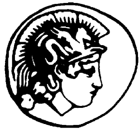

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Complex effects of CNQX on CA1 interneurons of the developing rat hippocampus

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Abstract

We have investigated the effect of the α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptor antagonist, 6-cyano-7-nitro-quinoxaline-2,3-dione (CNQX), on spontaneous $GABA_A$ receptor-mediated transmission in the hippocampal CA1 subfield. On average, simultaneous recordings from CA1 str. radiatum interneurons and pyramidal cells showed that CNQX application doubled the frequency of bicuculline sensitive spontaneous inhibitory postsynaptic currents (sIPSCs) without apparently changing their amplitude. However, despite the increase in sIPSC frequency, current-clamp recording showed that CNQX application was sufficient in most cases to depolarize interneurons to firing threshold. In contrast, CNQX application could not induce firing in pyramidal cells. In the presence of tetrado-toxin (TTX), CNQX increased interneuron membrane conductance, and depolarized interneurons from resting potentials. The axons of the studied interneurons ramify widely in the CA1 region and suggest that the cells of our sample are mostly involved with control of dendritic excitability.

Our results indicate that CNQX-induced increase of sIPSC frequency is not limited to excitatory cells, but also impacts GABA-ergic interneurons. However, despite the increase of sIPSC frequency, CNQX-induced depolarization is sufficient to selectively generate firing in interneurons and thus modify the network properties mediated by $GABA_A$ receptors in the hippocampus.
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Keywords: Quinoxaline derivatives; $GABA_A$ receptor; Bicuculline; Network; Dendrite; TTX

1. Introduction

Activation of $GABA_A$ receptors in the brain is involved in several functions ranging from control of cellular excitability to network synchronization and synaptic plasticity (Freund and Buzsaki, 1996). Spontaneous $GABA_A$ receptor-mediated activity in central networks ‘in vitro’ is often studied in the presence of glutamate receptor antagonists to suppress excitatory events. GABAergic input is most commonly isolated by a combination of blockers acting at α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) and NMDA receptors. A commonly used AMPA receptor antagonist, such as 6-cyano-7-nitro-quinoxaline-2,3-dione (CNQX), has been reported to increase GABAergic input to hippocampal pyramidal neurons and cerebellar granule cells (McBain et al., 1992; Brickley et al., 2001) with a mechanism that is unrelated to glutamate receptor block. In these studies, the effect of CNQX on spontaneous inhibitory postsynaptic currents (sIPSCs) was blocked or prevented by TTX, suggesting direct generation of firing in interneurons leading to increased sIPSC frequency on postsynaptic targets. However, the specific connectivity of GABAergic interneurons is complex (Freund and Buzsaki, 1996) and the question of whether CNQX-induced increase of inhibitory activity is restricted to pyramidal cells or is also present in GABAergic interneurons themselves has yet to be explored. In the CA1 hippocampus, specific classes of interneurons can innervate selectively pyramidal neurons, as in the case of axoaxonic cells (Somogyi et al., 1983), exclusively other interneurons (Acsady et al., 1996; Gulyas et al., 1996; Freund and Gulyas, 1997) or both interneurons and pyramidal cells (Freund and Buzsaki, 1996). Here, we directly address this point by comparing the effect

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of CNQX on sIPSC frequency in interneurons and pyramidal cells. In addition, we have characterized the effect of CNQX on membrane potential, action potential generation, and input conductance in anatomically identified str. radiatum interneurons. Our results indicate that CNQX alters spontaneous inhibition both in principal cells and interneurons by suprathreshold depolarization of presynaptic GABAergic interneurons.

2. Methods

2.1. Slice preparation

The procedure used to obtain slices is similar to the one described in Maccaferri and Dingledine (2002). Briefly, young rats (Sprague–Dawley, age P10–P16) were deeply anaesthetized using isoflurane and killed by decapitation, in accordance to NIH and Institutional protocols. The brain was quickly removed and placed into ice-cold 'cutting solution' of the following composition (mM): 130 NaCl, 24 $NaHCO_3$, 3.5 KCl, 1.25 NaH_2PO_4, 1 $CaCl_2$, 3 $MgSO_4$ and 10 glucose, saturated with 95% O2, 5% CO2, at pH 7.4. The hemisected brain was then glued onto the stage of a vibrating microtome (Leica, Germany) and sections of 300 μm thickness were cut and stored in an incubation chamber for about 1 h at room temperature before use. The composition of the artificial cerebrospinal fluid (ACSF) in the incubation and recording chamber was (mM): 130 NaCl, 24 $NaHCO_3$, 3.5 KCl, 1.25 NaH_2PO_4, 1.5 $CaCl_2$, 1.5 $MgSO_4$ and 10 glucose, saturated with 95% O2, 5% CO2, at pH 7.4. DMSO was added to the control solution at a concentration of 0.02% in order to maintain its concentration constant during CNQX application. Slices were then transferred, as needed, to the recording chamber and observed under an upright Zeiss Axioskop microscope (Zeiss, Germany) equipped with a 40X water immersion DIC objective coupled to an infrared camera system (Hamamatsu, Japan). Interneurons were located in CA1 str. radiatum or at the border between str. radiatum and str. lacunosum-moleculare.

2.2. Electrophysiological recordings

Conventional whole-cell current-clamp and voltage-clamp techniques were applied using two Axopatch-1D amplifiers (Axon Instruments, Union City, CA). Experiments were conducted at RT (~24°C). Pipettes used for current-clamp experiments (resistance ~5MΩ) were filled with the following solution (mM): 120 K-gluconate, 4 NaCl, 4 MgATP, 0.3 GTP, 10 HEPES and 0.5% biocytin to a pH of 7.2–7.3 and 285–295 mOsm, whereas voltage-clamp recordings were performed using electrodes filled with a solution of a different composition to minimize voltage dependent conductances. The filling solution was (mM): 120 CsCl (or 120 Cs-methasulfonate+4 NaCl) 4MgATP, 0.3 GTP, 5 QX-314, 10 HEPES, 0.5% biocytin to a pH of 7.2–7.3 and 285–295 mOsm. In the majority of the experiments sIPSC were recorded as inward currents at a holding potential of –70 mV using the CsCl based solution and with the constant presence of kynurenic acid (1 mM) in the bath to block ionotropic glutamate receptors. In the remaining cases, sIPSCs were recorded as outward currents at a holding voltage of 0 mV and the Cs-methasulfonate-low chloride electrode filling solution was used. No obvious differences were apparent in the results, and data were pooled together in the analysis.

2.3. Data analysis

Data were filtered at 5 kHz and digitized at 20 kHz using a Digidata 1200 A/D board. Analysis was performed using the pClamp (Axon Instruments, Union City, CA), Origin (MicroCal, Northampton, MA), Excel (Microsoft, Seattle, WA), Whole Cell Program (courtesy of Dr. J. Dempster, University of Strathclyde, UK) packages. sIPSC detection and measurement of peak amplitudes, averages and interevent intervals were performed using the Whole Cell Program. Statistical comparisons were performed used the appropriate Student's *t* test. Values are given as mean±SE.

2.4. Visualization of recorded cells and reconstruction.

Methods were similar to Maccaferri and Dingledine (2002). Briefly, slices were fixed for 1–10 days in a 4% paraformaldehyde PBS solution at 4°C. Endogenous peroxidase activity was removed by incubating the slices in 10% methanol, 1% H_2O_2 PBS solution. Biocytin staining was processed using an avidin-HRP reaction (Vectastain ABC Elite Kit) and axon visualization was improved by including $NiNH_4SO_4$ (1%) and $CoCl_2$ (1%) in the DAB solution. Slices were not resectioned, but directly mounted on the slide using an aqueous mounting medium (Vectashield). Alternatively, they were first dehydrated, and then mounted on the slide using a toluene solution (Permount). Slices were observed at 100× magnification and reconstructed using a camera lucida.

3. Results

3.1. CNQX modulation of sIPSCs in CA1 pyramidal cells and interneurons

In order to determine whether CNQX-induced potentiation of $GABA_A$-mediated synaptic transmission (McBain et al., 1992; Brickley et al., 2001) is specific to pyramidal cells or also affects interneurons, we per-

formed simultaneous double whole-cell patch clamp recordings from both cell types in the CA1 hippocampus. Spontaneous IPSCs were recorded in control conditions and in the presence of CNQX (10 μM). As shown in Fig. 1A, on the average, bath application of CNQX caused a large increased of the frequency of the recorded events. The frequency of sIPSCs was increased to 209 ± 42.5% of control in interneurons ($n = 12$, $p < 0.05$, Fig. 1A1) and to 258±49.8% in pyramidal cells ($n = 12$, $p < 0.05$, Figs. 1A2). These results confirm similar observations in other excitatory neurons such as CA3 pyramidal cells and cerebellar granules (McBain et al., 1992; Brickley et al., 2001). In addition, it provides for the first time evidence for CNQX-induced $GABA_A$-mediated synaptic transmission onto inhibitory hippocampal interneurons. CNQX-induced potentiation of sIPSCs in CA3 pyramidal and cerebellar granule cells is thought to depend on an increase of action potential generation in presynaptic GABAergic interneurons and has been shown to be blocked or prevented by TTX (McBain et al., 1992; Brickley et al., 2001). Consistent with this interpretation, on average, sIPSC amplitude showed no change in either pyramidal cells or interneurons during CNQX application. Peak amplitude of spontaneous events in the presence of CNQX was 105 ± 5.3% of control in interneurons ($n = 12$, $p > 0.05$, Fig. 1B1), which compared to a similar value of 110 ± 6.8% in pyramidal cells ($n = 12$, $p > 0.05$, Figs. 1B2). However a considerable degree of variability was present among the individual experiments (Fig. 1B2), which may be the result of a complex scenario (see discussion). The absolute values of sIPSC frequency and amplitude from each individual experiment are shown in Fig. 1C1 and 1C2. In interneurons, CNQX application increased the frequency of sIPSCs from 1.3 ± 0.5 Hz to 2.5 ± 0.8 Hz ($n = 12$, $p < 0.05$, Fig. 1C1, left), without apparently changing amplitudes, which were 65.9 ± 13.6 pA in control and 67.4 ± 13.7 pA after the addition of the drug ($n = 12$, $p > 0.05$, Fig. 1C2, left). In pyramidal cells, sIPSC frequency increased from 1.9 ± 0.4 Hz to 3.8 ± 0.8 Hz ($n = 12$, $p < 0.05$,

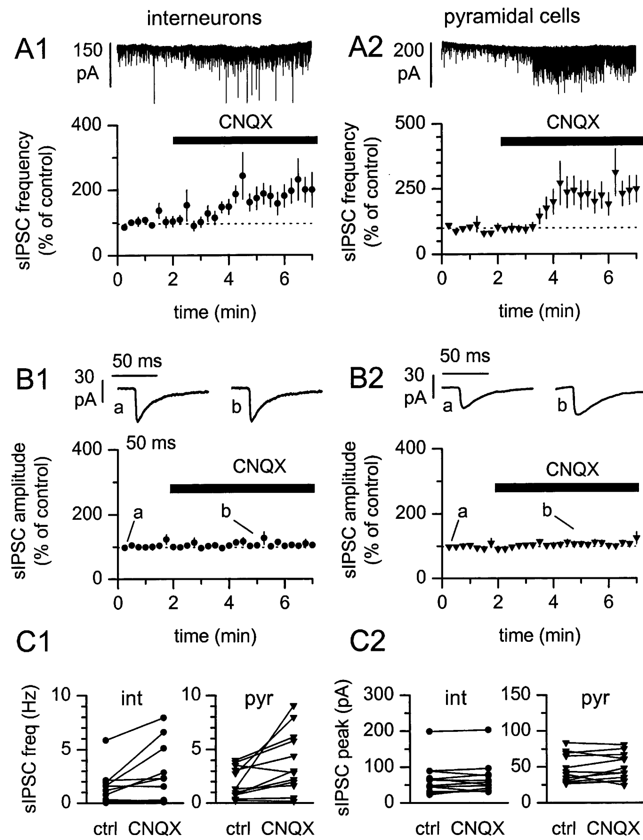

Fig. 1. CNQX enhances $GABA_A$-mediated synaptic transmission in interneurons and pyramidal cells by increasing spontaneous event frequency, but without affecting their amplitude. (A1) Time course of spontaneous IPSC frequency in interneurons (filled circle) during CNQX (10 μM, black bar) application: notice the clear increase in the event frequency. The upper inset shows a continuous current record at the same time scale. (A2) Identical type of analysis for pyramidal neurons (filled triangles) showed similar results. Analysis of the time course of sIPSCs amplitude during CNQX perfusion did not show significant changes either in interneurons (B1) or pyramidal cells (B2) when examined on the averaged dataset. Variability was present for individual results: see for example the increased amplitude of sIPSCs during CNQX application in trace 1A2. Insets show averaged traces of spontaneous events in control conditions (a) or in the presence of CNQX (b). Notice also the similarity in the kinetics of the sIPSCs in the two conditions. Absolute values of the results of frequency and amplitude analysis in interneurons (C1 and C2, left panel) and pyramidal cells (C1 and C2, right panel).

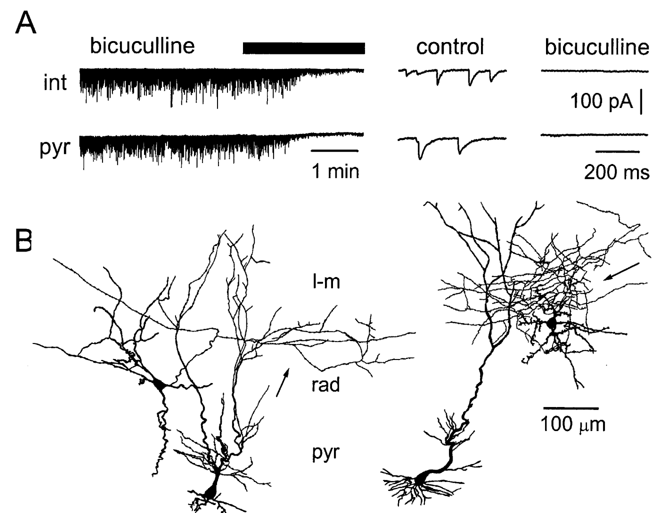

Fig. 2. After application of CNQX (10 μM), and in the presence of kynurenic acid (1 mM), spontaneous inward currents are mediated by $GABA_A$-type receptors. (A) Bicuculline (20 μM) completely blocks spontaneous events in interneurons and pyramidal cells recorded simultaneously. Extracts from the continuous record on the left are shown in the middle and right panels at two different time scales in control solution and in the presence of bicuculline. (B) Anatomical reconstructions of simultaneously recorded interneurons and pyramidal cells. The arrow points to the axon of the interneurons. Notice that in both cases the axon of the interneuron is mostly targeting layers associated with pyramidal cell dendrites. L-m=stratum lacunosum–moleculare, rad=-stratum radiatum, pyr=stratum pyramidale.

Fig. 1C1, right), wheras sIPSC amplitudes were 47.4 ± 5.8 pA in control and 49.6 ± 5.5 pA in the presence of CNQX (Fig. 1C2, right).

Besides GABA and glutamate, other neurotransmitters are known to affect cellular excitability in the hippocampus. For example, pyramidal cells and interneurons are known to express serotonin and acetylcholine receptors that could generate inward currents if synaptically activated (Freund and Buzsaki, 1996). Therefore, in order to exclude the possibility that CNQX triggered the appearance of other inward synaptic currents not mediated by $GABA_A$ receptors, we applied bicuculline at the end of six double recordings. As shown in Fig. 2A, the addition of bicuculline could virtually abolish spontaneous events in both interneurons and pyramidal cells. Interneurons selected for recordings had soma in str. radiatum and usually possessed multipolar dendritic extensions that could span different layers of the CA1 region, as shown by the reconstructions of two neuron pairs shown in Fig. 2B. Taken together, these results indicate that CNQX-induced modulation of $GABA_A$-mediated sIPSC in CA1 pyramidal cells and interneurons is similar to that previously described for CA3 pyramidal neurons and cerebellar granule cells (McBain et al., 1992; Brickley et al., 2001).

3.2. CNQX triggers firing in interneurons, but not in pyramidal cells

McBain et al. (1992) showed that CNQX application generates an inward current in interneurons, which could generate bursts of action currents and explain CNQX-induced increase of sIPSCs. We studied CNQX-induced firing activity in interneurons and pyramidal cells by recording from both cell types under current-clamp configuration. Cell membrane input resistance was monitored by the regular injection of negative current pulses. As shown in Fig. 3A, CNQX depolarized interneurons beyond firing threshold, whereas pyramidal cells remained silent (Fig. 3B). Similar results were obtained in 13 out of 19 interneurons and in all 16 pyramidal cells tested (Fig. 3C1). Injection of constant current to restore the original membrane potential allowed the measurement of membrane input resistance in both conditions. As shown in Fig. 3C2, from a control value of 866 ± 98.7 MΩ in interneurons and 143 ± 9.4 MΩ in pyramidal cells, input resistance decreased to 596 ± 74.1 MΩ and 129 ± 10.3 MΩ respectively in the presence of CNQX (n = 19 interneurons and n = 16 pyramidal cells). Thus, it appears that, despite CNQX-induced increase in the frequency of inhibitory events in interneurons (Fig. 1A1), CNQX can trigger firing in a vast proportion of interneurons and decrease their membrane input resistance.

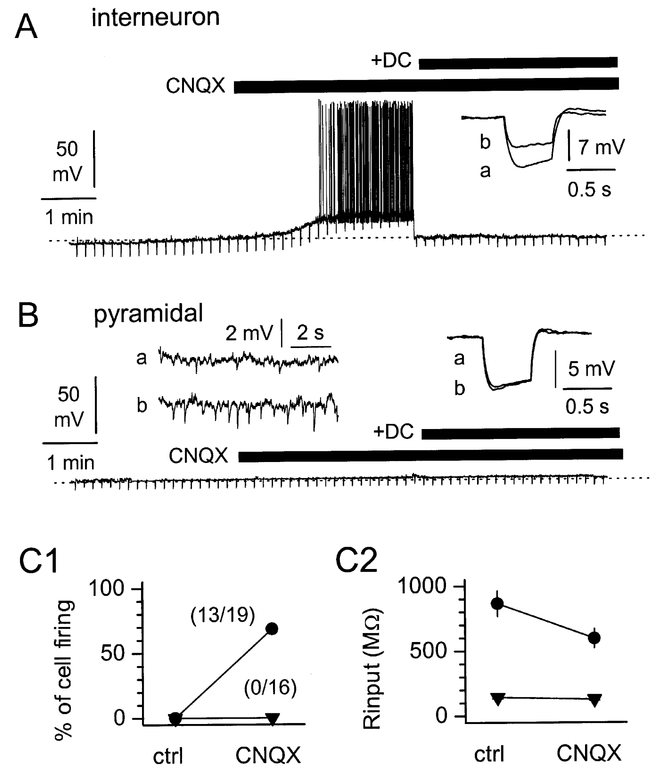

Fig. 3. CNQX can trigger firing in interneurons by opening a membrane conductance. (A) CNQX application (10 μM) on a resting interneuron causes suprathreshold depolarization and firing. DC current applied to restore the original membrane potential allows the comparison of membrane input resistance evaluated by a 10 pA, 500 ms current pulse. Average of 10 voltage deflection are shown superimposed in control condition (a) and in the presence of CNQX and DC (b). (B) Similar experiment performed on a pyramidal cell. Notice in the left inset, the increased frequency of spontaneous inhibitory postsynaptic potentials in CNQX (b), when compared to original conditions (a). The right inset is arranged as in (A): the current pulse injected was 30 pA amplitude and 500 ms duration. (C1) summary plot comparing CNQX-induced firing in interneurons (filled circles) and pyramidal cells (filled triangles). (C2) Average graph indicating the changes in membrane input resistance triggered by CNQX in pyramidal cells (filled triangles) and (interneurons filled circles).

3.3. CNQX-induced depolarization does not depend on network activity

We next examined CNQX-induced depolarization in interneurons in the presence of TTX to eliminate effects that are dependent on action potential generation and circuitry activation. At the beginning of the experiment, the firing pattern of the recorded interneuron was observed by injecting a depolarizing current step of 50 pA amplitude and 500 ms duration, and only then was TTX (1 μM) applied (Fig. 4A). The reason for the acquisition of the firing pattern at the start of the experiment was to obtain a positive control of TTX effect. In the presence of TTX, from a membrane potential of –60 mV, CNQX depolarized 9 out of 11 interneurons. The average measured depolarization was 7.8 ± 1.7 mV (n = 11) and was associated with a decrease in input resistance from

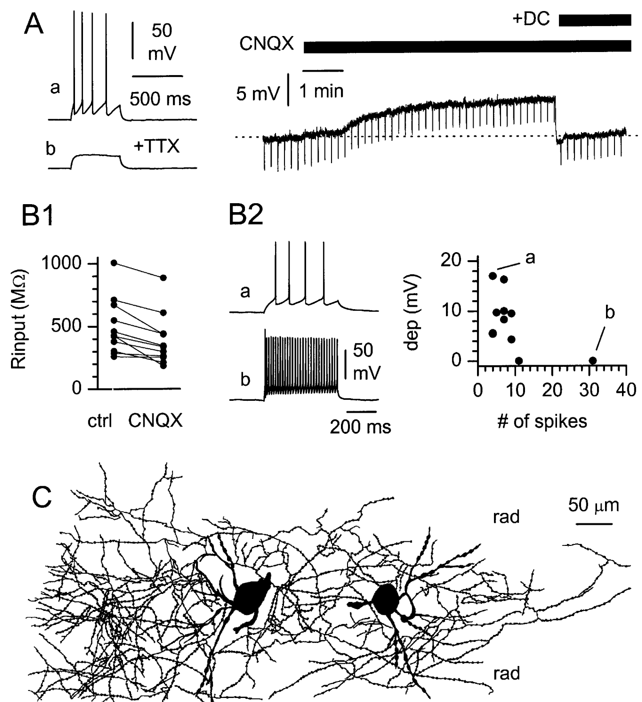

Fig. 4. CNQX-induced depolarization of Schaffer-associated interneurons does not depend on network communication. (A) Left: firing pattern of an interneuron and blockade of action potentials by TTX. Right: In the presence of TTX, CNQX depolarizes a resting interneuron and decrease its membrane input resistance. Black bars indicate the period of CNQX and DC application to restore the original membrane voltage. (B1) Summary plot of input resistance changes induced by CNQX in interneurons. (B2) CNQX-induced depolarization does not correlate with the excitability of the affected interneuron. Left panel: two traces showing different firing patterns in two interneurons producing few (a) and many (b) action potentials in response to the same 50 pA, 500 ms current pulse. Right: summary graph including all the data points. (C) Anatomical reconstruction of two Schaffer-associated interneurons recorded in the same slice showing a depolarizing response to CNQX application. Notice the dense, local arborization of the axon in stratum radiatum (rad).

497 ± 67.6 MΩ in control to 388 ± 61.7 MΩ in CNQX (n = 11, $p < 0.05$, Fig. 4B1). When CNQX-induced depolarization was plotted vs the number of spikes generated by the current step injected at the beginning of the experiment, no significant correlation was apparent (R = –0.55, $p > 0.05$), even though the two unresponsive interneurons appeared to be the ones with the higher number of spikes (Fig. 4B2). As shown in Fig. 4D from the reconstruction of two responsive str. radiatum interneurons recorded from the same slice, the axonal arborization was very dense and mainly restricted to str. radiatum.

4. Discussion

Since the initial description of CNQX-induced potentiation of $GABA_A$ mediated synaptic transmission (McBain et al., 1992), a few groups have reported an increased frequency of sIPSC in hippocampal pyramidal neurons (Martin et al., 2001), and other non-hippocampal cell types (Brickley et al., 2001; Stephens et al., 2001). Our work expands these observations by showing that CNQX enhancement of spontaneous $GABA_A$-mediated transmission is also present in hippocampal interneurons and that CNQX-induced potentiation occurs, on the average, to a roughly similar degree in interneurons and pyramidal neurons. This finding suggests a complex scenario where CNQX would have two opposing effects on GABAergic cells. First, as a consequence of CNQX blockade of synaptic AMPA receptors and potentiation of $GABA_A$-mediated transmission, cellular excitability would be strongly suppressed in interneurons. However, CNQX-dependent activation of a membrane conductance with a ~0 mV reversal potential (McBain et al., 1992) would generate direct membrane depolarization associated with increased cellular excitability. Our current clamp experiments show that CNQX-dependent depolarization predominates so that, overall, CNQX induces firing in interneurons and causes the increase of $GABA_A$-mediated transmission. We also show that CNQX-induced potentiation of $GABA_A$-mediated synaptic transmission in pyramidal cells and interneurons is mainly dependent on an increased frequency of spontaneous events, rather than a modulation of their amplitude. This point, however, should be addressed very carefully. Changes in amplitude of spontaneous events can be associated with bias of the detection threshold. An alternative analysis, based on measurements of the amplitude of the largest events, has been shown to produce more accurate results under some conditions (Stell and Mody, 2002). However, this type of analysis would be difficult to apply under our experimental conditions because CNQX-induced depolarization of interneurons is very likely to change firing rates, and trigger changes in probability of GABA release, due to short-term plasticity of GABAergic synapses (Pearce et al., 1995; Maccaferri et al., 2000; Gupta et al., 2000). The most parsimonious explanation for this result is that synaptic GABA release is uniformly augmented along the electrotonic length of the target neurons. Indeed, selective increase at specific postsynaptic domains would be likely to consistently alter the size and the kinetics of the averaged sIPSC (Maccaferri et al., 2000), which did not appear to be the case under our experimental conditions. Alternatively, selective recruitment of domain specific interneurons could be compensated by domain-specific differences in short-term plasticity. For example, perisomatic targeting neurons such as basket and axo-axonic cells have been shown to generate the largest amplitude unitary IPSCs (uIPSCs), when compared to dendritic targeting interneurons (Maccaferri et al., 2000). However, at the same time, short-term depression of uIPSCs during repetitive activation (Maccaferri et al., 2000) could reduce their proportional

contribution to the overall sIPSC population. It is important to keep in mind that our results are based on the averaged analysis of all the cells of our sample. Similar to the report by Brickley et al. (2001) a large level of heterogeneity was present in individual experiments, ranging from cells showing strong modulation of sIPSC frequency (and amplitude in our case: for example, the trace of Figs. 1 and 2 shows a clear increase of both frequency and amplitude of sIPSCs) during CNQX application to cells where these effects were minimal if not even opposite. The apparent lack of increase of sIPSC could also be the artefactual result of a decreased efficacy of voltage clamp conditions due to CNQX-induced increase in membrane conductance, especially at dendritic sites (Frerking et al., 1999). Under this scenario an actual increase of sIPSP size could be 'compensated' and masked by the changes in voltage-clamp conditions due to increased membrane conductance due to CNQX itself or to the increased sIPSC frequency (Frerking et al., 1999). In conclusion, increase in sIPSCs with no apparent change in amplitude is consistent with a presynaptic phenomenon, such as, for example, increased firing activity of presynaptic GABAergic interneurons.

At the concentration used in this study, CNQX would be most commonly expected to dramatically block AMPA and strongly reduce kainate receptor activity (Paternain et al., 1995, 1996; Bleakman and Lodge, 1998), whereas its action on NMDAR-mediated currents would be expected to be much weaker (Lester et al., 1989; Verdoorn et al., 1989). Nevertheless, it is unlikely that blockade of AMPA, kainate or NMDA receptors underlies the results observed in our work. Indeed, CNQX-induced potentiation of $GABA_A$-mediated transmission could be observed in experiments performed in the presence of the broad ionotropic glutamate receptor kynurenic acid, similarly to a report by Brickley et al. (2001) in cerebeller granule cells. Furthermore, other antagonists at NMDA or non-NMDA receptors have been reported to have no effect on sIPSCs (McBain et al., 1992; Stephens et al., 2001, but see also Brickley et al., 2001) and direct blockade of postsynaptic ionotropic receptors would decrease the value of membrane conductance, which is opposite to our experimental result.

A recent observation by Swanson et al. (2002), however, suggests that priming of heteromeric GluR5/KA-2 kainate receptors by dysiherbaine, a potent epileptogenic amino acid (Sakai et al., 2001), allows CNQX to gate the KA-2 subunit of the receptors, and therefore act as an agonist. If a similar set of conditions was to occur in native systems, then application of CNQX would be expected to produce effects similar to low doses of kainate on interneurons (Cossart et al., 1998; Frerking et al., 1998). Under this hypothesis, CNQX could isolate neurons expressing specific heteromeric kainate receptors and would be responsible for the increase of a kainate receptor-mediated conductance, which would generate firing in interneurons. In addition, it is important to mention that CNQX has been shown to act as an agonist also at GluR1 recombinant receptors containing the Lurcher mutation (Taverna et al., 2000). This finding suggests that, under specific circumstances, activation may be the main mode of action of CNQX. Glutamate receptors are known to interact with many accessory proteins (Garcia et al., 1998; Garner et al., 2000; Sheng, 2001): therefore it cannot be excluded that allosteric modulation of specific subunits can prime native receptors towards a state that is sensitive to CNQX activation. However, additional work is needed to confirm or to reject this hypothesis.

Although individual results showed a high degree of variability, CNQX-induced potentiation of $GABA_A$-mediated transmission was roughly similar in interneurons and pyramidal cells. This observation suggests that CNQX-sensitive interneurons target pyramidal cells and interneurons with a similar degree of connectivity, thus excluding a major involvement of selective types of interneurons which have been shown to innervate selectively only other GABAergic cells (Freund and Buzsaki, 1996). Indeed, our data indicate that CNQX depolarizes regular spiking interneurons with axonal arborization mainly restricted to str. rdiatum. These cells are likely to belong to the group that is referred to as Schaffer-associated interneurons (Vida et al., 1998; Cope et al., 2002). However, the possibility that within this anatomical group functional heterogeneity might be present cannot be discarded (Maccaferri and Dingledine, 2002). Increased firing in Schaffer-associated interneurons would explain the increase of sIPSC as the result of action potential-dependent GABA release onto the postsynaptic membrane domains of pyramidal cells and interneurons, which are located in str. radiatum. This is consistent with the recovered morphology of interneurons showing an increase in sIPSC (Fig. 2), which have a major dendritic localization in str. radiatum, but does not exclude that other specific hippocampal GABAergic cell types may be subjected to the same type of pharmacological modulation. If CNQX-induced potentiation of $GABA_A$-mediated transmission involved activation of kainate receptors, as previously discussed, then it could potentially involve a large variety of interneuronal types (Cossart et al., 1998).

In conclusion, our data clearly indicate that CNQX application on an intact network can modulate the activation of $GABA_A$ receptors, by a variety of direct and indirect effects impacting distinct cell types, such as pyramidal cells and interneurons. Although the detailed molecular mechanisms of CNQX-induced potentiation of $GABA_A$-mediated synaptic transmission remain unclear, an attractive speculation is that it depends on the activation of interneuronal kainate receptors in a specific functional state. The development of subunit-specific

drugs may be able to allow a direct test of this hypothesis in the future.

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