Activation of Kainate GLU$_{K5}$ Transmission Rescues Kindling-Induced Impairment of LTP in the Rat Lateral Amygdala

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The amygdala is a component of the limbic system that plays a central role in emotional behavior and certain psychiatric diseases. Pathophysiological alterations of neuronal excitability in the amygdala are characteristic features of temporal lobe epilepsy and certain (epilepsy accompanying) psychiatric illnesses such as anxiety and depressive disorders. The role of kainate receptors in the activity of synaptic networks, in brain function, and diseases is still poorly understood. Various kainate receptor subtypes have been shown to contribute to synaptic transmission and modulate presynaptic release of glutamate and γ-aminobutyric acid (GABA). Several lines of evidence point to the importance of GLU$_{K5}$ kainate receptors in epilepsy. In this study we investigated the role of specific GLU$_{K5}$ kainate receptor in the lateral nucleus of the amygdala (LA). The cellular mechanisms for emotional learning in the amygdala are believed to be the result of changes in synaptic transmission efficacy, similar to long-term potentiation (LTP). Here, we used both field potential and intracellular recordings in horizontal rat amygdala slices, and showed that LTP in the LA, induced by high-frequency stimulation of afferents running within LA, is impaired 48 h after the last induced seizure. This kindling-induced impairment was reversed by the specific kainate GLU$_{K5}$ agonist ATPA. Partial blockade of GABAergic transmission with the specific GABA$_A$ receptor antagonist SR95531 also significantly facilitated the induction of early LA-LTP, but only partially abolished the kindling-induced impairment of LA-LTP. This study shows that the stimulation of the GLU$_{K5}$ kainate receptor subtype rescues the kindling-induced impairment of LA-LTP at least within 48 h after the last seizure. Therefore, GLU$_{K5}$ kainate receptor subunits are involved in kindling-induced plasticity changes in the amygdala.
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INTRODUCTION

Temporal lobe epilepsy (TLE) is a common form of epilepsy and the most resistant to drug therapy (Pitkanen and Sutula, 2002). TLE patients often show emotional disturbances ranging from mild fear to pathological levels of anxiety and depression as well as memory impairment (Kalynchuk, 2000). Although these symptoms are well documented, little progress has been made in identifying their mechanisms. The amygdala is a key structure for emotional modulation of behavior as well as learning and memory (LeDoux, 2000; Maren, 2001). Synapses in the amygdala display long-term potentiation (LTP) (for review, see Chapman and Chattarji, 2000), and for many years LTP has been discussed as a model for emotional learning and memory.

Kindling is a widely studied animal model of TLE in which daily electrical stimulation of certain brain regions results in the gradual progression and intensification of limbic motor seizures (Goddard *et al*, 1969). These recurrent seizures induced by repeated electrical stimulation develop much faster in the amygdala than in the hippocampus (Goddard *et al*, 1969; McIntyre and Racine, 1986; Loscher, 1997). Although kindling has been extensively investigated in the context of its clinical relevance to epilepsy, limited numbers of studies have investigated plasticity changes after the kindling procedure. Recently, we have shown that kindling of the basolateral nucleus of the amygdala (BLA) resulted in a significant impairment in the overall magnitude of LTP in the lateral nucleus of the amygdala (LA) (Schubert *et al*, 2005). The observed impairment of LTP in kindled animals might be a result of functional changes such as the up- or downregulation of transmitter receptors, involved in mediation of plasticity in the amygdala.

Kainate receptors are hetero-oligomeric receptor channels composed of the subunits glutamate receptor GLU$_{K5}$, GLU$_{K6}$, GLU$_{K7}$, GLU$_{K1}$, and GLU$_{K2}$ (Huettner, 2003). They appear to play a special role in the regulation of synaptic

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network activity, such as mediation of excitatory synaptic transmission (Clarke and Collingridge, 2002; Li and Rogawski, 1998) or the modulation of neurotransmitter release (Frerking and Nicoll, 2000). GLU_{K5} kainate receptors are highly expressed in the adult amygdala in comparison to their expression in the hippocampus (Bettler *et al*, 1990; Li *et al*, 2001). Several lines of evidence point to the importance of GLU_{K5} kainate receptors in TLE: (1) GLU_{K5} kainate receptor antagonists prevent hippocampal seizures induced by pilocarpine or electrical stimulation in rats, both *in vitro* and *in vivo* (Smolders *et al*, 2002); (2) high concentrations of the selective GLU_{K5} kainate receptor agonist (RS)-2amino-3-(3-hydroxy-5-*tert*-butylisoxazol-4-yl) propanic acid (ATPA) induce spontaneous epileptiform bursting in rat amygdala slices (Li *et al*, 2001) and limbic status epilepticus when infused intravenously or when applied directly into the rat amygdala (Rogawski *et al*, 2003; Kaminski *et al*, 2004); (3) GLU_{K5} kainate receptors are one of the primary targets of the structurally novel anti-convulsant topiramate (Kaminski *et al*, 2004); and (4) expression of GLU_{K5} kainate receptors is elevated in epileptic temporal lobe regions in both humans and rats (Palma *et al*, 2002; Ullal *et al*, 2005).

γ-Aminobutyric acid (GABA)ergic transmission may also be important in TLE, as GABAergic interneurons seem to be involved in kindling-induced plasticity changes in the amygdala (Kamphuis *et al*, 1987; Callahan *et al*, 1991) and activation of kainate receptors modulates GABAergic synaptic transmission in the amygdala (Braga *et al*, 2003). Therefore, the aim of this study was to investigate whether the activation of GLU_{K5} kainate receptors and/or inactivation of $GABA_A$ receptors can rescue the kindling-induced impairment of LA-LTP.

We found that the specific GLU_{K5} kainate receptor agonist ATPA was able to completely reverse the kindling-induced inhibition of LA-LTP. However, the partial blockade of $GABA_A$ receptors with the antagonist SR95531 (6-imino-3-(4-methoxyphenyl)-1(6H)-pyridazinebutanoic acid hydrobromide) only partially reversed the kindling-induced impairment of LA-LTP.

METHODS

Animals and Treatment

Three groups of male Wistar rats (6-weeks old, 180–210 g) were used in this study: age-matched nonimplanted controls ($n = 26$ rats), age-matched sham-implanted controls ($n = 14$ rats), and age-matched kindled group of animals ($n = 14$ rats). Sham-implanted animals were used since the implanted electrode may induce marked biochemical and functional changes in the absence of kindling (Loscher *et al*, 1993, 1995). Animals were housed under standard laboratory conditions ($22 \pm 1^\circ C$, 60–65% relative humidity, 12/12 h alternate light–dark cycles, food and water *ad libitum*) for at least 1 week before being used for the experiments.

Under ketamine/rompun anesthesia (87 and 13 mg/kg, i.p.) bipolar stainless steel electrodes were implanted into the BLA (AP −2.3; L 5.0; H 8.5) (Paxinos and Watson, 1986). In some animals electrodes were also implanted in the CA1 region for EEG recordings. After a postsurgical
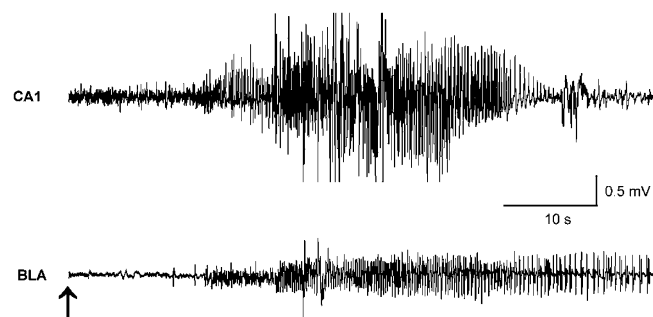
Figure 1 Kindling stimulus-associated EEG changes in the CA1 region of the hippocampus and the basolateral nucleus of the amygdala (BLA) in a rat reached stage-five seizures. The stimulus (60 Hz, 400 μA, I s) was delivered before the arrow. Stimulation resulted in intense activity in the hippocampus and amygdala. The kindling electrode was implanted into the left BLA in sham-implanted and kindled animals.

recovery period of 7–10 days, one group ($n = 14$ rats) was stimulated daily (five times per week) through the implanted electrode by a train of pulses with a duration of 0.1 ms at 60 Hz for 1 s (300–450 μA). The intensity used for stimulation was set to initially induce only a twitching of the eye ipsilateral to the stimulated amygdala. This threshold current was kept constant and delivered once per day until seven consecutive stage-five seizures with rearing and/or falling (Figure 1). Behavioral changes during kindling were scored according to the scale of Racine (1972). A mean of 14 ± 1 stimulations was required to evoke the first stage-five seizure. On these last seven stimulations, the mean duration of seizures was 43 ± 2 s. The mean age at the day of the *in vitro* experiment for the different groups did not differ significantly (nonimplanted = 87 ± 6 days; sham-implanted = 86 ± 5 days; kindled = 84 ± 4 days). There were also no significant differences between the time of electrode implantation and the time of the *in vitro* experiment between sham-implanted and kindled animals. The position of the stimulation electrode in the BLA was verified histologically. Electrodes in all kindled rats included in the study were located in the BLA. All experiments were carried out in accordance with the European Communities Council Directive of 24 November 1986 (89/609/EEC) and approved by the regional Berlin animal ethics committee (G0291/01). All efforts were made to minimize suffering and the numbers of animals used.

Preparation

The rats were anesthetized with isoflurane and decapitated. Their brains were rapidly removed and placed in ice-cold carbogenated artificial cerebrospinal fluid (ACSF, 129 mM NaCl; 3 mM KCl; 21 mM $NaHCO_3$; 1.25 mM Na_2HPO_4; 1.8 mM $MgSO_4$; 1.6 mM $CaCl_2$; and 10 mM glucose). The cerebellum was removed and a cut was made to divide the two cerebral hemispheres. Each hemisphere was fixed onto the perspex carrier of the vibroslicer (Campden Instruments, Silbey, UK) using a cyanoacrylat adhesive (Patex). Horizontal slices, 400 μm thick, contained the amygdala, the hippocampus, the entorhinal cortex, and parts of the piriform cortex. These horizontal brain slices preserve the majority of interconnections within the amygdala and

between the amygdala, the hippocampus, and the entorhinal cortex (von Bohlen und Halbach and Albrecht, 1998, 2002) and were prepared from nonimplanted control, sham-implanted, and kindled rats 48 h after reaching seven consecutive stage-five seizures.

The appropriate slices were placed in an interface chamber and allowed to equilibrate for 120 min at 34°C. They were superfused continuously with ACSF (1.5 ml/min). The pH was maintained at 7.4 by carbogenating and oxygenating the solution with 95% O_2 and 5% CO_2.

Extracellular Recording

Glass microelectrodes (Science Products, Hofheim, Germany) were filled with ACSF (tip resistance 1 MΩ) and placed in the caudoventral part of the LA. Bipolar stimulation electrodes were used to stimulate afferents running within the LA (Drephal *et al*, 2006). Single stimuli (duration 100 μs) were presented every 10 s. Signals of the evoked responses were amplified and filtered (bandpass: 0.1 Hz–3 kHz) by a preamplifier (World Precision Instruments, Sarasota, FL, USA), displayed on a storage oscilloscope, and fed via a CED laboratory interface (Cambridge Electronic Design, UK) to a computer for storage. The experimenter was not aware of the history of stimulation of each rat, and *in vitro* experiments were randomized with respect to the different groups of animals on a day-to-day basis.

An input/output (I/O) response curve was constructed by varying the intensity of single-pulse stimulation and, for each intensity, averaging six responses. The stimulus intensity that evoked a mean field potential (FP) equal to 50% of the maximum response was then used for all subsequent stimulations, ie tetanus and subsequent single- and paired-pulse stimulations. Drug-induced changes in baseline activity were considered.

Single stimuli (duration 100 μs) were presented every 10 s. To evaluate short-term synaptic interactions paired-pulse stimuli were delivered with interstimulus intervals ranging from 10 to 500 ms. Six consecutive impulses were averaged off-line for each interval. The relative amplitude of the second with respect to the first response amplitude was calculated and plotted as paired-pulse ratio against the interstimulus interval. Single stimuli were continually applied for a least 30 min and responses were monitored. Once a stable baseline of responses had been obtained for at least 20 min, high-frequency stimulation (HFS) was delivered as two trains at 100 Hz (1 s duration; 30 s apart). Subsequent responses to single stimuli were recorded for at least 60 min, and their amplitude quantified as percent change with respect to baseline. HFS was used because we found that θ- burst stimulation did not induce a reliable LTP in the LA in older rats (>8 weeks).

Intracellular Recordings

Stable intracellular recordings were made blind with sharp glass micropipettes (1.2 mm, World Precision Instruments) filled with 2 M potassium acetate (tip resistance: 96 ± 2 MΩ ($n = 66$)) in single cells of the lateral amygdala. Negative currents were initially injected by the use of a bridge amplifier (SEC05, npi electronic, Tamm, Germany), but after stabilization of the cells, no currents were applied. All membrane potential measurements were corrected for electrode offsets. To investigate the electric and firing properties of neurons, current injection steps (duration: 200 ms; frequency: 1 Hz) were applied from −900 to +900 pA in 100 pA increments. To analyze the frequency adaptation of neurons, current injection steps (duration: 1000 ms; frequency: 2 Hz) were applied from +50 to +900 pA in 50 pA increments.

Input resistance ($R_i = V/I_{pulse}$) was determined by passing an incremental series of current pulses (I_{pulse}) through the recording electrode and measuring the resultant voltage deflections (V—steady-state voltage). Membrane time constant τ was calculated as the time required for the voltage change across the membrane to reach $1/e = 0.37$ of its final value ($\tau = V^{(1-1/e)}$). After cell characterization, excitatory postsynaptic potentials (EPSPs) were induced by stimulation of intranuclear afferents by means of bipolar Pt-Ir electrodes. EPSPs were evoked by electric stimuli (100 μs in duration) delivered at a frequency of 0.1 Hz. The strength of the stimulus was adjusted to elicit EPSPs that were 50–60% maximum amplitude. To evaluate short-term synaptic interactions paired-pulse stimuli were delivered with interstimulus intervals ranging from 30 to 500 ms. LTP was induced by 2×100 Hz (HFS; see below). During the course of the LTP experiment, input resistance and membrane potential were continually measured. Only cells that remained within 10% from the initial values of membrane potential and input resistance were included for analysis.

Drug Application

All drugs were bath applied. Appropriate stock solutions were made and diluted with ACSF immediately before application. SR95531 (GABA$_A$ receptor antagonist), ATPA (kainate GLU$_{K5}$ agonist), and UBP296 ((RS)-3-(2-carboxybenzyl) willardiine; GLU$_{K5}$ receptor antagonist) were purchased from Tocris Cookson Ltd (UK). ATPA is a substituted analogue of AMPA and binds to GLU$_{K5}$ with high affinity and can also activate GLU$_{K6}$/GLU$_{K2}$ (Clarke *et al*, 1997). In all recordings we used ATPA at a concentration of 2 μM to get the same effect of ATPA (1 μM) on LTP as found in 8-week-old rats, ie a significant reduction of LA-LTP (Drephal, 2005). We did not observe significant changes in LA-LTP in 4-month-old rats when we used ATPA at a concentration of 1 μM. Recently, Wu *et al* (2007) have shown that in the anterior cingulate cortex at a minimum concentration of 3 μM, ATPA significantly increased the amplitude of evoked inhibitory postsynaptic currents. UBP296 has been shown to be highly selective against GLU$_{K5}$ subunits because it selectively depressed glutamate-induced calcium influx in cells containing GLU$_{K5}$ in homomeric or heteromeric forms (More *et al*, 2004). In drug-treated slices, HFS was induced 45 min after perfusing the slices with drugs. Control drug-free and drug-treated slices were measured alternately from the same animals.

Data Analysis

Data were collected and averaged with the software Signal 2 (Cambridge Electronic Design).

We defined the FP amplitude as the absolute DC voltage of a vertical line running from the lowest point of the FP to its intersection with a line running tangent to the points of FP onset and offset. This procedure showed less variability from test-to-test than the measurement of the peak-to-peak amplitude. In analysis of I/O curves we preferred the first peak-to-peak amplitude because these amplitudes correlated with results of intracellular recordings. In intracellular recordings the results obtained by measuring the slope of the positivity were similar to those obtained when measuring the amplitude.

The group data were then analyzed as follows: (1) the maximum positive EPSP or negative FP amplitudes data for each experiment were expressed as percentages of the baseline average (averaged over 10 min), (2) the time scale in each experiment was converted to time from the onset of HFS, and (3) the time-matched, normalized data were averaged across experiments and expressed as the means ($\pm$ SEM). Significance of differences between groups was calculated by the nonparametric Mann–Whitney test (Software GraphPad Prism 4) and assessed at $p < 0.05$. To express and compare changes of the EPSP or FP amplitudes between the animal groups, we averaged responses from the 55–60 min period after HFS.

RESULTS

Long-Term Potentiation: Extracellular Recordings

HFS induced in the LA a stable LTP ($70.2 \pm 5.8\%$ above baseline ($n = 11$ slices, eight rats)) in nonimplanted controls. Four to five weeks after implantation of the kindling electrode, stable LTP could also be evoked ($58.6 \pm 6.9\%$ ($n = 16$ slices, eight rats)), although the amplitude was significantly smaller compared to nonimplanted, age-matched controls ($p < 0.0001$). Kindling resulted in a significant impairment of LA-LTP during the postictal period as shown previously (Schubert et al, 2005). LTP in slices derived from kindled animals ($n = 11$ slices, seven rats) amounted to $38.0 \pm 4.6\%$ above baseline, thereby being significantly smaller than that obtained in the sham-implanted controls (Figure 2d and e; $p < 0.0001$).

For the first time we show that kainate GLU_{K5} stimulation significantly influenced HFS-induced LA-LTP. ATPA ($2 \mu M$) caused a reduced LTP in nonimplanted controls ($45.5 \pm 8.9\%$ ($n = 8$ slices, six rats), $p < 0.0001$; Figure 2a). This reduction in the magnitude of LA-LTP could be blocked by the specific GLU_{K5} kainate receptor antagonist UBP296 ($5 \mu M$; $64.7 \pm 10.9\%$ ($n = 6$ slices, three rats); Figure 2a). ATPA significantly enhanced LA-LTP in sham-implanted controls ($66.3 \pm 4.9\%$ ($n = 17$ slices, eight rats), $p = 0.0003$; Figure 2c) and LA-LTP in kindled animals (74.9 ± 14.0 ($n = 14$ slices, seven rats), $p < 0.0001$; Figure 2d and e). Its effect was significantly bigger in kindled animals in comparison with sham-implanted animals (Figure 2c–e). There were no significant differences between drug-free slices from nonimplanted controls and APTA-treated slices derived from kindled animals. The effect of ATPA on LTP was stronger on the ipsilateral, kindling electrode implanted side than on the contralateral side.

In contrast, as shown in Figure 2b, $GABA_A$ receptor antagonist SR95531 ($0.1 \mu M$) induced a strong increase in early LA-LTP in nonimplanted controls that declined within 60 min ($80.1 \pm 11.9\%$ ($n = 12$ slices, eight rats)). Thus, they significantly differed from LTP obtained in drug-free conditions ($p = 0.0022$). Similar results were obtained in sham-implanted animals ($64.2 \pm 7.8\%$ ($n = 9$ slices, eight rats), $p = 0.0189$; Figure 2c).

In kindled animals SR95531 did not significantly enhance LA-LTP in comparison to drug-free conditions (SR95531: $43.1 \pm 9.2\%$ ($n = 10$ slices, seven rats); Figure 2d). Note that the effect of SR95531 on LTP was stronger on the ipsilateral side than on the contralateral side suggesting a more intense process of epileptogenesis on the side where the kindling electrode was implanted.
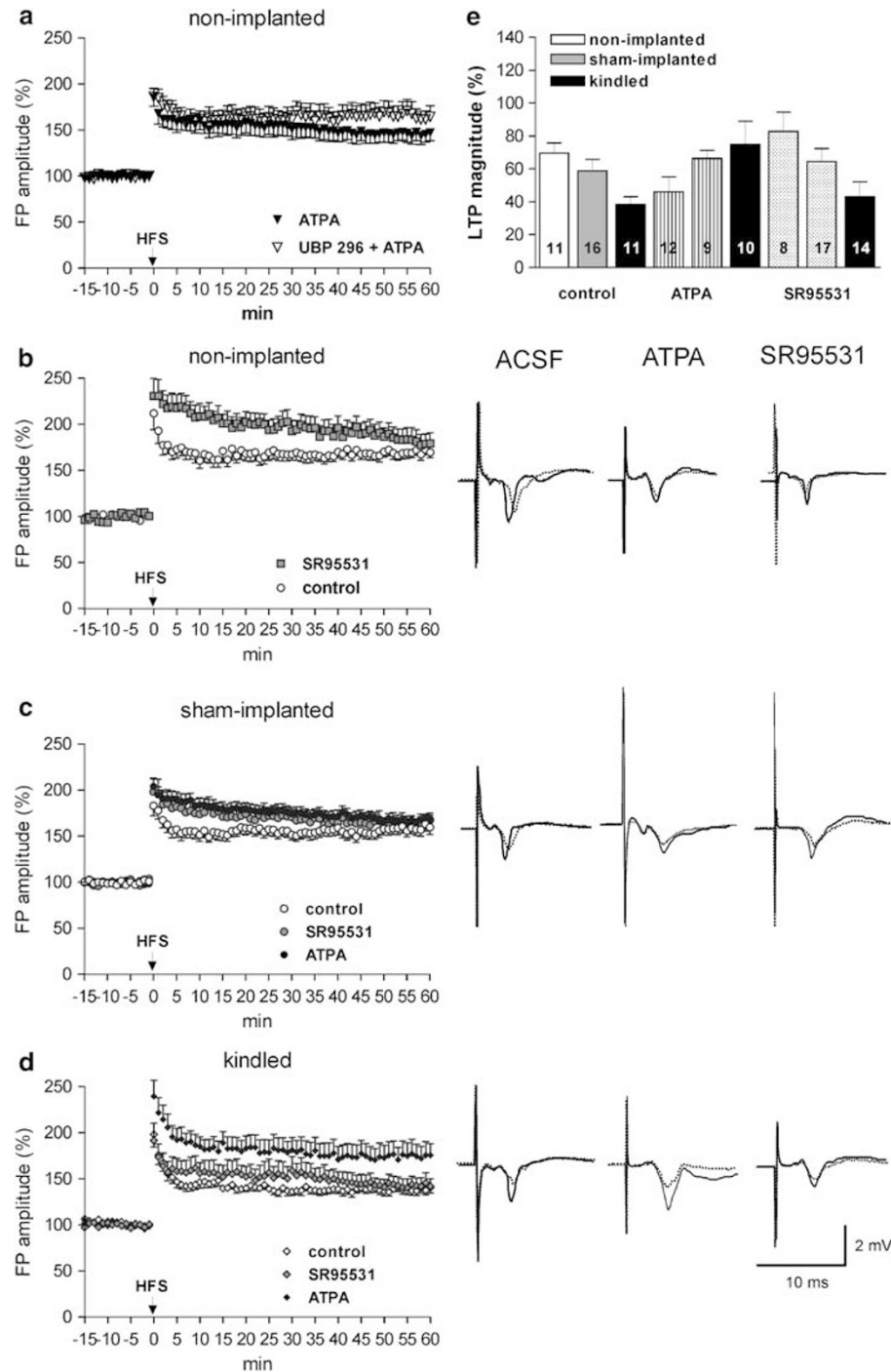
Long-Term Potentiation: Intracellular Recordings

As would also be predicted from extracellular recordings, intracellular responses to ATPA were considerably different in kindled slices from control slices. In ATPA-treated nonimplanted controls, HFS caused an insignificantly weaker potentiation of EPSP amplitudes in comparison to LA-LTP in sham-implanted animals (17.0 ± 4.3 ($n = 7$ cells, four rats) vs $25.5 \pm 8.6\%$ ($n = 9$ cells, six rats); Figure 3a). As shown for extracellular recordings ATPA caused a strong enhancement of LA-LTP in slices derived from kindled animals ($89.5 \pm 8.8\%$ ($n = 5$ cells, five rats); Figure 3a). The magnitude of this potentiation significantly differed from that obtained in slices derived from sham-implanted animals ($p < 0.0001$).

In sham-implanted controls HFS-induced LA-LTP in ATPA-treated slices was not significantly different from that in SR95531-treated slices (SR95531: $26.0 \pm 13.7\%$ ($n = 6$ cells, six rats); Figure 3c). The reduction of GABAergic transmission by SR95531 in kindled rats seemed to cause an occlusion of LA-LTP in intracellular recordings, since HFS did not enhance the amplitudes of EPSPs ($2.4 \pm 8.1\%$ ($n = 5$ cells, five rats); Figure 3c).

Intrinsic Cell Properties in Kindled Animals

Since we could demonstrate plasticity changes after the kindling procedure we were also interested in knowing whether the intrinsic properties show kindling-induced alterations. To determine the effects of the kindling procedure on the intrinsic properties of LA neurons, intracellular current-clamp recordings in the horizontal brain slice preparation were carried out. Data were collected only from the cells with stable resting membrane potentials (less than -60mV) and overshooting action potentials ($n = 66$). Recordings were made from eight nonimplanted, six sham-implanted, and seven kindled animals 48 h after the last stage-five kindled seizure. All recorded cells showed varying degrees of spike frequency adaptation. An example is shown in Figure 4a. They had relatively broad action potentials, typical for excitatory cells in the amygdala (Rainnie et al, 1991; McDonald, 1992; Pare et al, 1995).The mean resting membrane potential in nonimplanted animals was $-72.2 \pm 1.5 \text{mV}$ ($n = 33$). Action potentials were induced by current injection ($+100$ to $+900 \text{pA}$) from membrane potentials of $-56.4 \pm 1.4 \text{mV}$ and reached a mean amplitude of $66.0 \pm 0.7 \text{mV}$. The method for calculating neuronal R_i is illustrated in Figure 4b. R_i was calculated

Figure 2 Synaptic plasticity (extracellular recordings): LA-long-term potentiation (LTP) by stimulation of GLU_{K5} kainate receptor by (RS)-2amino-3-(3-hydroxy-5-*tert*-butylisoxazol-4-yl) propanic acid (ATPA, $2\,\mu M$) and partial blockade of $GABA_A$ receptor ($0.1\,\mu M$ SR95531). Data points represent averaged field potential (FP) amplitudes (mean ± SEM) normalized with respect to baseline values. Slices were derived from: (a) nonimplanted controls (control: $n = 11$ slices; ATPA: $n = 8$ slices; UBP296: $n = 6$ slices; 10 rats); (b) nonimplanted controls (controls: $n = 11$ slices; SR95531: $n = 12$ slices; eight rats); (c) sham-implanted controls (controls: $n = 16$ slices; ATPA: $n = 17$ slices; SR95531: $n = 9$ slices; eight rats), and (d) kindled animals after seven consecutive stage-five kindling-induced seizures (controls: $n = 11$ slices; ATPA: $n = 14$ slices; SR95531: $n = 10$ slices; seven rats); (e) bar histogram of data points in (a)–(d), as averaged 55–60 min after high-frequency stimulation (HFS) and normalized with respect to baseline (mean increase ± SEM). Right: representative examples of original traces are shown.

from the linear portion of the I–V curve. While the negative portion of the I–V curves was linear for all groups, the positive portion exhibited rectification. Intrinsic cell properties like resting membrane potential and time constant were not significantly different among our experimental animal groups. However, as shown in Figure 4c, R_i decreased in kindled animals in comparison with sham-implanted animals ($p < 0.0001$). In addition, the
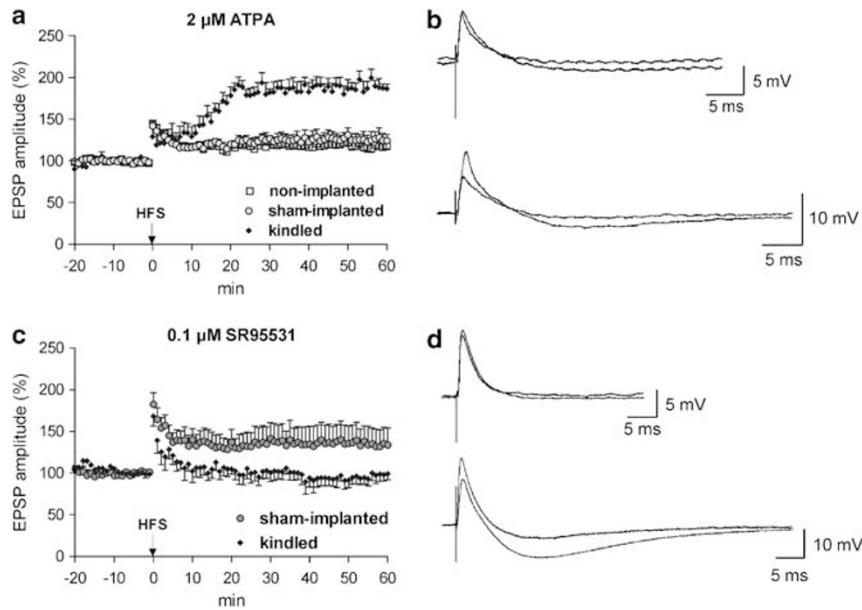
Figure 3 Synaptic plasticity (intracellular recordings): LA-LTP by stimulation of GLU$_{K5}$ kainate receptor by 2 μM (RS)-2amino-3-(3-hydroxy-5-*tert*-butylisoxazol-4-yl) propanic acid (ATPA, a) and partial blockade of GABA$_A$ receptor (c: 0.1 μM SR95531). Data points represent averaged amplitudes (mean ± SEM) of excitatory postsynaptic potentials (EPSPs) normalized with respect to baseline values. (a) Averaged EPSP amplitudes from nonimplanted (n = 7 cells, four rats), sham-implanted (n = 9 cells, six rats), and kindled animals (n = 5 cells, five rats). (c) Averaged EPSP from sham-implanted (n = 6 cells, six rats) and kindled animals (n = 5 cells, five rats). Application of high-frequency stimulation (HFS, 2 × 100 Hz, duration: 1 s, interval 30 s) at time 0. (b) and (d) are representative examples of original traces.

action potential amplitude in kindled animals also significantly decreased in comparison with sham-implanted animals ($p < 0.003$). Our study showed that the appearance of burst discharges in the LA 48 h after the last of seven consecutive stage-five seizure was much less common in contrast to recordings in the BLA 2–12 weeks after the last of three consecutive stage-five seizures (Rainnie *et al*, 1992) possibly due to other potassium and calcium concentrations in the ACSF used in our study. Burst discharges were observed in 3 of 33 cells from nonimplanted animals, 2 of 15 cells from sham-implanted, and only 1 of 18 cells from the kindled animals.

Basal Excitability After Kindling

FPs and EPSPs were elicited by brief electrical stimulation (100 μs) of intranuclear fibers running through the LA as described previously (Drephal *et al*, 2006; Kaschel *et al*, 2004). In contrast to data reported previously in the basolateral nucleus (Rainnie *et al*, 1992; Shoji *et al*, 1998), stimulation of fibers within the LA did not elicit epileptiform discharges during extra- and intracellular recordings.

The magnitude of responses obtained at different stimulus intensities was plotted as I/O curves. Neither in extracellular recordings nor in intracellular recordings the I/O relationship in the LA was significantly changed in dependence of the treatment at all stimulus intensities that had been tested (Figure 5a and c). This indicates that the basal excitability after kindling was not changed in the LA. It is to note that no different differences in I/O curves were found when FP amplitudes were determined by measurements of the amplitude between the first positivity and maximum of negativity, whereas the amplitude taken from the peak negativity to a tangent line from the FP onset to the

end of the positivity was increased in the kindled rat suggesting an GABA-mediated increase of the second positivity of population spikes.

ATPA-Induced Effects on Basal Transmission and Paired-Pulse Facilitation

Recently, it has been shown that basal transmission is also mediated by the involvement of GLU$_{K5}$ kainate receptor in the basolateral amygdala (Li and Rogawski, 1998). Basal transmission also depends on the strength of the GABAergic transmission within the amygdala (Chen and Lang, 2003). Therefore, we tested the effect of ATPA (2 μM) and SR95531 (0.1 μM) on basal transmission in both controls and kindled animals. Low concentrations were used to avoid epileptic discharges in the LA which appeared when 10 μM of ATPA and 0.2 μM of SR95531 were applied (data not shown).

ATPA affected slices in all groups by a reduction in the amplitude of FPs (nonimplanted: $-27.3 \pm 6.2\%$ (n = 8 slices, eight rats); sham-implanted: $-27.0 \pm 4.1\%$ (n = 16 slices, eight rats); kindled: $-20.5 \pm 3.9\%$ (n = 18 slices, seven rats)) and EPSP (nonimplanted: -19.8 ± 7.4 (n = 6 cells, four rats); sham-implanted: $-22.8 \pm 5.8\%$ (n = 11 cells, six rats); kindled: $-24.0 \pm 8.3\%$ (n = 13 cells, seven rats); Figure 5e). In addition, ATPA produced an overall shift to the right of I/O curve in kindled animals in both intracellular (kindled: n = 6 cells, six rats, $p = 0.0078$; Figure 5f) and extracellular recordings (kindled: n = 15 slices, $p = 0.0039$). ATPA did not change the I/O curve significantly in both nonimplanted and sham-implanted controls (data not shown).

To investigate pre- *vs* postsynaptic sites of action, we used a paired-pulse facilitation (PPF) protocol consisting of two stimuli of the same intensity delivered at different time intervals. No difference was observed in the PPF between
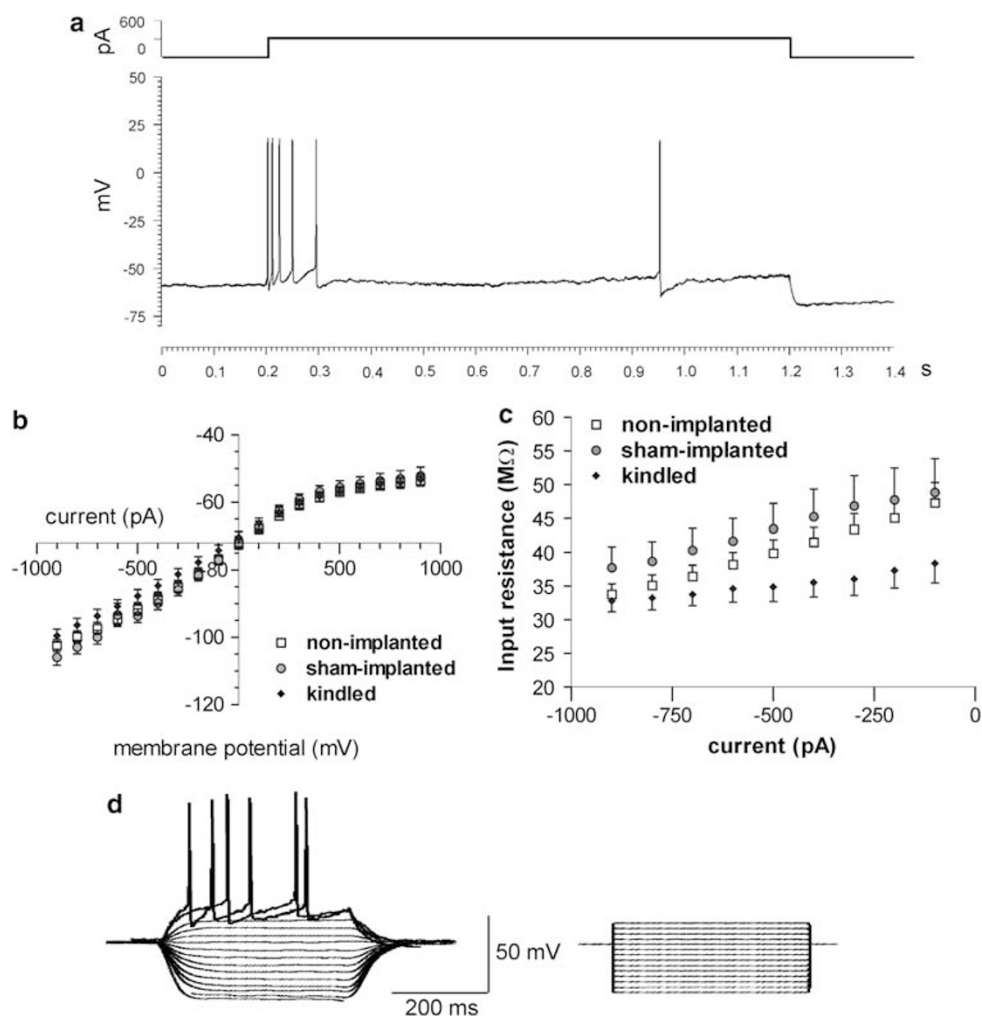
Figure 4 Intrinsic properties of LA neurons. (a) A depolarizing current injection (400 pA) elicited an initial burst of action potentials followed by accommodation. (b) Plot of current–voltage relationship. (c) Changes in the input resistance with varying intensity of current injection. (d) Direct current pulses (200 ms) ranging from -1000 to 400 pA in 100 pA increments were injected via the recording electrode. The graphs show superimposed voltage responses (left) to graded series of current pulses (right) applied from rest.

nonimplanted and sham-implanted controls (non-implanted: $n = 69$ slices, 25 rats; sham-implanted: $n = 92$ slices, 32 rats; $p = 0.5444$) or sham-implanted and kindled animals (kindled: $n = 90$ slices, 32 rats; $p = 0.5834$). As shown in Figure 5g and h in kindled animals PPF was significantly stronger in ATPA-treated slices as compared to drug-free slices (FP: $n = 18$ slices, seven rats; EPSPs: $n = 6$ cells, six rats; $p < 0.05$). In contrast, ATPA had no effect on the PPF in control groups.

Partial blockade of GABAergic transmission with SR95531 resulted in an increase of FP and EPSP amplitudes in kindled animals (FP: $+20.0 \pm 7.1\%$ ($n = 10$ slices, seven rats, $p < 0.0001$); EPSP: $+55.2 \pm 17.3\%$ ($n = 9$ cells, seven rats, $p < 0.0001$)). No significant changes of the baseline activity under SR95531 were observed in nonimplanted and sham-implanted animals.

For I/O curves we could demonstrate that SR95531 shifted the I/O relationship toward higher levels as compared to the same drug-free slices derived from kindled animals. No significant differences were found between drug-treated and drug-free nonimplanted and sham-implanted control animals in both recordings (data not shown).

DISCUSSION

Intra- and extracellular recordings of LA neurons were utilized to study the role of kainate GLU_{K5} receptors as modulators of synaptic transmission and plasticity in brain slices derived from nonimplanted, sham-implanted, and kindled animals 48 h after the last of seven consecutive stage-five seizures. The stimulation of GLU_{K5} kainate receptors by ATPA caused a decrease in LA-LTP in slices derived from nonimplanted and sham-implanted animals. However, ATPA rescues kindling-induced impairment of LA-LTP. The partial blockade of GABAergic transmission enhanced the induction of LA-LTP in nonimplanted controls, but did not compensate for kindling-induced changes in the magnitude of LTP.

Kindling-Induced Impairment of LTP

It is known that electroconvulsive treatment markedly inhibited HFS-induced LTP in the hippocampus (Anwyl et al, 1987). In TLE patients, NMDA-dependent LTP was strongly suppressed in the dentate gyrus in comparison to
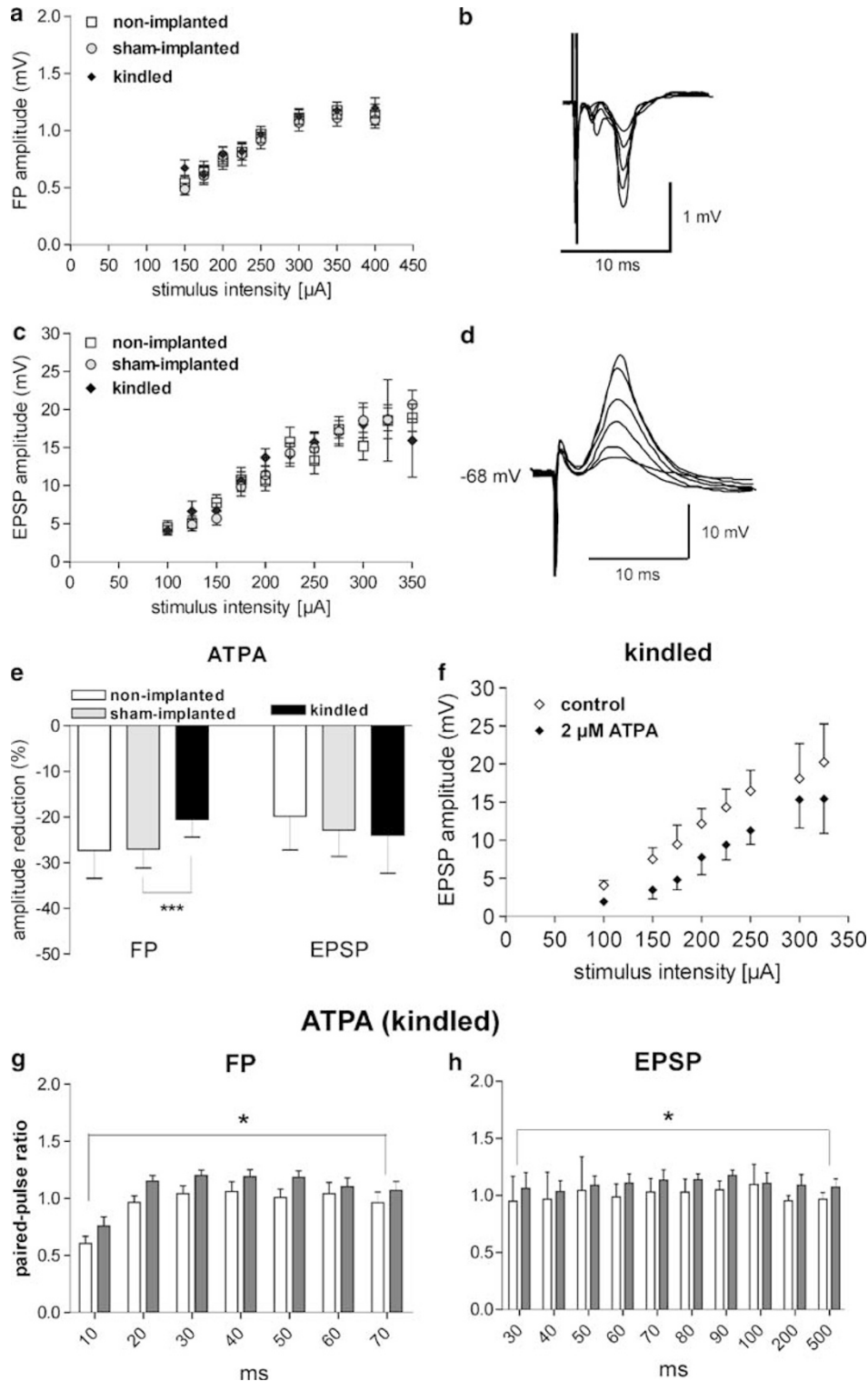
Figure 5 Effects of kindling, (RS)-2amino-3-(3-hydroxy-5-*tert*-butylisoxazol-4-yl) propanic acid (ATPA), and SR95531 on basal transmission and paired facilitation. (a) I/O curves of field potential (FP) amplitudes recorded in LA, as evoked by single stimuli applied at intra-amygdaloid fibers (nonimplanted: $n = 63$; sham-implanted: $n = 82$; kindled: $n = 94$). The I/O relationship in the LA was not significantly different between the animal groups. (b) Examples of original FP recordings. (c) I/O curves of excitatory postsynaptic potentials (EPSP) amplitudes recorded in LA neurons, as evoked by single stimuli applied at intra-amygdaloid fibers (nonimplanted: $n = 29$; sham-implanted: $n = 12$; kindled: $n = 17$). The I/O relationship in the LA was not significantly different between the animal groups. (d) Examples of original EPSP recordings. (e) ATPA induces a depression of FP and EPSP amplitudes in kindled animals. ***$p < 0.0001$. Slices were derived from sham-implanted controls (FP: $n = 16$ slices, eight rats; EPSP: $n = 11$ cells, six rats) and kindled animals (FP: $n = 18$ slices, seven rats; EPSP: $n = 13$ cells, six rats). (f) Effect of ATPA on input–output curves obtained from intracellular recordings. Slices were derived from kindled animals (EPSP: $n = 6$ slices, six rats). (g and h) Effects of ATPA on PPF in kindled rats. Both, FP (g, $n = 18$ slices, seven rats, $p < 0.05$) and EPSP amplitudes (h, $n = 6$ cells, six rats, $p < 0.05$) show a significant increase in PPF when ATPA was washed in.

nonepileptic tissue (Beck et al, 2000). In agreement with our previous results in the amygdala (Schubert et al, 2005) the repeated kindling procedure in the BLA in this study resulted in an impairment of LTP in the LA. In these previous experiments we have also shown that multiple spaced HFS resulted in stronger saturation of CA1-LTP in kindled animals in comparison to nonimplanted and sham-implanted rats. There is reason to question whether seizures induce a depression that masks LTP, or whether they saturate LTP. Our results show that ATPA 'rescues' LA-LTP in the kindled animal. This result argues against a simple saturation mechanism. Abraham and Huggett (1997) have shown for the CA1 region of the hippocampus that overstimulation transiently inhibits subsequent LTP induction through activation of voltage-gated calcium channels and NMDA receptors. We have recently shown that in horizontal slices LA-LTP induced by intranuclear stimulation is NMDA dependent (Drephal et al, 2006). One potential mechanism for the LTP inhibition might be a long-lasting reduction in NMDA receptor-mediated responses by prior stimulation. However, we have shown in the present study that the input resistance was decreased in the kindled animal. Therefore, our data support the hypothesis of a stronger GABA release in the kindled animal and in this way an impaired LTP rather than an occlusion.

Similar to the impairment of LA-LTP in kindled animals, the implantation of the electrode itself reduced the magnitude of LA-LTP. This situation could be explained by the fact that the presence of the stimulating electrode destroys the surrounding tissue (Goddard et al, 1969). Influences of electrode implantation into the BLA on overall excitability had been previously demonstrated (Blackwood et al, 1982; Loscher et al, 1995; Niespodziany et al, 1999). In addition, it has been shown that electrode implantation in the olfactory bulb induced an increase in benzodiazepine receptor density at the site of implantation as compared to nonimplanted rats (Ben Attia et al, 1992). The changes which were observed in sham-implanted controls support the view that the implantation of the electrode itself without any electrical stimulation provokes changes in transmission. Although the reasons for the decreased LTP in sham-implanted controls are not fully understood, our data substantiate that the choice of adequate controls is critical in functional studies on the kindling phenomenon especially using a position of the kindling electrode near the recording site.

Kindling Alters Intrinsic Properties of LA Neurons

In agreement with other studies (Rainnie et al, 1992; Shoji et al, 1998) intrinsic cell properties like resting membrane potential and time constant were not significantly different among our animal groups. However, in our study we found a decrease in input resistance and a change in the action potential height in kindled animals. The membrane resistance can be altered by addition of new, tonically active, synaptic inputs, converging on projection neurons and/or as a result of the addition or deletion of other open ionic channels. These obtained changes in our study seem to be short-lasting, since Rainnie et al (1992) and Shoji et al (1998) did not find significant changes in input resistance

and action potential amplitude. But, their studies were carried out either 2–12 weeks after the last of three consecutive stage-five seizures or 4–8 weeks after five consecutive stage-five seizures. The NMDA- and non-NMDA receptor-mediated glutamatergic transmission in the amygdala was always enhanced, 2–12 weeks after kindling (Gean et al, 1989; Rainnie et al, 1992; Shoji et al, 1998). In contrast, our changes in input resistance might be due to kindling-induced changes in kainate transmission. A decrease in input resistance of projection neurons in kindled slices suggests an increase in GABA release in the kindled animal possibly due to an upregulation of GLU_{K5} kainate receptors in GABAergic interneurons.

GLU_{K5} Stimulation Alters Basal Synaptic Transmission

In agreement with this hypothesis our results show that ATPA also caused a reduction in basal transmission in all animal groups. This reduction of basal transmission in nonimplanted controls is stronger than that observed at a concentration of $1 \mu M$ ATPA in younger rats (Drephal, 2005). At least in young control rats this ATPA-induced decrease in basal activity can be blocked by the coadministration of SR95531 ($0.1 \mu M$) and $1 \mu M$ ATPA (unpublished data). An ATPA-induced reduction in basal activity supports data that ATPA ($1 \mu M$) depresses the propagation of seizures generated in the opposite hippocampus by a convulsive agent (Khalilov et al, 2002). It is to consider that presynaptic kainate receptors modulate the release of glutamate and GABA and the synaptic transmission in a biphasic manner. Low concentrations of kainate receptor agonists potentiate, whereas high concentrations inhibit the release of neurotransmitters (Malva et al, 2003). As we used a low concentration of ATPA ($2 \mu M$), an enhanced GABAergic transmission to single stimuli would be expected as described by Braga et al (2003) in the BLA.

So far we know GLU_{K5} kainate receptors are present in the amygdala on somatodendritic sites of both pyramidal cells and interneurons (Aroniadou-Anderjaska et al, 2007). We suggest that the kindling procedure causes an upregulation of GLU_{K5} receptors together with other transmitter receptors (for instance of NMDA and/or mGLURs). Considering data from Braga et al (2003) this upregulation of GLU_{K5} receptors predominantly results in an enhancement of GABA release. This suggestion is also supported by the observed decrease in input resistance and in impaired LA-LTP after kindling. This enhanced inhibition may act as mechanism acting against processes provoking higher excitability induced by epileptogenesis. The mechanism by which ATPA modulates transmitter release is unclear because of the difficulty of studying presynaptic mechanisms and also, in part, because the pharmacological activation of GLU_{K5} receptors by bath application of ATPA probably affects synaptic transmission both directly and indirectly. Because of the ATPA-induced enhancement of PPF in the kindled animal our results suggest that the presynaptic release is altered by ATPA, whereas no changes were obtained in both control groups. Considering an upregulation of GLU_{K5} receptors in the kindled animal, increases in PPF likely indicate decreased probability of neurotransmitter release associated with presynaptic depression. Another consideration is that

alterations in PPF can result from postsynaptic changes (Bagal *et al*, 2005; Moult *et al*, 2006).

GLU$_{K5}$ Stimulation Alters Plasticity in Controls and Kindled Rats Differently

For the first time we have shown that low concentrations of ATPA caused a decrease of LA-LTP magnitude in male controls. This ATPA-induced decrease of LA-LTP could be blocked with the specific GLU$_{K5}$ kainate receptor antagonist UBP296. Our results support the conclusion that kainate GLU$_{K5}$ receptors might be involved in LA-LTP in agreement with other studies (Li *et al*, 2001). An ATPA-induced increase in GABA release in nonimplanted controls might not only cause a reduction of baseline activity as shown in our experiments but could also increase the threshold for LTP induction.

In contrast to controls, in the kindled animal LTP was enhanced by ATPA. It appears that the potentiation caused by ATPA in intracellular recordings of kindled animals has a nature rather different from HFS-induced LTP in controls. In fact, HFS causes a rapid short-term potentiation in both controls, which is not observed in kindled rats in the presence of ATPA. Instead, there is a slower-developing potentiation, which becomes evident minutes after HFS was delivered. It can be assumed that the recorded negative wave at least in part of the recordings reflects a summation of both EPSPs and synchronized action potentials (population spike component) (Doyere *et al*, 2003; Watanabe *et al*, 1995). Watanabe *et al* (1995) have carried out intracellular recordings of evoked potentials and confirmed that the latency of peak negative FPs (5–6 ms) corresponds well with that of intracellularly recorded action potentials, indicating that the extracellularly recorded sharp negativity is a population spike. The different time course of changes in EPSP and FP amplitudes in intracellular and extracellular recordings after HFS might therefore be due to recordings of population spikes in extracellular recordings. It is a commonly observed property of LTP that the potentiation of the population spike is disproportionately greater than potentiation of the EPSP.

The LTP-enhancing effect of ATPA in kindled animals also suggests an upregulation of GLU$_{K5}$ kainate receptors on projection neurons similar to that described for the kainate model of epilepsy in the hippocampus (Ullal *et al*, 2005). In addition, in the rat hippocampus following kainate-induced seizures (Bernard *et al*, 1999) and in human patients (Grigorenko *et al*, 1998; Kortenbruck *et al*, 2001), the level of editing of GLU$_{K5}$ kainate receptor is significantly altered. In ATPA-treated kindled rats the HFS caused a strong enhancement of LA-LTP. In the hippocampus it has been shown that the activation of postsynaptic kainate receptors only occurred at high frequency of mossy fiber afferents (Mulle *et al*, 1998). Therefore, it seems to be of essential relevance that one of the striking characteristics of GLU$_{K5}$ kainate receptor-mediated synaptic response is its remarkable enhancement by train stimulation (Li and Rogawski, 1998). The efficiency of kainate receptors in regulating network activity seems therefore to relay on their repetitive synaptic activation. Thus, kainate receptors appear to be involved in the temporal integration of excitatory signals (Pinheiro and Mulle, 2006). In addition, it can be supposed

that HFS in ATPA-treated kindled rats is accompanied with the reduction of GABA release. Considering an upregulation of GLU$_{K5}$ receptors after kindling, the strong depolarization by HFS in ATPA-treated slices will also cause a strong depolarization of GABAergic interneurons. The resulting reduction in GABA release might be due to the down-regulation of GABA receptors or by exhaustion of ready-to-fuse pool of synaptic vesicles. Moreover, extracellular GABA accumulation could activate presynaptic GABA$_B$ receptors, leading to reduced inhibition and the promotion of LTP. However, we cannot rule out kindling-induced affinity changes of GLU$_{K5}$ receptors. Thus, it is possible that excessive activation of GLU$_{K5}$ receptors induces epilepsy through a kindling process, analogous to NMDA receptor-dependent LA-LTP. As known, higher concentrations of ATPA than that we used in our experiments cause spontaneous synchronized bursting in BLA (Rogawski *et al*, 2003). In addition, it has been shown that GLU$_{K5}$ agonist ATPA can induce epileptogenic action (Kaminski *et al*, 2004). Therefore, it is likely that multiple mechanisms could explain that ATPA 'rescued' LA-LTP in the kindled animal. It is to note that Pinheiro and Mulle (2006) came to the conclusion that an understanding of the mechanisms by which kainate-receptor function is regulated during synaptic plasticity lags far behind similar studies for AMPA and NMDA receptors.

CONCLUSION

Our experiments show that the activation of the GLU$_{K5}$ kainate receptor subtype rescues the kindling-induced impairment of LA-LTP, at least within 48 h after the last seizure. Therefore, GLU$_{K5}$ kainate receptor subunits are involved in kindling-induced plasticity changes in the amygdala. In summary, changes in transmitter function might alter the optimal physiological conditions for LTP induction. They could disrupt the strength, pattern, or effectiveness of plastic changes related to learning. Our results demonstrate that impairment of LTP is due to changes in GABAergic and glutamatergic transmitter function. According to this hypothesis, kindling may represent an enduring form of metaplasticity (Abraham and Bear, 1996), which results in a shift away from the optimal 'settings' for learning. Amygdala kindling seems to cause a shift in the inhibition 'balance' between the projection neurons and GABAergic interneurons. Recent experiments by Smolders *et al* (2002) suggest that GLU$_{K5}$ kainate receptors-selective antagonists can block the induction of seizures by pilocarpine or electrical stimulation, and suppress preestablished seizure activity.

DISCLOSURE/CONFLICT OF INTEREST

The authors declare that except for income received from the primary employer no financial support or compensation

has been received from any individual or corporate entity over the past 3 years for research or professional service and there are no personal financial holdings that could be perceived as constituting a potential conflict of interest.

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