Histaminergic modulation of gamma oscillations in rat hippocampus

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1.ABSTRACT

Hippocampal network oscillations with distinct frequencies are likely to be involved in various cognitive functions and in storage of information and memory consolidation *in vivo*. Gamma oscillations (30-100 Hz) are thought to provide for binding of parallel processed information in the brain, contributing to cognition and the formation of memory. We investigated the effects of hypoxia, histamine and ERG channels on hippocampal gamma oscillations (γ) induced by kainate or by acetylcholine (ACh) in presence of physostigmine. Kainate-induced γ oscillations were reversibly blocked by 3 min hypoxia. The repetition of such hypoxic periods led to accumulative impairment of γ activities. By contrast, 6 min of hypoxia resulted in an almost complete and irreversible loss of γ oscillations. In studies on ACh induced γ I found that the power of γ was significantly increased by the H1 antagonist fexofenadine, and the H2 receptor agonist dimaprit, and reduced by the H2 receptor antagonist cimetidine. Kainate-induced γ was unaffected by H1 and H2 receptor modulation. These effects suggest an interaction between ambient histamine and acetylcholine. Depletion of histamine from their fibers by hypoxia and blockade of histamine uptake resulted in loss of the fexofenadine-mediated and cimetidine-mediated effects on acetylcholine-induced γ. We conclude that acetylcholine can cause histamine release from histaminergic fibers and that histamine then augments γ due to activation of H2 receptors. This compares to findings where application of a H2 agonist has a memory-enhancing effect that mostly concerns storage of information in working memory.

Next, we compared neuronal effects of astemizole, (an H1 and Kv 11 or ERG channel blocker) sertindole (an ERG channel blocker and dopamine ,serotonine blocker) and E4031, a selective blocker of ERG channels, in order to exclude potential side effects by the employed drugs. We found that astemizole and sertindole, but not the selective Kv11 channel blocker E4031, augmented γ oscillations in hippocampal slices induced by acetylcholine. Kainate-induced γ oscillations were only affected by astemizole. Evoked responses induced by *stratum radiatum* stimulation in area CA1 revealed that only E4031 augmented stimulus-induced synaptic responses and neuronal excitability and, hence, that the effect of astemizole on γ oscillations is related to its action on H1 receptor.

2.INTRODUCTION

The hippocampus is essential for the formation of spatial memory (Martin and Clark , 2007; Laczo et al. , 2010) and declarative memory (Morris et al., 1982; Henke et al., 1997) in animals and humans (Scoville and Milner, 2000). Memory functions of the hippocampus strongly depend on network properties. Oscillatory activity is a hallmark of neuronal network function in various brain regions including the olfactory bulb, thalamus, hippocampus and neocortex. The frequency of network oscillations covers more than three orders of magnitude, from circadian rhythms to slow oscillations in the delta (0.5-3 Hz) and theta (3-8 Hz) ranges to fast oscillations in the gamma (30-90 Hz) and ultrafast (90-200 Hz ripple) ranges (Buzsáki and Draguhn, 2004) under some conditions extending to 600 Hz (fast ripples; Bragin et al., 1999). Gamma frequency oscillations contribute to cognitive functions by binding parallel processed information (Uhlaas et al., 2011) and may also contribute to memory formation by providing a temporal structure for spike timing dependent plasticity and thereby for storage of information in the brain (Bartos et al., 2007), particularly since hippocampal neurons are sparsely firing and therefore synaptic plasticity required for memory formation may not depend on high frequency activation of synapses (Bartos et al., 2007). The power of gamma oscillations is correlated with the success of memory formation (Axmacher et al., 2010; Fell et al., 2001) and they are altered in several brain disorders such as Alzheimer's disease (AD) (Verret et al. 2012), schizophrenia (Kwon et al., 1999; Andersson et al., 2012; Rotaru et al., 2012 ; Papp et al., 2010 Uhlhaas, 2011) and epilepsy (Jefferys et al. 2012, ; Gupta et al. 2011, Andrade –Valenca et al., 2011) that result in decreased learning and memory performance as well as cognitive decline in patients. It has been demonstrated that O_2 availability is a key factor in processes which are related to this type of neuronal network oscillations (Verweij et al., 2007; Huchzermeyer et al., 2008) and that lowering O_2 concentration in the brain leads to an impaired short-term memory and rapid loss of consciousness (Hansen, 1985; Verweij et al., 2007). In hippocampal slices, γ can be induced by activation of muscarinic acetylcholine receptors (Fisahn et al., 1998), metabotropic glutamate receptors (Whittington et al., 1995), or ionotropic kainate receptors (Hajos et al. 2000; Hormuzdi et al., 2001). Histamine is,

like acetylcholine, in the central nervous system a neuromodulator acting through activation of G proteins (Brown et al., 2001) and like ACh is associated with the regulation of sleep and arousal (Haas et al., 2008) but has recently also been suggested to be important for learning and memory (van Ruitenbeek et al., 2010; Tsujii et al., 2010). Some of the antihistaminergic agents that bind to histamine receptors, such as astemizole, and antipsychotic drugs, such as sertindole, produce side effects in the heart such as long QT syndrome. These agents bind also to ERG channels, a group of potassium channels related to ether-a-go-go channels in Drosophila. Blockade of ERG K^+ channels by these drugs may change excitability in neurons and, for example, alter dopamine release in schizophrenic patients and/or affect network oscillations

3.AIM

The first aim of this study was to investigate the effects of transient hypoxia on kainate-induced γ oscillations and to test whether repetition of hypoxic periods leads to accumulative impairment of γ activities. This is also a way to deplete axonal fibers from outside the hippocampus from their transmitters (Moghaddam et al., 1987). The second aim of my study was to investigate the interaction between ambient histamine and acetylcholine by studying γ oscillations in rat hippocampus, induced by bath application of acetylcholine. Histamine receptor modulating drugs may also affect ERG K^+ channels which would alter neuronal excitability and γ oscillations in the hippocampus requiring analysis of specific ERG channel blockers.

METHODS

Electrophysiological experiments were performed on horizontal hippocampal slices (400 µM) of young adult Wistar rats. The slices were cut with a vibratome and immediately transferred into an interface-type recording chamber continuously perfused with warm ACSF. Usually slices were cut at an angle of 12° in the fronto-occipital direction with the occipital portion down. The slice recording chamber is designed to maintain isolated, living tissues in vitro in “interface” mode and allows long-term stable recordings to be made from the preparation. The interface chamber consisted of a plexiglas cylinder which was filled half with distilled water. In the lower

part there was a heating spiral and a perforated plastic tube that allows oxygenation. The cylinder was topped by the recording chambers, in which the brain slices were placed on a double layer of lens cleaning paper, which offered a large diffusion surface. In each of the 2 separated chambers there was place for 4-5 slices. Chambers were perfused with warmed, oxygenated ACSF with a flow rate of 1.8 ml/min delivered through polyethylene tubes into the recording chamber. In the lower

part of the cylinder, carbogen gas (95% O_2 and 5% CO_2) was warmed and saturated with water vapor. The gas was conducted to the perfusion chamber over the surface of the brain slices. The temperature in the perfusion chamber was kept at 34°C by using a thermostat, so that both, the gas mixture and the ACSF, had the desired temperature. The whole assembly was surrounded with a Faraday –cage, in order to reduce 50 Hz noise- artifacts . In this setting, brain slices could be kept viable for at least 10 hours .

Field potentials were recorded from *stratum pyramidale* of area CA1 and CA3b. Persistent γ oscillations were induced by bath application of kainate (100- 150 nM) and alternatively by ACh (10 µM) coapplied with physostigmine (2 µM). Hypoxic episodes were produced by switching the gas flow supplying the slice from 95% O_2- 5% CO_2 to 95% N_2- 5% CO_2 (Fleidervish et al., 2001; Gebhardt et al., 2002). Extracellular Ca^{2+} and K^+ concentrations were measured using double-barreled Ca^{2+}- or K^+-sensitive microelectrodes for DC-coupled recordings in the *stratum radiatum* (SR) of CA1 (Heinemann et al., 1977). In order to assess the effect of E4031 on presynaptic Ca^{2+} entry, stimulation trains (20 Hz, 2s) were applied to the SR in CA1 in the presence of glutamate receptor blockers DL-APV (50 µM), CNQX (25 µM) and MCPG (150 µM) (Ul Haq et al., 2012; Fano et al., 2012). The glutamate blockers were pre-washed at least for 30 min before application of E4031 (Fano et al., 2012). After each experiment the glutamate blockers and E4031 were washed out to confirm whether the effect was reversible (Fano et al., 2012). For input-output (IO) curves, stimulus intensities ranged from 1 to 10 V (Fano et al., 2012). For paired pulse experiments, two consecutive pulses were applied to the proximal SR of CA1 (inter-pulse interval: 50 ms, repeated every 30s) (Fano et al., 2012). Data analysis was performed off-line using spike 2 version 5 software (Cambridge Electronic Design, Cambridge, UK). In each experiment concerning gamma oscillations, peak power, peak frequency and halfband width of FP oscillations in the γ frequency band (20-80

Hz) were determined from 300 s raw data sections immediately before application and 60 min into drug application (Fano et al., 2011). For analyzing the effect of hypoxia on oscillation power, normalized peak power values were compared (Fano et al., 2007). In pharmacological experiments we took 5 min data sections during wash in of the drug (Fano et al., 2011). Main oscillation frequency was taken as the peak frequency determined from the respective power spectra. All numerical data were reported as mean ± standard error of the mean (SEM) , with “n” being the number of slices with usually one slice per animal. Statistical significance was determined by the Kolmogorov- Smirnow test, by the non-parametric paired t-test and by the Wilcoxon test. P values less than 0.05 were considered to indicate a significant difference between means. Drugs were administered through continuous bath perfusion. Fexofenadine and astemizole were first dissolved in dimethylsulfoxide (Fano et al., 2011) and diluted in ACSF to their final concentration (final dimethylsulfoxide concentration ≤ 0,2 %). All drug-containing solutions were freshly prepared before the experiment.

4.RESULTS

Effects of hypoxia on kainateinduced γ oscillations

Application of kainate (0.15 μM) reliably induced γ oscillations. After stabilization, such oscillations could be recorded for more than 3 hours without major changes in power or frequency. Oscillation amplitudes recorded in area CA3 were always 5-8 times larger than in area CA1 (Fano et al., 2007; Fig.1 (a)-(d)). Application of 95 % N_2 combined with 5 % CO_2 resulted in a rapid decline of oscillatory activity, which became apparent 40 s after onset of hypoxia (Fano et al., 2007; Fig. 1 (a)-(d)). Later on, γ oscillations showed an almost complete recovery in both areas (Fano et al., 2007; Fig. 1 (a)-(d)). Repeated hypoxia with intervals of 15 min between each hypoxic period led to an accumulative reduction in the power of oscillatory activity in area CA3 (Fano et al., 2007; Fig. 2(a)). This effect was less pronounced in area CA1 (Fano et al., 2007; Fig 2(a)). Furthermore, repeated hypoxia episodes also significantly reduced the peak frequency of γ oscillations (Fano et al., 2007; Fig. 2(b)). Simultaneous intracellular recordings showed that upon hypoxia, field-potential oscillations declined before the cells changed their membrane potential in either

hyperpolarizing or depolarizing direction (Fano et al., 2007; Fig. 3(a)-(b)). To test for effects of prolonged episodes of hypoxia on induced oscillations, we next induced hypoxia for 6 min. This resulted in spreading depression-like events in area CA1 and development of an hypoxic depolarization (Fano et al., 2007;Fig. 3(d)). Similar to 3 min hypoxia, a rapid depression of oscillatory activity occurred also in these experiments (Fano et al., 2007; Fig. 2 (c)- (d);3 (d); 4(c)). Recovery, however, was rather incomplete in area CA3 which was in contrast to a much better recovery observed in area CA1 (Fano et al., 2007; Fig. 2(c)- (d) ; 4(d)). Repeated application of hypoxia for 6 min, separated by 15 min intervals, resulted in an almost complete loss of kainate-induced γ oscillations in area CA3 already following the second repetition, (Fano et al., 2007; Fig. 2(c)- (d)) whereas in area CA1 some γ activity was left (Fano et al., 2007; Fig. 2(c)- (d)) indicating vulnerability of CA3 neurons.

Histaminergic modulation of* acetylcholine*- induced γ oscillations

As with kainate, also bath application of ACh (10 µM combined with 2 µM physostigmine) induced γ oscillations. The power was larger in area CA3 than in area CA1 and the activity in area CA3 preceded the activity in area CA1. Previous experiments had pointed to interactions between acetylcholine and histamine (Haas et al., 2008). We therefore tested for effects of the H1 receptor antagonist fexofenadine. The H1 receptor antagonist caused a near doubling of peak power and a slight decrease in peak frequency (Fano et al., 2011; Fig. 1(a)-(d)) and interestingly also a small harmonic oscillation at approximately 70 Hz (Fano et al., 2011; Fig.1 (b)). The effect of fexofenadine might indicate that histamine depresses cholinergic γ through an H1 receptor. We therefore tested for effects of the H1 receptor agonist, Trifluormethylphenylhistamine (TFMPH), which had no significant effects on γ oscillations (Fano et al., 2011; Fig.2(a)-(b)). We then tested for effects of histamine on H2 receptors. The H2 receptor antagonist cimetidine reduced γ power by 50% without any effect on frequency (Fano et al., 2011; Fig. 2 (c)-(d)), whereas the H2 agonist dimaprit nearly doubled γ power (Fano et al., 2011; Fig.2 (e)-(f)). Since we did not actively add histamine to the preparation, the effects of the antagonist of H1 and H2 receptors suggest that acetylcholine causes release of histamine from histaminergic fibers. Indeed, we found that kainate-induced γ oscillations were not affected by fexofenadine (Fano et al., 2011, data not shown). We therefore tried to

reduce histamine levels in hippocampal slices. It was previously shown that hypoxia leads to an increase in transmitter release for glutamate, γ- aminobutyric acid (Fleidervish et al., 2001) and dopamine (Moghaddam et al., 1987) with no major acute tissue damage if hypoxia was applied for less than 5 min. Similar data were obtained in carotid bodies where hypoxia led to spontaneous release of histamine along with dopamine (Koerner et al., 2004). We therefore tested for effects of transient hypoxia on γ in the presence of imipramine, which would reduce presynaptic uptake of histamine. As previously reported, hypoxia readily blocks γ activity (Fano et al., 2007; Huchzermeyer et al., 2008). This was also the case in our experiments when hypoxia was combined with imipramine (20 µM). After this treatment, γ could still be induced but they showed a reduced frequency and power (Fano et al., 2011; Fig. 3 (a), (e)). Interestingly, fexofenadine no longer augmented ACh-induced γ (Fano et al., 2011; Fig 3 (a)-(d)). This treatment may result in depletion of many transmitter pools and therefore may be not specific. We therefore tested whether the effect of the H2 receptor antagonist cimetidine was also prevented after the hypoxia-induced depletion of histamine from the slice. When slices were treated with hypoxia, the H2 antagonist cimetidine no longer decreased γ power (Fano et al., 2011; Fig. 3(e)-(h)).

Effects of astemizole, sertindole and E4031 on acetylcholine-induced gamma oscillations

After having demonstrated that histamine, through the activation of an H2 receptor or the block of an H1 receptor, has a strong influence on γ oscillations (Fano et al., 2011), we were interested in comparing the effects of two different antihistaminergic agents: astemizole and fexofenadine. Astemizole is an H1 receptor antagonist, as well as fexofenadine. These drugs were of particular interest to us not only because they are second generation antihistaminergic drugs with a long duration of action but also because they block Kv11 or ERG K^+ channels. The antipsychotic drug sertindole, apart from its effects on dopamine D2 and serotonin 5 -HT2 receptors, also blocks ERG K^+ channels. It was therefore important to be able to discriminate the effects of a potassium channel block from an histamine or dopamine receptor mediated effect on gamma oscillations. We therefore wanted to investigate the effect of a more specific ERG K^+ channel blocker and to compare these effects to the effects of sertindole and astemizole. Application of astemizole (30 µM) induced a large rise in power of ACh-

induced γ and a slight decrease in frequency (Fano et al.,2012; Fig. 1A, D). Peak power increased by a factor of two (Fano et al., 2012; Fig. 1A). The increase in peak power was associated with the increase in a harmonic peak (Fano e t al., 2012; Fig.1Ac). Sertindole also augmented ACh-induced γ oscillations (Fano et al., 2012; Fig 1B,D). The effect was smaller than that of astemizole. Peak frequency was unaffected (Fano et al., 2012; Fig. 1Bc). In contrast to astemizole and sertindole, E4031 did not affect the power and frequency of γ oscillations induced by ACh (Fano et al., 2012; Fig 1C-D).

Effects of astemizole, sertindole and E4031 on kainate-induced gamma oscillations

Astemizole also increased γ power when γ oscillations were induced by kainate (100 nM) (Fano et al., 2012; Fig. 2A,D). Astemizole reduced peak frequency significantly by ~3 Hz (Fano et al., 2012]; Fig. 2Ac). However, application of sertindole and E4031 did not have any significant effect, both on γ power and frequency (Fano et al., 2012; Fig. 2B-D).

Effects of astemizole, sertindole and E4031 on stimulus evoked responses

Kainate- and ACh -induced γ oscillations are generated in area CA3 and propagate to area CA1. By Schaffer-collateral stimulation an antidromically propagating action potential can be induced which then induces an excitatory field potential response in area CA3 with a superimposed population spike (Behrens et al., 2005). Astemizole and sertindole had no significant effect on the antidromically-induced population spikes (Fano et al., 2012; Fig. 3A-B) while E4031 significantly increased stimulus-induced antidromic responses when stimulus intensities were used which evoked sub-maximal responses (Fano et al., 2012; Fig.3C). Astemizole had a small augmenting effect on synaptically mediated secondary population spikes in area CA3 (Fano et al., 2012; Fig. 4A). This effect became significant for the stimulus intensity of 6 V which is above the stimulus intensity required for induction of maximal responses. Sertindole had no clear effect (Fano et al., 2012; Fig. 4B), while E4031 augmented the recurrent field excitatory postsynaptic potential (fEPSP) and the population spike over a wide range of stimulus intensities (Fano et al., 2012; Fig. 4C).

E 4031 effects on spontaneous activity in the hippocampus

Although there was a slight increase in the spontaneous baseline activity in area CA3, there was no appearance of recurrent epileptiform discharges or spontaneous events (Fano et al., 2012, data not shown). E 4031 had an augmenting effect on the amplitude of spontaneous sharp-wave-like events in area CA1 (Fano et al., 2012; Fig. 5A,C) without any effect on the incidence (Fano et al., 2012); Fig. 5B).

E 4031 effects on fEPSP in stratum radiatum of area CA1

Paired-pulse stimulation in *stratum radiatum* of area CA1 also evoked antidromic action potentials generated by Schaffer-collateral stimulation. The evoked response consists of an afferent volley and a field EPSP superimposed by a population spike (Fano et al., 2012; Fig. 6C). The afferent volley was unaffected by E4031 while the synaptically evoked response was strongly augmented (Fano et al., 2012; Fig. 6 D-E). Paired-pulse facilitation was reduced due to a large increase in the first response to paired pulse stimulation (Fano et al., 2012; Fig. 6F). This might indicate an effect on presynaptic terminals with increased Ca^{2+} entry due to a block of presynaptic K^+ currents. Therefore, I performed together with G Caliskan measurements on presumed presynaptic Ca^{2+} uptake.

E4031 effects on presynaptic CA^{2+} entry and extracellular K^+ accumulation

Repetitive stimulation (20 Hz, 2s) was used to evoke decreases in $[Ca^{2+}]_o$ due to cellular Ca^{2+} uptake. The presynaptic component of this Ca^{2+} uptake was isolated by blocking postsynaptic glutamate receptors with the application of CNQX, APV and MCPG. Changes in $[Ca^{2+}]_o$ were significantly reduced under this condition (Fano et al., 2012; Fig 7 B-C). However, these changes in $[Ca^{2+}]_o$ were not further reduced by E4031, confirming that E4031 had no effect on presynaptic Ca^{2+} entry (Fano et al., 2012; Fig. 7 B-C). E 4031 had also no effect on the kinetics of $[Ca^{2+}]_o$ changes (Fano et al., 2012; Fig. 7D). We didn´t find evidence for changes in $[K^+]_o$ concentration kinetics induced by E4031 either (Fano et al., 2012).

5.DISCUSSION

Effects of hypoxia on kainate-induced γ oscillations

The first goal of my research was to investigate the effects of hypoxia on γ oscillations and to understand the underlying mechanism. We found that:
(1) hypoxia rapidly suppressed kainate-induced γ oscillations in area CA3 and CA1 of adult rat hippocampal slices (Fano et al., 2007; Fig. 1(a)-(d)}; Fig. 3(a), (b), (d)). (2) The effects were partially reversible following hypoxia for 3 min but accumulated with repeated short term hypoxia (Fano et al., 2007; Fig. 2(a)-(b)). (3) The effects were more pronounced in area CA3 than in area CA1 (Fano et al., 2007; Fig. 1(a)-(d)}; Fig. 2(a)-(d)). (4) 6 min of hypoxia led to a transient anoxic depolarization after which γ oscillations remained almost completely blocked (Fano et al., 2007; Fig. 2(c)-(d); Fig. 3(d)). The generation of γ network oscillations depends on a functional interneuron network in which basket cell interactions are important in setting the oscillation frequency (Bartos et al., 2007). The suppression of such rhythmic activity can be due to effects on intrinsic membrane properties or to changes in synaptic interaction. It has been suggested that activation of ATP- dependent K^+ channels are the cause for loss of consciousness (Griesemer et al., 2002; Luhmann et al., 1993; Yamada et al., 2001). However, the increase of spontaneous transmitter release including glutamate, GABA and dopamine precedes the activation of ATP- dependent K^+ channels (Fleidervish et al., 2001; Gebhardt et al., 2002). Our research suggests that the increase in spontaneous transmitter release early during hypoxia, is responsible for the suppression of gamma activity and thereby for the rapid loss of consciousness after induction of ischemia or hypoxia. Kainate-induced γ oscillations are generated in area CA3 and propagate from this area to the hilus and to area CA1. The effects of repeated hypoxia for 3 min were cumulative in area CA3 and in the experiments with prolonged hypoxic periods, γ oscillations recorded in area CA3 were more affected than those in area CA1, suggesting that interneurons in area CA3 are particularly vulnerable to hypoxia.

Histamine effects on acetylcholine- induced γ oscillations

The main finding of this second part of my study was that γ oscillations induced by acetylcholine are modulated by histamine and this effect is likely mediated by H2 receptors. Histamine is a neuromodulator released from histaminergic neurons located in the tuberomamillary nucleus of the posterior hypothalamus. Active solely during waking, they maintain wakefulness and attention (Haas et al., 2008). We found that (1) the histamine H2 agonist dimaprit and H1 antagonist fexofenadine significantly increased the power of acetylcholine-induced γ (Fano et al., 2011; Fig.1(a)-(d); Fig. 2(e)-(f)). (2) The H1 receptor agonist TFMPH had no effect (Fano et al., 2011; Fig. 2 (a)-(b)). (3) The H2 antagonist cimetidine reduced the power of γ (Fano et al., 2011, Fig.2 (c)-(d)). We hypothesized that those effects are due to acetylcholine receptors on presynaptic terminals of histaminergic fibers, which would facilitate the release of histamine. In the presence of the H1 antagonist, more of the released histamine is affecting neuronal H2 receptors. To record directly from presynaptic terminals of histaminergic fibers is very difficult because of their small size and identification problems. It has been previously shown that hypoxia causes an increase in spontaneous transmitter release. We therefore used hypoxia to cause increased spontaneous release of histamine while preventing re-uptake of histamine from the extracellular space, thereby depleting fibers from their transmitter. We treated our slices with short periods of hypoxia which had no irreversible effects on cellular behavior both in the hippocampus and neocortex (Fano et al., 2007; Luhmann et al., 1993). Similarly to our previous experiments (Fano et al., 2007), γ recovered upon repeated hypoxia application. However, the H1 antagonist no longer augmented cholinergic γ (Fano et al., 2011; Fig.3(a)-(d)), and the H2 antagonist no longer reduced γ power (Fano et al., 2011; Fig 3 (e)-(h)). Our research demonstrates that acetylcholine might lead to a further enhancement of γ by augmenting the release of histamine from histaminergic fibers and therefore facilitating the storage of information in the hippocampus, while , application of H2 agonist has a memory-enhancing effect that mostly concerns storage of information in working memory. The histamine receptors are therefore potential targets for the development of pro-cognitive drugs to treat disorders such as AD, schizophrenia and attention deficit/hyperactivity disorder (ADHD) (Griebel et al., 2012; Weisler et al., 2012; Tiligada et al., 2011; Petroianu et al., 2006).

Effects of ERG channels on γ oscillations in rat hippocampal slices

Some of the effects of the H1 antagonist fexofenadine and the H2 agonist dimaprit on gamma oscillations induced by acetylcholine may be due to the block of ERG K^+ channels (Rajamani et al.; 2002). Therefore, we wanted to investigate the effects of a more specific ERG K^+ channels blocker (E4031) and compare them to effects of sertindole and astemizole which are also suspected to modify ERG K^+ channels. We found that (1) astemizole and sertindole increased ACh-induced γ oscillations while E4031 had no effect (Fano et al., 2012; Fig. 1A-C). (2) When γ were induced by kainate, astemizole still had an augmenting effect on peak power (Fano et al., 2012; Fig. 2A). This is consistent with a block of H1 receptors and activation of H2 receptors previously described by our lab (Fano et al., 2011). Interestingly, the effects of sertindole and astemizole were stronger on ACh-induced γ than on kainate-induced γ. This difference was already noted previously by us for other histamine receptor modifying agents suggesting that ACh might interfere with histamine release from histaminergic fibers (Fano et al., 2011). The effects of sertindole on γ oscillations may be related to the action on γ through dopaminergic and serotonergic receptors (Wojtowicz et al., 2009). The antipsychotic effect of sertindole is not likely due to a direct modulation of ERG K^+ channels. Rather, the blockade of ERG K^+ channels prolonged plateau potentials in bursting neurons (Shepard et al., 2007). This change in excitability would be expected to alter dopamine release.

In the hippocampus, ERG1 expression is relatively weak and appears to be concentrated in the pyramidal cells, interneurons and glial cells of area CA1 (Saganich et al., 2001). As interneurons are thought to play an important role in the generation of γ, we had hypothesized that E4031 would also affect γ. This was not the case and may be related to the weaker expression of ERG1 in area CA3 compared to area CA1 (Saganich et al., 2001), as area CA3 is the generator site for pharmacologically induced γ (Hájos and Paulsen, 2009). Moreover, Kv11 channels have slow kinetics which would not interfere with the frequency of γ oscillations (Shepard et al., 2007).

Effects of ERG channels on excitability in rat hippocampal slices

Current data from different regions of the brain suggest that the blockade of Kv11 channels could increase neuronal excitability. We found that (1) spontaneous sharp waves in area CA1 were increased in amplitude but not in incidence by E4031 (Fano et al., 2012; Fig. 5A-C), suggesting that ERG K^+ channels may also modulate neuronal excitability in the hippocampus. (2) In input-output (IO) curves from area CA3 astemizole and sertindole had no effect on antidromic population spike while E4031 significantly increased this response (Fano et al., 2012; Fig. 3A-C). The secondary evoked response due to the induction of EPSPs and action potentials in CA3 was also strongly augmented by E4031 but not affected by sertindole and astemizole (Fano et al., 2012; Fig. 4A-C). (3) Also in area CA1, postsynaptically-mediated field EPSP and population spikes were strongly enhanced by E4031 (Fano et al., 2012; Fig.6 A-B;D-E). (4) There were no alterations in presynaptic Ca^{2+} uptake following blockade of ERG K^+ channels (Fano et al., 2012; Fig.7 B-D). (5) The effect of E4031 on CA1 population spike was restricted to the first pulse as the second popspike was unaltered which resulted in decreased paired-pulse facilitation (Fano et al., 2012; Fig. 6D-F). Our findings suggest that long stimulation time may mask the effect of ERG K^+ channels blockade on presynaptic Ca^{2+} uptake or the effects of E4031 may be related to intracellular mobilization of Ca^{2+} causing an increased probability of transmitter release upon the first of two stimuli (Secondo et al., 2000). In conclusion, the role of ERG K^+ channels is probably not the modulation of γ but the modulation of excitability and transmitter release.

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7.Declaration of own contribute to the submitted publications

The contribution of the doctoral student Silvia Fano to the submitted publications presents as follows:

- **Publication 1: Fano S, Behrens CJ, Heinemann U**.
  Hypoxia suppresses kainate-induced γ-oscillations in rat hippocampal slices.
  Neuroreport 18: 1827-1831, 2007.

  Contribution : approx 70%, CJ Behrens helped with Corel draw and literature

  Detailed contribution: conducting the experiments (preparation of brain slices, electrophysiological recordings), data analysis, preparation of the figures, participation to the writing of the manuscript.

- **Publication 2: Fano S,Caliskan G, Behrens CJ, Heinemann U.**
  Histaminergic modulation of acetylcholine-induced γ-oscillations in rat hippocampus.
  Neuroreport 22 : 520-524, 2011.

  Contribution : approx 50%

  Detailed contribution: conducting the experiments (preparation of brain slices, electrophysiological recordings), data analysis, participation in the writing of the manuscript.

- **Publication 3: Fano S, Caliskan G, Heinemann U.**
  Differential effects of blockade of ERG channels on gamma oscillations and excitability in rat hippocampal slices.
  Eur J Neurosci : in press, 2012.

  Contribution : approx 45%

  Detailed contribution: conducting the experiments (preparation of brain slices, electrophysiological recordings),participation in writing of the manuscript, processing the peer review.

Prof.Dr. Uwe Heinemann Silvia Fano

8.List of own publications:

Publication 1 : Fano S, Behrens CJ, Heinemann U. Hypoxia suppresses Kainate-induced γ-oscillations in rat hippocampal slices. **Neuroreport**, 2007.

Publication 2: Fano S , Caliskan G, Behrens CJ, Heinemann U. Histaminergic modulation of acetylcholine-induced γ-oscillations in rat hippocampus. **Neuroreport,** 2011.

Publication 3: Fano S, Caliskan G, Heinemann U. Differential effects of blockade of ERG channels on gamma oscillations and excitability in rat hippocampal slices. **Eur J Neurosci**., 2012.

Further publications :

Abstracts:

1. **Fano S , Heinemann U.**
   Effects of Astemizole on rat hippocampal network oscillations.
   8th Meeting of the German Neurosciences Society, Göttingen, Germany. March 2009.

2. **Fano S, Heinemann U**.
   Effects of Astemizole on rat hippocampal network oscillations.
   Berlin Brain Days 2009, Berlin, Germany. December 2009**.**

3. **Fano S, Heinemann U.**
   Effects of histamine antagonists and potassium channels blockers on gamma oscillations.
   Berlin Neuroscience Forum, Liebenwalde, Germany. Juni 2010.

4. **Fano S, Heinemann U.**
   Histaminergic modulation of acetylcholine induced gamma oscillations in rat hippocampus in vitro.
   7th Forum of European Neuroscience, Amsterdam, Holland. July 20

9.Selbstständigkeitserklärung

„Ich, Silvia Fano, erkläre, dass ich die vorgelegte Dissertationsschrift mit dem Thema:

“Histaminergic modulation of gamma oscillations in rat hippocampus”

selbst verfasst und keine anderen als die angegebenen Quellen und Hilfsmittel benutzt, ohne die (unzulässige) Hilfe Dritter verfasst und auch in Teilen keine Kopien anderer Arbeiten dargestellt habe.“

Berlin, 23.10.2012 Silvia Fano

Paper 1

Fano S, Behrens CJ, Heinemann U. 2007. Hypoxia suppresses kainate-induced gamma-oscillations in rat hippocampal slices. *Neuroreport* 18: 1827-1831.
The original article is online avaible at
http://dx.doi.org/10.1097/WNR.0b013e3282f13e4f

Paper 2

Fano S, Caliskan G, Behrens CJ, Heinemann U. 2011. Histaminergic modulation of acetylcholine-induced γ-oscillations in rat hippocampus. *Neuroreport* 22: 520-524.
The original article is online avaible at
http://dx.doi.org/10.1097/WNR.0b013e32834889dd

Paper 3

Fano S, Caliskan G, Heinemann U.2012. Differential effects of blockade of ERG channels on gamma oscillations and excitability in rat hippocampal slices. 2012*. Eur J Neurosci*. 36: 3628-3635.
The original article is online available at
http://dx.doi.org/10.1111/ejn.12015