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Antagonism of spontaneous and evoked bursts by 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX) in the CA3 region of the in vitro hippocampus

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Superfusion of hippocampal slices with 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX) antagonized kainate-induced bursts and bursts of unknown origin in the CA3 region. CNQX also increased the latency of and eventually blocked evoked bursts which persist following kainate washout. In contrast, D-(–)-2-amino-7-phosphonoheptanoic acid did not alter burst latency or block bursts unless applied subsequent to CNQX. We conclude that the quisqualate type receptor has a prominent role in burst generation with a smaller contribution from *N*-methyl-D-aspartate receptors.

In the CA3 region of the in vitro hippocampal slice preparation a wide variety of convulsant agents and procedures (see ref. 10 for references) induce bursts consisting of a high frequency train of action potentials riding on a paroxysmal depolarizing shift. Glutamate and/or aspartate likely mediate a substantial portion of neurotransmission in this region[5] and as such it is not unreasonable to conclude that the excitatory amino acid receptors are important for burst generation. The *N*-methyl-D-aspartate (NMDA) receptor, one of the glutamate receptor subtypes[7], may participate in burst generation in the CA3 region but this participation is not essential for burst production[1,10].

Investigating the role of the other excitatory amino acid receptors in burst generation has been so far hampered by lack of suitable antagonists[5]. The availability of CNQX, with selectivity for the quisqualate type receptor[4,6,9] has partially rectified this situation. In the present report we have examined the effects of CNQX on burst generation in the CA3 region. A brief report on this material has been presented[8].

Male Wistar rats (80–125 g) were used. Transverse slices of hippocampus (500 μm) were cut and transferred to a submerged type recording chamber as described elsewhere[10]. The slices were superfused (2 ml/min) at a temperature of 33–34 °C. The artificial cerebrospinal fluid (ACSF) consisted of (mM): NaCl 126, KCl 3.5, $MgCl_2$ 1.3, NaH_2PO_4 1.2, $CaCl_2$ 2, $NaHCO_3$ 25 and glucose 11. The ACSF was gassed with 95% O_2/5% CO_2 and gave a pH of 7.3. The methods for stimulation, intracellular and extracellular recording have been presented elsewhere[10]. A 3-way tap system was used for drug application. Drugs used were: D-(–)-2-amino-7-phosphonoheptanoic acid (AP-7; CRB); 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX, Ferrosan); kainate (Sigma). Results are based on data from 16 slices.

Bath application of 200 nM kainate induced spontaneous and evoked bursts in the CA3 region[3,10] which were antagonized by CNQX (Fig. 1). At 2 μM CNQX the frequency of kainate-induced bursts was reduced to 6.5 ± 9% ($\bar{X}$ ± S.D.; n = 4) of control. Associated with the decline in burst frequency was a reduction in dV/dt of the burst rising phase (Fig. 1) to 27 ± 17% (n = 4) of control. At 3 μM CNQX, kai-

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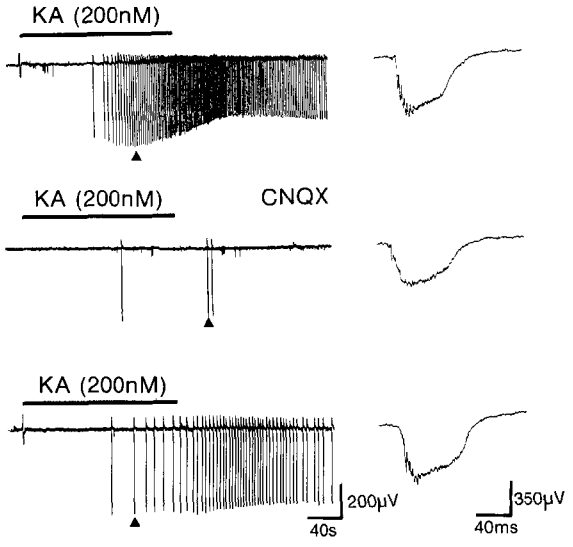

Fig. 1. CNQX antagonizes spontaneous bursts induced by kainate. Field potential recording in the apical dendrites of bursts induced by 200 nM kainate. Superfusion of 2 μM CNQX dramatically reduced the number of bursts. Partial recovery after 20 min wash (lower trace). Bursts recorded at the triangles are shown at a faster sweep on the right.

nate failed to induce bursts in 2 out of 3 slices. Full recovery of burst frequency was slow and was not complete with a 1 h wash.

In addition to inducing spontaneous and evoked bursts during superfusion, kainate alters transmissive processes such that spontaneous bursts persist and stimulation remains effective in evoking bursts long after kainate is washed out[3]. Both types of bursts were blocked by 0.5–1 μM CNQX (n = 5) within 5 min of changing the tap. As shown in Fig. 2B, CNQX increased the latency between stimulation and subsequent burst. The latency increased steadily during superfusion with CNQX until the evoked bursts were blocked. The average increase in latency was 77 ± 63% (n = 5, range 10–148%). The amplitude of the population EPSP declined as the latency to burst increased such that at the time of burst failure the EPSP amplitude was 81 ± 8% (n = 5) of the control.

In contrast to CNQX, application of AP-7 (n = 4) neither increased the latency to burst (Fig. 2A) nor blocked the spontaneous and evoked bursts resulting from prior exposure to kainate. However, when AP-7 was applied subsequent to CNQX exposure (n = 3), latency to burst increased (Fig. 2C) and the bursts eventually were blocked.

In 4 slices, stimulation evoked bursts prior to any drug manipulation. The origin of these bursts is unknown but may result from anoxia[2]. As with the persistent bursts which follow a brief exposure to kainate, CNQX (0.5–2 μM; n = 4) increased the burst latency until the bursts were eventually blocked (Fig. 3).

The effectiveness of CNQX in antagonizing kainate induced bursts and bursts of unknown origin is in sharp contrast to the absence of such antagonism by NMDA receptor antagonists[10] (and present results). The increased latency of evoked bursts, the reduced dV/dt of the burst rising phase and the block of both spontaneous and evoked bursts is consistent with a decline in the transmissive process generating the bursts. Binding and functional studies have shown CNQX to have a preferential action at the quisqualate receptor and no effect on input resistance, membrane potential or spike accommodation[4,6,9]. Taken together, the observations with CNQX suggests that the quisqualate receptor is of prime importance for

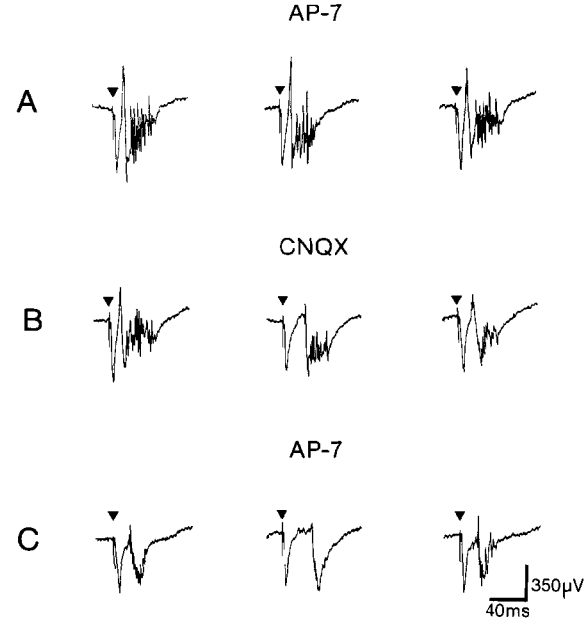

Fig. 2. CNQX, but not AP-7, increases the latency to burst unless AP-7 application follows CNQX. A,B,C: evoked bursts recorded from the same slice subsequent to brief exposure and washout of kainate. Control and wash records shown at left and right respectively. A: 5 min application of 20 μM AP-7 did not alter the latency between stimulation (triangles) and burst. B: 3 min application of 1 μM CNQX increased latency from stimulation to burst by 63%. C: following 1 h of wash, 20 μM AP-7 was reapplied and the latency to burst increased by 70%.

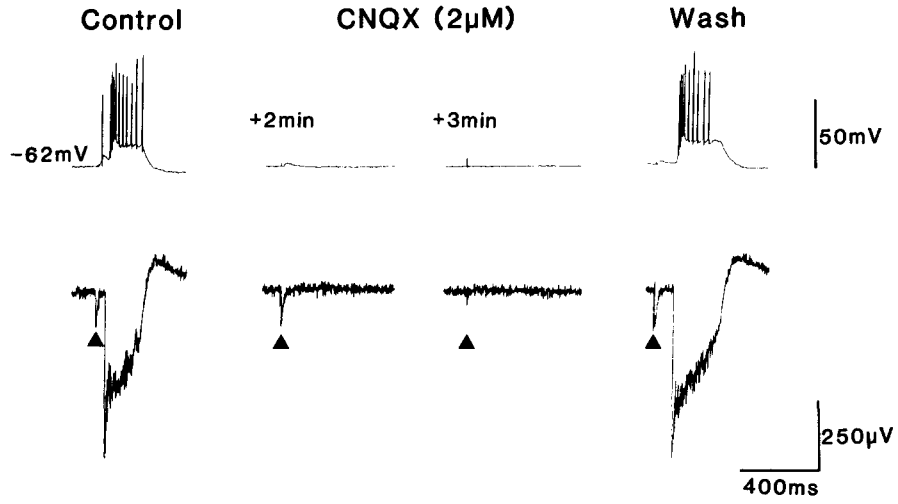

Fig. 3. CNQX blocks evoked bursts of unknown origin. Upper and lower traces are intracellular and extracellular recordings respectively. Stimulation indicated by triangles. Two min exposure to 2 μM CNQX reduced the mossy fibre EPSP and blocked the burst. By 3 min the EPSP was blocked. During wash both the EPSP and the burst recovered. Note the longer latency to burst during the wash.

burst generation in the CA3 region. Although the present data is consistent with other observations implying a postsynaptic site of action for CNQX[6,9], a presynaptic effect cannot at this time be excluded.

The observation that AP-7 blocks bursts suggests that NMDA receptors participate in the polysynaptic circuitry generating bursts in the CA3 region. However, the fact that AP-7 is only effective when the quisqualate receptor system has been compromised by prior exposure to CNQX suggests that NMDA has only a minor role in this process.

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