Pharmacology Biochemistry and Behavior, Vol. 65, No. 4, pp. 743–750, 2000

0091-3057/00/$–see front matter

ELSEVIER

PII S0091-3057(99)00267-1

Kindling Induced by Pentylenetetrazole in Rats is Not Directly Associated With Changes in the Expression of NMDA or Benzodiazepine Receptors

JOHN R. ATACK,* SUSAN M. COOK,* PETER H. HUTSON* AND SANDRA E. FILE†

*Merck Sharp & Dohme Research Laboratories, Neuroscience Research Centre, Terlings Park, Eastwick Road, Harlow, Essex, UK; and †Psychopharmacology Research Unit, Centre for Neuroscience, GKT School of Biomedical Sciences, King's College, London, UK

Received 20 October 1999; Revised 8 December 1999; Accepted 8 December 1999

ATACK, J. R., S. M. COOK, P. H. HUTSON AND S. E. FILE. *Kindling induced by pentylenetetrazole in rats is not directly associated with changes in the expression of NMDA receptors or benzodiazepine binding sites.* PHARMACOL BIOCHEM BEHAV **65**(4) 743–750, 2000.—Repeated injections of a subconvulsant dose of pentylenetetrazole (PTZ, 30 mg/kg IP three times weekly for 13 injections) in Wistar and hooded Lister rats resulted in kindled seizures, the extent of which varied between strains. Wistar rats achieved stage 4 of clonic–tonic seizures, whereas hooded Lister rats only reached stage 2 of convulsive waves axially through the body. Rats were killed 10 days after their final injection, and radioligand binding was used to measure the expression of NMDA receptors in cortex and hippocampus using [^{3}H]MK-801 and [^{3}H]L-689,560, the latter binding specifically to the NR1 subunit. [^{3}H]Ro 15-1788 measured expression of $GABA_A$–benzodiazepine binding sites containing α1, α2, α3, or α5 subunits. Specific analysis of $GABA_A$ receptors containing the α5 subunit, which are preferentially localized in the hippocampus, was assessed with [^{3}H]L-655,708. In the cortex, there was no effect of strain or treatment on the K_D or B_{max} of any of the ligands. Similarly, there was no effect of strain or treatment on hippocampal [^{3}H]L-689,560 or [^{3}H]Ro 15-1788 binding. However, in the hippocampus there was a significant, albeit modest, effect of treatment on the B_{max} of [^{3}H]MK-801 binding and the B_{max} and K_D of [^{3}H]L-655,708 binding, i.e., PTZ-treated rats had fewer [^{3}H]MK-801 and [^{3}H]L-655,708 binding sites (NMDA and α5-containing $GABA_A$ receptors, respectively), but, these reductions were significant only in the relatively seizure-insensitive hooded Lister strain. This suggests that the increased susceptibility to kindling in Wistar rats is not directly related to alterations in the expression of NMDA or $GABA_A$ receptors. © 2000 Elsevier Science Inc.

Strain differences Kindling Seizures Pentylenetetrazole NMDA receptors $GABA_A$ receptors α5 subunit Benzodiazepine binding site [^{3}]MK-801 [^{3}H]L-689,560 [^{3}H]Ro 15-1788 [^{3}H]L-655,708

KINDLING is the term used to describe the phenomenon whereby repeated administration of an initially subconvulsant electrical or chemical stimulus ultimately results in the generation of seizure activity (20). Examples of chemicals producing kindling in rats and mice include pentylenetetrazole, FG 7142, and picrotoxin (10).

Strain differences have been reported in the sensitivity to electrical brain stimulation in rats (20,50) and mice (32). Furthermore, within-strain differences in seizure threshold have been exploited to generate Fast and Slow kindling rats (51). With respect to chemical kindling, Wistar rats have been shown to develop more extensive seizures as a result of PTZ treatment than hooded Lister rats (4,16). Animals with differing kindling sensitivities offer a valuable tool for elucidating the molecular mechanisms underlying this differential vulnerability (34). Thus, changes directly associated with the propensity to kindle should be greater in strains that are more vulnerable.

Requests for reprints should be addressed to Dr. John R. Atack, Neuroscience Research Centre, Merck Sharp & Dohme Research Laboratories, Terlings Park, Eastwick Road, Harlow, Essex CM20 2QR, UK.

The increased susceptibility to seizure activity that occurs in kindling is a model of epilepsy and is long lasting and therefore presumably associated with chronic changes in neuronal excitability. In this regard, much attention has focussed on the excitatory glutamatergic neurotransmitter system (33). Thus, changes in glutamate release and glutamate binding have been reported after either electrical kindling (8,24,68) or chemical kindling with PTZ in rats (55–57) or mice (13). With respect to the NMDA subtype of the glutamate receptor family, NMDA receptor antagonists decrease the electrical and behavioral responses to electrical kindling in the rat amygdala (7,39,53) and inhibit kindling induced in rats or mice by PTZ or FG 7142 (12,19,60). More specifically, NMDA receptor antagonists act by inhibiting the development of kindling rather than suppressing convulsive activity in animals that have been fully kindled, implicating NMDA receptors in the mechanism underlying kindling (53). Furthermore, kindling alters the electrophysiological properties of NMDA receptors (27,40). In addition, MK-801 can block changes in gene expression that occur downstream of direct receptor activation following electrical stimulation of the rat hippocampus (44), underscoring the putative role of the NMDA receptor in the molecular mechanisms of kindling.

The NMDA receptor is a heteromeric complex consisting of NR1 and NR2 subunits (14). The NR1 subunit is ubiquitously expressed throughout the brain, and is essential for NMDA receptor function. In contrast, NR2 subunits (NR2A, -2B, -2C, and -2D) show heterogeneous patterns of expression and confer distinct pharmacological and electrophysiological properties to the NMDA receptor (14). The NMDA receptor contains separate binding sites for the coagonists glycine and glutamate as well as a binding site within the ion channel of the activated receptor, which can be labeled with [^{3}H]MK-801 (67). The glycine binding site, which is thought to be associated with the NR1 subunit, can be labelled using the selective ligand [^{3}H]L-689,560 (21,22).

Although the increased neuronal excitability, which is presumably the basis of kindling, may be associated with increased excitatory, and more specifically NMDA-mediated glutamate, neurotransmission, it may also be linked to decreased inhibitory neurotransmission (6). Compared with the glutamate neurotransmitter systems, the inhibitory GABA system has been less extensively studied. Nevertheless, pharmacological evidence shows that compounds that modulate GABAergic function via the $GABA_A$ receptor alter the susceptibility to PTZ-induced seizure activity in rats (3,11,31,60) and mice (13). In addition, the $GABA_B$ receptor has also been implicated in the development of PTZ-induced kindling in mice (18). Recently, electrophysiological studies of rats selected and bred according to their seizure thresholds following electrical stimulation of the amygdala have shown an alteration in paired-pulse depression but not long-term potentiation or long-term depression, consistent with differences in GABAergic rather than glutamate-mediated neurotransmission between these strains (51).

The $GABA_A$ receptor contains a binding site for the endogenous ligand GABA as well as the so-called convulsant (picrotoxin and TBPS) binding site and a number of modulatory sites that recognize barbiturates, neurosteroids, ethanol, and benzodiazepines (37). Cloning studies have identified 16 $GABA_A$ receptor subunits—α_{1-6}, β_{1-3},γ_{1-3}, δ, ϵ, π, and θ (2)—and in the mammalian CNS the majority of $GABA_A$ receptors are comprised of α, β, and γ subunits (37). The benzodiazepine binding site lies at the interface of an α and γ subunit, and because the γ2 subunit is the predominant γ subunit in the CNS, the benzodiazepine pharmacology of $GABA_A$ receptors in the brain is determined primarily by the α subunit present (37). Hence, the "classical" anxiolytic benzodiazepine agonists such as diazepam and chlordiazepoxide all bind to the benzodiazepine binding site of $GABA_A$ receptors containing either an α1, α2, α3, or α5 subunit. Collectively, this population of benzodiazepine binding sites can be labeled using the benzodiazepine antagonist [^{3}H]Ro 15-1788, which has equivalent affinity for these different subtypes of the $GABA_A$ receptor.

In contrast to [^{3}H]Ro 15-1788, [^{3}H]L-655,708 has 50–100-fold higher affinity for the benzodiazepine site of $GABA_A$ receptors containing an α5 rather than α1, α2, or α3 subunit and can, therefore, be used to selectively label this subpopulation of $GABA_A$ receptors (49). Furthermore, comparison of the properties of recombinant and native α5 subunit-containing $GABA_A$ receptors using [^{3}H]L-655,708 or [^{3}H]RY-80 (which has similar properties to [^{3}H]L-655,708) (59) have demonstrated a pharmacology of the native receptor consistent with an α5β3γ2 subunit composition (59,61). Although α5-containing $GABA_A$ receptors constitute only about 5% of whole brain benzodiazepine binding sites, this subtype is of particular interest because they are most highly expressed in the hippocampus, where they constitute around 20–25% of the total benzodiazepine receptor population (49,59,62). While the role of α5 subunit-containing $GABA_A$ receptors within the hippocampus is not known, the advent of radioligands selective for this subtype should help define their physiological and pathophysiological roles.

In the present study, radioligand binding of [^{3}H]MK-801, [^{3}H]L-689,560, [^{3}H]Ro 15788, and [^{3}H]L-655,708 was measured in the cortex and hippocampus of PTZ-kindled rats. To establish if any changes observed were related to the kindling phenomenon per se, radioligand binding was compared in control and PTZ-kindled rats of the Wistar and hooded Lister strain, because these strains show differences in the extent to which seizures can be kindled by PTZ (4,16).

METHOD

Animals

Male hooded Lister and Wistar rats (Harlan UK, Bicester) were housed in groups of five, with free access to food and water, in a room maintained at 22°C, with lights on from 0700–1900 h. At the beginning of the kindling procedure the rats were 8 weeks of age and weighed 240–260 g (Wistar) and 200–220 g (Lister), respectively. These experiments were conducted in compliance with the UK animal (Scientific Procedures) Act 1986 (License 90/00656).

Drugs and Chemicals

Pentylenetetrazole (Sigma, St. Louis, MO) was dissolved in saline and administered as detailed in the kindling section below. [^{3}H]MK-801 and [^{3}H]Ro 15-1788 were purchased from NEN and Amersham, respectively. [^{3}H]L-689,560 and [^{3}H]L-655,708 were synthesized in-house as described previously (21,49).

Kindling

Within each strain, rats were randomly allocated to the kindled and control (saline-injected) groups. Previous experiments had established no initial strain difference in sensitivity to an acute dose of PTZ (16), and therefore, the same subconvulsant dose (30 mg/kg IP) was used for both strains. After

each injection, the convulsive behavior was observed for 20 min, and classified as follows: stage 0—no response; stage 1—ear and facial twitching; stage 2—convulsive waves axially through the body; stage 3—myoclonic jerks, upright position with bilateral forelimb clonus; stage 4—clonic–tonic seizures; and stage 5—generalized clonic–tonic seizures, loss of postural control.

Rats received 13 injections of PTZ or saline, given three times weekly (on Mondays, Wednesdays, and Fridays). Ten days after the final kindling injection, the animals were killed by decapitation and the brains dissected and frozen.

Radioligand Binding

Radioligand binding was performed essentially as described elsewhere (21,22,49,67). Briefly, rats were killed and the whole cortex and hippocampus rapidly removed and frozen on dry ice. Tissues were subsequently thawed and homogenized in 10 volumes of 10 mM Tris/1 mM EDTA containing 0.32 M sucrose. The supernatant from a low-speed centrifugation (1000 × *g* for 15 min) was then pelleted at 48,000 × *g* for 30 min. This P2 pellet was then resuspended and washed four times in Tris/EDTA buffer before being frozen in a volume equivalent to the initial homogenate volume. On the day of the assay, the P2 pellet was washed a further four times, to ensure the complete removal of endogenous glycine, which interferes with the binding of [^{3}H]L-689,560 to the glycine binding site of the NMDA receptor (21,22).

Extensively washed membranes were then resuspended in Tris/EDTA at a final protein concentration of 1 mg/ml (35) and assayed as described previously (21,22,49,67). For each ligand, binding was measured at two different concentrations (3 and 10 nM for [^{3}H]MK-801 and [^{3}H]L-689,560, and 1 and 3 nM for [^{3}H]L-655,708 and [^{3}H]Ro 15-1788), and the apparent K_D and B_{max} was calculated for each sample using both the untransformed data (curve-fitting performed using XLFit (IDBS, Guilford, UK) with the Hill slope constrained to 1.0) and the data transformed for Scatchard analysis. The average of the values obtained by these two different methods was then used for subsequent statistical analysis.

Statistics

Because the rating scale for seizures provides only ordinal data, the kindling data were analyzed with Mann–Whitney *U*-tests. For the binding data, and because the groups were of unequal sizes, results were analyzed using a general linear model of regression, with strain and treatment as the two variables.

RESULTS

Kindling

As shown in Fig. 1 the Wistar and Lister strains reacted in a similar manner to the first PTZ injection, i.e., there was no evidence for a strain difference in response to an acute injection of PTZ. Both groups showed increasing seizure severity with repeated PTZ injections, but a significant strain difference emerged from the seventh injection onwards. The majority of the Wistar rats reached stage 4 of seizures, but a few reached stage 5. Only one of the Lister rats progressed past stage 2.

Receptor Binding

Tables 1 and 2 shows the K_D and B_{max}, respectively, for [^{3}H]MK-801, [^{3}H]L-689,560, [^{3}H]Ro 15-1788, and [^{3}H]L-655,708 binding in the cortex and hippocampus.

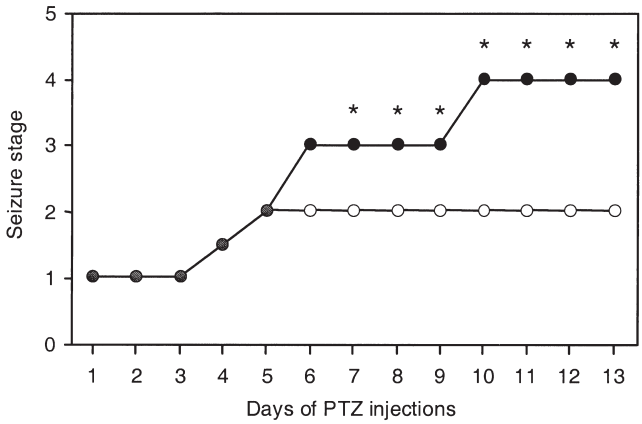

FIG. 1. Median seizure stage reached by hooded Lister (○) and Wistar (●) rats after 13 successive injections of PTZ (30 mg/kg IP), *$p < 0.01$ Wistar vs. Lister rats, Mann–Whitney *U*-test.

Cortex. There was no significant effect of either strain or PTZ treatment on any of the parameters measured in the cortex. Thus, hooded Lister and Wistar rats had the same affinity (K_D) and number (B_{max}) of NMDA receptors ([^{3}H]MK-801 binding), and more specifically the NRI subunit ([^{3}H]L-689,560 binding), with K_D values for [^{3}H]MK-801 and [^{3}H]L-689,560 around 4 and 8 nM, respectively and a B_{max}, which was similar for both ligands, in the region of 1500 fmol/mg protein. Likewise, the expression of the combined population of $GABA_A$ receptors containing an α1, α2, α3, or α5 subunit ([^{3}H]Ro 15-1788 binding) or $GABA_A$ receptors containing an α5 subunit ([^{3}H]L-655,708) was comparable between strains, with respective K_D values of about 1.9 and 1.6 nM, and B_{max} values of 500 and 45 fmol/mg protein, suggesting that in the

TABLE 1

COMPARISON OF K_D VALUES OF DIFFERENT RADIOLIGANDS IN CORTEX AND HIPPOCAMPUS OF VEHICLE-TREATED AND PTZ-KINDLED LISTER AND WISTAR RATS

	Cortex, K_D (nM)			
	Lister		Wistar	
Ligand	Vehicle (n = 8–11)	Kindled (n = 21–25)	Vehicle (n = 9–11)	Kindled (n = 19–22)
[^{3}H]MK 801	4.1 ± 0.2	4.3 ± 0.2	4.4 ± 0.3	3.8 ± 0.2
[^{3}H]L-689,560	8.6 ± 1.4	7.6 ± 0.5	6.7 ± 1.1	9.8 ± 0.8
[^{3}H]Ro 15-1788	2.0 ± 0.1	1.9 ± 0.1	1.8 ± 0.1	2.0 ± 0.1
[^{3}H]L-655,708	1.8 ± 0.5	1.5 ± 0.1	1.8 ± 0.4	1.7 ± 0.3
	Hippocampus, K_D (nM)			
	Lister		Wistar	
Ligand	Vehicle (n = 9–11)	Kindled (n = 20–25)	Vehicle (n = 8–11)	Kindled (n = 17–22)
[^{3}H]MK 801	2.2 ± 0.2	2.2 ± 0.1	2.1 ± 0.1	2.3 ± 0.2
[^{3}H]L-689,560	9.4 ± 2.1	8.1 ± 1.1	7.3 ± 2.1	8.1 ± 1.5
[^{3}H]Ro 15-1788	1.2 ± 0.1	1.4 ± 0.1	1.3 ± 0.1	1.4 ± 0.1
[^{3}H]L-655,708	1.1 ± 0.1	0.7 ± 0.1*	0.9 ± 0.2	0.7 ± 0.1

*$p < 0.05$ compared with vehicle-treated Lister rats.

TABLE 2

COMPARISON OF B_{max} VALUES OF DIFFERENT RADIOLIGANDS IN CORTEX AND HIPPOCAMPUS OF VEHICLE-TREATED AND PTZ-KINDLED LISTER AND WISTAR RATS

	Cortex, B_{max} (fmol/mg Protein)			
	Lister		Wistar	
Ligand	Vehicle (n = 8–11)	Kindled (n = 21–25)	Vehicle (n = 9–11)	Kindled (n = 19–22)
[^{3}H]MK 801	1289 ± 78	1396 ± 74	1396 ± 128	1299 ± 69
[^{3}H]L-689,560	1651 ± 310	1555 ± 101	1500 ± 154	1657 ± 130
[^{3}H]Ro 15-1788	503 ± 30	478 ± 25	514 ± 34	516 ± 22
[^{3}H]L-655,708	39 ± 5	44 ± 4	51 ± 6	46 ± 4
	Hippocampus, B_{max} (fmol/mg Protein)			
	Lister		Wistar	
Ligand	Vehicle (n = 9–11)	Kindled (n = 20–25)	Vehicle (n = 8–11)	Kindled (n = 17–22)
[^{3}H]MK 801	1392 ± 106	1114 ± 62*	1285 ± 81	1195 ± 63
[^{3}H]L-689,560	1784 ± 366	1606 ± 236	1535 ± 429	1670 ± 432
[^{3}H]Ro 15-1788	485 ± 52	530 ± 38	565 ± 44	505 ± 32
[^{3}H]L-655,708	118 ± 12	88 ± 7*	97 ± 8	81 ± 7

*$p < 0.05$ compared with vehicle-treated Lister rats.

cortex the α5 subtype represents around 10% of the total benzodiazepine binding site population. PTZ treatment did not alter the expression of any of these receptor subtypes in the cortex.

Hippocampus. There was no significant effect of strain on the K_D or B_{max} of hippocampal [^{3}H]L-689,560 or [^{3}H]Ro 15-1788 binding. Thus, control (vehicle-treated) rats of both strains had the same affinity and number of NMDA receptors ([^{3}H]MK-801 binding), approximately 2 nM and 1300 fmol/mg protein, and the expression of the NR1 subunit ([^{3}H]L-689,560 binding) was comparable across strains with K_D and B_{max} values in the region of 8 nM and 1600 fmol/mg protein.

Benzodiazepine binding sites labeled by [^{3}H]Ro 15-1788 had equivalent affinities (about 1.3 nM) and expression (B_{max} = approximately 500 fmol/mg) in the two control groups. The affinity of the α5 subunit-containing $GABA_A$ receptors identified using [^{3}H]L-655,708 binding was comparable across groups (around 0.9 nM). In the hippocampus of vehicle-treated animals, the expression of α5 subunit-containing $GABA_A$ receptors (about 100 fmol/mg protein) represents approximately 20% of the total benzodiazepine binding site population (500 fmol/mg protein). Regarding PTZ treatment, there was a significant effect of treatment (vehicle or PTZ) across strains on the number of [^{3}H]MK-801 binding sites ($F(1, 60) = 4.5$, $p < 0.05$) and the affinity ($F(1, 49) = 6.4$, $p < 0.05$) and number ($F(1, 49) = 6.0$, $p < 0.05$) of [^{3}H]L-655,708 binding sites. However, it can be seen in Figs. 2 and 3 that the major contributor to this treatment effect was within the Lister animals. Thus, relative to their respective vehicle-treated controls (Figs. 2 and 3), there were significant reductions in the B_{max} of [^{3}H]MK-801 binding (1392 to 1144 fmol/mg protein = 18% reduction) and the B_{max} (118 to 88 fmol/mg protein = 25% reduction) and K_D of [^{3}H]L-655,708 binding (1.1 to 0.7 nM = 36% increase in affinity) were significantly reduced in PTZ-treated Lister (but not Wistar) rats.

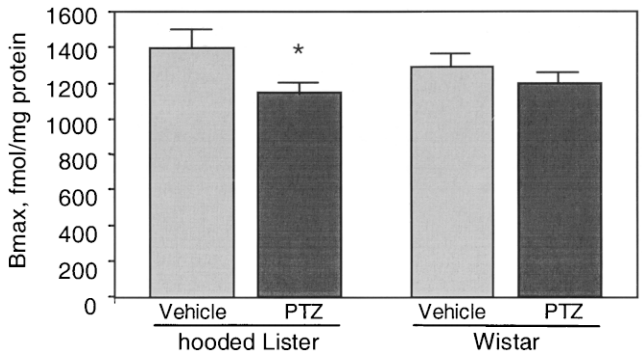

FIG. 2. B_{max} of [^{3}H]MK-801 binding in hippocampus of hooded Lister and Wistar rats killed 10 days after the final vehicle (saline) or PTZ (30 mg/kg IP) injection. *$p < 0.05$ PTZ-treated vs. saline-treated Lister rats. Values are mean ± SEM (n = 9–21/group).

DISCUSSION

Strain Differences in Seizure Activity

A strain difference in the susceptibility to develop seizures was first observed in rats (20,50), and has also been reported in mice (32). In the present study, hooded Lister and Wistar

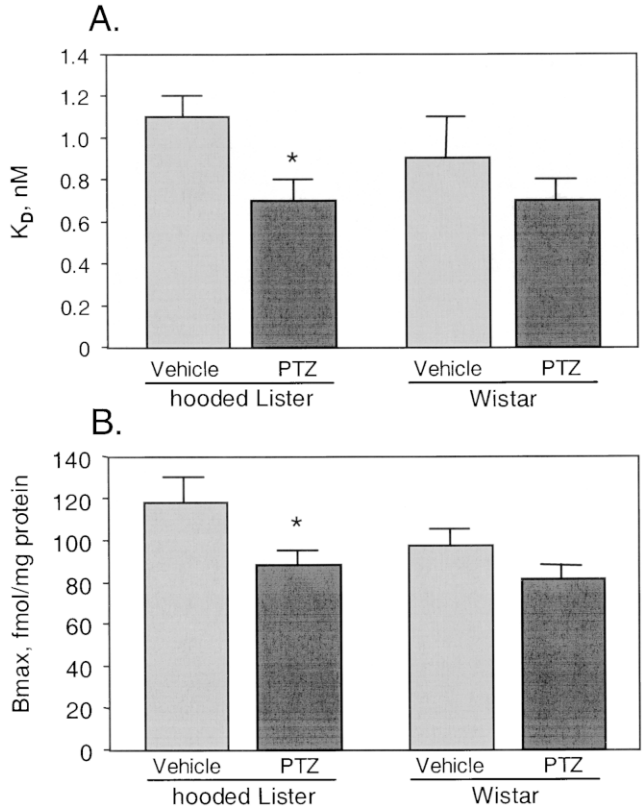

FIG. 3. (A) K_D and (B) B_{max} of [^{3}H]L-655,708 binding in hippocampus of hooded Lister and Wistar rats killed 10 days after the final vehicle (saline) or PTZ (30 mg/kg IP) injection. *$p < 0.05$ PTZ-treated vs. vehicle-treated Lister rats. Values are mean ± SEM (n = 11–23/group).

rats differed in their susceptibility to the kindling process in that the Lister strain reached an asymptote at stage 2, whereas the Wistar rats progressed to a median seizure of stage 4. A similar strain difference in the propensity for Lister and Wistar rats to develop PTZ-induced seizures has been reported previously, with the lowering of seizure threshold as a result of the chronic treatment still evident 1 week after the end of kindling (4,16).

It is possible that this strain difference in the susceptibility to develop seizure activity following repeated administration of PTZ is due to different pharmacokinetics of PTZ in these two strains. However, strain differences in the sensitivity of animals to electrical-induced kindling (20,32,34,50) indicate that there is an inherent, genetic basis to kindling susceptibility, which may be similar whether kindling is electrically or chemically induced. In addition, strain differences have also been observed in the behavioral consequences of kindling, with Wistar, but not Lister, rats showing an anxiolytic response 24 h after kindling (16).

More recently, intrastrain differences in vulnerability to develop seizures following electrical stimulation of the amygdala have been exploited to selectively breed Fast kindling and Slow kindling rats (51). Characterization of the molecular differences between not only these animals (26,36,46,51) but also strains of varying kindling vulnerability, offers the possibility of a novel means of establishing the specificity, and therefore pathophysiological significance, of such changes (34).

Ligand Binding Characteristics

There were no significant differences between the binding characteristics (K_D and B_{max}) of any of the ligands, in either cortex or hippocampus, between control (vehicle-treated) hooded Lister or Wistar rats. This suggests that the differences between strains with regard to their susceptibility to PTZ-induced seizures is not related to inherent differences in the expression of NMDA and $GABA_A$ receptor-associated neurotransmission.

The K_D values for [^{3}H]L-689,560 were similar in cortex and hippocampus (approximately 8 nM), and are within the range of previously published values (2–9 nM) (21,23,48). Similarly, the K_D for [^{3}H]MK-801 binding in the cortex is in agreement with published values (23,67). [^{3}H]MK-801 and [^{3}H]L-689,560 have approximately the same level of binding in both the cortex and hippocampus, consistent with previous data (42,67). The B_{max} values for [^{3}H]L-689,560 were slightly higher than the B_{max} values for [^{3}H]MK-801, in line with a previous report that the ratio of [^{3}H]L-689,560 to [^{3}H]MK-801 binding sites in rat brain membranes is 1.2 (23).

With respect to the total population of benzodiazepine binding sites labeled with [^{3}H]Ro 15-1788 (i.e., α1, α2, α3 plus α5 subunit-containing $GABA_A$ receptors), in both strains the number of binding sites in the cortex and hippocampus were comparable (62). However, the expression of [^{3}H]L-655,708 binding sites was greater in the hippocampus (around 100 fmol/mg protein) compared with the cortex (around 45 fmol/mg protein). Moreover, [^{3}H]L-655,708 binding in the hippocampus represented about 20% of the total number of benzodiazepine binding sites (i.e., [^{3}H]Ro 15-1788 binding), whereas in the cortex this number was about 10%, in agreement with previous data showing the higher expression of this $GABA_A$ receptor subtype in the hippocampus compared to the cortex (49,62). A similar preferential localization of the α5 subunit-containing $GABA_A$ receptors has been reported using either immunocytochemistry (17) or in situ hybridization of mRNA (66).

NMDA Receptor Binding in Kindling

In the more seizure-prone Wistar rats, cortical and hippocampal [^{3}H]MK-801 binding was unaltered 10 days after the final PTZ injection. These data suggest that the increased sensitivity of Wistar rats to PTZ-kindling is not a consequence of increased excitation due to over expression of NMDA receptors, and is consistent with unaltered levels of [^{3}H]MK-801 binding observed following electrical kindling of the rat (1).

On the other hand, in the Lister rats there was a significant 18% reduction in the number of [^{3}H]MK-801 binding sites. It is possible that a decrease in NMDA receptors in the Lister rats is an adaptive mechanism that renders this strain less vulnerable to NMDA-mediated glutamatergic excitation, resulting in their increased resistance to developing PTZ-induced kindled seizures.

There was no alteration in the number or affinity of [^{3}H]L-689,560 binding sites 10 days after the final PTZ injection in either strain. The lack of effect of PTZ-induced kindling on [^{3}H]L-689,560 binding, which specifically label the NR1 subunit of the NMDA receptor complex (22), is in agreement with previous reports that the hippocampal expression of NMDA receptor subunit genes is unaltered following kindling (25,28). More specific analysis of the splice forms of the NR1 subunit have shown that there is decreased expression of the isoforms containing the exon 21 cassette (29). However, this is transient, because it is seen 24 h but not 28 days after the last kindled seizure, suggesting that it is an acute response to the last seizure and is not associated with the long-lasting changes that underlie kindling (29).

Because NMDA receptors contain both an [^{3}H]MK-801 and a [^{3}H]L-689,560 binding site, one might expect that the number of these binding sites would change in parallel. This apparent inconsistency between [^{3}H]MK-801 and [^{3}H]L-689,560 binding could be methodological. Thus, the variability of the binding assay for [^{3}H]L-689,560 was much greater than that of the [^{3}H]MK-801 measurement, and therefore, the sensitivity of the [^{3}H]L-689,560 binding assay might not have been sufficient to reliably detect the relatively modest decrement (18%) in NMDA receptor expression that was detected using [^{3}H]MK-801.

In contrast to the present study, it has previously been reported that the binding of [^{3}H]glycine and [^{3}H]thienylcyclohexylpiperidine ([^{3}H]TCP)—ligands that bind to sites analogous to those labelled by [^{3}H]L-689,560 and [^{3}H]MK-801 in the present study—is increased following electrical stimulation of the amygdala (69). This discrepancy could be related to the use of chemical (PTZ; present study) vs. electrical (69) stimulation. Alternatively, these differences may be methodological in that the particular ligand chosen to label either the glycine, glutamate, or ion-channel site has a marked effect on the results observed (28).

PTZ has been reported to increase [^{3}H]glutamate binding in PTZ-kindled rats (55–57) and mice (13). This increase in hippocampal [^{3}H]glutamate binding was observed in Wistar, but not hooded Lister rats, implicating glutamate binding sites in the differential sensitivity of these strains to PTZ kindling (4). However, the increase in [^{3}H]glutamate binding is primarily associated with metabotropic glutamate and quisqualate- and kainate-sensitive glutamate receptors with no significant alteration in NMDA receptors (54). Thus, it would appear that PTZ-induced kindling is not directly associated with al-

tered NMDA receptor expression. Furthermore, NMDA receptor binding is not different in Fast and Slow kindling rats (46). Nevertheless, it remains possible that kindling could induce changes in subunit composition not detected by radioligand binding methods, although electrical kindling of the amygdala does not alter the long-term expression of hippocampal NMDA receptor subunit mRNAs (28) or proteins (30), and neither does electrical stimulation of the hippocampus (25,47). In addition, following PTZ kindling the phosphorylation state of NMDA receptor subunits could be altered, which would not change the expression of binding sites, but would alter functional aspects of NMDA receptor ion channels (27,40).

Benzodiazepine Receptor Binding in Kindling

Although the GABAergic system has been implicated in the development of kindling (see the introduction), the role of $GABA_A$ receptors is uncertain. Thus, after kindling, benzodiazepine binding sites have been reported to be increased (15,38,41,58,63,64) or decreased (52). Similarly, binding to the agonist (GABA) or convulsant (TBPS) binding site of $GABA_A$ receptors has been reported to be increased (58), decreased (9,12) or unchanged (64). Some of the discrepancies with respect to benzodiazepine binding may be related to methodological considerations, such as membrane preparation (41) or the choice of benzodiazepine site ligand (i.e., the agonist [^{3}H]flunitrazepam or the antagonist [^{3}H]Ro 15-1788) (65). In addition, these conflicting data may, in part, be related to the time at which animals are killed. Hence, changes may be observed acutely (i.e., within 24 h after the last kindled seizure), yet it is unclear whether these changes are an acute response to seizure activity per se or, in the case of chemical kindling, PTZ itself (43,52). Indeed, following electrical kindling, changes in [^{3}H]muscimol and [^{3}H]flunitrazepam, and more specifically Type I (i.e., α1 subunit-containing $GABA_A$ receptors) and Type II (i.e., α2, α3, and α5 subunit-containing receptors) benzodiazepine binding occur acutely, but not chronically (58). Similarly, changes in $GABA_A$ receptor subunit mRNAs, as well as [^{3}H]flunitrazepam binding, were seen acutely, but not 48 h after a single convulsant dose of PTZ (65). However, changes that are associated with the kindling process would be expected to be long lasting, and accordingly, in the present study, rats were killed 10 days after the final PTZ injection.

Of particular interest is the expression of α5 subunit-containing $GABA_A$ receptors, because they are preferentially localized in the hippocampus, a region of the brain particularly susceptible to seizure activity. In the present study, 10 days after the final PTZ treatment the total benzodiazepine population (i.e., α1, α2, α3, and α5 subunit-containing $GABA_A$ receptors), as measured using [^{3}H]Ro 15-1788, was unaltered in both the cortex and the hippocampus. However, when the α5 subunit expression was specifically measured using [^{3}H]L-655,708, there was a significant, 25% reduction in the number and an increased affinity of binding sites in the Lister rats, whereas in the Wistar rats there was a nonsignificant 16% decrease in the number of binding sites. It is not surprising that the reduction in the number of [^{3}H]L-655,708 binding sites is not reflected by changes in the number of [^{3}H]Ro 15-1788 binding sites, because the α5 receptor population is only a minor component of the total benzodiazepine binding sites that are measured using [^{3}H]Ro 15-1788. In addition, the changes in [^{3}H]L-655,708 binding are relatively small, and would only represent a reduction in [^{3}H]Ro 15-1788 binding of around 5%.

The basis of the altered affinity of α5 containing $GABA_A$ receptors is uncertain, but may reflect long-term changes in the receptor, which lead to an alteration in the affinity of this particular binding site. Such a change could result in allosteric changes in the receptor that may manifest themselves as an uncoupling of the GABA and benzodiazepine binding sites. In this regard, a single convulsant dose of PTZ has been reported to affect GABA and benzodiazepine binding site coupling (65), although no major change in uncoupling was observed following electrical kindling (58).

The cause of the reduction in the number of α5 subunit-containing $GABA_A$ receptors is unclear but it is presumably unrelated to the development of seizures per se, because these changes were observed in Lister rats, which are less vulnerable to seizures than the Wistar rats. Moreover, a single convulsive dose of PTZ affected a number of $GABA_A$ receptor subunit mRNAs but did not alter the expression of α5 mRNA (65), emphasizing that this $GABA_A$ receptor subunit is not associated either directly with seizure activity or the development of kindling.

Alterations in α5 subunit expression in the Lister rats may be related to PTZ-induced neuronal loss. Hence, hippocampal cell loss occurs following PTZ treatment (45), but may not be directly related to the development of seizure activity because piracetam was able to prevent PTZ-induced cell loss but did not alter the course of seizure development (45). If seizure activity can occur without cell loss, then it is possible that cell loss could occur without seizure activity [e.g., in enadoline-treated rats (5)], in which case the loss of α5 binding sites may be a nonspecific phenomenon related to a PTZ-induced loss of hippocampal CA1 neurons. The relationship between hippocampal cell loss and seizure activity remains unclear, but the measurement of hippocampal morphology in strains with differing seizure susceptibilities should establish whether this cell loss is related to PTZ treatment (e.g., occurs in Lister and Wistar rats) or is related to the development of seizure activity (in which case neuronal loss would be observed in Wistar but not Lister rats).

In Fast and Slow kindling rats, there was no difference in the level of hippocampal α5 subunit mRNA (46), nor were there any differences in α4, α6, β1–3, or γ1–3 subunit expression. However, in amygdala and paleocortical regions, Fast kindling rats had lower expression of the α1 subunit, the predominant adult subunit, whereas there was increased expression of the "developmental" subunits α2, α3, and α5. In contrast, in Slow kindling rats there was over expression of the α1 subunit and reduced expression of the α2, α3, and α5 subunits (46). Clearly, changes in $GABA_A$ receptor subunit composition occur in rats bred for their susceptibility to develop seizures following electrical stimulation of the amygdala. However, the specificity of these changes is unclear. For example, it would be interesting to know whether these changes also occur in fast and slow kindling rats bred on the basis of their vulnerability to chemical kindling.

CONCLUSIONS

Reductions in the expression of NMDA receptors ([^{3}H]MK-801 binding) and the α5 subunit containing $GABA_A$ receptors ([^{3}H]L-655,708 binding) were observed as a result of PTZ treatment in Lister rats. These changes are not due to seizure activity per se, because rats were killed 10 days after their last PTZ injection, nor are they directly related to the development of kindling, because they were observed in Lister rats but not the more kindling-susceptible Wistar rats.

On the other hand, it is possible that the decrement in NMDA receptors and α5 subunit containing $GABA_A$ receptors in Lister rats could be a protective mechanism. Clearly, however, the processes underlying PTZ-induced kindling are not related to an increased expression of either NMDA or $GABA_A$ receptors or, more specifically, α5 subunit-containing $GABA_A$ receptors.

Data in the present study suggest that PTZ-induced seizure activity is not directly associated with gross alterations in the expression of NMDA and $GABA_A$ receptors. However, this does not preclude the possibility that there may be altered expression in discrete regions of the cortex or hippocampus that cannot be detected using membrane-binding studies. In addition, functional aspects of the NMDA and $GABA_A$ receptors may be modified either as a result of altered subunit composition and/or phosphorylation, resulting in changes in NMDA, and/or GABA-mediated neuronal excitability. In this regard, it should be noted that although Fast- and Slow-kindling rats appear to have altered GABAergic function, as judged by an alteration in paired-pulse depression (51), the expression of $GABA_A$ receptors, as judged by [^{3}H]muscimol, binding was unchanged (51), although the subunit composition of these receptors is altered (46). Further studies of the neurochemical and molecular changes responsible for the differences in seizure susceptibility of Lister and Wistar rats as well as other strains should help clarify the mechanisms of seizure development and prove a valuable tool for the development of anticonvulsant drugs (34).

REFERENCES

1. Akiyama, K.; Yoneda, Y.; Ogita, K.; Itoh, T.; Daigen, A.; Sora, I.; Kohira, I.; Ujike, H.; Otsuki, S.: Ionotrophic excitatory amino acid receptors in discrete brain regions of kindled rats. Brain Res. 587:73–82; 1992.
2. Barnard, E. A.; Skolnick, P.; Olsen R. W.; Mohler, H.; Sieghart, W.; Biggio, G.; Braestrup, C.; Bateson, A. N.; Langer, S. Z.: International Union of Pharmacology. XV. Subtypes of γ-aminobutyric $acid_A$ receptors: Classification on the basis of subunit structure and receptor function. Pharmacol. Rev. 50:291–313; 1998.
3. Becker, A.; Grecksch, G.; Matthies, H.: The influence of diazepam on learning processes impaired by pentylenetetrazol kindling. Naunyn Schmiedebergs Arch. Pharmacol. 349:429–436; 1994.
4. Becker, A.; Krug, M.; Schröder, H.: Strain differences in pentylenetetrazol-kindling development and subsequent potentiation effects. Brain Res. 763:87–92; 1997.
5. Becker, A.; Braun, H.; Schröder, H.; Grecksch, G.; Höllt, V.: Effects of enadoline on the development of pentylenetetrazol kindling, learning performance, and hippocampal morphology. Brain Res. 823:191–197; 1999.
6. Bradford, H. F.: Glutamate, GABA and epilepsy. Prog. Neurobiol. 47:477–511; 1995.
7. Cain, D. P.; Desborough, K. A.; McKitrick, D. J.: Retardation of amygdala kindling by antagonism of NMD-aspartate and muscarinic cholinergic receptors: Evidence for the summation of excitatory mechanisms in kindling. Exp. Neurol. 100:179–187; 1988.
8. Cincotta, M.; Young, N. A.; Beart, P. M.: Unilateral up-regulation of glutamate receptors in limbic regions of amygdaloid-kindled rats. Exp. Brain Res. 85:650–658; 1991.
9. Corda, M. G.; Giorgi, O.; Longoni, B.; Orlandi, M.; Biggio, G.: Decrease in the function of the γ-aminobutyric acid-coupled chloride channel produced by the repeated administration of pentylenetetrazol to rats. J. Neurochem. 55:1216–1221; 1990.
10. Corda, M. G.; Giorgi, O.; Orlandi, M.; Longoni, B.; Biggio, G.: Chronic administration of negative modulators produces chemical kindling and $GABA_A$ receptor down-regulation. In: Biggio, G.; Costa, E., eds. GABA and benzodiazepine receptor subtypes. New York: Raven Press; 1990: 153–166.
11. Corda, M. G.; Orlandi, M.; Lecca, D.; Carboni, G.; Frau, V.; Giorgi, O.: Pentylenetetrazol-induced kindling in rats: Effect of GABA function inhibitors. Pharmacol. Biochem. Behav. 40:329–333; 1991.
12. Corda, M. G.; Orlandi, M.; Lecca, D.; Giorgi, O.: Decrease in GABAergic function induced by pentylenetetrazol kindling in rats: Antagonism by MK-801. J. Pharmacol. Exp. Ther. 262:792–800; 1992.
13. da Silva, L. F.; Pereira, P.; Elisabetsky, E.: A neuropharmacological analysis of PTZ-induced kindling in mice. Gen. Pharmacol. 31:47–50; 1998.
14. Dunah, A. W.; Yasuda, R. P.; Luo, J.; Wang, Y., Prybylowski, K. L.; Wolfe, B. B.: Biochemical studies of the structure and function of the *N*-methyl-D-aspartate subtype of glutamate receptors. Mol. Neurobiol. 19:151–179; 1999.
15. Fanelli, R. J.; McNamara, J. O.: Effects of age on kindling and kindled seizure-induced increase of benzodiazepine receptor binding. Brain Res. 362:17–22; 1986.
16. File, S. E.; Mabbutt, P. S.; Becker, A.; Gonzalez, L. E.; Fluck, E.: Short-term rebound anxiolytic effects and long-term changes in platelet benzodiazepine binding after pentylenetetrazol-kindling in two strains of rat. Anxiety 2:109–116; 1996.
17. Fritschy, J.-M.; Mohler, H.: $GABA_A$ receptor heterogeneity in the adult rat brain: Differential regional and cellular distribution of seven major subunits. J. Comp. Neurol. 359:154–194; 1995.
18. Getova, D.; Froestl, W.; Bowery, N. G.: Effects of $GABA_B$ receptor antagonism on the development of pentylenetetrazol-induced kindling in mice. Brain Res. 809:182–188; 1998.
19. Giorgi, O.; Orlandi, M.; Lecca, D.; Corda, M. G.: MK-801 prevents chemical kindling induced by pentylenetetrazol in rats. Eur. J. Pharmacol. 193:363–365; 1991.
20. Goddard, G. V.; McIntyre, D. C.; Leech, C. K.: A permanent change in brain function resulting from daily electrical stimulation. Exp. Neurol. 25:295–330; 1969.
21. Grimwood, S.; Moseley, A. M.; Carling, R. W.; Leeson, P. D.; Foster, A. C.: Characterization of the binding of [^{3}H]L-689,560, an antagonist for the glycine site on the *N*-methyl-D-aspartate receptor, to rat brain membranes. Mol. Pharmacol. 41:923–930; 1992.
22. Grimwood, S.; LeBourdelles, B.; Whiting, P. J.: Recombinant human NMDA monomomeric NR1 receptors expressed in mammalian cells form a high-affinity glycine antagonist binding site. J. Neurochem. 64:525–530; 1995.
23. Grimwood, S. G.; Le Boudelles, B.; Atack, J. R.; Barton, C.; Cockett, C.; Cook, S. M.; Gilbert, E.; Hutson, P. H.; McKernan, R. M.; Myers, J.; Ragan, C. I.; Wingrove, P. B.; Whiting, P. J.: Generation and characterization of stable cell lines expressing recombinant human *N*-methyl-D-aspartate receptor subtypes. J. Neurochem. 66:2239–2247; 1996.
24. Guela, C.; Jarvie, P. A.; Logan, T. C.; Slevin, J. T.: Long-term enhancement of K^+-evoked release of L-glutamate in entorhinal kindled rats. Brain Res. 442:368–372; 1988.
25. Kamphuis, W.; Hendriksen, H.; Diegenbach, P. C.; Lopes da Silva, F. H.: *N*-methyl-D-aspartate and kainate receptor gene expression in hippocampal pyramidal and granular neurons in the kindling model of epileptogenesis. Neuroscience 67:551–559; 1995.
26. Kokaia, Z.; Kelly, M. E.; Elmer, E.; Kokaia, M.; McIntyre, D. C.; Lindvall, O.: Seizure-induced differential expression of messenger RNAs for neurotrophins and their receptors in genetically fast and slow kindling rats. Neuroscience 75:197–207; 1996.
27. Köhr, G.; De Koninck, Y.; Mody, I.: Properties of NMDA receptor channels in neurons acutely isolated from epileptic (kindled) rats. J. Neurosci. 13:3612–3627; 1993.
28. Kraus, J. E.; Yeh, G.-C.; Bonhaus, D. W.; Nadler, J. V.; McNamara, J. O.: Kindling induces the long-lasting expression of a novel population of NMDA receptors in hippocampal region CA3. J. Neurosci. 14:4196–4205; 1994.

29. Kraus, J. E.; Nadler, J. V.; McNamara, J. O.: Regulation of alternative splicing of NMDAR1 in the kindling model. Mol. Brain Res. 41:97–104; 1996.
30. Kraus, J. E.; McNamara, J. O.: Measurement of NMDA receptor protein subunits in discrete hippocampal regions of kindled animals. Mol. Brain Res. 61:114–120; 1998.
31. Kubová, H.; Haugvicová, R.; Marěs, P.: Effects of NNC 711, a GABA uptake inhibitor, on pentylenetetrazol-induced seizures in developing and adult rats. Naunyn Schmiedebergs Arch. Pharmacol. 358:334–341; 1998.
32. Leech, C. K.; McIntyre, D. C.: Kindling rates in inbred mice: An analog to learning? Behav. Biol. 16:439–452; 1976.
33. Löscher, W.: Pharmacology of glutamate receptor antagonists in the kindling model of epilepsy. Prog. Neurobiol. 54:721–741; 1998.
34. Löscher, W.; Cramer, S.; Ebert, U.: Differences in kindling development in seven outbred and inbred rat strains. Exp. Neurol. 154:551–559; 1998.
35. Lowry, O. H.; Rosebrough, N. J.; Farr, A. L..; Randall, R. J.: Protein measurement with the Folin phenol reagent. J. Biol. Chem. 192:265–275; 1951.
36. McIntyre, D. C.; Kelly, M. E.; Dufresne, C.: FAST and SLOW amygdala kindling rat strains: Comparison of amygdala, hippocampal, piriform and perirhinal cortex kindling. Epilepsy Res. 35:197–209; 1999.
37. McKernan, R. M.; Whiting, P. J.: Which $GABA_A$-receptor subtypes really occur in the brain? Trends Neurosci. 19:139–143; 1996.
38. McNamara, J. O., Peper, A. M.; Patrone, V.: Repeated seizures induce long-term increase in hippocampal benzodiazepine receptors. Proc. Natl. Acad. Sci. USA 77:3029–3032; 1980.
39. McNamara, J. O.; Russell, R. D.; Rigsbee, L.; Bonhaus, D. W.: Anticonvulsant and antiepileptogenic actions of MK-801 in the kindling and electroshock models. Neuropharmacology 27:563–568; 1988.
40. Mody, I.; Heinemann, U.: NMDA receptors of dentate gyrus granule cells participate in synaptic transmission following kindling. Nature 326:701–704; 1987.
41. Niznik, H. B.; Burnham, W. M.; Kish, S. J.: Benzodiazepine receptor binding following amygdala-kindled convulsions: Differing results in washed and unwashed brain membranes. J. Neurochem. 43:1732–1736; 1984.
42. Pangalos, M. N.; Francis, P.T.; Foster, A. C.; Pearson, R. C. A.; Middlemiss, D. N.; Bowen, D. M.: NMDA receptors assessed by autoradiography with [^{3}H]L-689,560 are present but not enriched on corticofugal-projecting pyramidal neurones. Brain Res. 596: 223–230; 1992.
43. Paul, S. M.; Skolnick, P.: Rapid changes in brain benzodiazepine receptors after experimental seizures. Science 202:892–894; 1978.
44. Perlin, J. B.; Gerwin, C. M.; Panchision, D. M.; Vick, R. S.; Jakoi, E. R.; DeLorenzo, R. J.: Kindling produces long-lasting and selective changes in gene expression of hippocampal neurons. Proc. Natl. Acad. Sci. USA 90:1741–1745; 1993.
45. Pohle, W.; Becker, A.; Grecksch, G.; Juhre, A.; Willenberg, A.: Piracetam prevents pentylenetetrazol kindling-induced neuronal loss and learning deficits. Seizure 6:467–474; 1997.
46. Poulter, M. O.; Brown, L. A.; Tynan, S.; Willick, G.; William, R.; McIntyre, D. C.: Differential expression of α1, α2, α3, and α5 $GABA_A$ receptor subunits in seizure-prone and seizure-resistant rat models of temporal lobe epilepsy. J. Neurosci. 19:4654–4661; 1999.
47. Pratt, G. D.; Kokaia, M.; Bengzon, J.; Kokaia, Z.; Fritschy, J.-M.; Mohler, H.; Lindvall, O.: Differential regulation of *N*-methyl-D-aspartate receptor subunit messenger RNAs in kindling-induced epileptogenesis. Neuroscience 57:307–318; 1993.
48. Quirk, K.; Foster, A. C.; McKernan, R. M.: Characterization of the solubilized NMDA receptor complex from rat brain with [^{3}H]L-689,560, a glycine site antagonist. Mol. Membr. Biol. 11:17–21; 1994.
49. Quirk, K.; Blurton, P.; Fletcher, S.; Leeson, P.; Tang, F.; Mellilo, D.; Ragan, C. I.; McKernan, R. M.: [^{3}H]L-655,708, a novel ligand selective for the benzodiazepine site of $GABA_A$ receptors which contain the α5 subunit. Neuropharmacology 35:1331–1335; 1996.
50. Racine, R. J.; Burnham, W. M.; Gartner, J. G.; Levitan, D.: Rates of motor seizure development in rats subjected to electrical brain stimulation: Strain and inter-strain interval effects. Electroencephalogr. J. Clin. Neurophysiol. 35:553–556; 1973.
51. Racine, R. J.; Steingart, M.; McIntyre, D. C.: Development of kindling-prone and kindling-resistant rats: Selective breeding and electrophysiological studies. Epilepsy Res. 35:183–195; 1999.
52. Rocha, L.; Ackermann, R. F.; Engel, J., Jr.: Chronic and single administration of pentylenetetrazol modifies benzodiazepine receptor-binding: An autoradiographic study. Epilepsy Res. 24:65–72; 1996.
53. Sato, K.; Morimoto, K.; Okamoto, M.: Anticonvulsant action of a non-competitive antagonist of NMDA receptors (MK-801) in the kindling model of epilepsy. Brain Res. 463:12–20; 1988.
54. Schröder, H.; Becker, A.: The role of glutamate receptors in pentylenetetrazole kindling of rats—A neurochemical study. Neuropharmacology 35:A28; 1996.
55. Schröder, H.; Becker, A.; Lössner, B.: Glutamate binding to brain membranes is increased in pentylenetetrazole kindled rats. J. Neurochem. 60:1007–1011; 1993.
56. Schröder, H.; Schlichthaar, R.; Krug, M.: Specific [^{3}H]L-glutamate binding and [^{3}H]D-aspartate release in the hippocampus of rat after pentylenetetrazol kindling and long-term potentiation. Neuroscience 60:337–342; 1994.
57. Schröeder, H.; Becker, A.; Schröeder, U.; Hoellt, V.: ^{3}H-L-glutamate binding and ^{3}H-D-aspartate release from hippocampal tissue during the development of pentylenetetrazole kindling in rats. Pharmacol. Biochem. Behav. 62:349–352; 1999.
58. Shin, C.; Pedersen, H. B.; McNamara, J. O.: γ-Aminobutyric acid and benzodiazepine receptors in the kindling model of epilepsy: A quantitative radiohistochemical study. J. Neurosci. 5:2696–2701; 1985.
59. Skolnick, P.; Hu, R. J.; Cook, C. M.; Hurt, S. D.; Trometer, J. D.; Liu, R.; Huang, Q.; Cook, J. M.: [^{3}H]RY 80: A high-affinity, selective ligand for γ-aminobutyric $acid_A$ receptors containing *alpha*-5 subunits. J. Pharmacol. Expt. Ther. 283:488–493; 1997.
60. Stephens, D. N.; Weidmann, R.: Blockade of FG 7142 kindling by anticonvulsants acting at sites distant from the benzodiazepine receptor. Brain Res. 492:89–98; 1989.
61. Sur, C.; Quirk, K.; Dewar, D.; Atack, J.; McKernan, R.: Rat and human hippocampal α5 subunit-containing γ-aminobutyric $acid_A$ receptors have $\alpha5\beta3\gamma2$ pharmacological characteristics. Mol. Pharmacol. 54:928–933; 1998.
62. Sur, C.; Fresu, L.; Howell, O.; McKernan, R. M.; Atack, J. R.: Autoradiographic localization of α5 subunit-containing $GABA_A$ receptors in rat brain. Brain Res. 822:265–270; 1999.
63. Syapin, P. J.; Rickman, D. W.: Benzodiazepine receptor increase following repeated pentylenetetrazole injections. Eur. J. Pharmacol. 72:117–120; 1981.
64. Tuff, L. P.; Racine, R. J.; Mishra, R. K.: The effects of kindling in GABA-mediated inhibition in the dentate gyrus of the rat. II. Receptor binding. Brain Res. 277:91–98; 1983.
65. Walsh, L. A.; Li, M.; Zhao, T.-J.; Chiu, T. H.; Rosenberg, H. C.: Acute pentylenetetrazol injection reduces rat $GABA_A$ receptor mRNA levels and GABA stimulation of benzodiazepine binding with no effect on benzodiazepine binding site density. J. Pharmacol. Exp. Ther. 289:1626–1633; 1999.
66. Wisden, W.; Laurie, D. J.; Monyer, H.; Seeburg, P. H.: The distribution of 13 $GABA_A$ receptor subunit mRNAs in the rat brain. I: Telencephalon, diencephalon, mesencephalon. J. Neurosci. 12: 1040–1062; 1992.
67. Wong, E. H.; Knight, A. R.; Woodruff, G. N.: [^{3}H]MK-801 labels a site on the *N*-methyl-D-aspartate receptor channel complex in rat brain membranes. J. Neurochem. 50:274–281; 1988.
68. Wu, K.; Wasterlain, C.; Sachs, L; Siekevitz, P.: 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; 1990.
69. Yeh, G.-C.; Bonhaus, D. W.; Nadler, J. V.; McNamara, J. O.: *N*-methyl-D-aspartate receptor plasticity in kindling: Quantitative and qualitative alterations in the *N*-methyl-D-aspartate receptor-channel complex. Proc. Natl. Acad. Sci. USA 86:8157–8160; 1989.