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Research Report

Electrically evoked GABA release in rat hippocampus CA1 region and its changes during kindling epileptogenesis

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ABSTRACT

Previous findings on changes in K^+-induced GABA release from hippocampal slices during kindling epileptogenesis were reinvestigated using physiological electrical stimulation. For that purpose, a procedure was developed enabling neurochemical monitoring of GABA release locally in the CA1 region of rat hippocampal slices upon tetanic stimulation of Schaffer-collateral fibers. In the presence of a GABA reuptake blocker, subsequent application of short (3 s) pulses of 50-Hz stimuli induced a local transient increase in GABA release. In slices from fully kindled animals, 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, tetanically stimulated GABA release had returned to control levels. Application of the broad low-affinity $GABA_B$ 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, however, saclofen enhanced GABA release similarly as in control slices. We conclude that the transient increase in tetanus-induced GABA release during kindling epileptogenesis is seizure-related, and probably caused by temporarily impaired presynaptic $GABA_B$ receptors. The possible relevance of this finding for GABA transmission in epilepsy is discussed.

1. Introduction

The concept that enhanced excitability of neuronal networks in epilepsy is the result of a disturbed balance between glutamatergic and GABAergic neurotransmission is widely assumed (Meldrum, 1994; Bradford, 1995). In this context, a reduced GABAergic inhibition has been proposed as one possible mechanism leading to enhanced seizure susceptibility. Impairments in GABA release as well as GABA receptor function have been observed in hippocampus in a variety of animal models of epilepsy (Olsen and Avoli, 1997; Hirsch et al., 1999). 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, 1992; Zhao and 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 and Bertram, 1998). More recently, differences in vulnerability of certain types of interneurons and in their projection sites

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onto pyramidal neurons, i.e. soma versus dendrite, have been reported to underlie opposite changes in GABAergic inhibition in CA1 region in experimental epilepsy (Cossart et al., 2005).

Since GABAergic networks undergo complex rewiring and local postsynaptic GABA receptor redistribution in epileptic tissue (Cossart et al., 2005), a better insight in the plasticity of presynaptic GABA release during epileptogenesis requires direct monitoring of the latter. However, in CA1 region, reports on 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, 1991). In these studies the release of GABA was evoked by depolarization with high K^+, which will induce secretion of GABA from different releasable pools from neurons, i.e. vesicular and cytosolic, as well as from glial cells. As such, the changes in K^+-induced GABA release reported in these studies represent changes in the total capacity of these diverse pools, rather than changes in actual GABA release from locally excited GABAergic nerve endings. Therefore, targeted electrical stimulation of afferent fibers along with monitoring of local transmitter release near the projection site should give a more precise insight in presynaptic plasticity of GABAergic transmission (Klancnik et al., 1992).

In order to investigate changes in electrically evoked GABA release in CA1 region during epileptogenesis, we developed a procedure which combined targeted electric stimulation of Schaffer-collateral afferent fibers in hippocampus slices with local sampling of superfusates above CA1 region for GABA analysis. Physiological stimulation was approached by application of 3 s tetanus trains to the fibers. As model system for epileptogenesis, the kindling model of epilepsy was applied by which an epileptic focus was gradually generated in the hippocampus of rats by daily tetanic stimulation of the Schaffer-collateral fibers (Goddard et al., 1969; Kamphuis and Lopes da Silva, 1990). Since neuronal death is not prominent in this model, eventual changes in GABA release and/or receptor function due to cell loss were minimal. Release measurements were performed at different stages of kindling epileptogenesis (24 h and 4–5 weeks after establishing generalized seizures, respectively). As prominent regulators of transmitter release, involvement of $GABA_B$ receptors in altered GABA release during kindling was determined pharmacologically by application of the broad $GABA_B$ receptor antagonist saclofen (Kerr et al., 1989; Misgeld et al., 1995; Wu and Saggau, 1997). Previous electrophysiological studies have suggested a reduced function of both post- and presynaptic $GABA_B$ receptors in epileptic hippocampus CA1 region (Mangan and Lothman, 1996; Wu and Leung, 1997; Mangan and Bertram, 1998).

This study demonstrates local elevation of endogenous GABA release in CA1 region upon high-frequency stimulation of the Schaffer afferent fibers in hippocampus slices. This electrically evoked release of GABA is transiently enhanced during kindling epileptogenesis. This enhancement is most likely caused by a timely reduced regulation through $GABA_B$ receptors, possibly of the autoreceptor subtype. Part of this study has been previously reported in preliminary form (Zuiderwijk et al., 1997).

2. Results

2.1. *Electrically stimulated GABA release from CA1 region in hippocampal slices*

As shown in Fig. 1 (open circles), in control slices GABA levels collected near the hippocampus CA1 region under baseline conditions amounted to 10.7±1.7 nM (average of three prestimulation 1-min samples). These levels did not increase significantly upon local stimulation of Schaffer-collateral fibers by 3-s-lasting 50-Hz trains. Since stimulus-evoked GABA levels outside the slice could be masked by internal rapid clearance of local GABA release by GABA transporters, the experiments were repeated in the presence of the GABA reuptake inhibitor SK&F 89976-A (Yunger et al., 1984). Application of 10 μM SK&F 89976-A increased extracellular GABA levels significantly under baseline conditions to 17.4± 1.5 nM as compared to levels in the absence of the reuptake blocker (Fig. 1, closed circles; $p=0.03$, unpaired Student's *t*-test). In the presence of 10 μM SK&F 89976-A, repetitive stimulation of Schaffer-collateral fibers by 3-s pulses of 50 Hz applied every 20 s resulted in a significant increase in GABA release from the slices throughout the total 4-min-lasting stimulation period as compared to the pre-stimulation baseline levels. The increase was maximal (about twofold) during the first minute of stimulation (37.6±4.8 nM; $p=0.0001$, paired Student's *t*-test), declined thereafter and

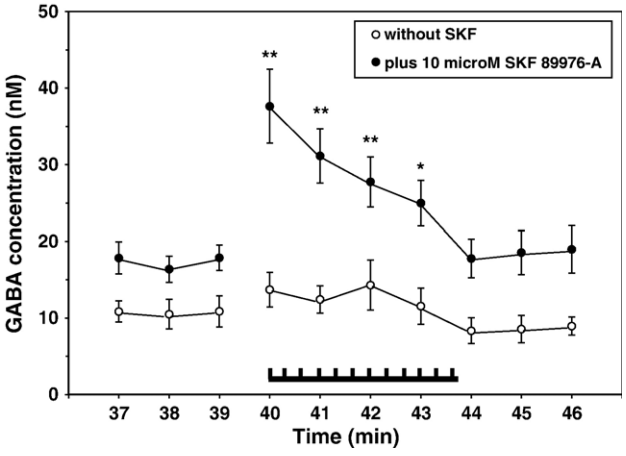

Fig. 1 – Effect of repetitive high-frequency stimulation (50 Hz) on GABA release from CA1 region in hippocampal slices. Measurements were performed in the absence (open circles, $n=5$) or presence (closed circles, $n=11$) of 10 μM SK&F 89976-A. Prior to and after high-frequency electrical stimulation, low-frequency (every 15 s) stimulation was applied at an intensity eliciting the maximal field postsynaptic potential amplitude (range 225–500 μA) to monitor the GABA release under baseline conditions. The 4-min period of 3-s-lasting high-frequency pulses applied at 20-s intervals is indicated by the notched bar. Values are expressed as mean sample concentrations±SEM. Comparisons were made between GABA levels during 50-Hz stimulation with levels before high-frequency stimulation ($^*p<0.05$, $^{**}p<0.01$, paired Student's *t*-test).