Plasticity of amino acid release and uptake during kindling epileptogenesis

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Chapter 3

Regulation of GABA release in rat hippocampus CA1 region during kindling epileptogenesis
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Abstract

GABA release induced by local tetanic stimulation of Schaffer-collateral fibres, was investigated during kindling epileptogenesis in the CA1 region of hippocampal slices. In slices from fully kindled animals, i.e. 24 h after the last generalized seizure, tetanically-stimulated GABA release was increased in comparison to control slices. In slices from long-term kindled animals, 4-5 weeks after the last seizure, stimulated GABA release was returned to control levels. Application of the broad GABA$_B$ receptor antagonist saclofen increased the tetanically-stimulated GABA release in control slices, but had no effect in fully kindled slices. In slices from long-term kindled animals, saclofen enhanced GABA release as in control slices. The selective GABA$_B$ autoreceptor antagonist CGP 52432 enhanced the tetanically-stimulated GABA release in control slices in a dose-dependent way, to equal values as induced by saclofen. We conclude that the transient tetanus-induced increased GABA release during kindling epileptogenesis is probably caused by impairment of presynaptic GABA$_B$ autoreceptors.

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Chapter 3

Introduction

There is compelling evidence that epilepsy is the result of a disturbed balance between glutamatergic and GABAergic neurotransmission (Meldrum, 1994; Bradford, 1995). A reduced GABAergic inhibition has been proposed as one possible mechanism leading to enhanced seizure susceptibility. As such, impairments in GABA release as well as GABA receptor function have been observed in hippocampus in a variety of animal models of epilepsy (Bradford, 1995). During epileptogenesis, opposite changes were reported in hippocampal CA1 region and the dentate gyrus with regard to GABA-mediated inhibition (Lopes da Silva et al., 1995). Generally, paired-pulse inhibition of local evoked field potentials was increased in dentate gyrus, but decreased in CA1 (Kamphuis et al., 1988, 1992a; Zhao & Leung, 1991, 1992). Consistent with these findings, GABA_A receptor function was found to be reduced in CA1 and enhanced in dentate gyrus (Titulaer et al., 1995; Gibbs et al., 1997; Mangan & Bertram, 1998). However, in CA1 region, both the direction and persistence of changes in GABA release observed during epileptogenesis were not consistent (Kapur et al., 1989; Jarvie et al., 1990; Kamphuis et al., 1990, 1991b). In these studies the release of GABA was evoked by high K^+ depolarization which makes it difficult to identify the source of released GABA and which synaptic pathways are involved. Furthermore, the nature of the depolarizing stimulus was very different from that occurring under physiological conditions (Klancnik et al., 1992).

In the CA1 region, GABA is released primarily from interneurons and exerts its inhibitory effect via two distinct classes of receptors: ligand-gated GABA receptors and G protein-coupled GABA_B receptors, mediating fast and slow postsynaptic inhibition, respectively (Nicoll et al., 1990; Thompson, 1994). In addition, presynaptic GABA_B autoreceptors on GABAergic neurons have been described controlling the release of GABA, whereas GABA_B heteroreceptors on glutamatergic afferents control the release of glutamate (Bowery et al., 1991; Thompson, 1994; Misgeld et al., 1995; Wu & Saggau, 1997). Recently, electrophysiological studies have indicated a reduced function of both post- and presynaptic GABA_B receptors in epileptic hippocampus, both in CA1 (Mangan & Lothman, 1996; Wu & Leung, 1997; Mangan & Bertram, 1998) and dentate gyrus (Haas et al., 1996; Buhl et al., 1997), indicating alterations in GABA release. However, these studies, measuring inhibitory postsynaptic potentials and currents, did not produce direct evidence for changes in GABA release as a consequence of reduced presynaptic autoinhibition. Moreover, there is no
consistency regarding whether changes occur in presynaptic $\text{GABA}_B$ auto- or hetero- receptors, postsynaptic $\text{GABA}_B$ receptors, or in a combination of these receptors.

In the present study we investigated the release of GABA from the CA1 region of hippocampal slices isolated from animals where an epileptic focus was generated by daily tetanic stimulation of the Schaffer-collateral fibres, the so-called kindling model of epilepsy (Goddard et al., 1969; Kamphuis & Lopes da Silva, 1990). In order to approach the physiological situation, GABA release was induced by electrical stimulation with 3-s tetanus trains of the Schaffer-collateral fibres. Release measurements were performed at different stages of kindling epileptogenesis (24 h and 4-5 weeks after establishing generalized seizures, respectively). Involvement of $\text{GABA}_B$ receptors in the regulation of GABA release during kindling was determined pharmacologically by application of the broad $\text{GABA}_B$ receptor antagonist saclofen, which does not discriminate between different $\text{GABA}_B$ receptor subtypes (Kerr et al., 1989; Misgeld et al., 1995). The $\text{GABA}_B$ receptor-mediated GABA release was studied in more detail by using the selective high-affinity $\text{GABA}_B$ autoreceptor antagonist CGP 52432 (Lanza et al., 1993).

This study demonstrates that the release of GABA is transiently enhanced during kindling epileptogenesis. This enhancement is most likely caused by a reduced regulation through $\text{GABA}_B$ autoreceptors. Part of this study has been previously reported in preliminary form (Zuiderwijk et al., 1997).

Materials and methods

Materials

SK&F 89976-A was kindly provided by Dr. Skidmore (Smith, Kline & Beecham, Welwyn, England). Saclofen was purchased from Tocris Cookson (Bristol, UK). CGP 52432 was derived from Novartis Pharma (Basel, Switzerland). Hypersil was from Shandon (Applied Science Group, Emmen, The Netherlands). Xylazine was from Bayer and ketamine from Aescoket (both from Aesculaap, Boxtel, The Netherlands). All other chemicals were of the purest grade available and were obtained from Sigma (Brunschwig, Amsterdam, The Netherlands) and Merck (Amsterdam, The Netherlands). All solutions were prepared with HPLC-
grade ultra-pure water generated by a "Milli-Q" purification system (Millipore, Bedford, MA, USA).

**Kindling procedure**

All experimental procedures were approved by the Animal Experiments Committee of the University of Amsterdam (March, 1997) according to the following criteria: (1) absence of alternatives for models of chronic epilepsy, (2) minimal suffering of the animals, (3) minimization of the number of animals used.

The kindling procedure used in this study was described in detail by Kamphuis et al. (1988). Briefly, male Wistar rats (230-300 g body weight) were implanted with stainless-steel, trimel insulated, electrodes in the CA1 area of the left dorsal hippocampus under ketamine/xylazine (4/3) anaesthesia (0.1 ml/100 g body weight, i.m.). The stimulation electrodes were placed in the Schaffer-collateral/commissural fibre pathway and the recording electrodes in stratum radiatum. After 1 week recovery, the rats were connected to a stimulation/recording device to enable kindling stimulations and local electroencephalographic recordings in freely moving conditions. The 31 implanted animals were divided into a non-stimulated control group \( n = 14 \) and a kindling group \( n = 17 \). The latter received, twice daily, kindling stimulations at an intensity suprathreshold for the induction of an afterdischarge (200-300 \( \mu \)A, 50 Hz, 1-2 s). The control group was handled in the same way as the kindling group throughout the experimental period, but did not receive tetanic stimulations. The animals of the kindling group were considered fully kindled after the occurrence of 7 generalized tonic-clonic class 5 convulsions (Racine, 1972). The release of GABA from hippocampal slices was studied in the following groups: a fully kindled group \( n = 10 \) in which release was measured 24 h after the 7\(^{th} \) class 5 seizure (along with this group, 8 animals of the control group were studied), and a so-called long-term kindled group \( n = 7 \) in which release was measured 28-33 days after the 7\(^{th} \) class 5 seizure (along with 6 animals of the control group). The animals of this long-term group were left unstimulated during this period, but it is known that a renewed kindling stimulation after such a long period results in a generalized convulsion, indicating that the excitability changes induced by kindling are long-lasting (Goddard et al., 1969).
**Preparation of hippocampal slices**

On the experimental day, the animals were anaesthetized with ketamine (0.1 ml/100 g body weight, i.p.) and decapitated. The left hippocampus was dissected out and 500 µm thick transversal slices from the dorsal hippocampus were cut with a tissue chopper. The slices were allowed to equilibrate for at least 1 h at room temperature in a holding chamber filled with artificial cerebrospinal fluid (a-CSF), containing (in mM) 124 NaCl, 3.5 KCl, 2 CaCl₂, 2 MgSO₄, 1.25 NaH₂PO₄, 26 NaHCO₃, 10 D-glucose, gassed with a mixture of 95 % O₂ and 5 % CO₂, at pH 7.4. A single slice was transferred to a recording chamber (1.0 ml volume), weighed down by a nylon grid, and submerged in continuously flowing a-CSF (1.0 ml/min) maintained at a temperature of 31-33°C.

**Electrical stimulation**

A bipolar stimulation electrode (stainless steel wires, 60 µm diameter) was positioned in the Schaffer-collateral/commissural fibre pathway near the CA1/CA3 border. A glass micro-electrode filled with a-CSF (resistance 1-5 MΩ) was placed in CA1 stratum radiatum to record extracellular field potentials, using an Axoclamp 1-D amplifier (Axon Instruments, Foster City, CA, USA) connected to a MacLab computer device (ADInstruments, Castle Hill, Australia). Biphasic stimuli (200 µs square pulse width) were delivered, using a PG 4000A stimulator (Neuro Data Instruments, Delaware Water Gap, PA, USA), at an intensity eliciting the maximal field postsynaptic potential amplitude (range 225-500 µA), at low-frequency (every 15 s) throughout the experiment to monitor the viability of the slice under baseline conditions. GABA release was evoked by 12 high-frequency trains (50 Hz, 300 µA, biphasic stimuli, 200 µs square pulse width) of 3 s duration applied at 20 s intervals, during a 4-min period.

**Measurement of GABA release**

In order to measure the local release of endogenous GABA, a small cannula (fused silica, i.d. 180 µm, o.d. 350 µm; Composite Metal Services, Hallow, UK) mounted on a micromanipulator, was placed under microscope guidance close to the recording electrode approximately 100 µm above the dendritic layers of CA1 pyramidal neurons. The cannula was connected to a peristaltic pump (model 205S; Watson-Marlow, Falmouth, UK) set at 35 µl/min. Following a 36-min
Chapter 3

stabilization period, 12 consecutive 1-min fractions were collected before, during and after 4 min of repetitive high-frequency stimulation. All collected samples were diluted to a total volume of 40 µl with a-CSF containing 1 % (wt/vol) trichloroacetic acid and 0.25 µM L-homoserine, as external standard, for amino acid content determination. To avoid amino acid metabolism, samples were kept cool (at 2°C) during the experiment by means of a recirculation cooler (B.Braun, Melsungen, Germany) and stored at -20°C until amino acid quantification. To prevent rapid clearance of local GABA release by GABA re-uptake carriers, all experiments were performed in the presence of 10 µM of the GABA re-uptake carrier blocker SK&F 89976-A (Yunger et al., 1984).

To investigate the regulation of electrically-stimulated GABA release by GABA_B receptors, the broad GABA_B receptor antagonist saclofen (250 µM) was added to the perfusion medium 10 min before the start of sample collection. From each animal, GABA release was measured in one hippocampal slice in the absence of saclofen and in another slice in the presence of saclofen. To investigate the subsynaptic location of the GABA_B receptor regulation of the stimulated GABA release, the high-affinity GABA_B autoreceptor antagonist CGP 52432 was applied at different concentrations in control experiments. These experiments were performed using two subsequent high-frequency trains of stimuli (S1 and S2) separated by 15 min, each consisting of three 50 Hz trains of 3 s during a 1 min period. a-CSF containing either CGP 52432, or saclofen was applied directly after S1 and during S2.

**Quantification of GABA**

GABA content was quantified by high-performance liquid chromatography (HPLC) after precolumn derivatization of a 25-µl sample with 50 µl 10 mM o-phthalaldehyde/0.4 % (vol/vol) mercaptoethanol in 100 mM Na-tetraborate (pH 10.5), as described previously (Verhage et al., 1989). The 12.5-cm-long, 4.6-mm-diameter column packed with Hypersil C-18 (3 µm particle size), was eluted isocratically with a buffer containing 0.1 M Na2HPO4, 1 mM Na2-ethylenediaminetetraacetate (EDTA), 0.3 % (vol/vol) tetrahydrofuran and 35 % (vol/vol) HPLC-grade methanol (pH 7.0). The o-phthalaldehyde derivates were detected by a Jasco (model FP-920) fluorimeter and evaluated by an on-line computerised data acquisition system (Gilson, Villiers le Bel, France). GABA levels were calculated by comparison with a standard sample of 0.25 µM of GABA.
Statistical analysis

GABA release from different experimental groups was statistically evaluated using two-factor analysis of variance (ANOVA) with repeated measures, followed by a post hoc unpaired Student's t-test applied to corresponding 1-min samples (Figs. 1 and 2). The S2/S1 ratios of GABA release were compared using an unpaired Student's t-test (Fig. 3). Values were considered significantly different when $P < 0.05$.

Results

Measurement of electrically-stimulated GABA release from hippocampal slices

GABA release stimulated by local application of high-frequency electrical stimuli to the Schaffer-collateral fibres in hippocampal slices could only be measured in the presence of $10 \mu M$ of the GABA re-uptake carrier blocker SK&F 89976-A (see Chapter 2). In control slices, in the presence of SK&F 89976-A, GABA release was increased significantly throughout the 4-min period of repetitive 50 Hz stimulation as compared to GABA levels before 50 Hz stimulation (Fig. 1, open circles). The increase was maximal (about 2-fold) during the first min of stimulation ($P = 0.0001$, paired Student's t-test), declined thereafter and reached pre-stimulus values immediately after termination of the 50 Hz stimulation period. This stimulation-induced GABA release was mainly of presynaptic exocytotic origin since $\text{Ca}^{2+}$ channel blockade with cadmium ($200 \mu M$) completely suppressed the enhancement of release (see Chapter 2). A significant increase in electrically-stimulated GABA release depended on the frequency of stimulation and could be clearly observed already at a frequency of 7.5 Hz. However, at 50 Hz, this increase was more pronounced and better reproducible. The electrically-stimulated GABA release measured occurred very locally in the CA1 region, since a cannula placed above the dentate gyrus region, at about 1 mm distance from the CA1 area, did not detect any changes in extracellular GABA upon stimulation of the Schaffer-collaterals (see Chapter 2).
Chapter 3

**GABA release is enhanced during kindling epileptogenesis**

GABA release from hippocampal slices was measured at two different stages of kindling epileptogenesis: at fully kindled stage, 24 h after the 7th class 5 seizure, and at long-term kindled stage, 28-33 days after the last class 5 seizure. All control experiments were pooled, since there was no significant difference between GABA release from control slices corresponding to the different kindled groups, neither under baseline conditions, nor during high-frequency electrical stimulation. Under baseline conditions (one stimulus every 15 s), the mean extracellular GABA level in control slices was 9.4 ± 1.0 nM (Fig. 1). This was slightly increased in slices from fully kindled animals to 13.0 ± 1.4 nM (P < 0.05, unpaired Student’s t-test) and returned to control values in slices from long-term

![GABA release graph](image)

**Fig. 1.** Effect of kindling on electrically-stimulated GABA release. GABA release was measured in slices from fully kindled animals (closed circles) (n = 10), and long-term kindled animals (triangles) (n = 7). Values of non-kindled control slices from the fully kindled and long-term kindled group were pooled into one control group (open circles) (n = 14). All experiments were performed in the presence of SK&F 89976-A (10 μM). Values are expressed as mean sample concentrations ± SEM. Comparisons were made between control and kindled groups, before, during and after 4 min of repetitive 50 Hz stimulation (indicated by the black bar), using repeated-measure ANOVA followed by an unpaired Student’s t-test at corresponding time points (*P < 0.05, **P < 0.01, ***P < 0.001).
kindled animals (8.6 ± 1.2 nM). During 4 min repetitive 50 Hz stimulation, the GABA release from fully kindled slices was increased significantly as compared to control slices (P = 0.0004, repeated-measure ANOVA). The increase was maximal during the first min of stimulation (28.2 ± 2.3 nM for fully kindled vs. 17.6 ± 0.9 nM for controls; Fig. 1), declined thereafter and reached pre-stimulus values after termination of 50 Hz stimulation. Since GABA levels from fully kindled slices were already increased under baseline conditions, we corrected for this effect by subtracting the GABA release under these conditions from that during 50 Hz stimulation. After baseline subtraction, 50 Hz-stimulated GABA release was still enhanced, although it was only significant during the first min of stimulation (15.2 ± 2.0 nM for fully kindled vs. 8.3 ± 1.1 nM for controls). 50 Hz-stimulated GABA release from long-term kindled slices did not significantly differ from control slices (17.4 ± 0.6 nM during the first min; Fig. 1). After termination of 50 Hz stimulation, GABA release from both fully kindled and long-term kindled slices, did not significantly differ from control slices.

**Regulation of GABA release by GABA_B receptors is impaired during kindling epileptogenesis**

We tested whether the regulation of electrically-stimulated GABA release was mediated by GABA_B receptors at different stages of kindling epileptogenesis. Because of the limited availability of kindled animals, we applied the low-affinity GABA_B receptor antagonist saclofen, which does not discriminate between different GABA_B receptor subtypes (Kerr et al., 1989; Misgeld et al., 1995). All control experiments were pooled, since there was no significant difference between GABA release from control slices corresponding to the different kindled groups, neither in the absence, nor in the presence of saclofen. Under baseline conditions, the application of 250 μM saclofen had no effect on extracellular GABA levels, neither in control, nor in kindled slices (Figs. 2A, B and C). In control slices, the observed increase in GABA release during 4 min of repetitive 50 Hz stimulation was further increased in the presence of 250 μM saclofen (P = 0.003, repeated-measure ANOVA; Fig. 2A). Maximal enhancement was observed during the first min of 50 Hz stimulation (peak values in the absence and presence of saclofen amounted to 17.6 ± 0.9 nM and 25.2 ± 2.4 nM, respectively), clearly indicating that 50 Hz-stimulated GABA release is regulated by GABA_B receptors. This regulation is frequency-dependent since under baseline conditions (0.067 Hz) no effects of saclofen were observed. In contrast to controls, the
Chapter 3

A

Control

GABA (nM)

--- 250μM Saclofen

--- Without Saclofen

Time (min)

36 37 38 39 40 41 42 43 44 45 46 47

50 Hz

B

Fully kindled

GABA (nM)

--- 250μM Saclofen

--- Without Saclofen

Time (min)

36 37 38 39 40 41 42 43 44 45 46 47

50 Hz

C

Long-term kindled

GABA (nM)

--- 250μM Saclofen

--- Without Saclofen

Time (min)

36 37 38 39 40 41 42 43 44 45 46 47

50 Hz
application of saclofen to slices from fully kindled animals did not induce any significant effect on GABA release during 50 Hz stimulation, indicating impaired GABA_B receptor function. Peak values in the absence and presence of saclofen amounted to 28.2 ± 2.3 nM and 27.9 ± 2.3 nM, respectively (Fig. 2B), being similar to the release values in the presence of saclofen in control slices (Fig. 2A). However, the application of saclofen to slices from long-term kindled animals enhanced GABA release during 50 Hz stimulation similarly to what was observed in control slices (peak values in the absence and presence of saclofen amounted to 17.4 ± 0.6 nM and 22.6 ± 1.8 nM, respectively; Fig. 2C). After termination of 50 Hz stimulation, the application of saclofen had no effect on extracellular GABA levels, neither in control, nor in kindled slices (Figs. 2A, B and C).

GABA release is regulated by presynaptic GABA_B autoreceptors

To investigate whether GABA_B autoreceptors are involved in the regulation of electrically-stimulated GABA release, we used the high-affinity GABA_B autoreceptor antagonist CGP 52432 (Lanza et al., 1993) in control experiments. These experiments were performed using two 50 Hz trains of stimuli, applied sequentially at an interval of 15 min, first in the absence (S1) and then in the presence (S2) of GABA_B receptor antagonists (see Materials and methods). Because the effects of saclofen on electrically-stimulated GABA release observed in the previous experiments were maximal during the first min of repetitive 50 Hz stimulation (see Fig. 2A), the duration of S1 and S2 was 1 min. The S2/S1 ratio in the absence of CGP 52432 was 1.05 ± 0.02. This ratio was increased by CGP 52432 in a dose-dependent manner, being maximal (1.33 ± 0.05) at a concentration of 100 μM (Fig. 3). At 200 μM CGP 52432, the S2/S1 ratio (1.16 ± 0.04; Fig. 3) was significantly reduced as compared to that at 100 μM CGP 52432 (P = 0.04, unpaired

Fig. 2. Effect of saclofen on electrically-stimulated GABA release in slices from kindled and control animals. From each animal, GABA release was measured in one hippocampal slice in the absence, and in another in the presence of saclofen (250 μM). All experiments were performed in the presence of SK&F 89976-A (10 μM). See Fig. 1 for groups and statistics. For reasons of comparison the data on GABA release without saclofen are replotted from Fig. 1.
Student's t-test). The S2/S1 ratio at 100 \mu M CGP 52432 was equal to that at 250 \mu M saclofen (1.36 \pm 0.09; Fig. 3), which was the concentration used in the kindling experiments. These results strongly indicate that the effects of saclofen on electrically-stimulated GABA release in the CA1 region of hippocampal slices are regulated by GABA_B autoreceptors.

**Fig. 3.** Effects of CGP 52432 and saclofen on electrically-stimulated GABA release in slices from control animals. Experiments were performed using two high-frequency stimuli (S1 and S2) separated by 15 min, each consisting of three 50 Hz trains of 3 s during a 1 min period. a-CSF containing either CGP 52432, or saclofen was applied directly after S1 and during S2. Data are expressed as the mean S2/S1 ratio \pm SEM (n = 4-10) of GABA release measured during S1 and S2. Comparisons were made between S2/S1 in the absence of GABA_B receptor antagonists and S2/S1 in the presence of different concentrations of CGP 52432, and between S2/S1 at 250 \mu M saclofen and S2/S1 at 100 \mu M CGP 52432 using an unpaired Student's t-test (**P < 0.01).

**Discussion**

In the present study we investigated changes in presynaptic GABA release during kindling epileptogenesis, and the involvement of GABA_B receptors in these changes. The main findings were: (1) Local tetanically-stimulated release of
GABA release during kindling

GABA from the CA1 region of hippocampal slices was enhanced at fully kindled stage (24 h after the last seizure), but restored to control level at long-term kindled stage (4-5 weeks after the last seizure). (2) Tetanically-stimulated GABA release was tonically regulated by presynaptic GABA$_B$ autoreceptors. (3) This GABA$_B$-mediated regulation was absent at fully kindled stage, resulting in enhanced GABA release, and recovered at long-term kindled stage.

**Changes in GABA release during kindling epileptogenesis**

This is the first study in which kindling-induced alterations in local exocytotic GABA release upon tetanic stimulation of afferent fibres were measured. Several other *in vitro* studies have demonstrated kindling-induced changes in GABA release from hippocampal slices evoked by depolarization with high K$^+$ (Liebowitz et al., 1978; Kapur et al., 1989; Jarvie et al., 1990; Kamphuis et al., 1990, 1991b). However, such a massive stimulus will drastically recruit GABA from its different releasable pools from neurons as well as glial cells, rather than triggering limited amounts of GABA release from locally excited GABAergic nerve endings. Therefore, K$^+$-induced GABA release represents total capacities of releasable pools, rather than actual GABA release (see Chapter 2).

In agreement with our finding of an increase in 50 Hz-stimulated GABA release at fully kindled stage, previous studies of Kamphuis et al. (1990, 1991b) using the same kindling protocol, observed an increase in K$^+$-stimulated Ca$^{2+}$-dependent release of GABA from CA1 slices at the same kindled stage. In contrast, at long-term kindled stage, 50 Hz-stimulated GABA release was restored to control values in our study, whereas K$^+$-stimulated GABA release was still enhanced (Kamphuis et al., 1990, 1991b). This contradictory result may be explained by the nature of the stimulus recruiting different releasable pools, as mentioned above. In agreement with our study, Jarvie et al. (1990) found no change in K$^+$-stimulated Ca$^{2+}$-dependent GABA release from CA1 slices 1 month after the last kindled seizure. The transient character of changes in GABA release, being enhanced at fully kindled stage and restored 4-5 weeks after the last seizure, indicates a seizure-related mechanism in the CA1 region. Under low-frequency (0.067 Hz) conditions, we observed an increase in GABA release at fully kindled stage. This increase is likely to be Ca$^{2+}$-independent since, in control experiments, extracellular GABA levels under these conditions were not altered by Ca$^{2+}$ channel blockade with cadmium (see Chapter 2). The increase might be related to the observation of Kamphuis et al. (1990) that CA1 slices of fully kindled animals
contained larger amounts of GABA, which could give rise to a larger spontaneous efflux of GABA from cytosolic compartments.

Recently, impaired functioning of hippocampal GABA transporters, both in patients with temporal lobe epilepsy and amygdala-kindled rats, has been reported (During et al., 1995). This would result in delayed clearance of released GABA from the extracellular space. Since in our study electrically-stimulated GABA release was measured in the presence of the GABA re-uptake blocker SK&F 89976-A, interference of eventual kindling-induced changes in GABA re-uptake activity with the observed changes in GABA release is rather unlikely.

**GABA\(_B\) receptor-mediated regulation of GABA release**

In control slices, the application of saclofen enhanced GABA release during high-frequency (50 Hz) stimulation, but did not affect GABA release under low-frequency conditions (0.067 Hz). This indicates that the activation of GABA\(_B\) receptors is frequency-dependent and may only play a role during periods of high-frequency stimulation. This is in line with other electrophysiological studies showing regulation of GABA release during repetitive stimulation at higher frequencies (Davies & Collingridge, 1993; Mott et al., 1993). It was demonstrated that presynaptic GABA\(_B\) receptors were not activated by spontaneous GABA release under basal conditions (Otis & Mody, 1992; Doze et al., 1995). Although our experiments were performed in the presence of the GABA re-uptake inhibitor SK&F 89976-A, the resulted increase in extracellular GABA was apparently not sufficient for activation of presynaptic GABA\(_B\) autoreceptors. Electrophysiological studies showed that GABA uptake inhibition can activate the action of GABA on both presynaptic GABA\(_B\) heteroreceptors and postsynaptic GABA\(_B\) receptors, but an effect on GABA\(_B\) autoreceptors was not reported (Thompson & Gähwiler, 1992; Isaacson et al., 1993). There is no firm evidence yet for presynaptic regulation of synaptic transmitter release by endogenous GABA acting on presynaptic GABA\(_B\) autoreceptors under basal conditions (Misgeld et al., 1995). In a biochemical study in cortical slices, it was shown that GABA\(_B\) receptor antagonists stimulated the release of GABA at low-frequency stimulation already, even in the absence of uptake inhibition (Waldmeier et al., 1993). However, GABA release in that study was evoked by massive electrical stimulation of the whole slice chamber, instead of local fibre stimulation as applied in our experiments.
It has been indicated that 2-OH saclofen (the hydroxylated analogue of saclofen) may act as weak partial agonist (Stuart & Redman, 1992; Caddick et al., 1995). An agonistic effect of saclofen in our experiments is, however, not likely, since this would result in a decrease, rather than the observed increase of stimulated GABA release (see Fig. 1). Although saclofen, which we applied in the kindling experiments, does not discriminate between GABA<sub>B</sub> receptor subtypes (Misgeld et al., 1995), the close similarity between the effects of saclofen and CGP 52432 in our control studies strongly suggests that also in the kindling experiments electrically-stimulated GABA release is selectively regulated by presynaptic GABA<sub>B</sub> autoreceptors. In our experiments, the enhanced GABA release at 100 µM CGP 52432 is likely to be mainly mediated by antagonism of GABA<sub>B</sub> autoreceptors, since at 200 µM CGP 52432 GABA release was reduced. This reduction may be explained by an antagonistic effect of CGP 52432 on GABA<sub>B</sub> heteroreceptors on glutamatergic terminals when applied at higher concentrations, which was also observed in other biochemical studies (Lanza et al., 1993; Bonanno et al., 1997). The subsequent elevation of extracellular glutamate could exert secondary reduction of GABA release via activation of metabotropic glutamate receptors on GABAergic interneurons (Gereau & Conn, 1995; Morishita et al., 1998). However, in our preparation we were not able to measure electrically-stimulated release of glutamate from the CA1 region, even when glutamate re-uptake was blocked with L-trans-pyrrolidine-2,4-dicarboxylate (Bridges et al., 1991), probably due to a relatively high background glutamate release from non-vesicular pools (data not shown).

In conclusion, our experiments with saclofen and CGP 52432 reveal biochemical evidence for a prominent role of GABA<sub>B</sub> autoreceptors in regulating GABA release in hippocampus CA1 region.

**Impaired GABA<sub>B</sub> autoreceptor function in epilepsy**

To our knowledge, this is the first study providing direct evidence for an impaired GABA<sub>B</sub> autoreceptor function during kindling epileptogenesis. Earlier electrophysiological studies have also indicated impairments in GABA<sub>B</sub> receptor-mediated processes during kindling (Asprondini et al., 1992; Buhl et al., 1997; Wu & Leung, 1997). However, in these studies postsynaptic events were determined, being rather indirect measures of eventual changes in presynaptic receptors. Moreover, there was no consistency regarding whether changes occurred in presynaptic GABA<sub>B</sub> auto- or hetero-receptors, or in a combination of these
receptors. This was probably due to the different kindling models used and different brain regions investigated. In CA1 region, Wu & Leung (1997) indicated a persistent reduction in GABA$_B$ autoreceptor activity, both directly after, and 3 weeks after partial hippocampal kindling. However, during partial kindling no convulsions were evoked, which was rather different from our study. Moreover, Wu & Leung (1997) used CGP 35348 at a very high concentration (1 mM) at which this antagonist acts probably on both auto- and hetero-receptors (Davies & Collingridge, 1993). Another kindling study showed a reduction in paired-pulse inhibition of monosynaptically evoked IPSCs in dentate granule cells, 1-2 days after fully kindling of the perforant path, but did not investigate the involvement of GABA$_B$ autoreceptors with selective antagonists (Buhl et al., 1997). A reduced GABA$_B$ heteroreceptor function has also been found in the basolateral amygdala, 4-8 weeks after amygdala kindling (Asprondini et al., 1992), but in this study it remained unclear whether the autoreceptor was altered as well.

The precise mechanism of the GABA$_B$ autoreceptor dysfunction is unknown. It is possible that the number of GABA$_B$ receptors was transiently decreased during kindling. However, although this has not yet been investigated in convulsive epilepsy models, no evidence for differences in the GABA$_B$ receptor density was found in a rat model of absence epilepsy (Marescaux et al., 1992). It is known that presynaptic GABA$_B$ receptor-mediated processes require linkage of the receptor to Ca$^{2+}$ channels via G proteins (Wu & Saggau, 1997; Bettler et al., 1998). Uncoupling between GABA$_B$ autoreceptors and G proteins, or a dysfunction at any level in the G protein cascade could explain the impaired GABA$_B$ receptor function.

The observation that transient impairment of presynaptic GABA$_B$ autoreceptor function results in enhanced GABA release, strongly suggests that this impairment may work as a compensatory mechanism to oppose the reduced postsynaptic GABA$_A$ receptor function in the CA1 region at fully kindled stage previously observed (Titulaer et al., 1994, 1995). The fact that the impaired GABA$_B$ receptor function was only apparent under tetanic stimulation conditions, could reflect its relevance to counteract the reduced GABA-mediated synaptic inhibition of excessive neuronal activation occurring during epileptic seizures. The recovery of GABA$_B$ receptor function 4-5 weeks after the last generalized seizure, indicates that the impairment is seizure-related. This opens up the possibility for therapeutical intervention during seizure-free periods. Selective blockade of presynaptic GABA$_B$ autoreceptors, might have
anticonvulsant potency by antagonizing the reduced GABAergic inhibition in the epileptogenic focus, thereby preventing the occurrence of new seizures.

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