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AUTORADIOGRAPHIC ANALYSIS OF $[^{35}\text{S}]$ -BUTYLBICYCLOPHOSPHOROTHIONATE BINDING IN KINDLED RAT HIPPOCAMPUS SHOWS DIFFERENT CHANGES IN CA1 AREA AND FASCIA DENTATA

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Abstract—We studied the binding of $[^{35}\text{S}]$ -butylbicyclophosphorothionate to the GABA_A receptor-mediated chloride channel in the CA1 area and fascia dentata of control and Schaffer collateral kindled rats, by means of semi-quantitative autoradiography. The $[^{35}\text{S}]$ -butylbicyclophosphorothionate binding was determined at three stages during kindling acquisition: (i) after six afterdischarges, (ii) after 14 afterdischarges and (iii) after the induction of fully kindled seizures. Furthermore, the binding was studied at the long-term stage, 28 days after the last generalized tonic-clonic seizure [Racine R. J. (1972) *Electroenceph. clin. Neurophysiol.* **32**, 281–294]. The binding was investigated at three $[^{35}\text{S}]$ -butylbicyclophosphorothionate concentrations, 4, 47.5 (K_D value) and 180 nM (B_{max} value).

A significant decrease in $[^{35}\text{S}]$ -butylbicyclophosphorothionate binding in the CA1 area (–6 to –20%) and hilar formation (–17 to –37%), in one or more of the three $[^{35}\text{S}]$ -butylbicyclophosphorothionate concentrations tested at the six and 14 afterdischarges and fully kindled stages was observed, but no significant changes at the long-term kindling stage were found. In contrast, the granular and molecular layers of the fascia dentata presented a significant increase in $[^{35}\text{S}]$ -butylbicyclophosphorothionate binding (+15 to +38%) at the 14 afterdischarges, fully kindled and long-term kindled stages.

These changes in $[^{35}\text{S}]$ -butylbicyclophosphorothionate binding are in line with the previously observed changes in the $[^3\text{H}]$ muscimol [Titulaer M. N. G. *et al.* (1993) *Neuroscience* **59**, 817–826 and $[^3\text{H}]$ flunitrazepam [Titulaer M. N. G. *et al.* (submitted)] binding, indicating that Schaffer collateral kindling stimulation is accompanied by changes of all three main GABA_A receptor binding sites: (i) the “cage” convulsant site associated with the chloride channel, (ii) the GABA_A agonist binding site and (iii) the benzodiazepine receptor agonist binding site. We hypothesize that this modification of GABA_A/benzodiazepine receptor complex binding may underlie the changes in GABA-mediated inhibition in the kindled rat hippocampus, as previously indicated in electrophysiological studies, [Kamphuis W. *et al.* (1988) *Brain Res.* **440**, 205–215], consequently contributing to the establishment of a kindling stimulation induced epileptogenic focus.

Application of short trains of high-frequency electrical pulses, mainly to the limbic cortex, elicits electroencephalographic focal seizure activity, i.e. epileptiform afterdischarges. Subsequent stimulations lead to a progressive increase and spread of the afterdischarges and can induce overt behavioural seizures.^{6,18,29} Once generalized tonic-clonic (class V)²⁹ seizures are elicited, the animal has reached the so-called fully kindled stage. Kindling of limbic cortical areas, such as the hippocampus and amygdala, is considered an appropriate model of human temporal lobe epilepsy and complex partial seizures.¹³ The kindling-induced state of hyperexcitability is long-lasting since animals, left unstimulated for as long as one year, will respond to resumed stimulations with

a class V seizure.²⁹ The mechanisms underlying kindling epileptogenesis are still unclear, but probably involve a shift in balance between excitatory and inhibitory neurotransmission.^{16,27}

Evidence for a modification of the GABAergic inhibition was obtained in several kindling studies.¹³ Field potential studies revealed a long-lasting decreased paired-pulse depression in the CA1 area but an increased paired-pulse depression in the fascia dentata,^{10,37,38} implying a long-lasting decreased GABAergic inhibition in the CA1 area and an increased GABAergic inhibition in the fascia dentata after kindling. These studies show that the inhibitory systems in the two hippocampal subareas respond differently to kindling stimulations. In addition, using single unit registrations in combination with micro-iontophoresis, a long-lasting decrease of the inhibitory effect of GABA on glutamate-elicited firing of hippocampal pyramidal neurons was reported,⁹ indicating a long-lasting decreased postsynaptic GABA_A receptor sensitivity in the CA1 area. The

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Abbreviations: AD, afterdischarge; CA1, cornu Ammonis area 1; EDTA, ethylenediaminetetra-acetate; FC, fully-kindled control group; FK, fully kindled; LC, long-term control group; LT, long-term kindled; SC, short-term kindled; TBPS, t-butylbicyclophosphorothionate.

observed electrophysiological^{10,37,38} and iontophoretic⁹ changes may be related to specific changes in GABA_A receptor complex binding sites in the two hippocampal subareas.

The GABA_A receptor is a hetero-oligomeric protein complex containing a central chloride channel that is opened when GABA occupies the GABA recognition site. In addition, the GABA_A receptor complex contains recognition sites for benzodiazepines, barbiturates and "cage" convulsants, such as picrotoxin and t-butylbicyclophosphorothionate (TBPS).²⁵ The latter are thought to bind to a site at, or close, to the chloride channel.^{8,33} A long-lasting and regional specific decrease in [³⁵S]TBPS binding after entorhinal kindling stimulation has been reported,²⁸ but there were no significant changes in the hippocampal area reported in this study. Here we report the results of a semi-quantitative autoradiographic study of the GABA_A receptor-mediated chloride channel binding site in the hippocampal kindling model, using three [³⁵S]TBPS concentrations.

EXPERIMENTAL PROCEDURES

Materials

[³⁵S]TBPS (110 Ci/ μ mol) was obtained from New England Nuclear (Dupont de Nemours, Netherlands). Muscimol, picrotoxin and TBPS were obtained from Sigma Chemical Co.

Animal and implantation procedures

Thirty-two male Wistar rats (250–300 g) were anaesthetized with sodium pentobarbital, and two bundles of isolated stainless-steel electrodes (80 μ m, diameter) were implanted in the left dorsal hippocampus under electrophysiological control. The recording electrodes were placed in the stratum radiatum of the CA1 area (coordinates: 2.4 mm posterior to bregma and 1.4 mm lateral to the midline). Stimulation electrodes were placed in the Schaffer collateral fibres (coordinates: 2.8 mm posterior to bregma and 2.4 mm lateral to the midline). The electrodes were fixed to the skull with dental acrylic cement, at the sites where an optimal field potential to Schaffer collateral fibre stimulation was obtained.¹¹

After implantation, the rats were individually housed in plastic cages with free access to food and water in a temperature-controlled room (20–22°C) with a 12 h light:12 h dark cycle (lights on at 7.00 a.m.). After a recovery period of one week, the rats were divided into a control group and a group that was to be kindled.

Kindling procedure

The kindling stimulus (200–300 μ A, 50 Hz, 0.1 ms duration biphasic pulses, 1 s) was applied twice daily. The animals in the control group were handled daily, in the same way as the kindled group.

In the course of kindling, the animals were randomly assigned to four groups. Two groups were killed 24 h after experiencing six afterdischarges (6AD, $n = 7$) or 14 afterdischarges (14AD, $n = 6$). A fully kindled group (FK, $n = 9$) was killed 24 h after the last class V seizure²⁹ and a long-term group (LT, $n = 9$) was killed 28 days after the last class V seizure. A "short-term" control group (SC, $n = 10$) was killed along with the 6AD and 14AD groups; "fully kindled" (FC, $n = 7$) and "long-term" control groups (LC, $n = 7$) were killed along with the FK and LT groups. The FK group received an average of 30 tetanic stimulations and

six class V seizures. The LT group received an average of 23 tetanic stimulations and six class V seizures.

Preparation of coronal brain sections

Rats were killed by decapitation and the brains were rapidly removed, dipped into ice-cold sucrose (0.32 M) and subsequently frozen by immersion in cooled (–30°C) isopentane. The brains were then stored at –60°C until sectioning.

The frozen brains were brought into a cryostat microtome (–20°C). Consecutive coronal sections (12 μ m) were cut from the hippocampal brain area (plates 33–36 of Paxinos and Watson²⁶) and thaw-mounted onto chrome-alum-gelatine-coated microscope slides. Four adjacent sections were mounted per slide. Mounted sections were air-dried for 10 min and subsequently stored at –20°C until incubation.

[³⁵S]t-Butylbicyclophosphorothionate binding assay

Sections were placed at room temperature for at least 45 min before incubation. All procedures were carried out at 21–22°C. The complete removal of endogenous GABA is essential for the detection of specific [³⁵S]TBPS binding.³³ The sections were therefore preincubated four times for 10 min in buffer (50 mM Tris-HCl + 200 mM NaCl + 1 mM EDTA, pH 7.4). Subsequently, the sections were quickly dried under a stream of cold air. High-performance liquid chromatography analysis of the GABA content of the tissue sections revealed that after preincubation the GABA content in the sections was reduced by more than 99%, compared to the GABA content in the unwashed sections (data not shown).

The sections were incubated with [³⁵S]TBPS for 3 h in buffer (containing no EDTA) at 22°C. Saturation isotherms were constructed with 12 concentrations of [³⁵S]TBPS ranging from 4 to 225 nM. For this, the sections were incubated with 4 nM [³⁵S]TBPS supplemented with different concentrations of unlabelled TBPS. After incubation, the sections were washed twice in buffer for 10 min, followed by a dip in ice-cold distilled water to remove residual salts. Non-specific binding was determined in the presence of 100 μ M picrotoxin and accounted for less than 5% of the total [³⁵S]TBPS binding. Sections were then quickly wiped off from the slides with filters (No. 6 Glasfaser Rundfilter, Schleicher and Schuell) and the radioactivity bound to the filter was determined by liquid scintillation counting (Packard, 1600 TR).

[³⁵S]t-Butylbicyclophosphorothionate autoradiography

The sections were incubated with 4 nM [³⁵S]TBPS and 10 nM [³⁵S]TBPS plus two concentrations of unlabelled TBPS (37.5 and 170 nM (estimated B_{max} value) concentration (Fig. 2B). After the incubation procedure, the sections were quickly dried under a stream of cold air. The sections were placed into light-tight cassettes and autoradiographs were obtained by apposing the slides to ³H-sensitive film (Hyperfilm, Amersham Corp., Arlington Heights, IL). Exposition time ranged from one to seven days, at room temperature. Each film contained a ³⁵S standard, with known radioactivity in pmol/mm². After exposure, the films were developed at room temperature (Kodak-D19, 4 min). Non-specific binding, determined in the presence of 100 μ M picrotoxin, was not detectable on the autoradiographs.

Data analysis

The autoradiographs were digitized and analyzed using an image analysing system (IBAS). The image analyser was connected to a Bosch TYK9BTV camera, equipped with a chainycon tube mounted on a Zeiss microscope. The microscope was equipped with planapo objectives (magnification 1.25 times 1.6) and connected to a light source with stabilised power supply. First, a shading image of the film proper

was taken and the 100% transmission level was set by adjusting the illumination intensity. Second, the autoradiographic image of the dorsal hippocampus was loaded into the IBAS system. Third, the strata oriens and radiatum of the CA1 area, and the granular and molecular layers and hilus of the fascia dentata, were outlined by hand. The outlined areas are shown in Fig. 1A.

In order to study the relation between the amount of radioactive ligand bound to the sections and the grey values obtained via densitometric analysis of the autoradiograms, a ³⁵S calibration scale was established according to the procedure described previously.¹⁵ Quantification of the outlined areas was performed as follows: the image analyser scaled the mean grey values of the ³⁵S calibration scale into the known radioactivity in pmol/mm², and a calibration curve was established. Subsequently, the mean grey values of the measured areas were determined and scaled into specific ligand bound in pmol/mm², by using the previously established ³⁵S calibration curve.

Only two sections of the four sections on a slide were used for analysis (in general the two middle sections), since the variation between animals was found to be larger than the variation between two different sections of one animal. No statistical differences were found between the data from the ipsi- and contralateral sites (paired Student's *t*-test) and further analysis was therefore carried out on the mean grey values of both hemispheres.

Statistics

All results are presented as relative changes in relation to binding in the control animals (mean % difference ± S.E.M.). Because stratum oriens, pyramidale and radiatum of the CA1 area, on the one hand, and the granular and molecular layers of the upper and lower blade of the fascia dentata on the other, appeared to react in a similar way to kindling stimulation, we decided to test the null hypothesis (i.e. no difference between 6AD, 14AD, FK or LT groups and the corresponding controls) using a two-way ANOVA with repeated measurements, with treatments and the two hippocampal subareas as variables. The ANOVA was carried out on the original raw data both for controls and treatment groups. Values of *P* less than 0.05 were considered significant. The data of the hilus were analysed using Student's *t*-test.

RESULTS

[³⁵S]*t*-Butylbicyclophosphorothionate binding in the rat hippocampus

We observed the highest [³⁵S]TBPS binding in stratum oriens of the CA areas. Intermediate binding was present in the molecular layers of the fascia dentata and in stratum radiatum of the CA1–CA3 areas, whereas the hilus showed very low binding (Fig. 1B). This distribution of [³⁵S]TBPS binding is in agreement with the previously described distribution of [³⁵S]TBPS binding sites in the rat hippocampal formation.²⁵

[³⁵S]*t*-Butylbicyclophosphorothionate binding kinetics

Scatchard analysis of the equilibrium saturation curve (Fig. 2A) in the concentration range from 4 to 225 nM indicated the presence of one specific binding affinity of [³⁵S]TBPS for the GABA_A receptor-mediated chloride channel in coronal rat brain sections. Computer-assisted non-linear least square curve fitting of the Scatchard plot²² (Fig. 2B) gave

an estimated *K_D* value of 47.5 nM (regression line correlation coefficient: *r*² = 0.97, *n* = 3).

[³⁵S]*t*-Butylbicyclophosphorothionate binding in kindled rat hippocampus: semi-quantitative autoradiography

Table 1 presents the binding of the 4, 47.5 and 180 nM [³⁵S]TBPS concentrations to the GABA_A receptor-mediated chloride channel in the two hippocampal subareas, the CA1 area (stratum oriens and stratum radiatum) and the fascia dentata (granular and molecular layers of the upper and lower blades of the fascia dentata, and hilus), at the 6AD, 14AD, FK and LT stages. The results are presented as relative changes (%) in relation to control binding ± S.E.M. The main findings are the following.

CA1 area. Stratum oriens and radiatum of the CA1 area showed a significant decrease in the 4 nM binding after 6AD (*c.* 10%) and 14AD (*c.* 15%). The 47.5 nM binding was significantly decreased, but only by 6% at the 6AD stage. The 180 nM binding was significantly decreased by 10–20% at the FK stage. No significant changes at 28 days after the last class V seizure were observed, at any of the three concentrations tested.

Fascia dentata. The [³⁵S]TBPS binding was studied separately in the granular/molecular layers of the upper and lower blades of the fascia dentata (see Fig. 1A). However, both areas responded, in general, in a similar way to kindling stimulations. We observed a significant increase in the 4 nM binding (*c.* 35%) and the 47.5 nM binding (15–33%) at the FK stage. Furthermore, we observed a significant increase in the 180 nM binding after 14AD (*c.* 25%) and at LT (*c.* 32%). Apparently, the changes in TBPS binding observed in the fascia dentata appeared somewhat later in the course of kindling as compared to the CA1 area, where changes were already observed at 6AD.

Hilus. The hilus showed a significant decrease (by 36%) in the 4 nM binding at the 14AD stage. The 47.5 nM binding was significantly decreased after 6AD (17%) and 14AD (18%), and at the FK stage (34%). the 180 nM binding was significantly decreased (by 36%) 24 h after the last class V seizure. There was no significant change present at the LT stage.

DISCUSSION

Main findings

There are two main findings in this study. First, kindling stimulations induce time-dependent changes in [³⁵S]TBPS binding in the hippocampal formation. Second, the changes in [³⁵S]TBPS binding are specific to the subareas. We observed a decreased binding in one or more of the three [³⁵S]TBPS concentrations tested in the CA1 area and hilus after 6AD and 14AD, and at the FK stage, whereas the granular and molecular layers of the fascia dentata presented an

Fig. 1. (A) Outlined hippocampal areas for analysis of the $[^{35}\text{S}]\text{TBPS}$ binding with the aid of an image analysing system (IBAS). 1, Stratum oriens; 2, stratum radiatum (CA1 area); 3 and 4, granular and molecular layers of the upper and lower blade of the fascia dentata, respectively; 5, hilus (fascia dentata, FD). See text (Experimental Procedures) for explanation of the procedure of analysis and quantification. (B) Direct photographic print of an autoradiogram showing the distribution of $[^{35}\text{S}]\text{TBPS}$ binding sites in the rat hippocampus. Stratum oriens of the CA areas showed the highest binding. High binding was also present in the granular and molecular layers of the fascia dentata. Stratum radiatum of the CA1-CA3 areas showed intermediate binding, whereas the hilar formation presented very low binding.

Fig. 2. (A) Saturation plot of the [35 S]TBPS binding (4–200 nM) to cryostat-mounted coronal brain sections (12 μ m) from three control rats. Specific binding is given in c.p.m./two sections $\pm$ S.E.M. The binding assay was carried out as described previously (see Experimental Procedures). Bound radioactivity was measured by liquid scintillation counting. (B) Scatchard analysis of the specific [35 S]TBPS binding. Non-linear curve fitting indicated the presence of one binding affinity with an estimated K_D value of 47.5 nM (regression line correlation coefficient: $r^2 = 0.97$, $n = 3$).

increase in one or more of the three [35 S]TBPS concentrations at the 14AD, FK and LT stages.

Kindling-induced changes in [35 S]*t*-butylbicyclopophosphorothionate binding

Scatchard analysis of the saturation data indicated a single population of binding sites for the binding of [35 S]TBPS to the GABA $_A$ receptor-mediated chloride channel site in coronal rat brain sections of control animals, with an estimated K_D value of 47.5 nM (Fig. 2B). A K_D value of approximately 21–29 nM in coronal rat brain sections was reported previously.⁵ However, K_D values in the range of 37–170 nM³³ were also reported, depending on the assay conditions used.

As there is only one class of [35 S]TBPS binding sites under our experimental conditions (Fig. 2B), we

made an effort to interpret the observed changes in binding of the 4, 47.5 (estimated K_D value) and 180 nM (estimated B_{max} value) [35 S]TBPS concentrations, in terms of specific changes in binding characteristics. Two general patterns of significant changes in [35 S]TBPS binding can be distinguished: (i) that of the CA1 area and hilus, and (ii) that of the fascia dentata. At the 6AD and 14AD stages, the two former areas presented significant decreases in the lower concentrations of [35 S]TBPS binding (4 and 47.5 nM). In contrast, at the FK stage, decreases were observed in the 180 nM (CA1) or 47.5 and 180 nM (hilus) binding. This indicates that at the early kindling stages, the decreases in [35 S]TBPS binding were at, or below, the K_D concentration, suggesting a decrease primarily in binding affinity, rather than binding site density. At the FK stage, the decrease in

Table 1. Relative changes in [³⁵S]t-butylbicyclophosphorothionate binding in relation to control binding in the two hippocampal subareas CA1 area and fascia dentata (upper and lower blade of the granular and molecular layers, and hilus)

CA1 area			Fascia dentata		
6AD					
4 nM	str. or.	-13.2 ± 2.4	upp. bl.	0.8 ± 5	hilus -12.2 ± 9
	str. rad.	-7.8 ± 3.4		low. bl.	
47.5 nM	str. or.	-5.8 ± 3.1	upp. bl.	-1 ± 4.9	-17.2 ± 3.9*
	str. rad.	-5.9 ± 4		low. bl.	
180 nM	str. or.	-0.6 ± 2.6	upp. bl.	2.9 ± 4	-7 ± 8.6
	str. rad.	1.3 ± 5.4		low. bl.	
14AD					
4 nM	str. or.	-12.4 ± 4.6	upp. bl.	1.2 ± 5.5	hilus -36.9 ± 4.7**
	str. rad.	-18 ± 4.7		low. bl.	
47.5 nM	str. or.	-4.2 ± 5.2	upp. bl.	8.4 ± 6.8	-17.3 ± 4.3*
	str. rad.	0.2 ± 5.5		low. bl.	
180 nM	str. or.	2 ± 5.6	upp. bl.	26.7 ± 4.0	1.3 ± 5.8
	str. rad.	6.8 ± 3.7		low. bl.	
FK					
4 nM	str. or.	2.2 ± 9.4	upp. bl.	32.2 ± 10.6	hilus 0 ± 10.4
	str. rad.	0.1 ± 8.9		low. bl.	
47.5 nM	str. or.	0.5 ± 5.1	upp. bl.	15.3 ± 9.5	-34.1 ± 7*
	str. rad.	-9.4 ± 7.3		low. bl.	
180 nM	str. or.	-10.4 ± 4.2	upp. bl.	2.1 ± 6.4	-35.7 ± 4.5*
	str. rad.	-20.6 ± 5.7		low. bl.	
LT					
4 nM	str. or.	0.4 ± 4.7	upp. bl.	10.9 ± 6.4	hilus 13.9 ± 11.7
	str. rad.	-1.7 ± 6.2		low. bl.	
47.5 nM	str. or.	-6.4 ± 7.4	upp. bl.	6.2 ± 12.5	-0.8 ± 17.5
	str. rad.	-5.3 ± 10.3		low. bl.	
180 nM	str. or.	14.3 ± 3.3	upp. bl.	28.4 ± 8.5	31.8 ± 12
	str. rad.	16.6 ± 6.6		low. bl.	

Statistical comparison in the CA1 area and granular and molecular layers of the fascia dentata was done by two-way ANOVA with repeated measurements (see text). The data of the hilar region of the fascia dentata were analyzed using Student's *t*-test. Data are means ± S.E.M. for the following number of experiments: 6AD group, *n* = 6; 14AD group, *n* = 7; FK and LT groups, *n* = 9; SC group, *n* = 10; FC and LC groups, *n* = 7. **P* ≤ 0.05, ***P* ≤ 0.01. str. or., stratum oriens; str. rad., stratum radiation; upp. bl., upper blade; low. bl., lower blade.

the 180 nM binding (both in the CA1 area and hilus) indicates a decrease in binding site density. In the fascia dentata, changes did not appear until the 14AD stage. At this stage and at the LT stage, we observed an increased 180 nM binding, indicating an increase in binding site density. However, this increase was not found at the FK stage. Surprisingly enough, we observed an increase in the 4 and 47.5 nM binding at this stage, suggesting an increase in binding affinity. However, it is obvious that Scatchard analysis, including more [³⁵S]TBPS concentrations, is required to substantiate these interpretations.

Using quantitative autoradiography, long-lasting and regional-specific changes in the 4 nM [³⁵S]TBPS binding have been reported in entorhinal kindled rat brain.²⁸ However, there were no significant changes observed in the hippocampal formation in this study. This may be explained by the fact that entorhinal kindling causes no significant change in the GABA_A receptor-mediated chloride channel binding site in the hippocampal formation, although there are reports of changes in [³H]muscimol²⁴ and [³H]flunitrazepam²³ binding in the fascia dentata, induced by entorhinal kindling stimulations. Furthermore, close examination of the data presented in the study of Petrask and co-workers²⁸ reveals a tendency towards an

increased 4 nM [³⁵S]TBPS binding in the molecular layers of the fascia dentata, in agreement with our observations.

Kindling-induced changes in [³⁵S]t-butylbicyclophosphorothionate binding and GABAergic inhibition

The observed decrease in [³⁵S]TBPS binding in the CA1 area at the FK stage closely parallels our previous observations of a hippocampal kindling-induced decrease in [³H]flunitrazepam binding³⁴ and decrease in high/intermediate and low-affinity [³H]muscimol binding at this stage.³⁵ Furthermore, the increase in [³⁵S]TBPS binding in the granular and molecular layers in the fascia dentata is in agreement with the previously observed increases in [³H]muscimol³⁵ and [³H]flunitrazepam³⁴ binding in this hippocampal subarea.

The changes in opposite directions for the two hippocampal subareas may underlie the electrophysiologically observed gradual decrease of paired-pulse depression in the CA1 area and increased paired-pulse depression in the fascia dentata.^{10,38} In agreement with these alterations, we recently observed a decreased muscimol-stimulated ³⁶Cl⁻ uptake in rat synaptoneurosomes obtained from the CA1 area, in contrast to an increase in fascia dentata

synaptoneurosome, in kindled animals 24 h after the last class V seizure, indicating bidirectional changes in GABA_A receptor-mediated function in these subareas as well.³⁶

The changes in paired-pulse depression are still present two to four weeks after the last kindled seizure in both the CA1³⁷ and fascia dentata.³⁸ In line with these observations, Kamphuis *et al.*⁹ reported a long-lasting decrease in the responsiveness of pyramidal CA1 neurons to GABA and we found a long-lasting decrease in the low-affinity [³H]muscimol binding in the CA1 area and increase in the fascia dentata,³⁵ 28 days after the last kindled seizure. However, we only observed a significant increase in the 180 nM [³⁵S]TBPS binding in the granular and molecular layers of the fascia dentata at the LT stage (Table 1).

The decreased GABA_A receptor-mediated chloride channel binding in the CA1 area at the FK stage may be related to the previously reported long-lasting increased GABA exocytosis.^{12,14} At an early kindled stage (6AD), a decrease in [³⁵S]TBPS binding was found in the CA1 area and hilus, while no change in GABA exocytosis¹⁴ was detected. However, at the 14AD stage an increased GABA release was encountered.¹⁴ This may act as a compensatory mechanism to overcome the decrease in [³⁵S]TBPS (and [³H]flunitrazepam³⁴ and [³H]muscimol³⁵) binding. In contrast, it may also reinforce the changes in GABA_A receptor-mediated chloride channel binding, since it has been shown that chronic exposure to GABA may cause down-regulation of GABA_A receptor binding,¹⁹ GABA-gated chloride currents,⁷ and of GABA_A receptor subunit mRNA expression levels.²⁰ However, a more detailed time course analysis of the

reported changes is necessary in order to gain more insight into a possible causal relationship between these events.

The increased [³⁵S]TBPS binding in the granular and molecular layers of the fascia dentata at the LT stage is in agreement with the long-lasting increased low-affinity [³H]muscimol³⁵ binding and [³H]flunitrazepam³⁴ binding in this hippocampal subarea. These changes may be related to the process of mossy fibre synaptic reorganization,² which may also involve outgrowth of GABAergic interneurons⁴ and subsequently the formation of new GABA_A receptors. However, the latter observation was made following kainate injection and further studies are necessary to determine whether this also occurs following hippocampal kindling. In contrast, the decrease in [³⁵S]TBPS binding in the hilus may be related to a kindling-induced specific loss of hilar neurons.^{3,31} However, this is not very likely since the loss of hilar neurons is permanent, whereas the [³⁵S]TBPS binding presents a non-significant increase in binding at the LT stage. Moreover, the heterogeneous population of neurons present in the hilus¹ makes it difficult to interpret these data, or to relate them to other studies that also showed distinct spatio-temporal patterns of changes in various cell types and neurotransmitter systems after kindling.^{17,21,30–32}

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