.e., 0.2 mg/kg, although sham-kindled rats tended to exhibit moderate hypothermia which was not seen in kindled rats, the difference between both groups being statistically significant (Fig. 5). All behavioral effects were rapid in onset and long-lasting; maximum behavioral alterations were seen between 1 and 2 h after drug administration. At most time points, rats were not able to pass the rotarod test (not illustrated), indicating marked motor impairment. Comparison of data in kindled, sham kindled and naive (non-implanted) control rats showed that the electrode-implanted kindled and nonkindled groups tended to exhibit more intense stereotyped behaviors than non-implanted control (Fig. 2), whereas most other recordings were similar between groups (Fig. 1, Fig. 3–5).

At the doses used, the competitive NMDA receptor antagonist D-CPPene induced a similar severity of ataxia as MK-801, with no difference between groups (Fig. 1). Again, rats were not capable of passing the rotarod test at most time points after administration (not illustrated). Induction of motor impairment was rapid with marked ataxia seen after 15 min; maximal motor impairment was obtained between 30 and 120 min after drug administration. Differences between groups were obtained with respect to stereotyped behaviors (Fig. 2), circling (Fig. 3), and locomotion (Fig. 4). When D-CPPene, 15 mg/kg, was administered to amygdala-kindled rats 7 days after a fully kindled seizure, kindled rats exhibited significantly more intense stereotyped behaviors than the two control groups (Fig. 2). The same difference was seen when D-CPPene, 15 mg/kg, was injected in kindled rats about 180 days after the last fully kindled seizure, indicating a long-lasting or permanent alteration in sensitivity of amygdala-kindled rats to

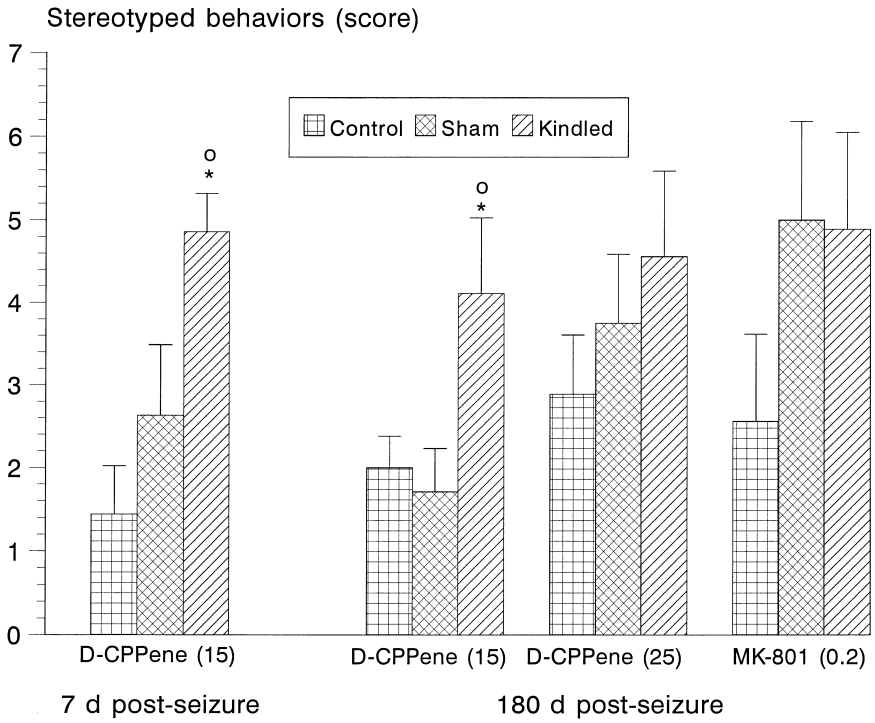

Fig. 2. Stereotyped behaviors (head weaving, piano playing, stereotyped sniffing, or stereotyped face washing) induced by D-CPPene and MK-801 in amygdala kindled and nonkindled rats. Drugs were injected in fully kindled rats after either 7 or 180 days following the last kindled seizure; doses in mg/kg are indicated in brackets. Two groups of age-matched nonkindled controls were used together with the kindled rats; one group ('sham') was electrode-implanted, the other ('control') was not electrode-implanted. The intensity of stereotyped behaviors was scored at 15, 30, 60, 120 and 240 min after i.p. drug injection. Each score was summed over the five observation periods and the individual summed scores were used for calculation of group means + S.E.M. Group sizes were 7–9 (kindled), 7–9 (sham) and 9 (control), respectively. Evaluation of data by ANOVA indicated significant differences among the group means in case of the two experiments with 15 mg/kg D-CPPene ($P = 0.0129$ and 0.0318, respectively); results from post-hoc analysis are indicated by circles (significant difference to control; P at least < 0.05) or stars (significant difference to sham; P at least < 0.05).

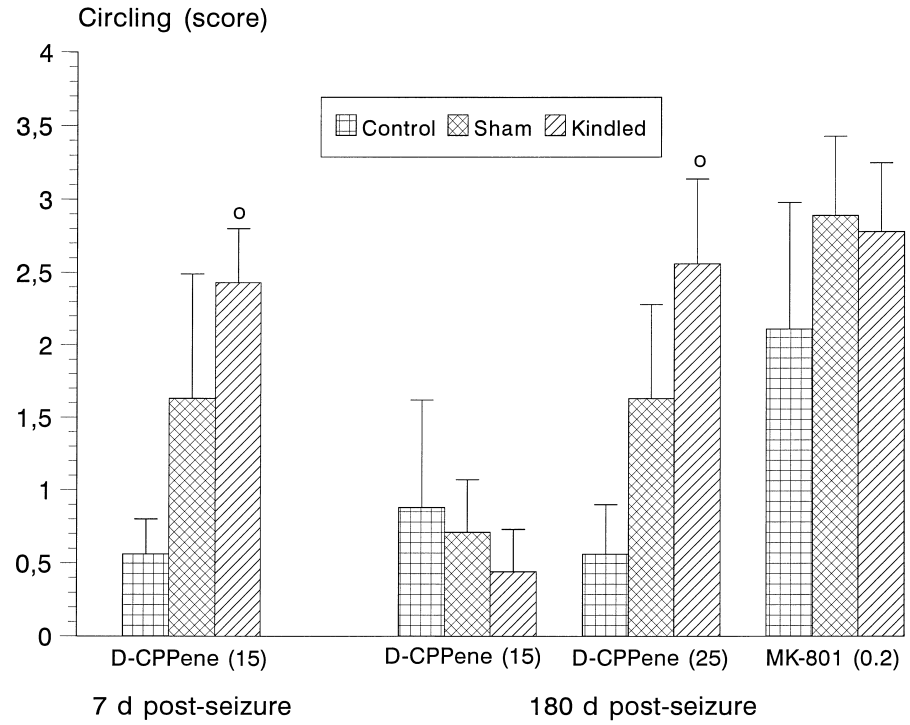

Fig. 3. Circling induced by D-CPPene and MK-801 in amygdala kindled and nonkindled rats. Drugs were injected in fully kindled rats after either 7 or 180 days following the last kindled seizure; doses in mg/kg are indicated in brackets. Two groups of age-matched nonkindled controls were used together with the kindled rats; one group ('sham') was electrode-implanted, the other ('control') was not electrode-implanted. The intensity of circling was scored at 15, 30, 60, 120 and 240 min after i.p. drug injection. Each score was summed over the five observation periods and the individual summed scores were used for calculation of group means + S.E.M. Group sizes were 7–9 (kindled), 7–9 (sham) and 9 (control), respectively. Evaluation of data by ANOVA indicated significant differences among the group means in case of the experiment with 15 mg/kg D-CPPene 7 days post-seizure ($P = 0.0184$) and the experiment with 25 mg/kg D-CPPene 180 days post-seizure ($P = 0.0180$); results from post-hoc analysis are indicated by circles (significant difference to control; $P < 0.05$).

this adverse effect. When the dose of D-CPPene was increased to 25 mg/kg, differences in intensity of stereotypies between groups were no longer significant (Fig. 2).

Similar to stereotyped behaviors, circling, which usually occurred during the first hour after injection, was significantly increased in kindled rats 7 days after the last seizure, whereas the same dose of D-CPPene did not induce such a difference 180 days after the last seizure (Fig. 3). By increasing the dose of D-CPPene to 25 mg/kg, the difference between kindled rats and controls became similar to the difference observed with 15 mg/kg 7 days after the last seizure (Fig. 3).

In contrast to MK-801, the effects of D-CPPene on locomotion were biphasic with depressed forward locomotion in the open field after 15 and 30 min, but increasing locomotion thereafter (Fig. 6). In the experiment with 15 mg/kg at 7 days after last seizure, the summed scores for locomotion were not different among the groups (Fig. 4). However, when the time course of locomotion was compared, electrode-implanted groups tended to exhibit more intense hyperlocomotion at 60 and 120 min after administration than non-implanted controls (Fig. 6). This difference from naive controls became more marked when experiments were performed 180 days after the last seizure (Fig. 4) but, because of the large variation, differences among the groups were not statistically significant. With both 15 and 25 mg/kg, electrode-implanted rats exhibited hyperlocomotion that almost reached that obtained with MK-801, whereas no hyperlocomotion was seen in non-implanted controls (Fig. 4). Body temperature was decreased by D-CPPene without any indication of differences among the groups (Fig. 5). Interestingly, the decrease in body temperature induced by D-CPPene 7 days following the last seizure was more pronounced than the hypothermic effect observed 180 days after last seizure (Fig. 5), which could be due to the age difference of rats at either 7 or 180 days after last kindled seizure. This could also be involved in the different pattern of effects on locomotion observed at 7 days and 180 days (Fig. 4).

3.2. Pentylenetetrazole kindled rats

In contrast to the behavioral differences found with D-CPPene in amygdala-kindled rats, no such differences were seen in pentylenetetrazole kindled rats (Figs. 7 and 8). Although there was a tendency to more intense stereotyped behaviors or circling, and less intense hypolocomotion in some of the experiments in pentylenetetrazole kindled rats (Fig. 7), the difference from sham controls was not significant. However, kindled rats did not exhibit

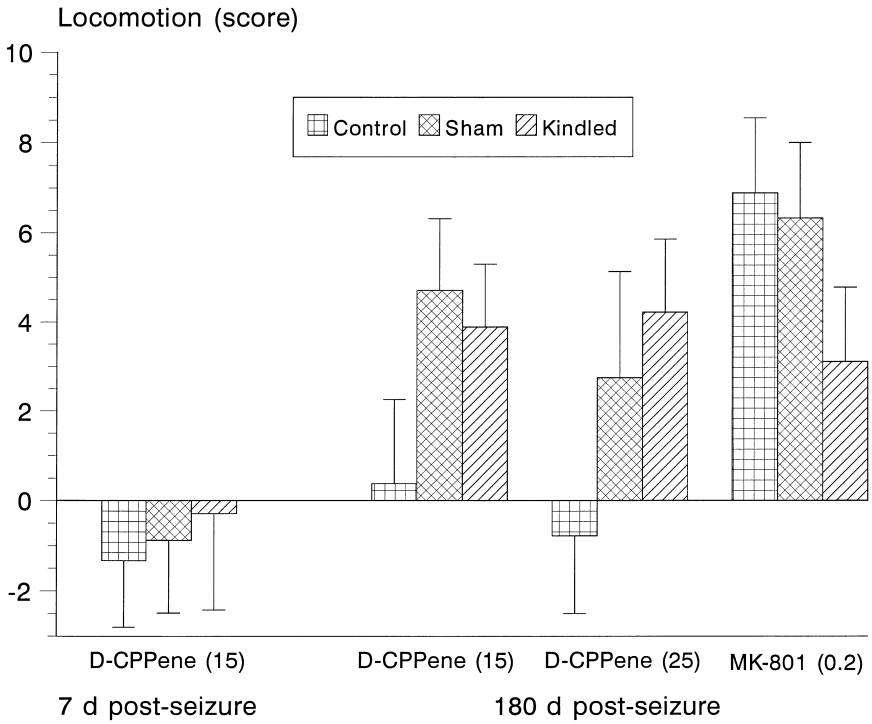

Fig. 4. Alterations in locomotion induced by D-CPPene and MK-801 in amygdala kindled and nonkindled rats. Hyperlocomotion is indicated by positive scores and hypolocomotion by negative scores. Drugs were injected in fully kindled rats after either 7 or 180 days following the last kindled seizure; doses in mg/kg are indicated in brackets. Two groups of age-matched nonkindled controls were used together with the kindled rats; one group ('sham') was electrode-implanted, the other ('control') was not electrode-implanted. The intensity of hypo- or hyperlocomotion was scored at 15, 30, 60, 120 and 240 min after i.p. drug injection. Each score was summed over the five observation periods and the individual summed scores were used for calculation of group means + S.E.M. Group sizes were 7–9 (kindled), 7–9 (sham) and 9 (control), respectively. Evaluation of data by ANOVA indicated no significant differences among the group means.

the same extent of hypothermia after D-CPPene than sham kindled rats, the difference being significant in the two experiments with 15 mg/kg D-CPPene (Fig. 8).

4. Discussion

In our previous experiments with the competitive NMDA receptor antagonist CGP 37849, this drug was found to produce a PCP-like behavioral syndrome (ataxia, hyperlocomotion, stereotypies) in amygdala-kindled rats when tested about 4 days after the last seizure, whereas the amphetamine-like behavioral alterations of the syndrome (hyperlocomotion, stereotypies) were only infrequently seen in nonkindled (nonimplanted) rats, indicating that kindling had induced a hypersensitivity to these adverse effects (Löscher and Hönack, 1991c). Hyperlocomotion and stereotyped behaviors induced by CGP 37849 in kindled rats could be counteracted by pretreatment with the dopamine antagonist haloperidol, the α_1-adreceptor antagonist prazosin, and the 5-HT_{1A} receptor ligand ipsapirone, indicating that enhanced release or function of dopamine, noradrenaline, and serotonin is involved in these adverse effects of the competitive NMDA receptor antagonist (Löscher and Hönack, 1991c). Indeed, CGP 37849, at doses which induce stereotyped behaviors, has been shown to increase dopamine and serotonin turnover in several brain regions, including the basal ganglia, and to increase noradrenaline in the striatum of rats (Löscher et al., 1993a). Since recent studies on dopaminergic function suggest that electrical kindling induces a lasting hyperdopaminergic malfunction in the rat brain (Adamec, 1990), the enhanced susceptibility of kindled rats to amphetamine-like adverse effects of CGP 37849 could relate to a hypersensitivity of kindled rats to dopaminergic effects of NMDA receptor antagonists (Löscher and Hönack, 1991c). The present study now shows that a similar hypersensitivity of amygdala kindled rats can be demonstrated for the competitive NMDA receptor antagonist D-CPPene, and that this hypersensitivity is long-lasting if not permanent once kindling has been established.

One argument against our previous findings with CGP 37849 (Löscher and Hönack, 1991c) was that data of kindled rats were compared with data of age-matched but not electrode-implanted nonkindled controls, leaving the possibility that the difference between kindling and control was not due to kindling but to mere electrode implantation. Indeed, electrode implantation has been shown to induce a number of functional and neurochemical alterations in rats that could alter drug actions (e.g., Blackwood et al., 1982;

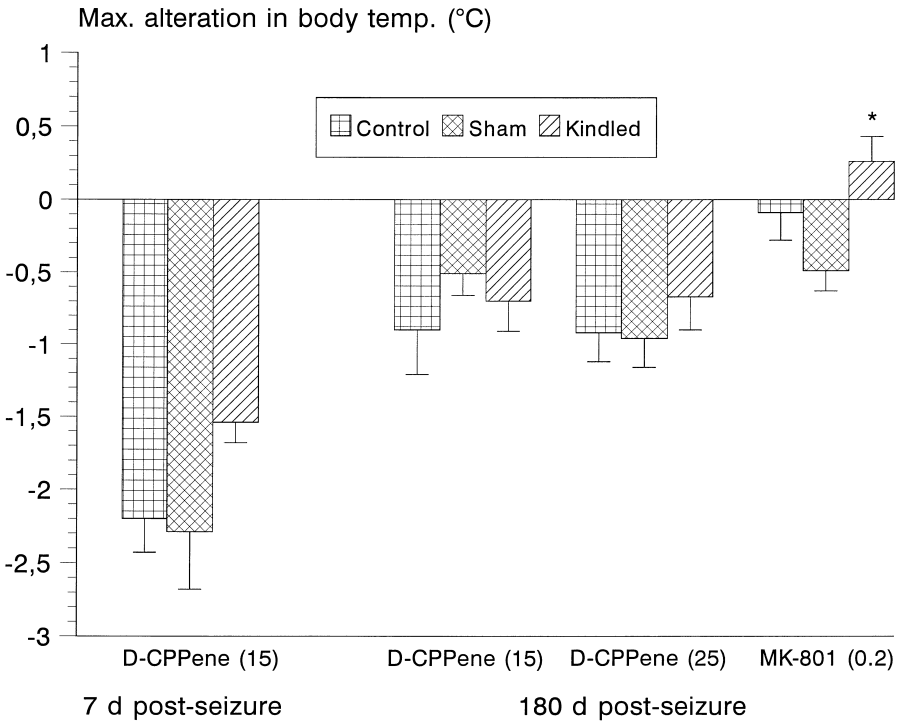

Fig. 5. Alterations in rectal body temperature induced by D-CPPene and MK-801 in amygdala kindled and nonkindled rats. Data are shown as maximal alteration in post-drug values from predrug control for each group of rats. Drugs were injected in fully kindled rats after either 7 or 180 days following the last kindled seizure; doses in mg/kg are indicated in brackets. Two groups of age-matched nonkindled controls were used together with the kindled rats; one group ('sham') was electrode-implanted, the other ('control') was not electrode-implanted. Body temperature was measured before and 15, 30, 60, 120 and 240 min after i.p. drug injection. The maximum alteration (mean ± S.E.M.) from predrug control was usually recorded 2 h after drug administration. Group sizes were 7–9 (kindled), 7–9 (sham) and 9 (control), respectively. Evaluation of data by ANOVA indicated significant differences among the group means in case of the experiment with MK-801 ($P = 0.0153$); results from post-hoc analysis are indicated by star (significant difference to sham; $P < 0.01$).

Babb and Kupfer, 1984; Benattia et al., 1992; Löscher et al., 1993b; Löscher et al., 1995). We therefore used both electrode-implanted and non-implanted rats as controls in the present study. The data demonstrate that the enhanced susceptibility of kindled rats to stereotypies induced by D-CPPene is not seen in electrode-implanted nonkindled rats so that the hypersensitivity of kindled rats to this amphetamine-like adverse effect cannot be explained by electrode-implantation. On the other hand, there was a tendency for increased hyperlocomotion in response to D-CPPene in both groups of implanted rats when compared to non-implanted controls.

To further address the potential problem of electrode implantation as a bias in such behavioral studies, we used another method of kindling, i.e., chemical kindling with pentylenetetrazole. In contrast to amygdala kindling, pentylenetetrazole kindling did not lead to enhanced susceptibility of kindled rats to stereotyped behaviors induced by D-CPPene, substantiating that chemical kindling and electrical kindling do not share the same functional brain alterations (Löscher, 1998a). Interestingly, the only difference between pentylenetetrazole kindled and sham kindled rats was that kindled rats exhibited a significantly lower hypothermal response to D-CPPene, which was not seen in amygdala kindled rats.

In contrast to the findings with D-CPPene, the effects of the uncompetitive high affinity NMDA receptor antagonist MK-801 were comparable in amygdala kindled and nonkindled rats, although there was a tendency that electrode implantation may enhance the intensity of stereotyped behaviors in response to MK-801. In recent experiments with a lower dose (0.1 mg/kg) of MK-801, this drug caused significantly more intense hyperlocomotion in kindled than in nonkindled, non-implanted controls (Löscher and Hönack, 1991c), which was not seen in the present experiments with 0.2 mg/kg.

Since MK-801 and competitive NMDA receptor antagonists such as CGP 37849 in duce comparable alterations in dopamine and serotonin metabolism in various brain regions of rats (Löscher et al., 1993a), a hyperdopaminergic malfunction alone does not explain why kindled rats show an increased susceptibility to amphetamine-like stereotyped behavioral effects of competitive but not uncompetitive NMDA receptor antagonists. One likely explanation for this apparent difference between competitive and uncompetitive NMDA receptor antagonists is that electrical kindling not only activates NMDA receptor-mediated excitation and increases the number of NMDA receptors in the hippocampus (cf., McNamara, 1994; Bradford, 1995; Rogawski, 1995) but also induces a long-last-

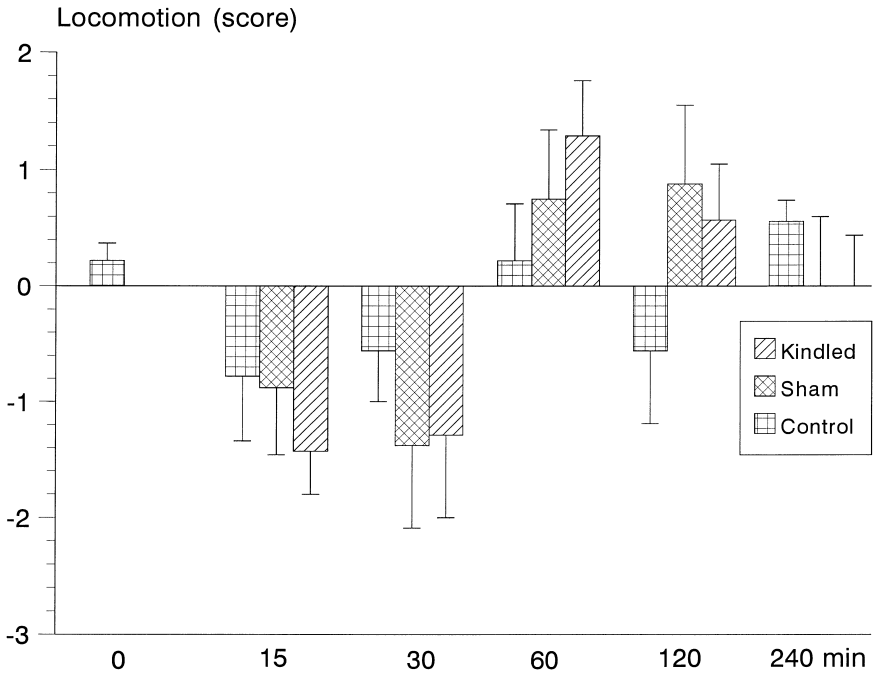

Fig. 6. Alterations in locomotion induced by D-CPPene, 15 mg/kg i.p., in amygdala kindled and nonkindled rats. Hyperlocomotion is indicated by positive scores and hypolocomotion by negative scores. D-CPPene was injected in fully kindled rats 7 days following the last kindled seizure. Two groups of age-matched nonkindled controls were used together with the kindled rats; one group ('sham') was electrode-implanted, the other ('control') was not electrode-implanted. The intensity of hypo- or hyperlocomotion was scored before and 15, 30, 60, 120 and 240 min after i.p. drug injection. Data are shown as means ± S.E.M. Group sizes were 7–9 (kindled), 7–9 (sham) and 9 (control), respectively. Evaluation of data by ANOVA indicated no significant differences among the group means.

ing expression of a novel population of hippocampal NMDA receptors with altered sensitivity to competitive NMDA receptor antagonists (Kraus et al., 1994). When studied 28 days after the last fully kindled seizure, there was a 2.8-fold increase in the number of binding sites for the competitive NMDA receptor antagonist [^{3}H]CPP (3-(2-carboxypiperazine-4-yl)-propyl-1-phosphonate) in CA3 area of the hippocampus compared to nonkindled controls, whereas no change was seen in the binding of another competitive NMDA receptor antagonist, [^{3}H]CGS 19755 (*cis*-4-(phosphonomethyl)-2-piperidinecarboxylate), indicating that kindling induced the expression of a novel population of NMDA receptors that is recognized by [^{3}H]CPP but not by [^{3}H]CGS 19755 (Kraus et al., 1994). If this receptor is involved in the enhanced susceptibility of kindled rats to amphetamine-like behavioral adverse effects of D-CPPene and CGP 37849, it might be possible to develop competitive NMDA receptor antagonists that do not share this unfavorable side effect profile, at least in the kindling model. In view of the fact that the data on CGP 37849 in kindled rats predicted the enhanced susceptibility of epileptic patients to adverse effects of D-CPPene (Löscher and Schmidt, 1994), competitive NMDA receptor antagonists lacking enhanced amphetamine-like side effects in kindled rats might be interesting candidates for further development. In this respect, it should be noted that alterations in the pharmacology of NMDA receptor antagonists in amygdala kindled rats were not only found with CGP 37849 (Löscher and Hönack, 1991c) and D-CPPene (present study), but also with the uncompetitive, low-affinity NMDA receptor antagonists memantine and dextrorphan (Löscher and Hönack, 1990; Löscher and Hönack, 1993), and the glycine/NMDA receptor antagonist (+)-HA-966 ((+)-3-amino-1-hydroxypyrrolid-2-one) (Wlaź et al., 1994).

Since in the clinical trial on D-CPPene in epileptic patients, plasma D-CPPene levels were approximately twice that expected from normal volunteer data, the discrepancy between volunteers and patients in the frequency and severity of adverse effects may be explained by pharmacokinetic differences between the two populations, e.g., due to the co-medication of epileptic patients with standard antiepileptic drugs (Sveinbjornsdottir et al., 1993). However, as mentioned in Section 1, volunteers tolerated doses up to 2000 mg/d, i.e., twice as high as the maximum doses given in epileptic patients, thus pointing to pharmacodynamic factors such as increased susceptibility to NMDA blockade as the reason for the unexpected adverse effects in epileptic patients. This is substantiated by the finding that there was no clear relationship between plasma concentration and the severity of side effects in epileptic patients (Sveinbjornsdottir et al., 1993). In this respect, it is also interesting to note that dose-response studies with CGP 37849 in kindled and nonkindled rats showed that while this drug induced no stereotyped behaviors such as head weaving even at a high ataxiogenic dose of 40

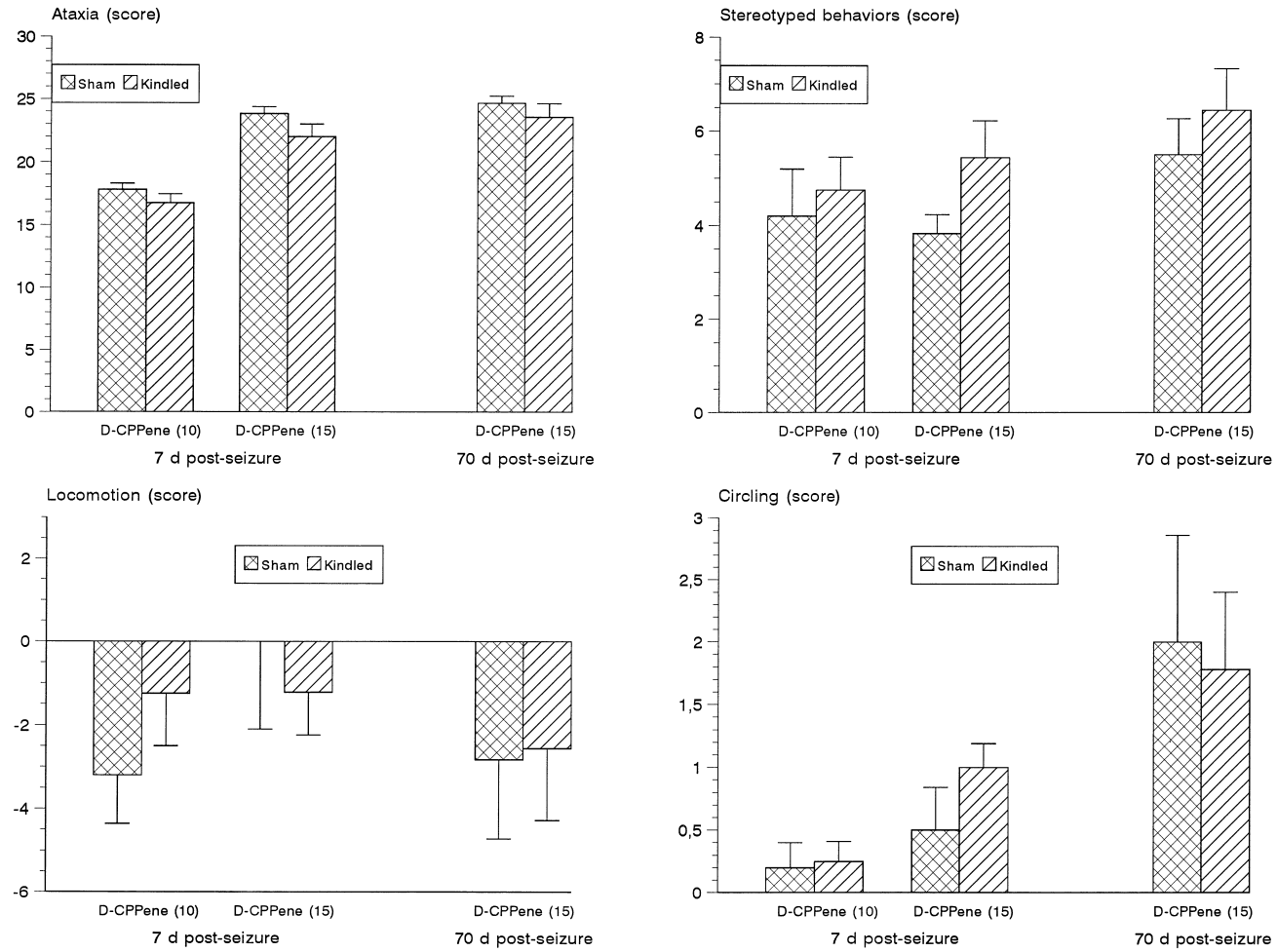

Fig. 7. Ataxia, stereotyped behaviors (head weaving, piano playing, stereotyped sniffing, or stereotyped face washing), alterations in locomotion, and circling induced by D-CPPene in pentylenetetrazole kindled and nonkindled ('sham') rats. D-CPPene was injected in fully pentylenetetrazole kindled rats after either 7 or 70 days following the last kindled seizure; doses in mg/kg are indicated in brackets. The intensity of ataxia was scored at 15, 30, 60, 120 and 240 min after i.p. drug injection. Each score was summed over the five observation periods and the individual summed scores were used for calculation of group means + S.E.M. Group sizes were 8–9 (kindled) and 5–6 (sham), respectively. Evaluation of data by *U*-test did not indicate any significant differences between kindled and sham rats.

mg/kg in nonkindled rats, all age-matched kindled rats exhibited intense stereotypies already at 20 mg/kg, again indicating that this difference between kindled and nonkindled rats cannot be explained by pharmacokinetics (Löscher and Hönack, 1991c). One might argue that drug penetration into the brain is altered by effects of kindled seizures on blood–brain barrier function, but this is unlikely to explain the present findings on D-CPPene in kindled rats 6 months after the last fully kindled seizure.

A further difference between volunteer and patient studies with D-CPPene was that, while volunteers received the drug alone, epileptic patients received a combined (add-on) treatment with D-CPPene added to the already existing treatment with standard antiepileptics such as carbamazepine, phenobarbital, phenytoin, valproate, or clobazam (Sveinbjornsdottir et al., 1993). Thus, pharmacokinetic or pharmacodynamic interactions between D-CPPene and standard antiepileptics may have been involved in the increased adverse effects observed in epileptic patients. In this respect, previous experiments with combined treatment of mice with D-CPPene and conventional antiepileptics are of interest (Zarnowski et al., 1994). While D-CPPene, 1 mg/kg, did not influence plasma levels of carbamazepine, diazepam, phenytoin, phenobarbital, or valproate, combined treatment increased motor impairment in response to single drug treatment, except in case of car bamazepine. However, in most cases the increase in adverse effects was purely additive (Zarnowski et al., 1994), which cannot explain the severe adverse effects of combined treatment with D-CPPene and antiepileptics in epileptic patients, requiring hospitalization in six of eight cases (Sveinbjornsdottir et al., 1993). The findings of the present study and previous (Löscher and Hönack, 1991a,b,c) studies on competitive NMDA receptor antagonists in kindled rats seem to provide another reason for the severe adverse effects observed in patients with focal

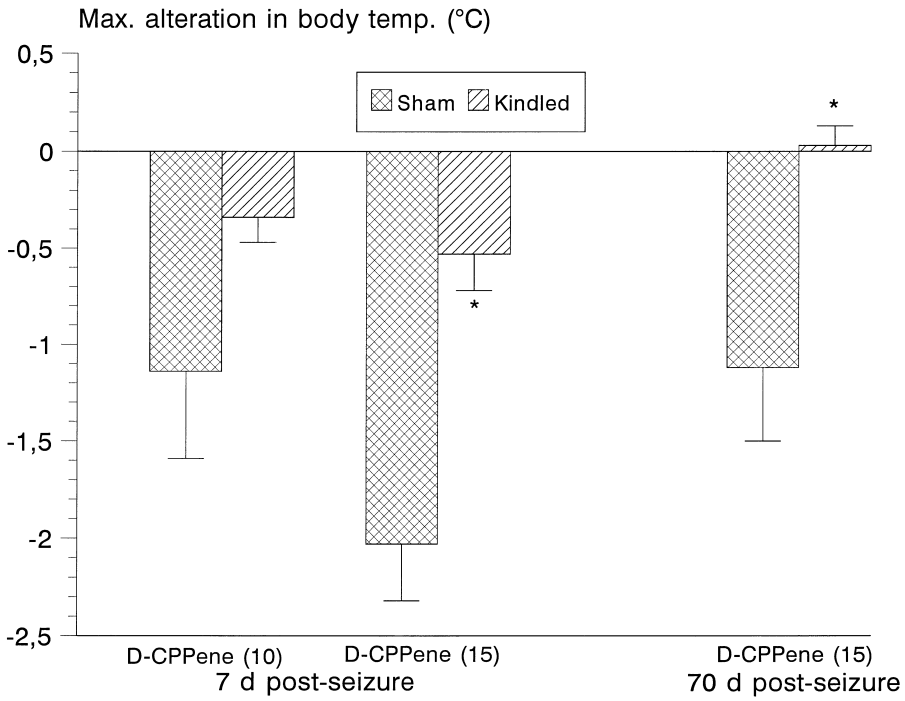

Fig. 8. Alterations in rectal body temperature induced by D-CPPene in pentylenetetrazole kindled and nonkindled ('sham') rats. Data are shown as maximal alteration in post-drug values from predrug control for each group of rats. D-CPPene was injected in fully pentylenetetrazole kindled rats after either 7 or 70 days following the last kindled seizure; doses in mg/kg are indicated in brackets. Body temperature was measured before and 15, 30, 60, 120, and 240 min after i.p. drug injection. The maximum alteration (mean ± S.E.M.) from predrug control was usually recorded 2 h after drug administration. Group sizes were 8–9 (kindled) and 5–6 (sham). Evaluation of data by *t*-test indicated significant differences between groups in case of the two experiments with 15 mg/kg D-CPPene ($P = 0.0005$ and 0.004, respectively), which are indicated by stars. The P value from the two-tailed *t*-test of the experiment with 10 mg/kg D-CPPene was 0.0585.

epilepsy treated with both D-CPPene and antiepileptic drugs.

Whereas our present and previous studies indicate that limbic epileptogenesis as induced by amygdala kindling renders the brain more susceptible to some PCP-like behavioral effects induced by competitive NMDA receptor antagonists such as D-CPPene or CGP 37849, studies in genetically epilepsy-prone rats did not indicate an enhanced susceptibility of these animals to such effects of NMDA receptor antagonists, including CGP 37849 or CPPene (De Sarro and De Sarro, 1992; De Sarro and De Sarro, 1993; De Sarro et al., 1996). As suggested by the latter authors, the history of epileptogenesis of genetically epilepsy-prone rats with audiogenic seizures where auditory pathways are mostly involved is different from that of kindled rats where limbic structures are principally involved (De Sarro and De Sarro, 1992). This may render audiogenic seizure-susceptible rats less susceptible to PCP-like adverse effects of competitive NMDA receptor antagonists. In fact, in these epilepsy-prone rats with generalized tonic–clonic seizures upon audiogenic stimulation, CPPene did not induce marked excitatory effects, such as increased locomotion, stereotyped behaviors or circling, in combination with a pronounced ataxia, as noncompetitive NMDA receptor antagonists did (De Sarro and De Sarro, 1993). These differences between kindled and audiogenic seizure-susceptible rats may indicate that patients with different forms of epilepsy (e.g., focal vs. generalized) will exhibit different susceptibility to certain PCP-like adverse effects of competitive NMDA receptor antagonists. If one assumes that kindling by pentylenetetrazole is a model of another type of epilepsy than amygdala kindling (Löscher, 1998a), the lack of increased PCP-like adverse effects of D-CPPene in pentylenetetrazole kindled rats would seem to substantiate that different types of epilepsy may differ in their sensitivity to adverse effects in response to competitive NMDA receptor antagonists.

In conclusion, the present study substantiated and extended previous observations that amygdala kindling increases the sensitivity to some PCP-like adverse effects induced by competitive NMDA receptor antagonists. Both changes at the level of NMDA receptors and downstream from these receptors, e.g., at the level of dopamine/serotonin turnover in the basal ganglia, which is under glutamatergic influence, may be involved in this clinically relevant functional alteration caused by kindling-induced epileptogenesis. We are currently performing experiments in which dopamine and serotonin turnover and release are studied by striatal microdialysis in kindled and sham-kindled rats to determine whether the hypersensitivity of kindled rats to some adverse effects of D-CPPene is related to an enhanced monoamine turnover in the kindled brain.

Irrespective of the mechanisms of this phenomenon, the available data indicate that models with chronic brain dysfunction, such as amygdala kindling, should be involved in the characterization of adverse effect profiles of novel NMDA receptor antagonists (Wlaź and Löscher, 1998; Löscher, 1998b,c).

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