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RESEARCH NOTE

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Sensitivity and density of glutamate receptor subtypes in the hippocampal formation are altered in pentylenetetrazole – kindled rats

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Abstract Kindling induced by 13 intraperitoneal injections of 40 mg/kg pentylenetetrazole (PTZ) over a period of 4 weeks resulted in a significant long-lasting increase in both the convulsive susceptibility of animals to the convulsant and the density of the specific [^{3}H]-L-glutamate binding sites in the hippocampus. The quisqualate- and kainate-sensitive [^{3}H]-L-glutamate binding sites were increased 24 h after the final PTZ injection, whereas the *N*-methyl-D-aspartate (NMDA)-sensitive sites had only a tendency to be enhanced. Furthermore, we investigated [^{3}H]-L-glutamate binding on metabotropic receptors and found a significant increase in the hippocampus following PTZ kindling. In addition, in hippocampal tissue of kindled rats (±)-1-aminocyclopentane-*trans*-1,3-dicarboxylic acid (*trans*-ACPD)-stimulated inositol phosphate formation is increased. It can be concluded that the increase in metabotropic glutamate receptor (mGluR) density may be the expression of a specific enhancement in susceptibility of the glutamatergic systems to this excitatory amino acid developing in the course of PTZ-induced kindling.

Key words Chemical kindling · Glutamate binding sites · NMDA · mGluR · Inositol triphosphate

Introduction

Kindling is among the most prominent forms of enduring changes in neuronal excitability after repeated applications of an initially subconvulsive electrical or chemical stimulus to animals, and results in the progressive development of behavioral convulsive activity (Goddard et al. 1969; Mason and Cooper 1972). This enhanced seizure susceptibility represents a long-lasting – probably lifelong – altered neuronal excitability (Cain 1989) and is dependent on activation of glutamate receptors. These receptors are crucial for fast synaptic signal transduction in the central nervous system and play an important role in processes of neuronal plasticity such as long-term potentiation (LTP), learning and memory, as well as in neurodegenerative diseases including ischemia and seizure-related brain damage (Bliss and Collingridge 1993; Meldrum 1994; Green and Greenamyre 1996).

Chemically induced kindling in rats was first described by Mason and Cooper (1972) using pentylenetetrazole (PTZ) as convulsant. However, the question whether the mechanisms underlying electrically and chemically induced kindling are similar or different remains open. Previously we have shown that L-glutamate binding was increased in the hippocampus and cortical regions of PTZ-kindled rats (Schröder et al. 1993, 1994). In this context, an altered *N*-methyl-D-aspartate (NMDA)- and non-NMDA glutamate receptor density and/or receptor subtype gene expression as well as an increased glutamate release in target neuron populations has been measured after electrically induced kindling (Geula et al. 1988; Wu et al. 1990; Kraus et al. 1996). To our knowledge there are no data concerning L-glutamate subreceptor characterization in the hippocampal formation of PTZ-kindled rats.

The aim of the present study was therefore, to determine L-glutamate binding to receptor subtypes in the hippocampal formation of PTZ-kindled rats in comparison with saline-treated controls. In addition, *trans*-ACPD-stimulated inositol phosphate formation was measured.

Materials and methods

PTZ kindling

For all procedures followed, ethical approval was sought prior to the experiments according to the requirements of the National Act on the Use of Experimental animals (Germany). The experiments were performed using 8-week-old male Wistar rats (Schönwalde, Germany). Over a period of 4 weeks, animals were intraperitoneally inject-

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ed with subconvulsive doses of PTZ (40 mg/kg, each second day) as described by Becker et al. (1992). After injections the seizure activity was scored for 20 min (Becker et al. 1992).

The animals were considered to be kindled after having received 13 PTZ injections and after suffering at least three consecutive stage 4 or 5 seizures (clonic-tonic seizures). One week after the last PTZ kindling injection these rats received a final PTZ injection (35 mg/kg, i.p.) to check the persistence of enhanced susceptibility to the convulsant. Only animals showing stage 4 and more seizures were used for the binding assay. Controls received saline alone.

To produce an acute PTZ seizure, chronic saline-treated controls were administered with a single convulsive dose of PTZ (60 mg/kg, i.p.). Controls received saline.

Binding experiment

One day after the final PTZ or saline injection, animals were decapitated, hippocampi were dissected out and crude membrane fractions were prepared. [^{3}H]-L-glutamate (specific activity: 1.43 TBq/mmol, NEN-Dupont) binding was assayed in membrane fractions from hippocampi as described by Schröder et al. (1993). The membranes were incubated in 30 mM TRIS-HCl buffer (pH 7.4) containing 2.5 mM $CaCl_2$ for 40 min at 37 °C. The following incubation procedures were carried out: (i) an excess of unlabelled quisqualate and kainate (NMDA-sensitive sites), (ii) an excess of unlabelled NMDA and kainate (quisqualate-sensitive sites) or (iii) an excess of unlabelled NMDA and quisqualate (kainate-sensitive sites) were added (all of them 100 µM) to limit [^{3}H]-L-glutamate binding to the corresponding binding sites. Nonspecific binding (about 25%) was determined in the presence of 100 µM L-glutamate. The metabotropic glutamate binding sites were detected by incubation of [^{3}H]-L-glutamate with an excess of NMDA and 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX; 100 µM) saturating the ionotropic binding sites (Becker et al. 1996). Nonspecific binding was determined in the presence of 10 µM *trans*-ACPD. The reaction was terminated by rapid filtration through GF/A glass fiber filters. The washed filters were taken for liquid scintillation counting.

In a proportion of the probes the specific binding of [^{3}H] MK 801 and [^{3}H] CGP 39653 to hippocampal synaptic membranes of control and kindled rats was determined by the method described above using 100 µM unlabelled ligand for the determination of the non-specific binding.

[^{3}H]inositol phosphate formation

The dissected hippocampi of saline- and PTZ-treated rats were sliced (300×300 µm) with a McIllwain tissue chopper. The slices were incubated in carbon-gassed medium (mM: 4.7 KCl, 24.9 $NaHCO_3$, 10 glucose, 118.5 NaCl, 2 $MgSO_4$, 2 $CaCl_2$, 1 KH_2PO_4, pH 7.4) at 37 °C; aliquots of gravity-packed slices were preincubated in medium containing 2.5 µCi *myo*[2-^{3}H]inositol (specific activity 21 Ci/mmol, NEN Dupont) to label inositol lipids. After a further 90 min the medium was replaced with medium containing 10 mM LiCl and 100 µM *trans*-ACPD. Twenty minutes later the incubation was stopped by addition of 10% perchloric acid. After sonication and centrifugation the resulting pellet was used for protein determination as described by Lowry et al. (1951). The neutralized (5% KOH) supernatants were transferred to BioRad anion exchange resin AG 1×8 columns and eluted according to Berridge and Irvine (1984).

Statistical analysis

Data were calculated as means±standard error of the means (SEM). Statistical comparisons were made using the Mann-Whitney *U*-test with significance being set at the $P<0.05$ level.

Results

The development of PTZ-induced kindling is shown in Fig. 1. From the seventh up to last PTZ injection "fully" kindled rats responded with stage 4 or 5 seizures.

The specific [^{3}H]-L-glutamate binding to hippocampal synaptic membranes displayed a significant enhancement in kindled rats compared with controls (Table 1). However, acute application of a convulsive dose (stage 5) of PTZ (60 mg/kg i.p.) did not alter the density of glutamate binding sites (saline control, 2229±139 fmol/mg protein; PTZ-treated rats, 2172±127 fmol/mg protein; n=6).

The density of [^{3}H]-L-glutamate binding sites was assayed in the presence of an excess of the corresponding unlabelled drugs that differentiated the binding at NMDA, quisqualate and kainate binding sites, respectively. The quisqualate- and kainate-selective [^{3}H]-L-glutamate binding to membrane fractions of kindled rats was significantly increased in the hippocampus; the NMDA fraction showed only a tendency to be enhanced (Table 1).

The specific binding of [^{3}H]-MK 801 and [^{3}H]CGP 39653 [207±12/224±5 and 71±6/75±6 fmol/mg protein, saline/PTZ respectively) to hippocampal synaptic membranes of control and kindled rats remained unchanged.

To differentiate the quisqualate-selective [^{3}H]-L-glutamate binding the hippocampal membranes were incubated in the presence of NMDA and CNQX, using *trans*-ACPD for the determination of the nonspecific binding. The me-

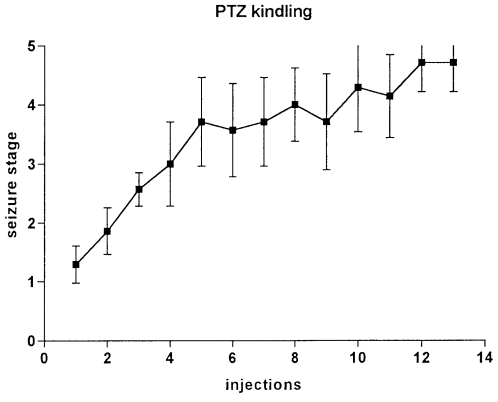

Fig. 1 Development of the susceptibility of rats to pentylenetetrazole (PTZ; 40 mg/kg, i.p.) in the course of kindling (means±SEM, n=7)

Table 1 The specific total, NMDA-, quisqualate- and kainate-sensitive [^{3}H]-L-glutamate binding to crude synaptic membranes of hippocampus of saline-treated (control) and PTZ-kindled rats (in fmol/mg protein, mean±SEM, n=6)

	Saline	PTZ
Total	3816±215	4608±243*
NMDA	1012±183	1242±221
Quisqualate	2731±231	3191±165*
Kainate	913±113	1199±115*

*$P<0.05$ saline compared with PTZ

Table 2 The total and metabotropic receptor [^{3}H]-L-glutamate binding to synaptic membranes (in fmol/mg protein, mean±SEM, *n*=6) and the *trans*-ACPD-stimulated [^{3}H]inositol phosphate formation in hippocampal slices after PTZ kindling (in dpm/mg protein, mean±SEM, *n*=7)

	Saline	PTZ
[^{3}H]-L-glutamate binding		
Total	2539±258	3038±266*
mGluR	1796±123	2123±105*
Inositol triphosphate formation		
Control	5394±363	5433±338
trans-ACPD	7042±501†	8257±513†
	130%	152%

* $P<0.05$ saline compared with PTZ
† $P<0.05$ *trans*-ACPD effect of saline compared with PTZ

tabotropic [^{3}H]-L-glutamate binding as well as the total specific [^{3}H]-L-glutamate binding to hippocampal membrane fractions of kindled rats was shown by this method to be significantly increased (Table 2).

In a further series of experiments (Table 2) it was shown that *trans*-ACPD stimulated inositol phosphate formation was significantly higher in hippocampal slices from kindled rats (152%) in comparison with saline-treated animals (130%).

Discussion

We have previously found a long-lasting increase in glutamate binding that was detectable 9 weeks after the completion of PTZ kindling (Schröder et al. 1994). In this study the principal finding was that the quisqualate- and kainate-sensitive glutamate binding sites were increased in crude synaptic membranes isolated from the hippocampus of kindled rats as compared with controls. Furthermore, the discriminated glutamate receptor (mGluR) binding sites from the quisqualate-sensitive [^{3}H]-L-glutamate binding were significantly enhanced in response to PTZ kindling. The enhancement of the measured total glutamate receptor density may be crucially related to an increase in density of the metabotropic receptor demonstrated by the *trans*-ACPD competition. This conclusion is supported by the enhancement of mGluR-mediated *trans*-ACPD-stimulated inositol phosphate formation after PTZ kindling (Table 2). The metabotropic receptors were classified into three groups (Pin and Duvoisin 1995), coupled either positively with phospholipase C or negatively with adenylate cyclase. Our data can be discussed as group I receptor (mGluR1 and 5) alterations.

The activation of presynaptically located mGluR I has been shown to enhance glutamate release from brain tissue (Cozzi et al. 1996). In earlier studies on rat hippocampus preparations following PTZ kindling we described a significant augmentation of L-glutamate-modulated K^+-stimulated [^{3}H]-D-aspartate release by glutamate itself (Schröder and Becker 1996). On the other hand, postsynaptic mechanisms acting by means of a reduction in K^+ conductances and/or a reduction in inhibitory postsynaptic potentials via postsynaptic receptors can not be excluded. The expression pattern of mGluRs in the hippocampus provides clues to the subtypes of mGluRs that contribute to a large number of functions as well as neurodegenerative events (Fotuhi et al. 1994). After ischemia, for example, phosphoinositide turnover in the hippocampus increases 6-fold (Seren et al. 1989).

The PTZ kindling phenomenon is associated with an enhanced activity state of the metabotropic receptors of the glutamatergic transmission systems. Since acute applications of convulsive doses of PTZ did not alter the specific glutamate binding to hippocampal membranes, the kindling-induced enhancement of glutamate receptor density is assumed to be a correlate of plastic changes of synaptic connectivity due to kindling.

The enhancement in kainate-sensitive binding sites in response to PTZ kindling is difficult to discuss because their specific functional role is still a matter of debate. The molecular entities as well as the functional properties are more diverse than previously believed.

In electrically kindled rats, neurophysiological and binding data clearly demonstrated an involvement of NMDA-type glutamate receptors (Wu et al. 1990; Cain 1989; Kraus et al. 1996). In this respect, we did not find any differences at any time point in the NMDA-sensitive [^{3}H]-L-glutamate or [^{3}H]CGP 39653 and [^{3}H]MK 801 binding sites in hippocampal tissue following PTZ kindling, indicating a different role of the glutamate receptor subtypes in the development of chemically and electrically induced kindling. Further experiments are necessary to clarify the changes in glutamate receptor subtype expression by use of "in situ" or immunohistochemical investigations.

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References

Becker A, Grecksch G, Rüthrich HL, Pohle W, Marx B, Matthies H (1992) Kindling and its consequences on learning in rats. Behav Neural Biol 57:37–43

Becker T, Gombos G, de Barry J (1996) Differential ontogenetic pattern of metabotropic [^{3}H]-L-glutamate receptors in normal and granule cell-deficient mouse cerebellum. Exp Brain Res 107:361–366

Berridge MJ, Irvine RF (1984) Inositol triphosphate: a novel second messenger in cellular signal transduction. Nature 325:315–321

Bliss TVP, Collingidge GL (1993) A synaptic model of memory: long-term potentiation in the hippocampus. Nature 361:31–39

Cain DP (1989) Long-term potentiation and kindling: how similar are the mechanisms? Trends Neurosci 12:6–10

Cozzi A, Lombardi G, Leonardi P, Carfi M, Moroni F (1996) Modulation of transmitter output "in vivo" by mGluR agents. Neuropharmacology 35:A10

Fotuhi M, Standaert DG, Testa CM, Penney JB, Young AB (1994) Differential expression of metabotropic receptors in the hippocampus and entorhinal cortex of the rat. Mol Brain Res 21:283–292

Geula C, Jarvie PA, Logan TC, Slevin JT (1988) Long-term enhancement of K^+-evoked release of L-glutamate in entorhinal kindled rats. Brain Res 442:368–372

Goddard GK, McIntyre DC, Lech CK (1969) A permanent change in brain function resulting from daily electrical stimulation. Exp Neurol 25:295–330

Green JG, Greenamyre JT (1996) Bioenergetics and glutamate excitotoxicity. Prog Neurobiol 48:613–634

Kraus JE, Nadler JV, McNamara JO (1996) Regulation of alternative splicing of NMDAR1 in the kindling model. Mol Brain Res 41:97–104

Lowry OH, Rosebrough RN, Farr AL, Randall RJ (1951) Protein measurement with the folin phenol reagent. J Biol Chem 193:265–275

Mason CR, Cooper RM (1972) A permanent change in convulsive threshold in normal and brain-damaged rats with repeated small doses of pentylenetetrazole. Epilepsia 13:663–674

Meldrum B (1994) The role of glutamate in epilepsy and other CNS disorders. Neurology 44:14–23

Pin JP, Duvoisin R (1995) The metabotropic glutamate receptors: structure and functions. Neuropharmacology 33:715–717

Schröder H, Becker A (1996) The role of glutamate receptors in pentylenetetrazole kindling of rats; a neurochemical study. Neuropharmacology 35:A28

Schröder H, Becker A, Lössner B (1993) Glutamate binding to brain membrane is increased in pentylenetetrazole-kindled rats. J Neurochem 60:1007–1011

Schröder H, Schlichthaar R, Krug M (1994) Changes of glutamatergic synaptic mechanisms after PTZ kindling and LTP. Neuroscience 60:337–342

Seren MS, Aldinio C, Zannoni A, Leon A, Nicoletti F (1989) Stimulation of inositol phospholipid hydrolysis by excitatory amino acids is enhanced in brain slices from vulnerable regions after transient global ischemia. J Neurochem 53:1700–1705

Wu K, Wasterlain C, Sachs L, Siekewitz P (1990) Effect of septal kindling on glutamate binding and calcium/calmodulin-dependent phosphorylation in a postsynaptic density fraction isolated from rat cerebral cortex. Proc Natl Acad Sci USA 87:5298–5302