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# Pentylenetetrazol-kindling Modulates Stimulated Dopamine Release in the Nucleus Accumbens of Rats

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BECKER, A., G. GRECKSCH, W. THIEMANN AND V. HÖLLT. *Pentylenetetrazol (PTZ)-kindling modulates stimulated dopamine release in the nucleus accumbens of rats.* PHARMACOL BIOCHEM BEHAV **66**(2) 425–428, 2000.—Kindling-induced activation of dopaminergic neurones in the nucleus accumbens in pentylenetetrazol (PTZ)-kindled rats was studied using microdialysis. Dopamine (DA) release after PTZ challenge was measured: (1) two weeks and (2) ten weeks after kindling completion and (3) two weeks after a kindling procedure with diazepam (DZP) treatment. In (1) a significant increase in DA concentration was found after PTZ challenge and this increase was still evident 10 weeks after kindling completion (2). Coadministration of DZP in the course of kindling development inhibited the increase in sensitivity of the accumbens dopaminergic system (3). © 2000 Elsevier Science Inc.

Pentylenetetrazol Kindling Microdialysis Dopamine Rat

KINDLING represents an animal model of human epilepsy that is characterized by a high degree of clinical relevance (7,10). Beside the convulsive component of epilepsy it reflects secondary alterations in behavior such as learning and memory which are detectable up to 6 weeks after kindling completion (1–3). Complex modifications in central excitability should well correlate with specific alterations in neurophysiologic and neurochemical parameters. Indeed, after pentylenetetrazol (PTZ)-kindling a specific type of long-term potentiation in the hippocampus of PTZ-kindled rats that is related to the kindling status but not to the severity of seizures (9) as well as a long-lasting increase in glutamate binding (still detectable 9 weeks after kindling completion) and increased glutamate concentrations in the brain were found (11–14).

Recently, Dazzi et al. (5) investigated mesocortical, accumbens, and nigrostriatal dopaminergic neurones with the transversal microdialysis technique. Four days after the last chronic administration of PTZ, the basal extracellular concentration of dopamine (DA) was increased. In reaction to a subconvulsive challenge dose of PTZ, DA concentrations were significantly increased in the prefrontal cortex and the nucleus accumbens (Nacc) relative to that in the control group. Alterations in dopaminergic neuronal regulation in these structures would partly explain impairments in learning performance in kindled rats and increased locomotor activity after challenge with subconvulsive PTZ doses.

However, in the study by Dazzi et al. (1997) rats from both groups (i.e., saline-injected control rats and PTZ-kindled rats) were challenged with 20 mg/kg PTZ (5). By definition, the term "kindling" also includes a long-lasting, if not permanent, hyperexcitability and increased seizure activity. In consequence, kindled rats will exhibit more severe seizures in reaction to challenge than control animals. One might argue that the differences in DA release as measured in the study are due to different levels of seizure severity in reaction to the same challenge dose.

Mesolimbic structures are important in the modulation of learning processes and motor functions. To investigate DA release in the Nacc in kindled and saline-injected rats in more detail, the animals were challenged at different times (i.e., 2 or 10 weeks) after kindling completion with equieffective PTZ doses. Additionally, rats were kindled with diazepam administration prior to each PTZ injection were subjected to microdialysis.

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## METHOD

Ethical approval was sought according to the requirements of the National Act on the Use of Experimental Animals (Germany).

### *Animals*

Experiments were carried out with male Wistar rats (Shoe:Wist[Shoe], Tierzucht Schönwalde GmbH) aged 7 weeks at the beginning of the experiments. The animals were kept under controlled laboratory conditions (light regime of 12h light/12 h dark, light on at 0600 h, temperature 20 ± 2°C, 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 per cage (Macrolon IV).

### *Experiments*

Three experiments were carried out. In Experiment 1, PTZ-stimulated DA release in the Nacc was measured 2 weeks after kindling completion to test whether changes in dopaminergic regulation are also detectable in Wistar rats. Experiment 2 was aimed at measuring the persistence of increased sensitivity of dopaminergic systems. For that purpose, animals were kindled as described below and microdialysis was performed 10 weeks after kindling completion. Finally, in Experiment 3 we measured stimulated DA release in kindled rats with diazepam (DZP) pretreatment during kindling. Previously, it was shown that DZP prevents PTZ induced seizures and kindling related cell loss but not the kindling-induced learning deficit. This experiment was done 2 weeks after kindling completion. At this time DZP was expected to have been cleared. Sham-kindled rats were challenged with 30 mg/kg PTZ whereas kindled rats were challenged with 20 mg/kg PTZ. Resultant seizures were scored as described below.

Per group 5–6 animals were used.

### *Kindling*

For kindling a dose of 37.5 mg/kg body weight PTZ (Carl Roth GmbH), i.e., $ED_{16}$ related to clonic seizures, was injected intraperitoneally (IP) in a volume of 1 ml/100 g body weight once every 48 h. After each injection the convulsive behavior was observed for 20 min. The resultant seizures were classified as follows:

Stage 0: no response
Stage 1: ear and facial twitching
Stage 2: myoclonic jerks without rearing
Stage 3: myoclonic jerks, rearing
Stage 4: turn over into side position, clonic-tonic seizures
Stage 5: turn over into back position, generalised clonic-tonic seizures

In total, rats received 13 kindling injections. The animals were considered to be kindled after reaching at least 3 consecutive stage 4 or stage 5 seizures. Control animals received the same number of sal injections, injection volume was 1 ml/100 g body weight.

In Experiment 3, the animals were dosed with 2.5 mg/kg diazepam (Faustan, Arzneimittelwerk Dresden GmbH) IP 30 min prior to the kindling or sham-kindling injection.

### *Implantation*

Rats were anaesthetised with pentobarbital (40 mg/kg IP) and placed in a Kopf stereotactic frame. A loop dialysis probe (outer diameter 0.6 mm, 2-mm tip length, molecular weight cut-off 4.000, Cuprophan hollow fibre, Akzo Nobel Faser AG) was inserted into the right Nacc according to the coordinates relative to bregma: AP + 2.7 mm, lateral 1.7 mm, and ventral 7.2 mm with bregma 2 mm above lambda. The probe was fixed to the skull with dental cement. It was attached via a fluid swivel (CMA/120) to a CMA/100 Microdialysis Pump.

### *Microdialysis*

Dialysis was performed after a recovery period of 24 h with sterile Ringer solution at a flow rate of 0.5 μl/min overnight and it was increased to 1.0 μl/min the next morning. After a 60 min period, samples were collected every 20 min and analysed within 2 min after collection by Gynkotek HPLC with electrochemical detection (working electrode was set at 850 mV against Ag/AgCl reference electrode). Separation was achieved on a reverse phase YMC HPLC Column (size 150 × 3.0 mm, 5 μm 120A packing, YMC Europe GmbH). The mobile phase (flow rate 0.4 ml/min, Gynkotek High Precision Pump Model 300) contained 1.3 mM octanesulphonic acid, 3.2 mM phosphoric acid, 10 mM $NH_4H_2PO_4$, 0.1 mM Na-EDTA and 27 w% methanol. Detection limit for DA under these conditions was 5 fmol/injection.

At completion of the experiments, the animals received an chloral hydrate overdose. The animals were transcardially perfused with formaldehyde (8%) and the brains were removed. Cryostat sections were cut to verify the probe location.

### *Statistical Analysis*

Seizure scores were analyzed by the Mann/Whitney U-test. DA baseline was defined as a difference $< 5\%$ between two consecutive samples preceding the sample that was collected immediately before the experimental treatment. In order to assess effects of the treatment, DA concentrations were represented as 100% of the baseline value. For statistical analysis, these data and kindling scores were subjected to analysis of variance with repeated measures and post hoc Student-Newman-Keuls test (SPSS+ software). A probability of 0.05 was used as the threshold for statistical significance.

## RESULTS

Repeated injections of PTZ resulted in increasing seizure activity culminating in generalized clonic-tonic seizures (Fig. 1). Animals pretreated with DZP reached lower seizures scores ($F[1, 15] = 11.68$, $p = 0.004$).

Kindled rats challenged with 20 mg/kg PTZ and control rats challenged with 30 mg/kg PTZ reacted with similar seizure scores (Fig. 2, inserts). The differences are insignificant (Experiment 1: $U = 7.5$, $p = 0.31$; Experiment 2: $U = 12.5$, $p = 0.56$; Experiment 3: $U = 11.5$, $p = 0.82$).

There were no significant differences in basal DA levels between the experimental groups. In Experiment 1, DA concentrations differed significantly ($F[1, 7] = 21.56$, $p = 0.002$). In reaction to PTZ challenge, DA release was elevated 40 min after challenge and the climax was 80 min after challenge. Afterwards, DA release decreased gradually, Fig. 2 (1). In Experiment 2 similar curves were measured 10 weeks after kindling completion ($F[1, 7] = 10.84$, $p = 0.009$), Fig. 2 (2). That means that changes in increased sensitivity of the dopaminergic system in Nacc is still evident. The difference between the kindled group in Experiment 1 and Experiment 2 is insignificant ($F[1, 7] = 0.4$, $p = 0.543$).

As expected, pretreatment with 2.5 mg/kg diazepam depressed the occurrence of seizures after repeated PTZ in-

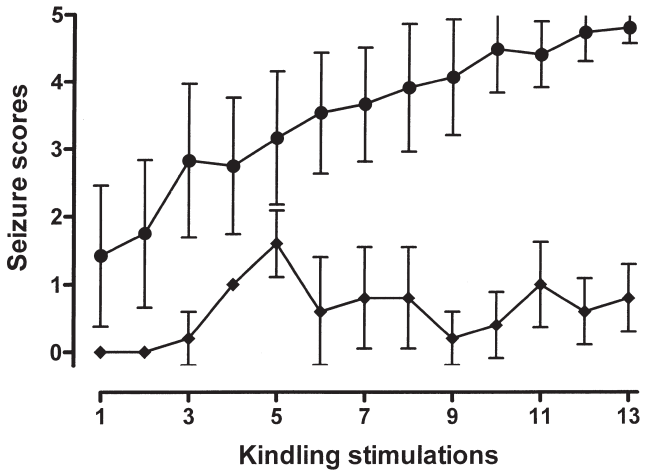


FIG. 1. Development of kindled seizures in rats. (black circle) Prior to each PTZ injection rats received saline ($n$ =11). (black diamond) Prior to each PTZ injection rats received 2.5 mg/kg diazepam ($n$ =6) mean seizure stage ± SEM, $F_{1,15}$= 11.68, $p$ = 0.004.

jections. These animals did not differ in DA release compared to saline pretreated kindled rats ($F$[1, 7] = 0.85, $p$ = 0.5), Fig. 2 (3).

## DISCUSSION

In reaction to PTZ challenge DA release in the Nacc of kindled rats increased and the maximum was +31.2 ± 8.7% relative to basal value, Fig. 2 (1). This corresponds well with data by Dazzi et al. (1997) (5) who found a maximum increase of +36%. Comparing kinetic parameters of PTZ-stimulated DA increase, there is a high degree of concordance although in the study by Dazzi et al.(1997) (5) maximum output was 20 min after challenge whereas in our study maximum peak was 80 min after PTZ challenge. It is speculated that different rat strains contribute to this difference. Strain differences in encephalic dopaminergic neuronal regulation and the period between kindling completion and the microdialysis might also explain similar basal DA levels as measured in present study.

It was hypothesized that at least two different mechanisms contribute to an enhancement of DA output, i.e., decreased inhibitory modulation mediated by $GABA_A$ receptors and enhancement of excitatory systems (4,6,8). It was shown that L-glutamate binding was increased up to 9 weeks after kindling completion in the hippocampus and cortical regions of rats (13) caused by an increase in metabotropic glutamate receptor density (12). This long-lasting enhancement in susceptibility of the glutamatergic system might explain the increased DA release in the Nacc of kindled rats as found in our experiments 10 weeks after kindling completion, Fig. 1 (2).

DZP coadministration in the course of kindling induction potently suppressed motor seizures (1,3) and as shown in Fig. 1 (3), there was no increase in DA release after PTZ challenge. Interestingly, co-adminstration of DZP during kindling initiation did not counteract worsened shuttle-box learning (1,3). This suggests that kindling-impaired shuttle-box performance seems to be independent of increased sensitivity of dopaminergic accumbens systems.

It might be argued that DZP pretreatment inhibited the developmental component of kindling. However, to obtain similar seizure scores in kindled rats a lower PTZ challenge dose was used. Moreover, it was shown that kindling-induced

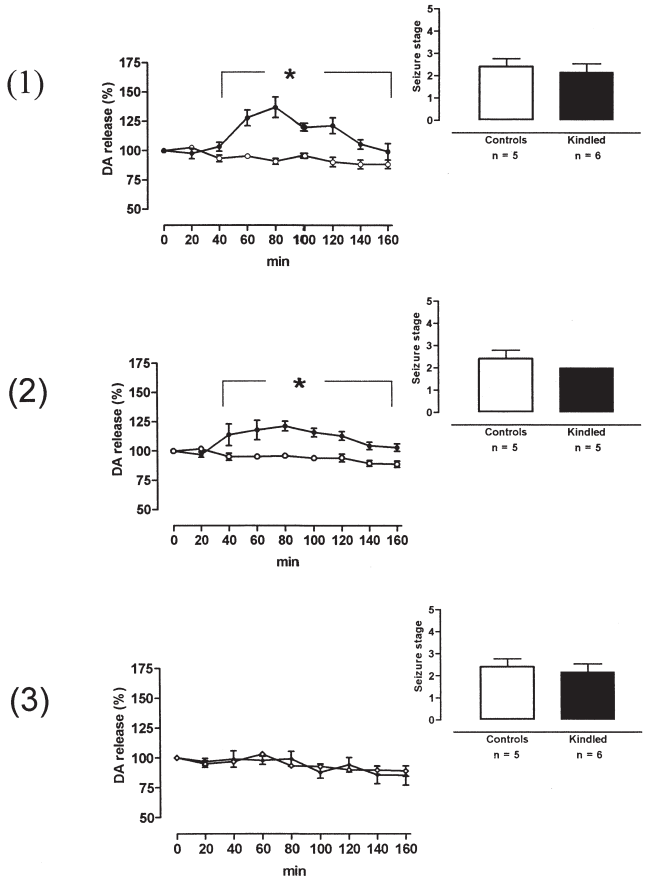


FIG. 2. Time-course of pentylenetetrazol (PTZ)-stimulated dopamine release (percentage of basal values ± SEM) in the Nucleus accumbens of freely moving kindled (black circle) and control (white circle) rats. Insert: seizure stage (mean ± SEM) in reaction to PTZ challenge either 20 mg/kg in kindled or 30 mg/kg in control (open bar) rats. Data are presented as means ± SEM: (1) dialysis was performed 2 weeks after kindling completion; (2) dialysis was performed 10 weeks after kindling completion; and (3) prior to each kindling stimulation rats were dosed with 2.5/kg Diazepam (black diamond) and control rats (white diamond) received the identical number of Diazepam and saline injections. Dialysis was performed two weeks after kindling completion. * $p < 0.05$ (Student-Newman-Keuls test).

potentiation effects occurred regardless of the PTZ pretreatment (3). This clearly suggests that DZP is actually depressing PTZ-induced seizures but it is not capable to suppress all components of the kindling process.

In conclusion, the increase in dopamine release in the Nacc of kindled rats is not due to different levels of seizure severity in reaction to challenge. Moreover, this enhanced sensitivity of Nacc dopaminergic system is detectable up to 10 weeks after kindling completion. DZP given prior to each PTZ kindling injection inhibited kindling effects on dopaminergic systems in the Nacc. This suggests that modulation of mesolimbic dopaminergic systems might partly contribute to learning impairments associated with kindling.

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