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Effects of the NMDA receptor antagonist D-CPPene on extracellular levels of dopamine and dopamine and serotonin metabolites in striatum of kindled and non-kindled rats

Heidrun Potschka, Maren Fedrowitz, Wolfgang Löscher *

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

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Abstract

Electrical kindling in rats has previously been shown to cause a hypersensitivity to amphetamine-like behavioral effects of competitive NMDA receptor antagonists such as D,L-(*E*)-amino-4-methyl-5-phosphono-3-pentenoic acid (CGP 37849), D-(*E*)-2-amino-4-methyl-5-phosphono-3-pentenoic acid (CGP 40116), or 3-(2-carboxypiperazine-4-yl)propenyl-1-phosphonate (SDZ EAA 494; D-CPPene). From this observation, it was concluded that kindling-induced epileptogenesis enhances the potential of competitive NMDA receptor antagonists to induce such unwanted adverse effects, predicting that such drugs may induce more severe side effects in epileptic patients than in healthy volunteers, which was confirmed in clinical trials. In the present study, we thought to examine the biochemical basis for the enhanced susceptibility of kindled rats to amphetamine-like behavioral effects of NMDA receptor antagonists by measuring extracellular levels of dopamine, the dopamine metabolites dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA), and the serotonin (5-hydroxytryptamine, 5-HT) metabolite 5-hydroxyindoleacetic acid (5-HIAA) in the striatum of awake, behaving rats, using in vivo microdialysis. When administered systemically, D-CPPene, 15 mg/kg i.p., caused more intense stereotyped behaviors in kindled than in non-kindled rats. While there was no significant alteration in extracellular dopamine, in both groups of rats HVA and 5-HIAA significantly increased. In kindled rats, basal levels of HVA and the increase in HVA in response to D-CPPene were higher compared to non-kindled animals. When administered intrastriatally via the microdialysis probe, D-CPPene, 10 μM, significantly increased dopamine, HVA and 5-HIAA, which was associated with stereotyped behaviors. Again, these behaviors were more intense in kindled rats. The data indicate that a competitive NMDA receptor antagonist at high, behaviorally active doses induces increases in striatal dopamine and presumably also 5-HT release, which most likely underlie the amphetamine-like behavioral effects of such a drug. Kindling enhances the sensitivity to these behavioral effects, which could be related to a more marked dopamine and 5-HT release. Thus, in order to avoid false predictions for the clinical situation, it is important to study the behavioral and biochemical effects of NMDA receptor antagonists not only in naive, healthy animals but also in animals that mimic the disease for which a drug is developed.

Keywords: Glutamate; Epilepsy; Caudate–putamen; 5-HT (5-hydroxytryptamine, serotonin)

1. Introduction

Non-competitive and, at high doses, competitive NMDA receptor antagonists induce a behavioral syndrome in rodents which consists of amphetamine-like (e.g., hyperlocomotion, stereotypies) and barbiturate-like (e.g., motor impairment) symptoms (Willetts et al., 1990). The same amphetamine-like stereotyped behaviors are also observed when competitive or non-competitive NMDA receptor antagonists are injected into the dorsal striatum (caudate–putamen) or the nucleus accumbens (Schmidt, 1986; Schmidt and Bury, 1988; Imperato et al., 1990; Alkhatib et al., 1995). The hyperlocomotion and the stereotyped behaviors induced by NMDA receptor antagonists can be blocked by dopamine D_1 and D_2 receptor antagonists, α_1-adrenoceptor antagonists, and partial agonists/antagonists of 5-hydroxytryptamine (5-HT) receptors of the 5-HT_{1A} subtype (Löscher and Hönack, 1991b, 1992, 1993; Behrens and Gattaz, 1992; Ouagazzal et al., 1993; Alkhatib et al., 1995; Corbett et al., 1995; Lapin and Rogawski, 1995; Mathe et al., 1996), indicating that these

* Corresponding author. Tel.: +49-511-953-8721; Fax: +49-511-953-8581; E-mail: wloscher@pharma.tiho-hannover.de

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behaviors are mediated by increased monoaminergic activity. Indeed, using conventional brain homogenate assays, both non-competitive and competitive NMDA receptor antagonists have been reported to increase dopamine and serotonin metabolism in several brain regions of rats, including striatum and nucleus accumbens which are thought to mediate stereotyped behaviors (Nabeshima et al., 1984; Hiramatsu et al., 1989; Rao et al., 1990a,b; Löscher et al., 1991, 1993; Ping et al., 1992; Bowers and Morton, 1994; Dai et al., 1995; Hadjiconstantinou et al., 1995; Wedzony et al., 1996).

Using microdialysis as a means to determine extracellular levels of monoamines and their metabolites in rats in vivo, non-competitive NMDA receptor antagonists such as phencyclidine (PCP), dizocilpine (MK-801) or memantine were shown to increase dopamine levels in striatum and/or nucleus accumbens after systemic or local administration (Carboni et al., 1989; Chapman et al., 1990; Imperato et al., 1990; Steinpreis and Salamone, 1993; Kiss et al., 1994; Spanagel et al., 1994; Mathe et al., 1996; Miller and Abercrombie, 1996), although some studies on MK-801 did not find such an effect (Hubner and Pert, 1990; Whitton et al., 1992a). In several of these microdialysis studies, the dopamine metabolites homovanillic acid (HVA) and dihydroxyphenylacetic acid (DOPAC) were increased by the non-competitive NMDA receptor antagonists, too (Steinpreis and Salamone, 1993; Spanagel et al., 1994; Mathe et al., 1996). Furthermore, one study reported increased extracellular levels of 5-HT in the striatum after systemic administration of MK-801 (Whitton et al., 1992b). In contrast to non-competitive NMDA receptor antagonists, only a few studies examined the effect of competitive NMDA receptor antagonists on extracellular dopamine or dopamine metabolite levels in striatum or nucleus accumbens (Gruen et al., 1990; Imperato et al., 1990; Whitton et al., 1994a,b; Waters et al., 1996). While two reports described that local application of DL-2-amino-5-phosphonovaleric acid (AP-5) and 3-(2-carboxypiperazine-4-yl)propenyl-1-phosphonate (CPPene) increase dopamine levels in striatum (Gruen et al., 1990; Imperato et al., 1990), a more recent study found that D-AP-5 decreased dopamine in this brain region (Whitton et al., 1994a). 5-HT and its metabolite 5-hydroxyindoleacetic acid (5-HIAA) were increased by local application of D-AP-5 to the striatum (Whitton et al., 1994b). Systemic administration of CGS 19755 (*cis*-4-[phosphonomethyl]-2-piperidine carboxylic acid) decreased striatal dopamine levels but increased striatal levels of the 5-HT metabolite 5-HIAA (Waters et al., 1996). At least part of the different results between microdialysis studies on NMDA receptor antagonists could be due to the fact that some studies used anesthetized animals, because anesthesia has been shown to alter the effects of NMDA receptor antagonists on dopamine metabolism in the striatum (e.g., Chapman et al., 1990). Furthermore, the dose of the NMDA receptor antagonist is certainly important in explaining the different results because some studies used these drugs at doses below those inducing behavioral effects.

The aims of the present microdialysis study in conscious, behaving rats were twofold. First, we were interested to determine the effect of the highly selective and clinically used competitive NMDA receptor antagonist D-CPPene (SDZ EAA 494; 3-(2-carboxypiperazine-4-yl)propenyl-1-phosphonate; cf., Herrling et al., 1997) at behaviorally active doses on dopamine and dopamine and 5-HT metabolites in the striatum, both after systemic and local application. Second, because amygdala kindling induces a hypersensitivity to the amphetamine-like behavioral effects of competitive NMDA receptor antagonist such as D-CPPene (Löscher and Hönack, 1991a,b; Löscher, 1998; Wlaz et al., 1998), we wished to examine whether kindling also changes the effect of D-CPPene on extracellular monoamine and metabolite levels in the striatum.

2. Materials and methods

2.1. Animals

Adult female Wistar rats (Winkelmann Versuchstierzucht, Borchen, F.R.G.) were used. The animals were obtained from the breeder at an age of about 10 weeks and adapted to the laboratory conditions for at least 1 week before being used for experiments. 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.). Standard laboratory chow (Altromin 1324 standard diet) and tap water were allowed ad libitum. All experiments were done in the forenoon to minimize the bias of circadian variations.

2.2. Electrode implantation and kindling

At an age of about 12 weeks, a bipolar electrode was implanted into the right basolateral amygdala under anesthesia with chloral hydrate as described elsewhere (Wlaz et al., 1998). Two weeks following electrode implantation, one subgroup of electrode-implanted rats was kindled as described previously (Wlaz et al., 1998). Another electrode-implanted subgroup was not kindled but used as sham control. Some age-matched non-implanted rats served as naive controls.

2.3. Implantation of guide cannulae and microdialysis procedure

Following about 1 year after implantation of the kindling electrodes, i.e., at an age of about 15 months (body weight 275 to 325 g), guide cannulae were implanted into the striatum of kindled and age-matched non-kindled rats.

This period between the two implantations was chosen because we were interested in prolonged (if not permanent) alterations in behavioral and neurochemical alterations in response to kindling. To assure that all kindled rats were still fully kindled after this long period without amygdala stimulations, a stimulation with 500 μA was applied via the amygdala electrode, which induced focal and secondarily generalized (stage 5) seizures in all kindled rats. One day later, surgical procedures were performed under chloral hydrate anesthesia (380 mg/kg). A guide cannula (CMA/12 polyurethane, Carnegie Medicine, Stockholm, Sweden) was implanted in the right striatum (ipsilateral to the kindling electrode) with the tip of the cannula positioned at rostral +0.6, lateral −2.8 and ventral −3.9 mm to bregma, coordinates according to the atlas of Paxinos and Watson (1986). The cannula was held in place with additional anchor screws and dental acrylic cement, which was applied to the exposed skull surface.

Microdialysis experiments were performed following a recovery period of at least 7 days after surgery. The microdialysis probe (CMA/12, 3 mm polycarbonate membrane, cut off 20 kD; Carnegie Medicine) was lowered through the guide cannula to a depth of −6.9 according to bregma, and the rat was placed in a freely moving system. Fourteen to 16 h after insertion, perfusion of the probe was started using Ringer's solution (mM 147 Na^+, 2.3 Ca^{2+}, 4.0 K^+ and 155.6 Cl^-, pH 6.0). The inflow to the probe was driven by a CMA/100 microinjection pump, and each 40 μl outflow sample was collected in a polypropylene tube containing 5 μl of 0.25 M perchloric acid using a CMA/170 refrigerated fraction collector (4°C). The flow rate was 2 μl/min. After 2 h of perfusion to stabilize baseline, six samples were collected over a subsequent period of 2 h. Mean values of these six samples were defined as baseline level for calculation of drug effects. At the end of baseline sampling, rats were injected with D-CPPene (15 mg/kg) intraperitoneally (i.p.) and further 15 samples were collected. This dose was chosen on the basis of recent experiments showing a significant difference in intensity of stereotyped behaviors in kindled vs. non-kindled rats following 15 mg/kg D-CPPene (Wlaz et al., 1998). In this respect, it is also interesting to note that doses of about 10 mg/kg and above are needed to exert anticonvulsant effects in fully kindled rats (Dürmüller et al., 1994).

At least 10 days after this first experiment, a second microdialysis experiment was done in the same rats, but D-CPPene was given locally instead of systemically. All other experimental details were as described above. At the end of the baseline sampling phase, the perfusion medium was changed to Ringer's solution containing 10 μM D-CPPene, and further 12 samples were collected. This concentration of D-CPPene was based on previous experiments of Imperato et al. (1990) with CPPene, in which the perfusion of the striatum with 10 μM induced marked increases in extracellular dopamine.

In another group of non-electrode implanted, naive controls rats, a control experiment was performed with systemic (i.p.) administration of vehicle (saline) instead of D-CPPene. All experimental details were as for the experiment with systemic injection of D-CPPene.

When experiments were finished, rats were perfused and correct positioning of guide cannulae and probes (and kindling electrodes) was verified by histological examination. All rats used for the present experiments had a correct placement of probes in the dorsal striatum (caudate–putamen).

2.4. High pressure liquid chromatography (HPLC) analysis

Dialysate samples were stored at 4°C until analysis, which was done as rapidly as possible after sampling (within a maximum of 60 min). For analysis, we used a modification of a method previously described by us for the concomitant determination of monoamines and their metabolites (Löscher et al., 1993). Samples were injected without further purification into a high performance liquid chromatography apparatus equipped with a reverse phase microbore column (Nucleosil 120 C18, pore size 80 A, 3 μm, BAS, West Lafayette, IN, USA) and an electrochemical detector (LC4C with UniJet cell, BAS) with a glassy carbon electrode. The working electrode was set at 0.675 V with respect to an Ag/AgCl reference electrode, range 1.0 nA. The composition of the mobile phase was 0.3 mM EDTA, 0.44 mM octyl hydrogen sulfate, 10 mM NaCl, 100 mM citric acid monohydrate, 5.5% acetonitrile; pH was adjusted at 3.0. The mobile phase was pumped with a CMA/250 LC Pump (Carnegie Medicine) at a flow rate of 0.08 ml/min. Retention times under these conditions were as follows: DOPAC, 2.31 min; dopamine, 3.03 min; 5-HIAA, 4.43 min; HVA, 5.71 min; 5-HT, 8.78 min.

The limit of detection of the microbore HPLC method used was about 0.5 nM for dopamine and HVA and 1 nM for 5-HT, DOPAC and 5-HIAA, which is comparable with previously published microbore systems (e.g., Carter, 1994). Thus, the fact that extracellular dopamine levels in the striatum were below detection limit in several rats of this study (see Section 3) was not due to a low sensitivity of the analytical procedure, but to uncommonly low dopamine levels in such rats, which may be due to the strain, age and sex of the animals used in this study. Endogenous levels of 5-HT in the dialysis samples could not be measured because they were generally below the detection limit. The amount of the dialysate compounds was determined by comparison of the area under curve values with those obtained with standards, which were run with each experiment. Variability of the method was determined by analyzing 10 times the same sample containing 20 nM of each compound, which gave coefficients of variation between 4.6 and 5.4%.

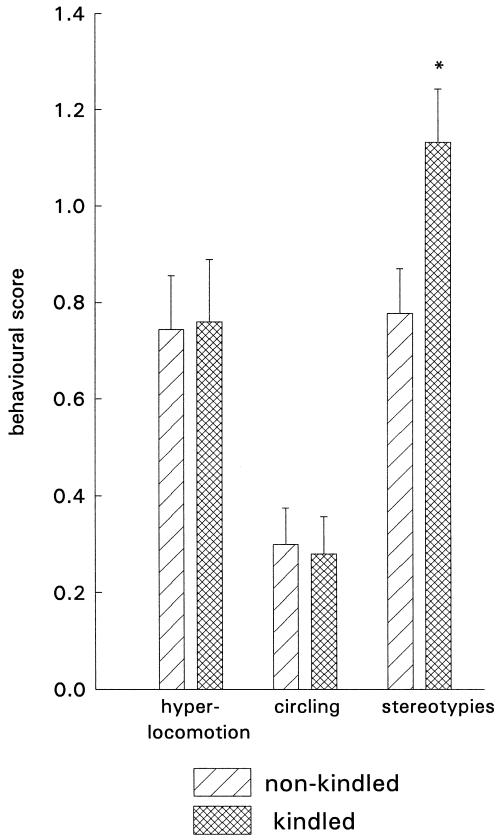

Fig. 1. Behavioral alterations following i.p. D-CPPene application (15 mg/kg i.p.) in non-kindled ($n = 6$) and kindled ($n = 5$) rats. Intensity of behaviors was scored every 20 min according to dialysate sampling. For each animal, the scores from all time points were averaged and these average scores were used for calculation of group means (±S.E.). Significant differences (Mann–Whitney *U*-test) between groups are marked by asterisk ($P < 0.05$).

For each microdialysis probe used for the in vivo experiments, in vitro recovery was determined by dialysis of a stirred solution of 100 nM dopamine, DOPAC, HVA, 5-HT and 5-HIAA. Recovery values were as follows: dopamine, 20%–35%; DOPAC, 22%–38%; HVA, 35%–50%; 5-HT, 20%–35%; and 5-HIAA, 30%–45%.

The ability of the in vivo microdialysis method to measure dopamine release was verified in preliminary experiments in rats with amphetamine (5 mg/kg i.p.), the monoamine oxidase inhibitor pargyline (75 mg/kg i.p.), the dopamine uptake blocker nomifensine (5 μM; added to the perfusion after the baseline period), and high K^+ (50 mM; added to the perfusion after the baseline period). As could be expected from previous studies (e.g., Zetterström et al., 1988), extracellular levels of dopamine were increased by all these pharmacological manipulations.

2.5. Behavioral effects

Behavioral effects of D-CPPene were scored during microdialysis. The animals were observed every 20 min for about 5 min. Head weaving (swaying movements of the upper torso from side to side for at least one complete cycle, i.e., left–right–left), stereotyped sniffing, face washing, circling, and hyperlocomotion were scored using an intensity rank scale where 0 = absent, 1 = equivocal, 2 =

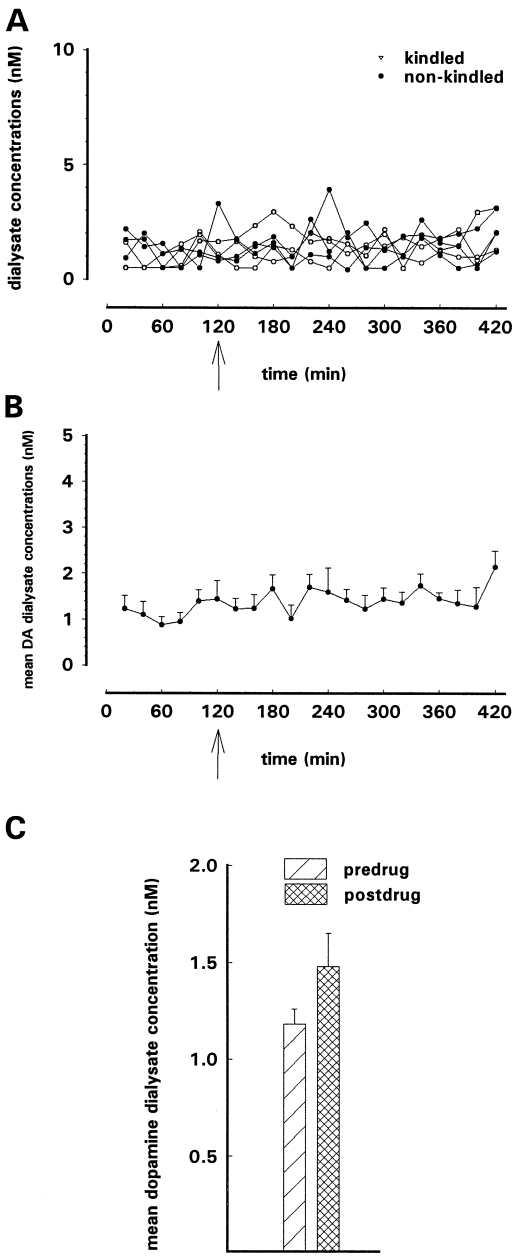

Fig. 2. Effect of i.p. D-CPPene application (15 mg/kg i.p.) on extracellular concentrations of dopamine in the striatum of non-kindled ($n = 3$) and kindled ($n = 3$) rats. In **A**, individual levels of the 6 rats are shown. Time of injection is marked by an arrow. Results are shown as nM absolute dialysate concentrations. In case that a value was below the detection limit of 0.5 nM, the symbol of this value is set at this detection limit. In **B**, means ± S.E. of these individual values are illustrated, using values of all six rats (kindled and non-kindled) for calculation of mean data. Analysis of these data by ANOVA did not indicate any significant difference between means. In **C**, data are presented as mean predrug values and mean postdrug (41–300 min post application) values (mean ± S.E.M.) of the six rats. Pre- and postdrug data did not differ significantly.

present and 3 = intense (Löscher and Hönack, 1992). Ataxia could not be reliably scored in the bowl with the freely moving system used for the microdialysis experiments.

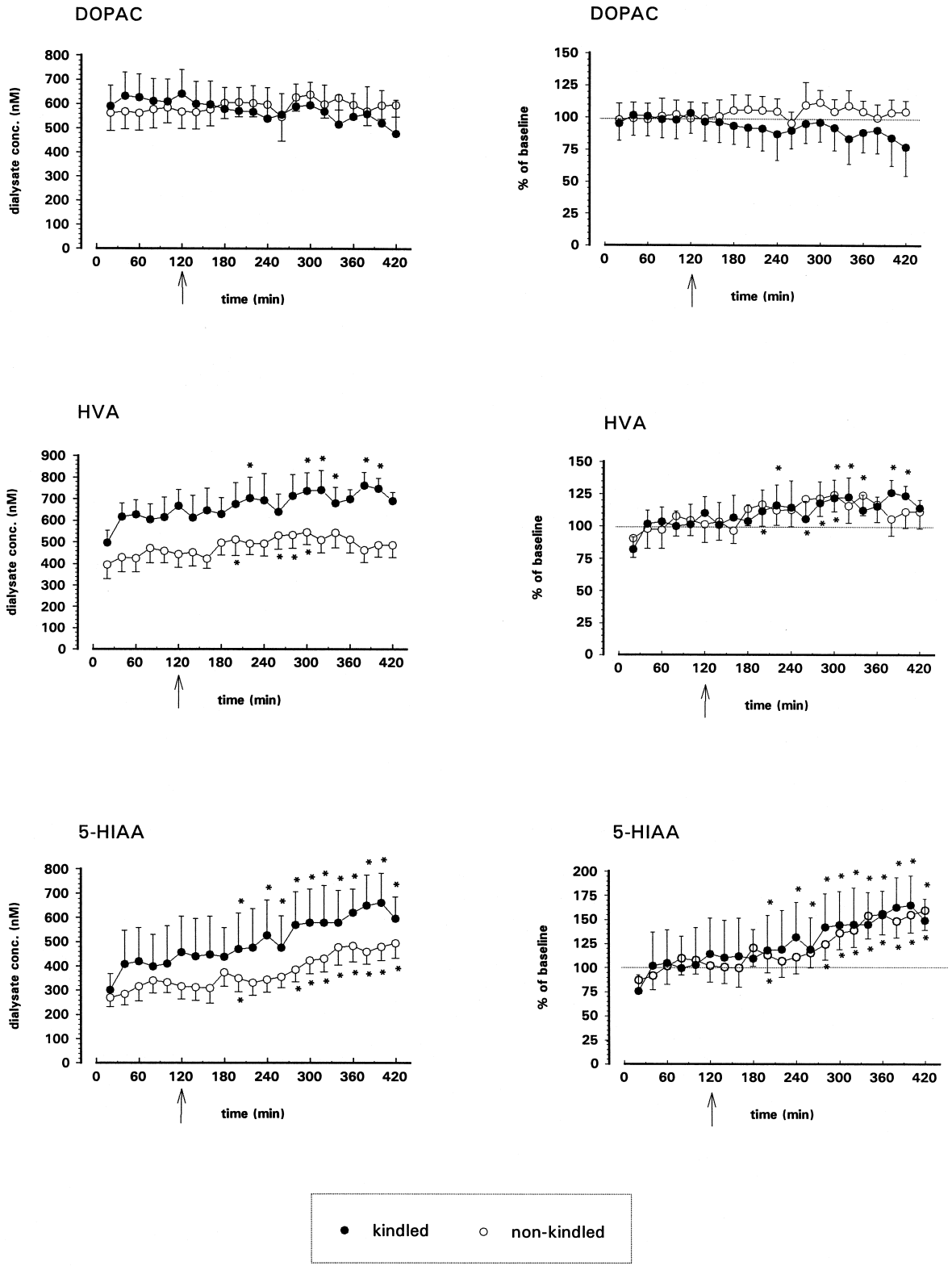

Fig. 3. Effect of D-CPPene injection (15 mg/kg i.p.) on striatal extracellular concentration of DOPAC, HVA and 5-HIAA of kindled and non-kindled rats. Time of injection is marked by an arrow. Results are shown as nM absolute dialysate concentrations (mean ± S.E.M.; $n = 5$ for the kindled group and $n = 6$ for the non-kindled group) as well as mean percent variation ± S.E.M. vs. mean baseline values (set at 100%). Significant differences (ANOVA and Student's *t*-test) of postdrug values in comparison to mean baseline values are marked by asterisk ($P < 0.05$).

2.6. Statistics

Because some sham kindled rats lost their electrode assembly during the long period between kindling and microdialysis and because the remaining sham kindled rats did not seem to differ in their microdialysis data from naive controls, sham kindled and naive control rats were combined to one control group. Group comparisons for behavioral data were done by the Mann–Whitney *U*-test, while all other group comparisons were done by Student's *t*-test for paired or unpaired data. In cases of more than two sets of data, statistical evaluation of neurochemical data within the same group of rats was done by analysis of variance (ANOVA) for paired data. In cases that ANOVA indicated a significant difference between means, Student's *t*-test for paired data was used to compare postdrug data to mean baseline (predrug) data. In order to reduce the number of multiple comparisons, only selected sets of postdrug data were compared with mean predrug data. A $P < 0.05$ was considered significant.

2.7. Drugs

D-CPPene was kindly provided by Novartis (Basle, Switzerland). For systemic administration, the drug was freshly dissolved in distilled water and injected i.p. at a volume of 3 ml/kg. For local application, the drug was freshly dissolved in Ringer's solution.

3. Results

3.1. Effects of systemic administration of D-CPPene

In both control and kindled rats, D-CPPene, 15 mg/kg i.p., induced the typical PCP-like behavioral syndrome with ataxia, hyperlocomotion and stereotyped behaviors, i.e., head weaving, stereotyped sniffing, face washing and grooming. Most of these behavioral effects were rapid in onset (within 10 to 20 min) and were observed over the whole 5 h period of observation. While the intensity of hyperlocomotion and circling was about the same in both groups of rats, differences were observed in stereotyped behaviors (Fig. 1). Thus, when all scores rated in each group were averaged, kindled rats showed significantly more intense stereotypes than non-kindled rats (Fig. 1). When scores of the various observation times were summed and used for mean calculations, mean score for stereotypies was 17.0 ± 1.18 in kindled vs. 11.7 ± 2.82 in non-kindled rats, again showing the higher sensitivity of kindled rats to these behavioral effects of D-CPPene. This was also indicated by the time course of stereotypies, because maximum scores were reached much more rapidly in kindled rats (after 80–100 min following drug injection) than in non-kindled rats (after 280–300 min).

Extracellular dopamine levels in the striatum before and after D-CPPene were below detection limit in several rats and could only reliably measured in three controls and three kindled rats (Fig. 2A). In order to allow statistical evaluation of the effect of D-CPPene on dopamine levels, data of kindled and non-kindled rats were averaged (Fig. 2B). Comparison of postdrug and predrug values by ANOVA did not indicate any significant difference between means. When average predrug values were compared with average postdrug values, there was a trend for slightly increased postdrug values (Fig. 2C) which, however, was not significant.

In contrast to dopamine, its metabolites HVA and DOPAC and the 5-HT metabolite 5-HIAA could be reliably measured in all rats, thus allowing group comparisons before and after injection of D-CPPene. As shown in Fig. 3, DOPAC levels in the baseline (predrug) period were similar in kindled and non-kindled rats and were not significantly affected by D-CPPene, although there was a trend to postdrug decreases in the kindled group. Predrug levels of HVA in kindled rats were significantly higher than those in non-kindled rats (Figs. 3 and 4). Baseline levels of 5-HIAA also tended to be higher in kindled rats,

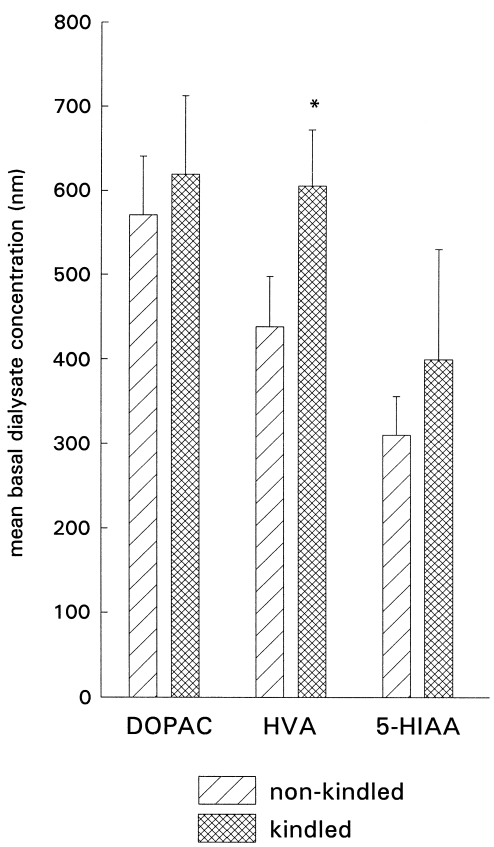

Fig. 4. Basal extracellular concentrations of DOPAC, HVA, and 5-HIAA in the striatum of non-kindled ($n = 6$) and kindled ($n = 5$) rats. Data are presented as mean + S.E.M. values for each group. Significant differences (Student's *t*-test) between groups are marked by asterisk ($P < 0.05$).

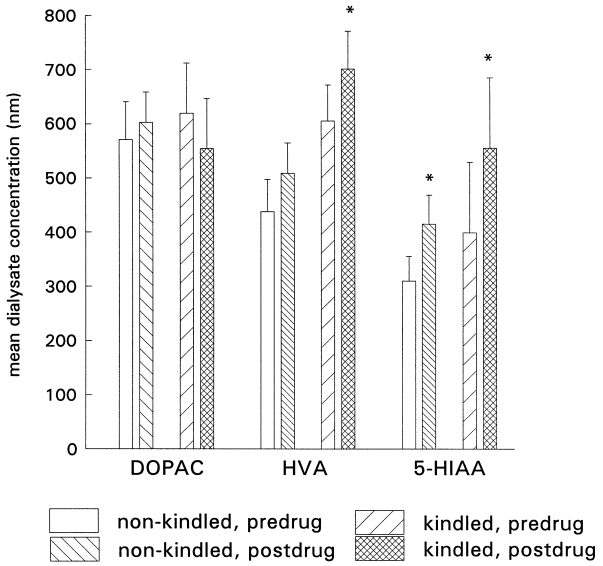

Fig. 5. Effect of i.p. D-CPPene application (15 mg/kg) on extracellular concentrations of DOPAC, HVA, and 5-HIAA in the striatum of non-kindled ($n = 6$) and kindled ($n = 5$) rats. Data are presented as mean predrug values and mean postdrug (41–300 min post application) values ($\pm$S.E.M.) for each group. Significant differences (paired Student's *t*-test) between predrug and postdrug mean values within each group are marked by asterisk ($P < 0.05$). Comparisons between postdrug data of the control group and the kindled group showed that HVA in the kindled group after D-CPPene was significantly higher ($P < 0.05$; unpaired *t*-test) than postdrug HVA levels in controls.

but the difference to control was not significant (Figs. 3 and 4). Compared to within group baseline values, D-CPPene significantly increased extracellular levels of HVA and 5-HIAA in the striatum (Fig. 3). When all pre- and postdrug data were averaged for within group and between group comparisons (Fig. 5), average postdrug 5-HIAA levels were significantly increased in both groups of rats, while HVA was significantly increased only in the kindled group. Furthermore, the postdrug HVA levels in the kindled group were significantly higher than postdrug HVA levels in the control group.

In order to be sure that the increase in monoamine metabolite levels observed after D-CPPene was not secondary to i.p. injection alone or the mere duration of the experiment, a control experiment with vehicle injection in six additional non-kindled rats was undertaken. As shown in Fig. 6, DOPAC, HVA and 5-HIAA levels were stable throughout the experimental period and were not affected by injection of vehicle. Dopamine could only be measured in one of these animals, not indicating any increases over the duration of the experiment (not illustrated).

3.2. Effects of intrastriatal administration of D-CPPene

Continuous administration of D-CPPene (10 μM) via the microdialysis probe induced stereotyped behaviors (head weaving, stereotyped sniffing, face washing and grooming) in both groups of animals, but head weaving and stereotyped sniffing were more frequent in kindled rats (being observed in all kindled rats) than in non-kindled rats (observed in only two animals). Whereas face washing and stereotyped grooming were rapid in onset (starting within 20 min after onset of drug application), head weaving and stereotyped sniffing started after about 40 min in kindled but 80 min in non-kindled rats. Maximum scores

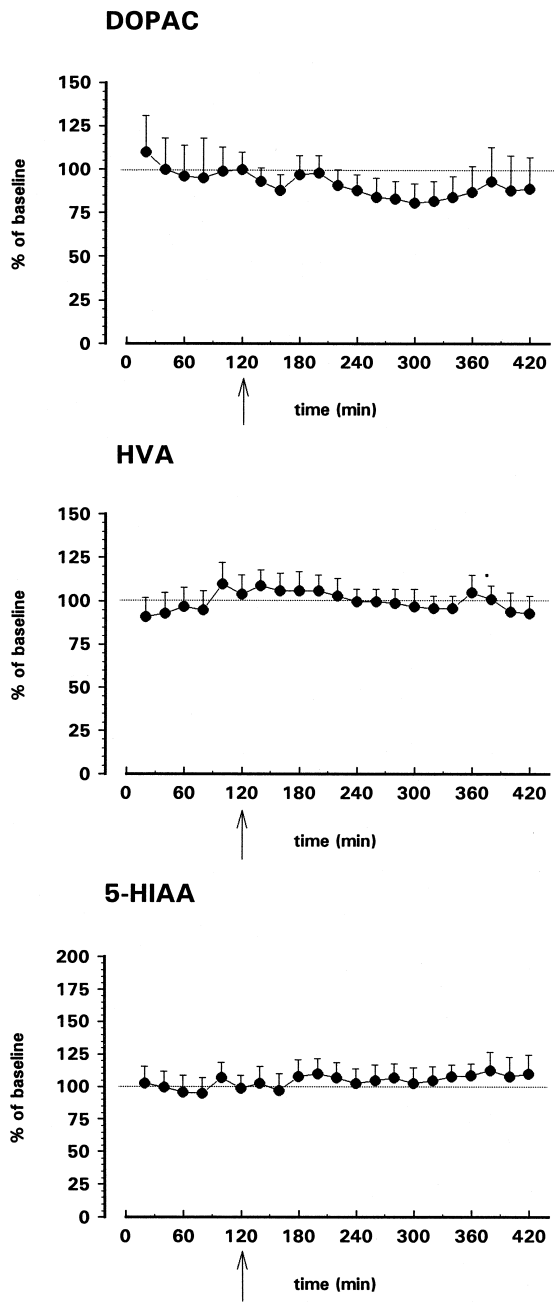

Fig. 6. Effect of i.p. injection of a physiological saline solution (3 ml/kg) at 120 min on the extracellular concentration of DOPAC, HVA and 5-HIAA in the striatum of freely moving rats. Time of injection is marked by an arrow. The results are shown as mean percent variation $\pm$S.E.M. vs. mean baseline values ($n = 6$ for HVA and 5-HIAA values; $n = 5$ for DOPAC values because DOPAC baseline values were stable in only five rats). ANOVA revealed no statistically significant differences of postinjection values in comparison to baseline values.

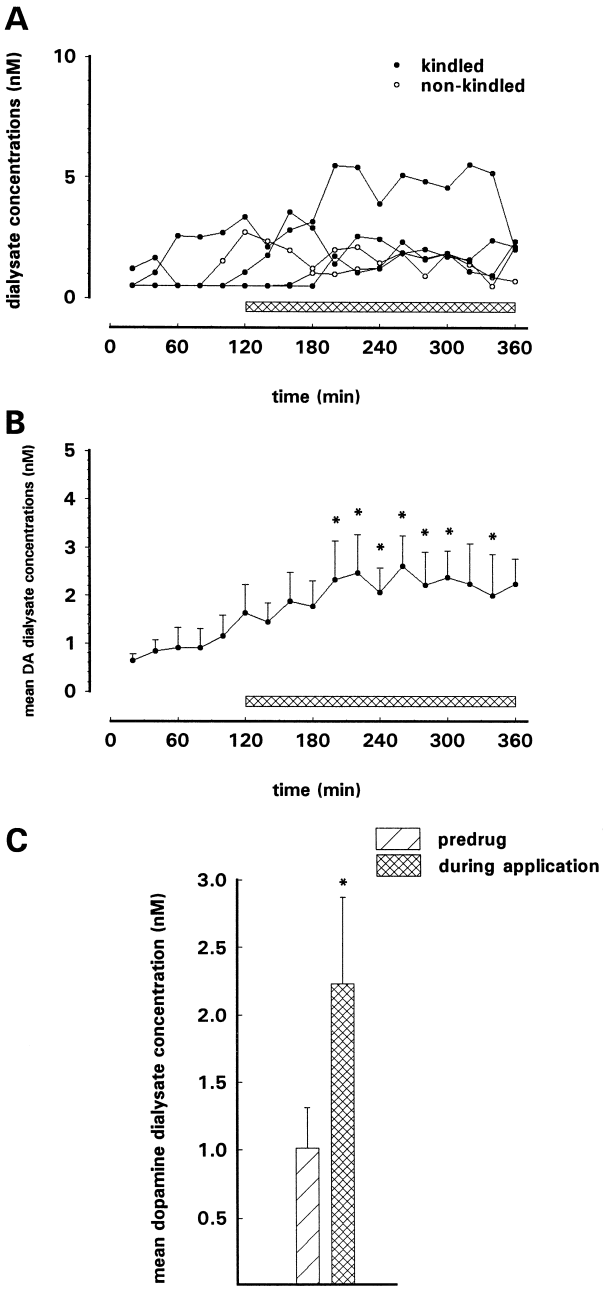

Fig. 7. Effect of D-CPPene infusion (10 μM) into striatum via the microdialysis probe on striatal extracellular concentration of dopamine in the striatum of non-kindled ($n = 2$) and kindled ($n = 3$) rats. In **A**, individual levels of the five rats are shown. Time period of infusion is marked by the horizontal cross-hatched bar. Results are shown as nM absolute dialysate concentrations. In case that a value was below the detection limit of 0.5 nM, the symbol of this value is set at this detection limit. In **B**, means ± S.E. of these individual values are illustrated, using values of all five rats (kindled and non-kindled) for calculation of mean data. Analysis of these data by ANOVA indicated significant differences to baseline in response to drug application; individual differences to mean baseline value are marked by asterisk ($P < 0.05$). In **C**, data are presented as mean predrug values and mean drug (41–240 min after onset of application) values (mean ± S.E.M.) of the five rats. Predrug and drug data differed significantly ($P < 0.05$).

of the stereotyped behaviors were reached after about 80–120 min. The intensity of the stereotyped behaviors induced by intrastriatal drug application were lower compared to those seen after systemic administration. Head weaving and stereotyped sniffing were more frequent and more intense in kindled than in non-kindled rats; cumulative scores were 3.0 ± 0.84 in kindled vs. 0.6 ± 0.4 in non-kindled rats, the difference being significant ($P = 0.037$).

Dopamine could only be reliably measured in two controls and three kindled rats (Fig. 7A). In order to allow statistical evaluation of the effect of D-CPPene on dopamine levels, data of kindled and non-kindled rats were averaged (Fig. 7B). Comparison of drug and predrug values by ANOVA indicated significantly increased dopamine levels in response to drug application. When average predrug values were compared with average drug values, there was a significant increase by 125% in the drug phase (Fig. 7C).

In addition to dopamine, local application of D-CPPene significantly increased HVA and 5-HIAA levels in the striatum in both groups of rats (Fig. 8). The relative

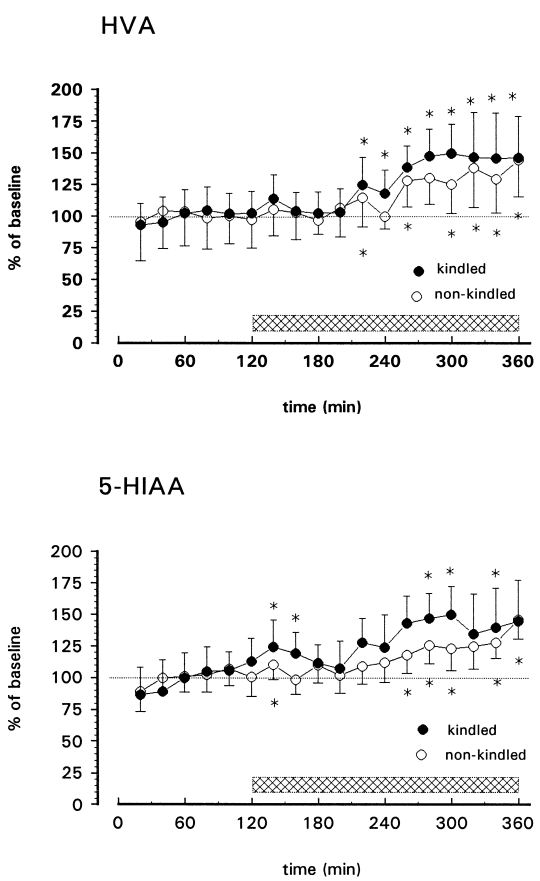

Fig. 8. Effect of D-CPPene infusion (10 μM) into striatum via the microdialysis probe on striatal extracellular concentration of HVA and 5-HIAA of kindled and non-kindled rats. Time period of infusion is marked by the horizontal cross-hatched bar. Results are shown as mean percent variation ± S.E.M. vs. mean baseline values ($n = 5$ for both groups). Significant differences (ANOVA and Student's *t*-test) of values during drug infusion in comparison to mean baseline values are marked by asterisk ($P < 0.05$).

increases above baseline appeared to be more marked in kindled rats (Fig. 8). As in the experiments with systemic administration of D-CPPene, DOPAC levels were not increased by drug application (not illustrated).

4. Discussion

There is some controversy as to whether competitive NMDA receptor antagonists increase dopamine turnover in striatum or nucleus accumbens. Several studies, using systemic injection of DL-(*E*)-2-amino-4-methyl-5-phosphono-3-pentanoic acid (CGP 39551) or CGS 19755, intracerebroventricular (i.c.v.) injection of (±)-3-(2-carboxypiperazin-4-yl)propyl-1-phosphonic acid (CPP), AP-5 or 2-amino-7-phosphonoheptanoate (AP-7), or intrastriatal application of AP-5 found no increases of dopamine or dopamine metabolites (Rao et al., 1991; Bubser et al., 1992; Whitton et al., 1994b; Waters et al., 1996), whereas other studies, using systemic administration of CGP 39551, D,L-(*E*)-amino-4-methyl-5-phosphono-3-pentenoic acid (CGP 37849), or perfusion of striatum and nucleus accumbens with AP-5 or CPPene found an increase in dopamine, dopamine metabolites or dopamine turnover in striatum or nucleus accumbens (Gruen et al., 1990; Imperato et al., 1990; Löscher et al., 1993). Furthermore, increased 5-HT and/or 5-HIAA levels in striatum were reported after CGP 37849, CGS 19755, or D-AP-5 (Löscher et al., 1993; Whitton et al., 1994a; Waters et al., 1996). With respect to the apparent differences between studies in terms of dopamine metabolism or release, it is important to note that all studies which reported increases after competitive NMDA receptor antagonists injected these drugs at doses inducing stereotyped behaviors.

In the present microdialysis study, using systemic or local administration of D-CPPene at doses inducing amphetamine-like stereotypies, a significant increase in extracellular levels of HVA and 5-HIAA was observed in non-kindled and kindled rats, while DOPAC levels were not affected. In the experiment with systemic administration of D-CPPene, no clear effect on extracellular dopamine levels was observed, but the very low levels of dopamine in the rats used for the present study did not allow to reliably measure dopamine in all animals.

HVA is formed extraneuronally either by the action of catechol-*O*-methyltransferase and monoamine oxidase from dopamine via 3-methoxytyramine or by catechol-*O*-methyltransferase from DOPAC. Under control conditions, HVA is mainly formed from DOPAC (Westerink and Korf, 1976), which is produced intraneuronally from dopamine by the action of monoamine oxidase and is thought to be an accurate index of intraneuronal dopamine metabolism (Wood et al., 1987). Microdialysis studies have shown that a major portion of extracellular DOPAC is derived from an intraneuronal pool of newly synthesized dopamine so that extracellular DOPAC levels do not necessarily increase when dopamine release is enhanced (Zetterström et al., 1988; Santiago and Westerink, 1991). Small but functionally relevant increases in dopamine release may be missed by striatal microdialysis, because released dopamine may undergo inactivation processes before reaching the dialysis probe (Pani et al., 1990). Thus, one explanation of the present data after systemic drug administration is that D-CPPene did increase dopamine release (as indicated by increased extracellular HVA), but did not affect dopamine synthesis (as indicated by unchanged DOPAC). In line with this interpretation, we found significant HVA *and* dopamine (but not DOPAC) increases during local (intrastriatal) application of D-CPPene. The latter finding confirms previous experiments of Imperato et al. (1990) who found that perfusion of the striatum with CPPene (1 and 10 μM) significantly increased extracellular levels of dopamine, which was associated with stereotypic behavior.

With respect to dopamine metabolites, drug-induced increases in metabolite concentrations cannot directly be translated to changes in the production rate of these metabolites, because many centrally acting drugs can inhibit the efflux of HVA and DOPAC from the rat brain (Westerink and Kikkert, 1986). However, such an effect is unlikely to explain the present findings with D-CPPene because only HVA but not DOPAC increased in response to this drug, and intrastriatal application of D-CPPene increased HVA *and* dopamine, strongly indicating that the elevation of HVA resulted from increased dopamine release.

In addition to increased release of dopamine, an alternative explanation for the stereotypies seen after intrastriatal and systemic administration of D-CPPene in rats could be an increased serotonergic activity. In line with recent microdialysis experiments showing increased extracellular levels of 5-HIAA after systemic administration of CGS 19755 (Waters et al., 1996) and increased 5-HT and 5-HIAA levels after intrastriatal application of D-AP-5 (Whitton et al., 1994a), we found that D-CPPene significantly increased 5-HIAA after both routes of administration, possibly indicating enhanced synaptic release of 5-HT. In this respect it is important to consider that part of the behaviors, e.g., hyperlocomotion and head weaving, induced by NMDA receptor antagonists are also seen after 5-HT precursors and 5-HT receptor agonists, and that these NMDA receptor antagonist-induced behaviors can be blocked by 5-HT_{1A} receptor antagonists and partial agonists (Löscher and Hönack, 1991b, 1992, 1993).

How can the alterations in functional indices of striatal monoaminergic neurotransmission after administration of NMDA receptor antagonists be explained? With respect to the effects of local (intrastriatal) application of NMDA receptor antagonists seen in the present and previous studies (Gruen et al., 1990; Imperato et al., 1990; Kiss et al., 1994; Whitton et al., 1994a), one explanation is that NMDA receptors can mediate a modulatory inhibitory

control over 5-HT and dopamine release in the striatum, most likely indirectly through activation of a neuronal system exerting an inhibitory action on presynaptic release processes. Indeed, it has been shown that glutamate or NMDA can cause a reduction of 5-HT release in the striatum, probably through activation of γ-aminobutyrate(GABA)-ergic interneurons, and that this effect is blocked by a NMDA receptor antagonist (Becquet et al., 1990). With respect to dopamine release in the striatum, NMDA receptors on dopaminergic nerve terminals are thought to exert a tonic excitatory effect on dopamine release (e.g., Krebs et al., 1991; Moghaddam and Bolinao, 1994), although this view is debated, particularly because of the effects of NMDA receptor antagonists on striatal dopamine release (e.g., Keefe et al., 1993). One possible explanation for increases in striatal dopamine release in response to NMDA receptor antagonists was given by Carlsson and Carlsson (1990) who proposed that inhibition of glutamatergic transmission in the striatum by a NMDA receptor antagonist may lead to a decreased activity in the GABAergic striatonigral neurons, thereby resulting in a decreased inhibition of the nigrostriatal dopaminergic neurons and hence an increased dopamine release in the striatum. In addition to the substantia nigra, the ventral tegmental area could be involved, too. This view is corroborated by the recent finding that both MK-801 and the competitive NMDA receptor antagonist D-(*E*)-2-amino-4-methyl-5-phosphono-3-pentenoic acid (CGP 40116; the biologically active (*R*)-enantiomer of CGP 37849) in doses inducing behavioral effects increased the activity of dopaminergic neurons in the ventral tegmental area and substantia nigra (Wedzony et al., 1996). On the other hand, MK-801 but not ±-CPP or CGS 19755 were found to increase firing rate or burst firing in ventral tegmental area and substantia nigra, although it is not clear whether the competitive antagonists were given at behaviorally active doses (French et al., 1993; Murase et al., 1993).

Another explanation for striatal increases in dopamine release following local or systemic administration of NMDA receptor antagonists would be increased striatal 5-HT release, because 5-HT increases in the striatum have been shown to increase striatal dopamine release (Benloucif and Galloway, 1991). The parallel increase in dopamine/HVA and 5-HIAA seen in the present experiments with D-CPPene would be in line with such a mechanism. A quite distinct explanation for dopamine increases and associated behaviors was given recently by the observation that NMDA receptor antagonists *increase* glutamate release (Moghaddam and Adams, 1998) and may therefore produce an increase of dopamine release by potentiating glutamatergic neurotransmission at non-NMDA receptors on striatal dopaminergic terminals (Wang, 1991). Finally, denervation hypersensitivity linked to activation of dopamine turnover may be involved in effects of NMDA receptor antagonists on monoaminergic neurotransmission in the striatum (Reith et al., 1998). In addition to such mechanisms relating behavioral effects of NMDA receptor antagonists to monoaminergic systems, both non-competitive and high doses of competitive NMDA receptor antagonists can induce locomotor stimulation in monoamine-depleted rodents, indicating mechanisms unrelated to monoaminergic systems (Carlsson and Carlsson, 1990).

In the present study, kindled rats appeared to be more sensitive to D-CPPene's behavioral and neurochemical effects than non-kindled rats. We have recently reported that D-CPPene induces more intense stereotyped behaviors in amygdala kindled than in non-kindled rats and that this hypersensitivity of kindled rats is long-lasting, being present for at least 6 months after establishment of kindling (Wlaz et al., 1998). From this observation, which substantiated previous observations of our group with CGP 37849 (Löscher and Hönack, 1991b), its active (*R*)-enantiomer CGP 40116, and other NMDA receptor antagonists (cf., Löscher, 1998), we concluded that epileptogenesis as initiated by kindling induces a hypersensitivity of kindled rats to dopaminergic effects of NMDA receptor antagonists, which is consistent with the lasting dopaminergic supersensitivity in the kindled rat brain suggested by various other experimental observations (cf., Adamec, 1990). This conclusion is substantiated by the present observations, because kindled rats had higher baseline HVA levels and reached significantly higher HVA levels after systemic administration of D-CPPene than non-kindled rats. There was also a tendency for more marked increases in 5-HIAA. To our knowledge, this is the first microdialysis study in amygdala kindled rats showing alterations in the dopaminergic system and its pharmacological sensitivity in the dorsal striatum. Strecker and Moneta (1994) have previously reported that hippocampal kindling increases extracellular dopamine in the nucleus accumbens. More recently, Dazzi et al. (1997) showed that chemical kindling with pentylenetetrazole significantly increases basal extracellular concentrations of dopamine in the striatum and nucleus accumbens. Thus, together with the present data on HVA in amygdala kindled rats these studies indicate that kindling enhances the basal activity of dopamine neurons in the ventral and dorsal striatum of rats and suggest that these neurons contribute to the central alterations associated with experimental epilepsy. This conclusion based on data from in vivo microdialysis is in contrast to previous studies examining in vitro dopamine release from slices of dorsal and ventral striatum of kindled rats in which no alterations in electrically or chemically stimulated release of dopamine were found (Mintz et al., 1992; Ohmori et al., 1992).

Several previous reports have shown long-lasting increases in dopamine D_2 receptor densities in the nucleus accumbens and striatum after kindling (Csernansky et al., 1988a,b; Janowsky et al., 1991), which was recently confirmed using in situ hybridization of the mRNA for dopamine D_2 receptors (Gelbard and Applegate, 1994). These data may explain why the dopamine-releasing drug

methamphetamine and the dopamine D_1/D_2 receptor agonist apomorphine induce markedly more intense stereotypies in kindled than in non-kindled rats (Adamec, 1990) and may also explain the increased behavioral sensitivity of kindled rats to the moderate increase in dopamine release induced by NMDA receptor antagonists. Based on such data, Adamec (1990) has proposed that kindling induces a long-lasting hyperdopaminergic malfunction that is relevant to epileptic psychosis.

In conclusion, the present in vivo microdialysis study on D-CPPene in awake, behaving rats substantiates that competitive NMDA receptor antagonists at high, behaviorally active doses induce similar increases in striatal dopamine and presumably also 5-HT release than non-competitive NMDA receptor antagonists, which most likely underlie the amphetamine-like behavioral effects of such drugs. Kindling enhances the sensitivity to these behavioral effects, which could be related both to a more marked dopamine and 5-HT release and to an increased dopamine D_2 receptor density in the striatum of kindled rats. We currently extend our microdialysis studies on NMDA receptor antagonists to the nucleus accumbens. While effects of NMDA receptor antagonists on neurotransmitters in the striatum may be useful in the treatment of basal ganglia disorders such as Parkinson's disease (Carlsson and Carlsson, 1990; Di Chiara et al., 1994), they may cause unacceptable adverse effects in other brain diseases, particularly when the disease itself predisposes the brain to such adverse effects, as suggested for epilepsy (Löscher, 1998; Löscher and Hönack, 1991a; Löscher and Schmidt, 1994) and, more recently, for stroke (Löscher et al., 1998). Indeed, at doses that had been well tolerated in phase I studies in healthy volunteers, competitive NMDA receptor antagonists such as D-CPPene or CGS 19755 induced severe adverse effects in patients with epilepsy or stroke (Sveinbjornsdottir et al., 1993; Grotta et al., 1995), confirming the prediction from kindling and stroke rat models (Löscher, 1998; Löscher et al., 1998). Thus, as corroborated by the present experiments, it is important to study behavioral and biochemical effects of drugs not only in naive, healthy animals but also in animals that mimic the disease for which a drug is developed.

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