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Research report

Excitatory and inhibitory control of epileptiform discharges in combined hippocampal/entorhinal cortical slices

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Abstract

We examined whether epileptiform activity can be induced and prevented by mild reduction of $GABA_A$ receptor-mediated inhibition and non-NMDA receptor-mediated excitation, respectively, in different regions of combined hippocampal/entorhinal cortical slices from juvenile rats (P15–21). We used the receptor antagonists bicuculline ($GABA_A$) and CNQX (non-NMDA) as tools to investigate the sensitivities of the CA1, the subiculum (SUB) and the medial entorhinal cortex (MEC) for generating epileptiform discharges upon extracellular stimulation. We found that low concentrations of bicuculline (<3.5 μM) were enough to induce epileptiform discharges in the three regions. These discharges were similar to those observed under high concentrations of bicuculline (>10 μM) and consisted of stereotyped population bursts, recorded both extra- and intracellularly. Interestingly, the CA1 and SUB were more susceptible to generate discharges compared to the MEC in the same slices. We also found that non-NMDA excitation was critical in controlling discharges, as they were blocked by CNQX in a concentration-dependent manner. The sensitivity of the CA1 region to CNQX was lower than that of the SUB and MEC. Based on these regional differences, we show that epileptiform activity can be pharmacologically isolated within the CA1 region in the hippocampal–entorhinal circuitry in vitro.

Theme: Disorders of the nervous system

Topic: Epilepsy: basic mechanisms

Keywords: Hippocampus; Entorhinal cortex; Partial disinhibition; Epileptiform discharges; Bicuculline; CNQX

1. Introduction

The differential role of the hippocampal and entorhinal cortical regions in experimental epileptogenesis is not completely understood [21,33]. The entorhinal cortex participates in the amplification and reverberation of prolonged discharges in the hippocampal–entorhinal cortical loop [3–6,33,43], but under certain circumstances can be considered as an independent epileptogenic region [4,21,38]. In the juvenile as well as in adult tissue, the hippocampus and entorhinal cortex appear to have different susceptibility for generating and transmitting epileptiform activity, which is mainly controlled by GABAergic inhibition and glutamatergic excitation [12,15,23,48]. The removal of fast GABAergic inhibition uncovers the involvement of polysynaptic excitatory circuits in the development of epileptiform discharges [29]. However, the effect of a partial blockage of $GABA_A$ receptor-mediated inhibition remains unexplored and little is known about the excitatory control of epileptiform activity under this condition. This is especially important, as differences in susceptibility between regions can be better detected when the inhibitory and excitatory systems are only slightly unbalanced (low concentrations of receptor antagonists) [8,9,45,46].

Here we addressed the following two issues: (a) whether epileptiform discharges in vitro can be differently induced by mild reductions of fast GABAergic inhibition (partial disinhibition) in the hippocampal versus the entorhinal cortical regions, and (b) how does non-NMDA receptor-mediated excitation control epileptiform discharges under partial disinhibition? We explored these questions in the hippocampus and entorhinal cortex from juvenile rats of 3 weeks of age (postnatal days P15–P21) because of its

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greater susceptibility to generate epileptiform discharges [15,35,36,51]. We used different concentrations of the receptor antagonist bicuculline ($GABA_A$ receptors) and CNQX (non-NMDA receptors) to explore how epileptiform discharges were induced and prevented in different regions of the hippocampal–entorhinal cortical formation: the CA1, the subiculum (SUB) and the medial entorhinal cortex (MEC).

2. Materials and methods

2.1. Preparation of slices

Animal care and use was in accordance with the guidelines of the Universidad Complutense de Madrid. Experiments were performed on tissue taken from 15- to 21-day-old Wistar rats. Rats were decapitated under ether anesthesia, the brain removed, and chilled in 4 °C oxygenated (95% O_2–5% CO_2) artificial cerebrospinal fluid (ASCF: 125 mM NaCl, 3 mM KCl, 0.9–1 mM $MgSO_4$, 1.2 mM NaH_2PO_4, 2 mM $CaCl_2$, 22 mM $NaHCO_3$, 10 mM glucose). Slices (350 μm) including the hippocampus and adjacent entorhinal cortex were prepared using a tissue chopper, according to previously published criteria [22]. Slices were maintained in ACSF at room temperature for 1 h before being transferred to a submerged-type recording chamber (flow rate of 1–1.5 ml/min, 32–34 °C) attached to an upright microscope (4× lense). Once in the recording chamber, slices were tested by stimulation of the Schaffer collaterals, while extracellular or whole-cell recordings were made at the CA1/subiculum border. Slices were accepted for study only if reliable responses were recorded.

2.2. Electrophysiological stimulation and recordings

Monopolar stimulation was applied via tungsten electrodes (WPI Instruments). Stimulation (10–20 trials) consisted of square-wave pulses of 0.1 ms duration and 0.5–1 mA amplitude. Extracellular field potential recordings were performed using patch-clamp pipettes filled with 2 M NaCl. Patch pipettes were made of borosilicate glass capillaries (outer diameter 1.2 mm, inner diameter 0.69 mm; Harvard Apparatus Ltd.). Signals were amplified and filtered at 3 kHz.

Somatic whole-cell recordings were performed using an Axoclamp 2B amplifier in current-clamp mode (Axon). Patch pipettes had a resistance of 4–6 MΩ when filled with intracellular solution containing (in mM): 140 KCl, 0.9 $MgCl_2$, 1 NaCl, 1 EGTA, 5 HEPES, 2 K_2ATP, pH 7.3 adjusted with KOH (osmolarity was between 290 and 300 mosM). In some experiments ($n=6$), a low chloride intracellular solution was tested (in mM): 131 K-gluconate, 6 KCl, 0.9 $MgCl_2$, 1 NaCl, 1 EGTA, 5 HEPES, 2 K_2ATP, pH 7.3 adjusted with KOH and osmolarity between 290 and 300 mosM. We found no difference between these two intracellular solutions when recording epileptiform discharges under low (1.5–5 μM) and high (>5 μM) concentrations of bicuculline. Reagents (all from Sigma) were applied via bath superfusion at several concentrations: bicuculline methiodide, from 1.5 to 10 μM; 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX), from 0.1 to 10 μM; and DL-2-amino-5-phosphonopentanoic acid (AP5), from 50 to 100 μM. In experiments using low concentrations of bicuculline, the slices were incubated for at least 35 min before recordings were made (see Section 3). All signals were digitized at 16-bit resolution and acquired using Spike 2 software (Cambridge Electronic Design).

2.3. Criteria for characterizing epileptiform discharges under different antagonist concentrations

Epileptiform discharges are network-driven events that can be defined both by extracellular and intracellular criteria [25]. In extracellular recordings, epileptiform discharges appeared as stereotyped all-or-none bursts of population spikes. Simultaneous whole-cell recordings revealed that they consisted of bursts of action potentials riding on a large depolarizing envelope (reversal potential near 0 mV). The amplitude of epileptiform discharges was estimated by the voltage difference between the resting membrane potential (RMP) and the peak of the depolarizing envelope. Duration was defined at half-amplitude.

The dose-dependent effect of bicuculline was examined by combining extracellular and intracellular criteria. Extracellular recordings at the CA1 were performed simultaneously with whole-cell recordings at the CA1, SUB and MEC. We first tested a given slice electrophysiologically under control conditions, and then bicuculline was added at different concentrations (1.5–10 μM). Two to five different bicuculline concentrations were tested per slice. Concentration thresholds of bicuculline were defined as the concentration at which more than 50% of extracellular stimuli evoked stereotyped epileptiform discharges. The role of non-NMDA excitation was examined by adding CNQX at several concentrations (0.1–10 μM) to previously bicuculline-treated slices (2.5, 3, 3.5, 5 and 10 μM).

2.4. Sectioning experiments

In order to investigate the origin of the spontaneous epileptiform activity recorded in a particular region we performed a number of sectioning experiments. Isolation of a particular region (MEC and CA1) was performed using dissection scissors, after which the two parts were separated and new recordings were performed. Cutting of the subicular input to the MEC was made at the border of the parasubiculum from the alveus to the hippocampal fissure. The CA1 region was isolated by cutting Schaffer collaterals at the CA2/CA1 border and separating the CA3 region.

2.5. Data analysis

All results are given as the mean±S.D., the number of slices being indicated in every case. Results were compared using the Student's *t*-test or the ANOVA test, being significantly different if $P<0.05$.

3. Results

3.1. Epileptiform discharges in the hippocampal–entorhinal cortex in control conditions and under high doses of bicuculline

Under control conditions, extracellular stimulation of the Schaffer collaterals evoked excitatory postsynaptic potentials (EPSPs) in cells from the CA1 (8.2±4.2 mV amplitude and 37±9 ms duration; $n=5$) and SUB (6.3±3.2 mV and 39±18 ms; $n=9$). Comparable, or stronger, stimulation did not elicit any synaptic response in the MEC ($n=13$). However, both spontaneous and evoked epileptiform discharges were recorded in the CA1, SUB and MEC at high doses of bicuculline (10 μM, $n=10$; Fig. 1Aa). Intracellularly, epileptiform discharges consisted of depolarizations of 20.3±4.2 mV amplitude and 340±190 ms duration ($n=10$). Prolonged afterdischarges, also called seizure-like events [6,11,32], were evoked by extracellular stimulation in three of ten slices (Fig. 1Ab). Intracellularly, afterdischarges were initiated by an epileptiform discharge of 24.2±4.8 mV amplitude and followed by rhythmic population bursts lasting 25.8±6.5 s ($n=3$). The NMDA receptor antagonist AP5 (50 μM) prevented the occurrence of evoked afterdischarges ($n=3$; not shown), but not of epileptiform discharges ($n=6$; Fig. 1B,C). Epileptiform discharges were blocked by the non-NMDA receptor antagonist CNQX (10 μM, $n=10$) in the CA1, SUB and MEC (Fig. 1B,C).

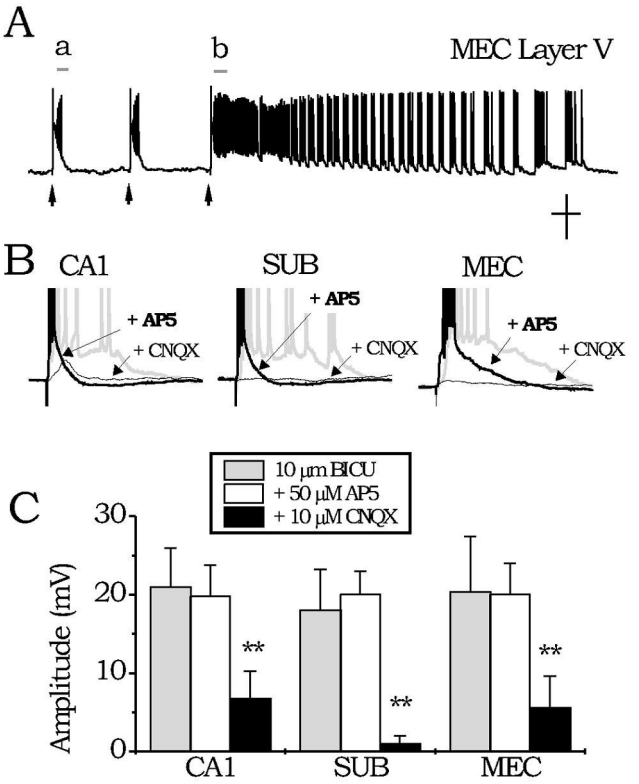

Fig. 1. Epileptiform discharges under high concentrations of bicuculline. (A) Epileptiform discharges (a) evoked at layer V of the medial entorhinal cortex (MEC) by stimulation of the Schaffer collaterals (vertical arrowheads) under 10 μM bicuculline. In three of ten slices, prolonged afterdischarges (b) were also evoked by extracellular stimulation. (B) Epileptiform discharges under 10 μM bicuculline evoked at the CA1, subiculum (SUB) and MEC by extracellular stimulation of the Schaffer collaterals, CA1 alveus and SUB, respectively (gray traces). Discharges were blocked by the non-NMDA receptor antagonist CNQX (10 μM, thin black traces). The NMDA receptor antagonist AP5 (50 μM) did not block epileptiform discharges (thick black traces). (C) Effects of CNQX and AP5 at the CA1, SUB and MEC from $n=6$ slices. ** Statistically different, $P<0.05$. Calibration bars: vertical, 50 mV in A and 25 mV in B; horizontal, 500 ms in A and 75 ms in B. Spikes in B are truncated.

3.2. Effect of low doses of bicuculline

Extracellular stimulation of the Schaffer collaterals under 3.5–4 μM bicuculline ($n=11$ slices) resulted in epileptiform discharges in the CA1, SUB and MEC, similar to those recorded under 10 μM (Fig. 2A). In these regions, the bicuculline-induced effect reached a plateau after 30 min for concentrations <5 μM ($n=5$, Fig. 2B,C), whereas for concentrations >5 μM the effect was faster (7–12 min, $n=16$; not shown). Thus, experiments involving low concentrations of bicuculline were performed 35–40 min after its application. After this delay, simultaneous extracellular and whole-cell recordings showed both evoked and spontaneous epileptiform discharges, spreading from the CA1 to the MEC (layer V) via the subicular complex, with latencies of 6.2±1.2 ms (CA1), 14.1±3.2 ms (SUB) and 51.7±5.6 ms (MEC; data from $n=5$ slices under 5 μM bicuculline, Fig. 2D). Afterdischarges were never observed under doses of bicuculline <5 μM ($n=16$ slices), indicating that they might require a greater imbalance of fast GABAergic inhibition.

3.3. Bicuculline-dependence of evoked and spontaneous epileptiform discharges

Increasing doses of bicuculline were used to determine the sensitivity of each region to generating epileptiform discharges upon stimulation of the Schaffer collaterals. In the CA1, discharges were evoked at bicuculline concentrations that ranged between 2 and 2.5 μM from slice to slice ($n=10$; Table 1, Schaffer stimulus). In Fig. 2E, it can be seen that the number of evoked extracellular spikes showed a characteristic threshold in correlation with the amplitude of the intracellular response (data from $n=5$ slices). The duration of the response increased monotoni-

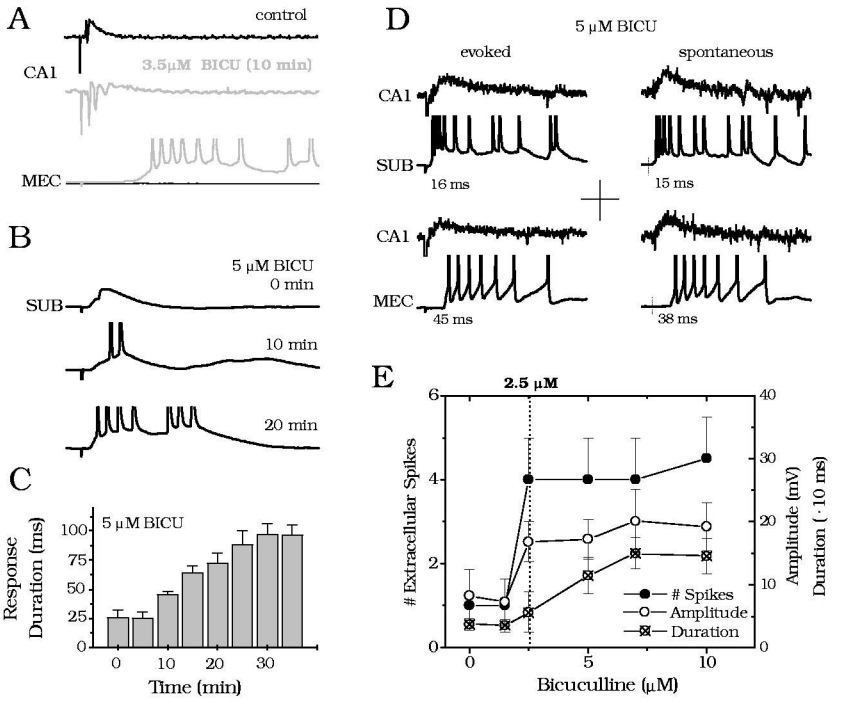

Fig. 2. Epileptiform discharges under concentrations of <5 μM bicuculline. (A) Simultaneous extracellular (CA1) and whole-cell recordings (MEC) in control (black traces) and under 3.5 μM bicuculline (gray traces). (B) Bicuculline time-dependent effects at the subiculum (SUB). (C) For concentrations <5 μM, the bicuculline-induced effect reached a plateau after 35 min (n=5). (D) After 35 min, spontaneous and evoked epileptiform discharges could be recorded simultaneously at the CA1–SUB and CA1–MEC. (E) Epileptiform discharges were evoked at the CA1 at doses of bicuculline >2.5 μM after extracellular stimulation of the Schaffer collaterals (data from n=5 slices). Both the number of extracellular spikes and the intracellular amplitude of the corresponding discharge showed a characteristic all-or-none behavior at 2.5 μM bicuculline in these slices (vertical dotted line). Discharge duration, recorded intracellularly, increased as the bicuculline concentration increased, being maximal for doses >7 μM. Calibrations bars: vertical, 50 mV for whole-cell recordings and 2 and 1 mV for field potentials in A and D; horizontal, 25 ms in A and B and 80 ms in D.

cally as the concentration of bicuculline increased, and reached a plateau at concentrations >7 μM.

In the SUB, epileptiform discharges were evoked at slightly higher bicuculline concentrations (2.5–3 μM, n= 18; Table 1, Schaffer stimulus). This threshold difference from that of the CA1 was conspicuous, because the sharp concentration threshold in the SUB was statistically different from that in the CA1 at each slice (n=18, P<0.05; see Fig. 3A). In the MEC, still higher concentrations of bicuculline (3.5–4 μM) were required to evoke epileptiform discharges (n=16; Table 1, stimulus at Schaffer Stimulus; and Fig. 3B).

Table 1
Concentration thresholds of bicuculline (μM) underlying interictal discharges at the CA1, SUB and MEC

	CA1	SUB	MEC
Schaffer stimulus (n)	2–2.5 (10)	2.5–3 (18)	3.5–4 (16)
Spontaneous (n)	2–2.5 (7)	– (18)	3–3.5 (9)
Isolated areas (n)	– (4)	–	3–3.5 (3)
Local stimulus (n)	2–2.5 (15)	2–2.5 (13)	3–3.5 (13)

Values are concentrations of bicuculline required to evoke interictal discharges in each region according to extracellular and intracellular criteria. The number of slices is given in parentheses. SUB, subiculum; MEC, medial entorhinal cortex.

Spontaneous epileptiform discharges appeared only in the CA1 and MEC, and at concentrations higher than 2–2.5 μM (n=7) and 3–3.5 μM (n=9), respectively (Table 1). We did not record spontaneous discharges initiated in the SUB (n=18). Spontaneous discharges initiated in the CA1 were dependent on the CA3 input, as they were eliminated after isolating the CA1 from the CA3 region (n=4; see Methods). On the other hand, cutting of the subicular input to the MEC did not eliminate spontaneous epileptiform discharges in this region (n=3, Table 1).

3.4. Regional thresholds of bicuculline concentration

The above data suggested that epileptiform activity appeared throughout the hippocampal–entorhinal circuitry depending on the bicuculline concentrations. However, the regional thresholds could have been underestimated because of the polysynaptic nature of discharge spreading from the CA1 to the MEC (see bicuculline thresholds for generating spontaneous increasing discharges in Table 1). Therefore, in a new set of experiments, we examined the effect of repositioning the stimulation electrode throughout the slice, while making simultaneous whole-cell record-

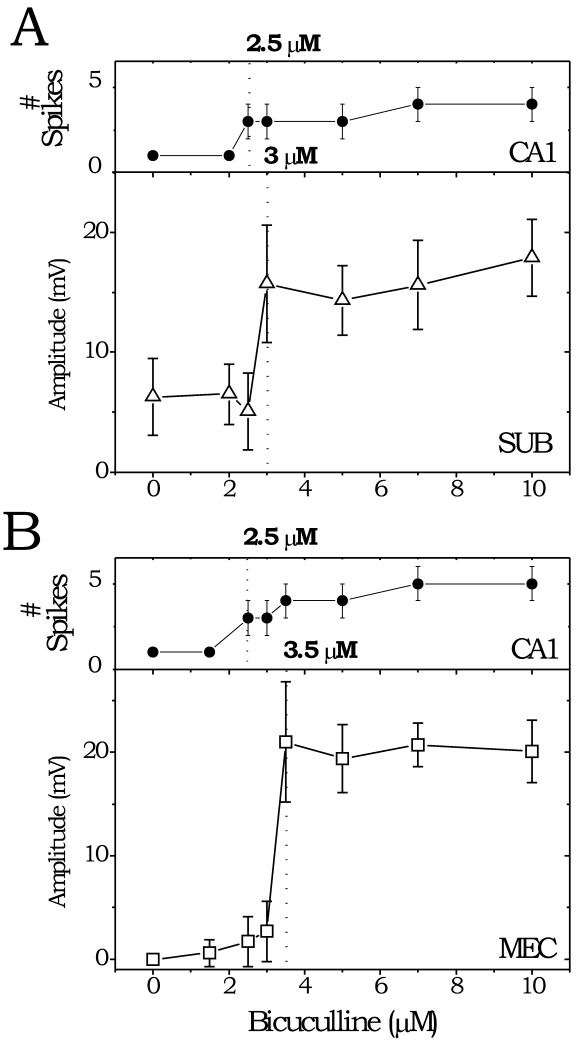

Fig. 3. Concentration thresholds of bicuculline at the SUB and MEC following extracellular stimulation of the Schaffer collaterals. (A) Simultaneous extra (CA1) and whole-cell (SUB) recordings, showing how epileptiform discharges were evoked at the CA1 under 2.5 μM bicuculline and at the SUB under 3 μM bicuculline (data from $n=6$ slices). (B) Simultaneous extra (CA1) and whole-cell (MEC) recordings, showing a bicuculline threshold of 3.5 μM at the MEC (data from $n=5$ slices).

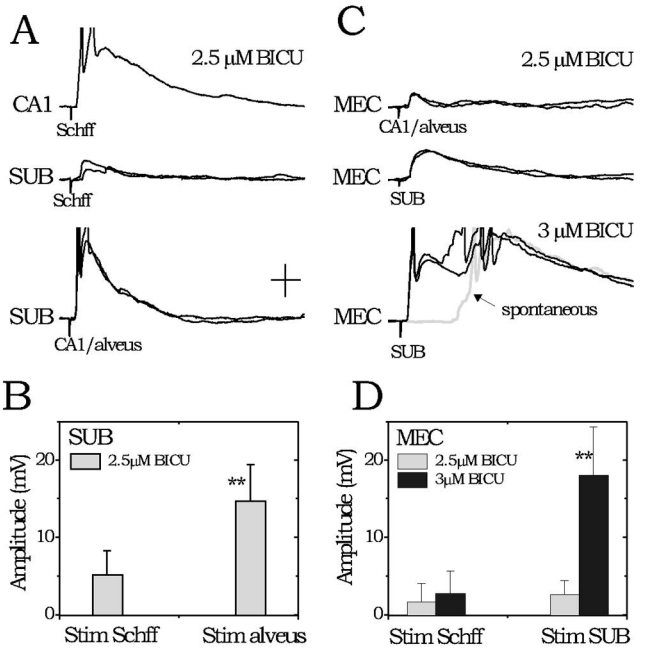

Fig. 4. Effects of local extracellular stimulation on bicuculline thresholds for epileptiform discharges. (A) Extracellular stimulation of the Schaffer collaterals (Schff) evoked discharges at the CA1 (upper trace), but not at the SUB, under 2.5 μM bicuculline (two consecutive traces are shown in the SUB). Repositioning of the stimulation electrode at the CA1 alveus (CA1/alveus) resulted in discharges at the SUB (lower traces). (B) All-or-none behavior of the SUB response amplitude under 2.5 μM bicuculline while stimulating the CA1/alveus (Stim alveus), compared with stimulation of the Schaffer collaterals in the same slices (Stim Schff; data from $n=4$ slices). (C) Same slice as in A. Extracellular stimulation at the CA1/alveus evoked EPSPs in the MEC under 2.5 μM bicuculline (upper traces), which persisted when the stimulation electrode was repositioned at the SUB (middle traces, same cell). Nevertheless, increasing the bicuculline concentration to 3 μM resulted in both the generation of epileptiform discharges after stimulation at the SUB (lower trace), as well as spontaneous discharges (gray). (D) All-or-none behavior of the MEC response amplitude under 3 μM bicuculline, but not under 2.5 μM, while stimulating at the SUB (Stim SUB), compared with stimulation of the Schaffer collaterals in the same slices (Stim Schff; data $n=5$ slices). ** Statistically different, $P<0.05$. Calibration bars: vertical, 10 mV; horizontal, 50 ms.

ings. Bicuculline concentrations of 2, 2.5, 3 and 3.5 μM were tested.

As described above, epileptiform discharges were evoked in the CA1, but not in the SUB, at 2–2.5 μM bicuculline upon stimulation of the Schaffer collaterals ($n=15$; Fig. 4A, upper two traces). However, repositioning of the stimulation electrode to the CA1 alveus (i.e. the direct input to the SUB) resulted in epileptiform discharges in the SUB under similar bicuculline concentrations at each slice ($n=13$; Fig. 4A, lower trace, and Fig. 4B). On the other hand, neither extracellular stimulation of the CA1 alveus nor repositioning of the stimulation electrode to the SUB (i.e. the direct input to the MEC), evoked discharges in the MEC under 2–2.5 μM bicuculline ($n=13$; Fig. 4C, upper two traces, same slice as in A). Epileptiform discharges were evoked in the MEC of each slice by stimulation at the SUB only if bicuculline was increased to 3–3.5 μM ($n=13$; Fig. 4C, lower trace, and Fig. 4D). Interestingly, spontaneous discharges in the MEC appeared at these concentrations (Fig. 4C, gray trace) and persisted after cutting the subicular input to the MEC, as described above (Table 1).

3.5. Effect of CNQX on epileptiform discharges under low and high doses of bicuculline

We then examined the effect of non-NMDA excitation on epileptiform discharges recorded at the CA1, SUB and MEC. To this purpose, we tested the effect of different concentrations of CNQX on the bicuculline-mediated

epileptiform discharges upon stimulation of the Schaffer collaterals ($n=8$), CA1 alveus ($n=10$) and SUB ($n=8$).

We found that blockage of epileptiform discharges by CNQX depended on the bicuculline concentration. Under bicuculline concentrations of 2.5 and 3.5 μM, low concentrations of CNQX (1–2 μM) substantially reduced or blocked both spontaneous and evoked discharges in the CA1, SUB and MEC ($n=7$; Fig. 5A, left). However, the same doses of CNQX had little effect on epileptiform discharges at 5 and 10 μM bicuculline ($n=8$; Fig. 5A, right). In these cases CNQX concentrations >5 μM were required to block discharges. Interestingly, under low concentrations of bicuculline, higher concentrations of CNQX (3–5 μM) were required to block discharges in the CA1, compared to those in the SUB and MEC in the same slices (1–2 μM, $n=8$; Fig. 5B). We used this difference in CNQX sensitivity between the CA1 and the SUB and MEC to pharmacologically isolate epileptiform discharges within the CA1 region (Fig. 5C, $n=5$).

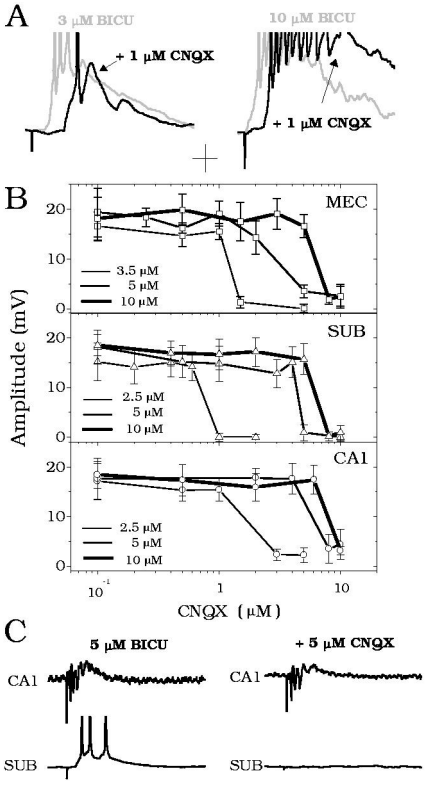

Fig. 5. Dose-dependent blockage of non-NMDA excitation. (A) Blockage of epileptiform discharges by CNQX depended on the concentration of bicuculline. CNQX at 1 μM substantially reduced discharge duration under 3 μM bicuculline, having little effect on interictal discharges under 10 μM bicuculline (whole-cell recordings at MEC). (B) CNQX blockage of epileptiform discharges under 2.5, 3.5, 5, and 10 μM bicuculline at the MEC ($n=8$ slices), SUB ($n=8$) and CA1 ($n=8$). (C) Isolation of epileptiform discharges under 5 μM bicuculline at the CA1 by adding 5 μM CNQX. Note how discharges are blocked at the SUB, but not at the CA1. Calibration bars in A: vertical, 10 mV; horizontal, 50 ms. Calibration bars in C: vertical, 2 mV (CA1) and 40 mV (SUB); horizontal, 100 ms.

4. Discussion

The main result shown here is that subtle pharmacological manipulations uncovered regional differences in the control of the hyperexcitability in the CA1, SUB and MEC from juvenile rats. More specifically, we found different low concentrations of bicuculline underlying discharges in the CA1 and SUB, compared to the MEC in the same slices. Similarly, the non-NMDA excitatory control of epileptiform discharges was different, the sensitivity of the CA1 region to CNQX being lower than that of the SUB and MEC. Based on these regional differences, we showed that epileptiform discharges could be isolated within the CA1 hippocampal region.

4.1. Regional differences between the hippocampus and entorhinal cortex from juvenile rats under mild reduction of inhibition and excitation

Different in vitro studies have explored the properties of epileptiform activity in the hippocampus using the slice preparation from juvenile rats. They have reported that epileptiform discharges similar to those present in adult tissue, can be reliable recorded in rats and rabbits of the third to four postnatal weeks [15,26,35,42,49]. In many of these experimental models, discharge susceptibility is greater in juvenile compared to adult tissue [15,36,51].

A main question underlying this study was whether epileptiform activity can be differently induced by mild reductions in $GABA_A$ receptor-mediated inhibition in the hippocampal versus entorhinal cortical regions. The blockade of $GABA_A$ receptor-mediated inhibition is often used in experimental models of epilepsy [1,18,39,47]. In the hippocampal formation, the majority of these models completely impaired fast GABAergic inhibition by using high concentrations of bicuculline or picrotoxin in both, juvenile and adult tissue [1,18,32,36,47]. Nevertheless, under mild reductions of fast GABAergic inhibition regional differences in the inhibitory control of epileptiform activity could be better detected [8,45,46], especially in tissue from juvenile rats [15,49].

We found that bicuculline concentrations between 2 and 2.5 μM in the CA1 and SUB and 3 and 3.5 μM in the MEC were enough to induce epileptiform discharges in vitro, similar to those recorded under higher concentrations (>10 μM). The bicuculline concentration data can be related to the degree of disinhibition through the dose dependency of bicuculline antagonism of $GABA_A$ receptors. Binding studies show that half-blockage of $GABA_A$ receptors (IC_{50}) is between 2.7 and 5 μM bicuculline [40,55] and electrophysiological recordings demonstrate that fast IPSPs are substantially reduced or blocked by 10 μM bicuculline [7,11,17], whereas 1 μM bicuculline slightly reduces $GABA_A$ receptor-mediated conductances by no more than 20% [2]. Therefore, the concentration thresholds of bicuculline we found suggest that a 30–40%

reduction in inhibition at the CA1 and SUB, and >50% at the MEC, were enough to induce epileptiform discharges similar to those recorded in the absence of fast inhibition.

Epileptiform discharges under low and high concentrations of bicuculline were mainly mediated by non-NMDA excitation since they were blocked by CNQX at concentrations of 10 μM [1,22,23,34] (Fig. 1B,C). NMDA excitation partially modulated discharges by reducing their duration (Fig. 1A,B), as previously reported [23,34,50]. Because of its potentiality in blocking epileptiform discharges, we further explored the effect of CNQX in controlling discharges under low and high concentrations of bicuculline. We found a differential effect of CNQX in epileptiform discharges evoked in the CA1, SUB and MEC under low concentrations of bicuculline (2.5–3.5 μM). Discharges were prevented at the SUB and MEC by 1–2 μM CNQX whereas a higher concentration was required in the CA1 (3–5 μM CNQX). The IC_{50} of CNQX is between 2.5 and 3.5 μM [54], and non-NMDA mediated EPSPs are blocked by 10–20 μM CNQX [10,20]. This indicates that a reduction of less than 50% in non-NMDA excitation is required to prevent epileptiform discharges at the SUB and MEC, while more than 50% is needed in the CA1 region. In fact, we showed that epileptiform discharges could be pharmacologically isolated within the hippocampal CA1 region (Fig. 5C).

4.2. In vitro epileptiform discharges under low concentrations of bicuculline and CNQX

In spite of the regional differences, we found that epileptiform activity can be induced in the CA1, SUB and MEC under partial disinhibition. Computational simulations of realistic hippocampal networks have explored the mechanisms underlying epileptiform discharges in the presence of inhibition [45,46]. These studies showed that there is a critical value of the fast inhibitory conductance (threshold value) at which population activity qualitatively changes from asynchronous to synchronous. Around that threshold value, subtle fluctuations in the degree of inhibition lead to large changes in population activity. Computational simulations also showed that epileptiform discharges induced in both, disinhibited and partially disinhibited networks are prevented if excitation is reduced beyond a particular threshold value [16,45]. This threshold value is larger in disinhibited compared to partially disinhibited networks [45].

We found that low concentrations of CNQX (1–2 μM) significantly reduced or blocked epileptiform discharges under low concentrations of bicuculline, having virtually no effect on epileptiform discharges under high bicuculline concentrations (Fig. 5A). This is in accordance with theoretical predictions from computational models of realistic networks [45]. In agreement with these models, we also found that epileptiform activity can be induced under partial disinhibition and estimated the concentration thresholds underlying this behavior in our preparation. Our data is therefore giving experimental support to the idea advanced by computational studies that a subtle imbalance between inhibition and excitation underlies epileptiform activity under partial disinhibition [45,46]. According to this idea, epileptiform discharges can occur in the presence of inhibition if non-NMDA excitation is strong enough to mediate synchronization (see Fig. 2A) [8,16,31,45,53]. Even under reduced fast GABAergic inhibition, polysynaptic excitation is released because it is strongly controlled by the inhibitory system [8,31,20,29,30]. However, if non-NMDA excitation were slightly reduced (low concentrations of CNQX), the synchronized capability of excitatory circuits would decrease and the remaining inhibition would be powerful enough to control network hyperexcitability (see Fig. 5A, left) [45]. This situation does not occur when fast inhibition is completely blocked (high concentrations of bicuculline). In that case, the fast inhibitory control of polysynaptic excitatory circuits is eliminated, and a subtle reduction of non-NMDA excitation has little effect in controlling hyperexcitability (see Fig. 5A, right) [16,45].

4.3. Different discharge susceptibility of the hippocampal and entorhinal cortical regions

The regional differences in the inhibitory and excitatory control of hyperexcitability could be related to the different susceptibility of hippocampal and entorhinal cortical regions for generating and sustaining in vitro epileptiform activity in both juvenile and adult tissue. The hippocampus is among the structures most susceptible to initiating seizures [39]. In the majority of in vitro models, hippocampal epileptiform discharges are generated in the CA3 region, and subsequently propagate to the CA1 [4,6,35,52]. Only under certain conditions, the CA1 region can generate epileptiform activity in isolation [18,27]. This difference from the CA3 region is mainly due to the lack of recurrent excitatory synapses and of intrinsically bursting cells in the CA1 region [28,44]. Nevertheless, although CA1 is not especially prone to initiating discharges, it successfully mediates their transmission throughout the hippocampus [4,6,19,52]. We found that the CA1 region had the highest bicuculline sensitivity and the lowest CNQX sensitivity, which may constitute a basis for its propensity to transmit CA3 generated epileptiform activity.

The subiculum, on the contrary, exhibits a higher CNQX sensitivity than that of the CA1, although their concentration thresholds of bicuculline were similar. The subiculum is abundantly interconnected with the pre- and para-subicular regions [13,24]. Together, they constitute the subicular complex, which differ in their intrinsic properties [13,41] and anatomical connectivity [24] from the hippocampal networks. Electrophysiological studies have shown that the subicular complex is able to participate in the generation of epileptiform discharges under

certain conditions [12,14,15]. We did not record spontaneous epileptiform discharges initiated in the subiculum, which however acted as a gateway for discharges to spread to the medial entorhinal cortex (Fig. 2D). This strategic position of the subiculum as the principal output of the hippocampus, together with its intrinsic susceptibility to transmitting epileptiform discharges in the presence of inhibition, would support its role in controlling the propagation of epileptic activity to other cortical regions.

Finally, the propagation of epileptiform discharges to the entorhinal cortex requires a higher reduction in inhibition compared with those required in the SUB and CA1. Under this condition, discharges generated in the hippocampal regions successfully spread to the MEC via the subicular complex. Interestingly, spontaneous discharges can be initiated in the MEC at similar bicuculline thresholds, being subsequently prevented by reducing non-NMDA excitation in more than 50%. The role of the entorhinal cortex in the initiation and amplification of epileptiform activity has been reported in several experimental configurations, including 4-AP [3,4,34], zero-Mg^{2+} [15,22,48] and repetitive stimulation [37,42]. We have shown that under conditions of sufficient inhibitory impairment ($>$50%), the entorhinal cortex could be considered as an independent epileptogenic area and, consequently, is likely to contribute to more complex epileptic scenarios, such as prolonged self-sustained discharges [6,21,33,38,43].

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