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Epileptiform bursts elicited in CA_3 hippocampal neurons by a variety of convulsants are not blocked by *N*-methyl-D-aspartate antagonists

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Intracellular and extracellular recordings from CA_3 hippocampal neurons in vitro were used to study the ability of several NMDA (*N*-methyl-D-aspartate) receptor antagonists to suppress epileptiform bursts induced by NMDA and convulsants not thought to act at NMDA receptors. The antagonists, APV (D-2-amino-5-phosphonovalerate), AP-7 (D,L-2-amino-7-phosphonoheptanoate) and CPP (D,L-3-[(±)-2-carboxypiperazin-4-yl-]-propyl-1-phosphonic acid), blocked the spontaneous and evoked bursts induced by NMDA. CPP, but not APV or AP-7, prevented the development of bursts induced by Mg-free medium. The NMDA antagonists failed to block bursting induced by kainate, 7 mM K^+, mast cell degranulating peptide, anoxia or spontaneous bursting. In some cases the NMDA antagonists induced spontaneous bursts or enhanced burst frequency, a proconvulsant effect. It is concluded that activation of NMDA receptors is sufficient but not necessary for burst generation in the CA_3 region.

INTRODUCTION

In both man and experimental animals, the hippocampus is particularly prone to seizure disorders[18]. Within the hippocampus, the CA_3 region is well known for its pacemaker activity[19] and its ability to generate paroxysmal events following application of convulsive agents[37] or electrical stimulation of afferent pathways[33]. Recently it has been suggested that the activation of the *N*-methyl-D-aspartate (NMDA) receptor may contribute to the induction of epileptiform bursting[12]. Furthermore, it has been proposed that NMDA antagonists may be effective anticonvulsant agents. This proposal has been tested in several animal models of epilepsy with conflicting results. Thus, audiogenic[21], photogenic[26], chemical[9,10], kindled seizures[30] and NMDA-induced myoclonic seizures[35] are reduced or blocked by prior application of NMDA receptor antagonists. However, in other seizure models[35] NMDA receptor antagonists are without effect.

In the present paper, using intracellular and extracellular recording techniques, we have compared the effects of NMDA antagonists on epileptiform discharges induced in the CA_3 region by NMDA and other convulsants which are not thought to act by means of NMDA receptor activation. The rationale behind this approach was to test the hypothesis that NMDA antagonists are efficient in blocking epileptiform events induced in the same system by different mechanisms.

MATERIALS AND METHODS

Hippocampal slices were prepared from Wistar rats (90–200 g) as described in detail elsewhere[17]. Briefly, the animals were killed by a heavy blow, the brain rapidly removed, the hippocampus dissected

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free and sliced transversely on a McIllwain tissue chopper at a nominal thickness of 500 μm. Slices were maintained at room temperature in a holding chamber until required. Recordings were made from completely submerged slices at a bath temperature at 33–34 °C. The artificial cerebrospinal fluid (ACSF) had the following composition (mM): NaCl 126; KCl 3.5; $CaCl_2$ 2; $NaHCO_3$ 25; $NaHPO_4$ 1.2; $MgCl_2$ 1.3; glucose 11. Equilibrating the ACSF with 95% O_2/5% CO_2 gave a pH of 7.3–7.4. Drugs were dissolved in the ACSF and were applied via a 3-way tap system. Unless otherwise indicated in the text, the NMDA antagonist to be tested was superfused 5–10 min before addition of the convulsant drug. The flow (2.75 ml/min) and dead volume were such that the dead time before a drug reached the bath was 20–30 s. Equilibrium in the chamber was apparently reached within 2–3 min.

Intracellular recordings were made with microelectrodes filled with 4 M potassium acetate or 2 M potassium methylsulfate. Extracellular recordings from the pyramidal cell layer or dendritic region were made with glass micropipettes filled with 4 M NaCl. Slices were stimulated at the outer edge and just below the upper blade of the granule cell layer. As both the mossy fiber pathway and the temporoammoniac pathways were stimulated, the stimulation is referred to simply as 'stimulation'. Bipolar etched tungsten electrodes or twisted nichrome wires were used for stimulation (10–100 μs and 5–130 V). A standard preamplifier with an active bridge (Axoclamp II) was used for intracellular recording and current injection. Intracellular and extracellular recordings were displayed on a Nicolet 3091 digital oscilloscope. Oscilloscope traces were sent to a computer for storage and from the computer to a Brush recorder.

The drugs used were: D,L-2-amino-7-phosphono-

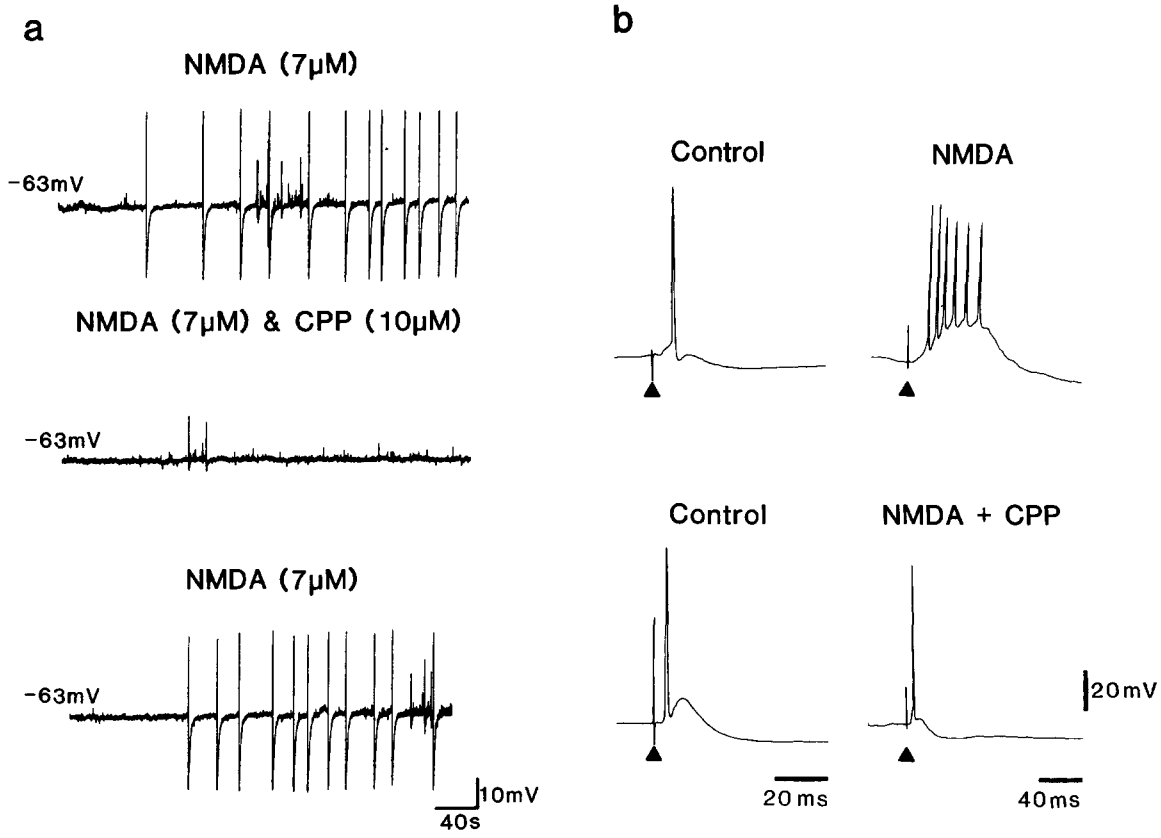

Fig. 1. CPP blocks spontaneous and evoked bursts induced by NMDA. The traces in a and b are from different neurons. a: bursts induced by NMDA (upper trace). Superfusion with CPP prevented the occurrence of NMDA-induced bursts (middle trace). Fifteen min after wash of CPP, NMDA again induced spontaneous bursts (lower trace). b: upper panel, stimulation (solid triangle) evoked an action potential in control medium (left) and burst in 10 μM NMDA (right); lower panel, stimulation in the presence of 10 μM CPP + 10 μM NMDA (right) evoked only an action potential as in control ACSF (left).

heptanoate (AP-7), Sandoz); D-2-amino-5-phosphonovalerate (APV; Cambridge Research Biochemicals); D,L-3-[(±)-2-carboxypiperazin-4-yl-]propyl-1-phosphonic acid (CPP; Sandoz); kainic acid (Sigma); mast cell degranulating peptide (MCD, Dr. Lazdunski); NMDA (Cambridge Research Biochemicals). Drugs were dissolved in water and kept frozen until use at which time they were diluted further. The preparation of MCD has been described previously[5]. Mg-free ACSF was made by eliminating $MgCl_2$ without replacement.

RESULTS

Stable intracellular recordings (>2 h) were made from 42 CA_3 pyramidal neurons. Only neurons that had resting membrane potentials greater than −57 mV and action potential amplitudes greater than 70 mV were included in the present study. The apparent input resistance as measured in a subset of these neurons by hyperpolarizing current pulses (500 ms) was 77 ± 26 MΩ ($\bar{X}$ ± S.D.; n = 21). Intracellular and concomitant extracellular recordings were made in 32 neurons. In 35 additional slices only field potentials were recorded.

Effects of NMDA antagonists on spontaneous and evoked bursts induced by NMDA

In agreement with Anderson et al.[2], superfusion of NMDA (5–10 μM; 3–5 min) induced spontaneous

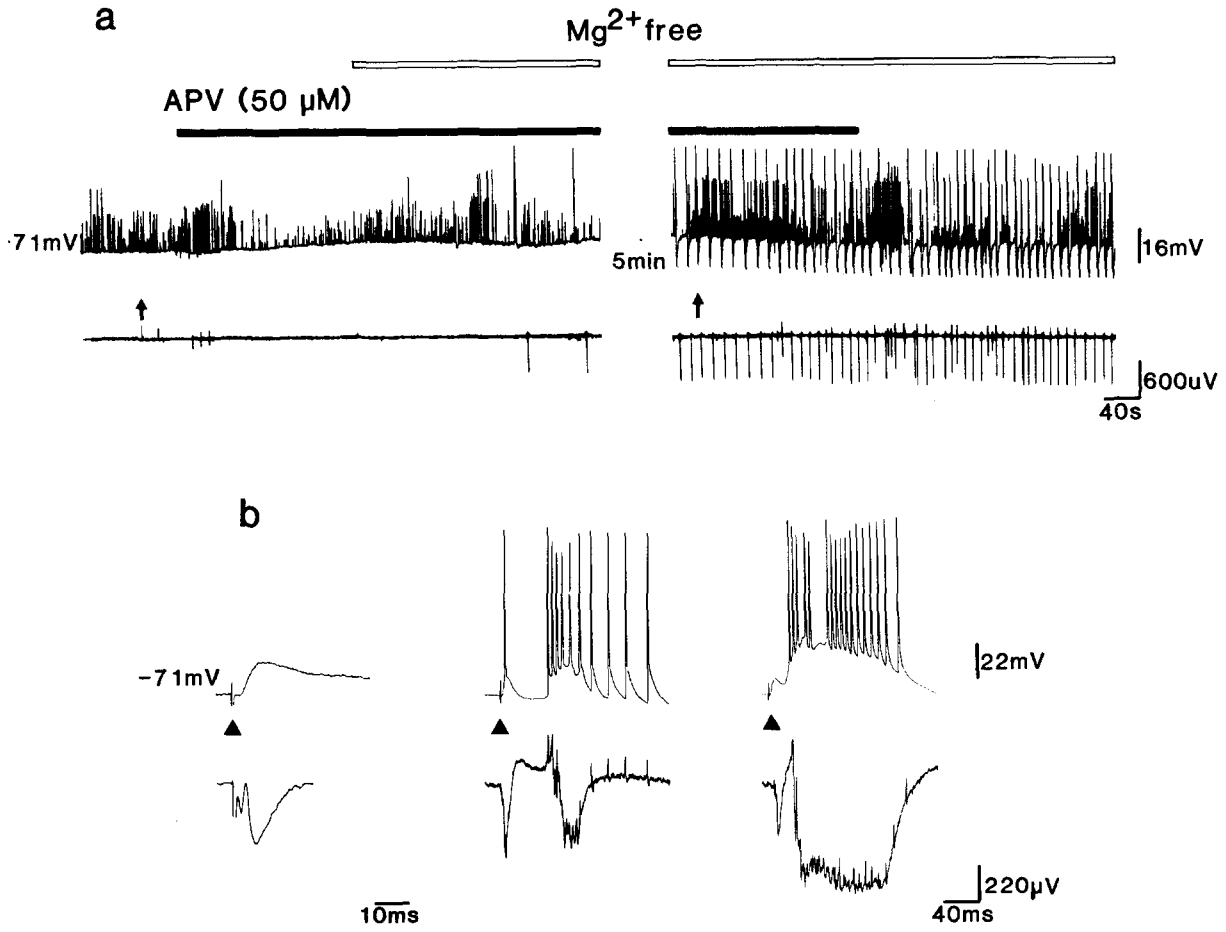

Fig. 2. APV fails to prevent spontaneous bursts induced by Mg-free ACSF. Upper and lower traces in a and b are intracellular and extracellular recordings, respectively. a: superfusion of APV (solid bar) before and during Mg-free ACSF (open bar) failed to prevent the development of bursts. A slight increase in burst frequency was observed following washout of APV. b: activity evoked by stimulation at the time indicated by the arrows in a. The last trace was taken after return to Mg-containing medium. APV did not block the evoked burst (middle trace) although it shortened the duration of the PDS and increased the latency to burst when compared to the burst evoked in ACSF (last trace).

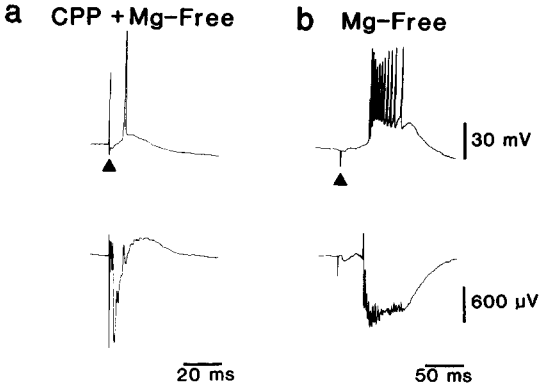

Fig. 3. CPP prevents the induction of evoked bursts by MG-free ACSF. Upper and lower traces in a and b are intracellular and extracellular recordings, respectively, evoked by stimulation (triangles). a: stimulation applied 25 min after superfusing the slice with Mg-free medium containing 20 μM CPP failed to evoke a burst. b: 7 min after wash in Mg-free ACSF stimulation evoked a burst. Note that the stimulus intensity was reduced from 100 V in a to 8 V in b.

bursts (Fig. 1) in 22 out of 24 CA_3 neurons. With intracellular recordings, the bursts consisted of a paroxysmal depolarizing shift (PDS) of 10–20 mV amplitude and 60–150 ms duration at the resting membrane potential. Riding on the PDS were 4–15 fast action potentials. Concomitant with the intracellular burst was an extracellular field potential which was negative in the stratum radiatum and positive in the stratum pyramidale. As described elsewhere, the PDS is generated by a giant EPSP (Neuman et al., in preparation). In addition to the spontaneous bursts, stimulation evoked an all or none PDS during and shortly after NMDA application (Fig. 1). These evoked bursts were similar to the spontaneous bursts but shorter in duration. On washout of NMDA, the spontaneous bursting rapidly subsided in over 80% of the neurons. In the remainder, the bursting persisted

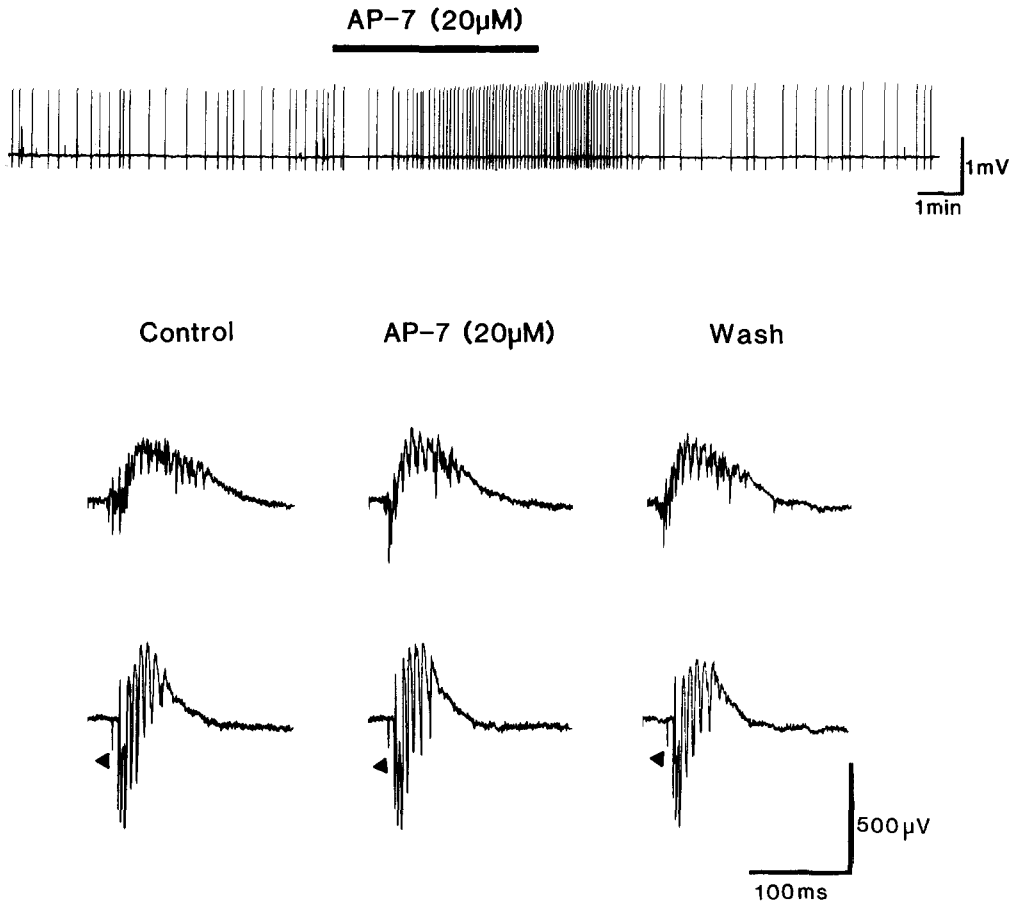

Fig. 4. AP-7 fails to block bursts induced by MCD. Upper trace: persistent spontaneous bursting induced by exposure to MCD (3 μM) for 5 min. Application of AP-7 (solid bar) increased the frequency of bursting. Lower traces from different slice. AP-7 reduced both the duration of spontaneous bursts and the period of spiking (middle trace). AP-7 did not block the evoked bursts (bottom trace) although it did reduce the number of spikes in the burst.

but at a lower frequency than during exposure to NMDA. The ability to evoke bursts ceased in 8 out of 11 neurons on NMDA washout.

In 6 out of 8 slices, superfusion of the NMDA antagonists, APV (10–20 μM; $n = 2$), AP-7 (10–30 μM, $n = 2$) or CPP (10–30 μM; $n = 4$), prevented the development of spontaneous and evoked bursts induced by NMDA (Fig. 1). In the remaining two cases, APV and AP-7 dramatically reduced the burst frequency (0.57 to 0.02 and 0.4 to 0.02 Hz, respectively).

The addition of NMDA antagonists to slices bursting in the presence of 10 μM NMDA rapidly abolished these bursts. Thus, AP-7 (20 μM; $n = 4$) and CPP (20 μM; $n = 4$) stopped the bursting in 107 ± 24 and 59 ± 14 s, respectively. It should be noted that these values include the dead time of the superfusion system (20–30 s).

Effects of NMDA antagonists on the spontaneous and evoked bursts induced by Mg-free ACSF

Reducing the concentration of Mg^{2+} in the bathing medium leads to the development of spontaneous and evoked bursts in CA_3 neurons[1,28,29,32]. Thus, spontaneous bursts appeared 10.5 ± 5 min after switching to Mg-free ACSF ($n = 14$). Both the intracellular and extracellular recordings of these bursts were similar in time course, amplitude and number of action potentials to those induced by NMDA. However, the frequency of bursting in Mg-free ACSF was slower than in 10 μM NMDA (0.11 ± 0.09 and 0.23 ± 0.12, respectively).

Superfusing slices with APV or AP-7 (30–50 μM, $n = 8$) 5–30 min before and continuous with Mg-free ACSF did not prevent the development of either the spontaneous or evoked bursts (Fig. 2). Indeed, the latency to the start of bursting after the addition of

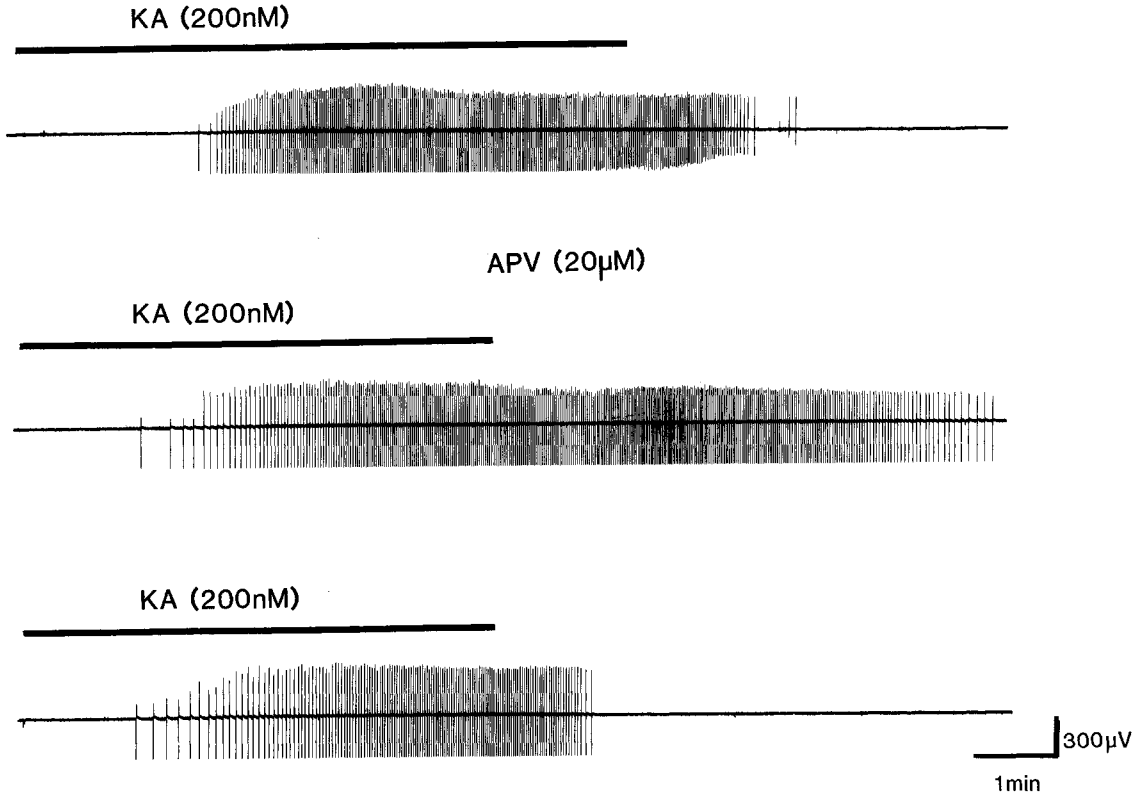

Fig. 5. APV prolongs the time to washout of bursts induced by kainate. Field recordings from one slice. Kainate-induced spontaneous bursting which ceased within 2 min of kainate wash (upper panel). In the presence of APV, bursts induced by kainate continued over 6 min after switching to drug-free ACSF (middle panel). After wash out of APV, the bursting induced by kainate (bottom trace) again subsided shortly after starting the wash.

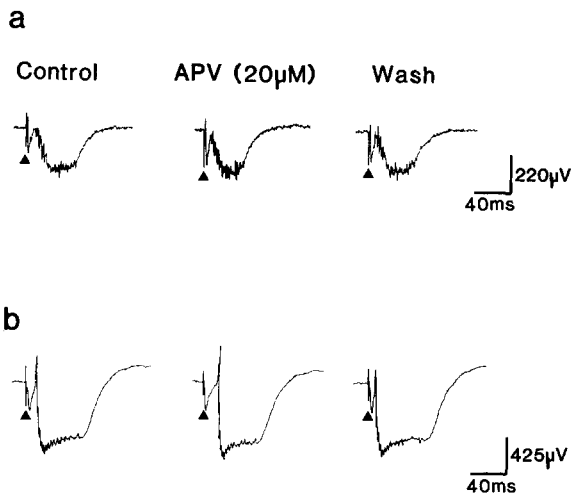

Fig. 6. APV fails to block the evoked bursts induced by prior exposure to kainate or 7 mM K^+. a: 5-min exposure to kainate led to a long-term change in excitability such that stimulation continued to evoke bursts (left trace) after prolonged washing in drug-free ACSF. APV failed to block evoked bursts although the duration was reduced (middle trace). After wash, the burst duration returned to control values (right). b: 10 min exposure to 7 mM K^+, like kainate, led to a long-term change in excitability apparent on washout (left). APV increased the latency from stimulus to burst and shortened the burst duration but did not block the evoked burst (middle). During wash the burst duration returned toward the control value (right).

Mg-free ACSF was actually reduced by APV and AP-7 to 6.1 ± 2 min. During wash it became apparent that the antagonists had reduced the amplitude and duration of the PDS along with the number of spikes in the superimposed burst (Fig. 2).

In contrast to APV and AP-7, CPP (20–30 μM) prevented the development of both spontaneous and evoked bursts in 6 out of 8 slices exposed to Mg-free ACSF (Fig. 3). During the washout of CPP to Mg-free ACSF, spontaneous bursting began within 4–6 min of switching the tap (not shown).

Effects of NMDA antagonists on spontaneous and evoked bursts induced by kainate, MCD and 7 mM K^+

Spontaneous and evoked bursts are readily induced in the CA_3 region of the hippocampus by kainate[4], MCD[6] (a toxin isolated from bee venom), or by raising the extracellular K^+ concentration[4,24,31]. Brief exposure to MCD results in spontaneous bursting which persists, despite prolonged washing of the slices in drug-free ACSF[6].

The NMDA antagonists neither prevented the development of spontaneous bursts or evoked bursts induced by kainate, MCD or by 7 mM K^+ nor reduced/blocked the persistent spontaneous bursts induced by MCD (Figs. 4–6). This data is summarized in Table I. The individual antagonists did not differ with respect to altering the bursting induced by these agents, so the observations have been grouped. In the case of MCD and kainate, the NMDA antagonists increased the frequency of bursting in 4 out of 10 and 2 out of 6 slices, respectively (Figs. 4 and 5).

TABLE I

Frequency of spontaneous bursts (Hz)

The number of slices is given in parentheses.

	Control[1]	*NMDA antagonists**
Kainate (200 nM)	0.47 ± 0.21 (7)	0.57 ± 0.29 (8)
MCD** (2–3 μM)	0.18 ± 0.07 (9)	0.16 ± 0.34 (13)
K^+ (7 mM)	0.28 ± 0.34 (5)	0.13 ± 0.046 (5)

* APV (10–50 μM), AP-7 (10–30 μM) or CPP (10–30 μM); note some slices were used to test more than one antagonist.

** These values include bursts present during MCD and persistent bursting which follows wash, see text.

Fig. 5 shows that the application of kainate-induced burst activity which rapidly (<2 min) subsided after return to control ACSF. However, during the superfusion of APV the cessation of spontaneous bursting during the wash of kainate increased to 6 min. In the same slice, AP-7 (20 μM) and CPP (10 μM) prolonged the bursting during kainate wash to 17 and 18 min, respectively (not shown). This potentiating effect was not due to the consecutive application of kainate, since in the absence of NMDA antagonists the wash-out of kainate-induced bursting was not altered by consecutive applications to the toxin. Despite the failure of NMDA antagonists to prevent or block the spontaneous bursts induced by the above agents, they typically reduced the duration of spontaneous bursts and shortened the period of spiking (Fig. 4).

Effects of NMDA antagonists on the evoked bursts produced by brief anoxic episodes

Repeated, brief (3–5 min) exposure of slices to ACSF saturated with 95% N_2 and 5% CO_2 resulted, after 2–5 trials, in the persistent (>4 h) ability to evoke bursts by afferent stimulation for the duration of the recording following the last anoxic episode[4a]. In slices demonstrating such long-term burst behavior, the application of APV (30 μM, n = 3), AP-7 (20

μM, $n = 3$) and CPP (10 μM, $n = 2$), only slightly reduced the amplitude and duration of the PDS and the number of spikes; in no case did the antagonists block the evoked bursts (Fig. 7) and as shown in Fig. 7, AP-7 induced bursts (reversibly).

In 4 slices, spontaneous and/or evoked bursts were present before any drug manipulation. These bursts may have resulted from anoxic damage during slice preparation[11]. APV (30 μM) and AP-7 (20 μM) were ineffective in blocking the evoked bursts or reducing the frequency of spontaneous bursts. However, they typically reduced the amplitude and duration of the PDS. The effect of APV on such evoked bursts is shown in Fig. 8.

DISCUSSION

From these experiments it is clear that NMDA receptor antagonists are only entirely effective in blocking spontaneous bursts induced by NMDA but have little effect on bursts induced by other agents or procedures. Although in keeping with several recent studies[2,3,20,28,32] the antagonists reduced the duration of the bursts induced by convulsant agents or procedures, they clearly neither prevent the development nor block the bursts once they have been induced.

Effects of NMDA and Mg-free medium

Prior exposure to APV, AP-7 and CPP prevented or dramatically reduced the development of spontaneous paroxysmal activity induced by NMDA. Furthermore, once bursting had developed in NMDA, the addition of CPP or AP-7 rapidly abolished the bursts. Similar results were obtained by Anderson et al.[1], who found that the specific NMDA antagonist APV prevented the development of spontaneous bursts induced by NMDA or by trains of electrical stimulation.

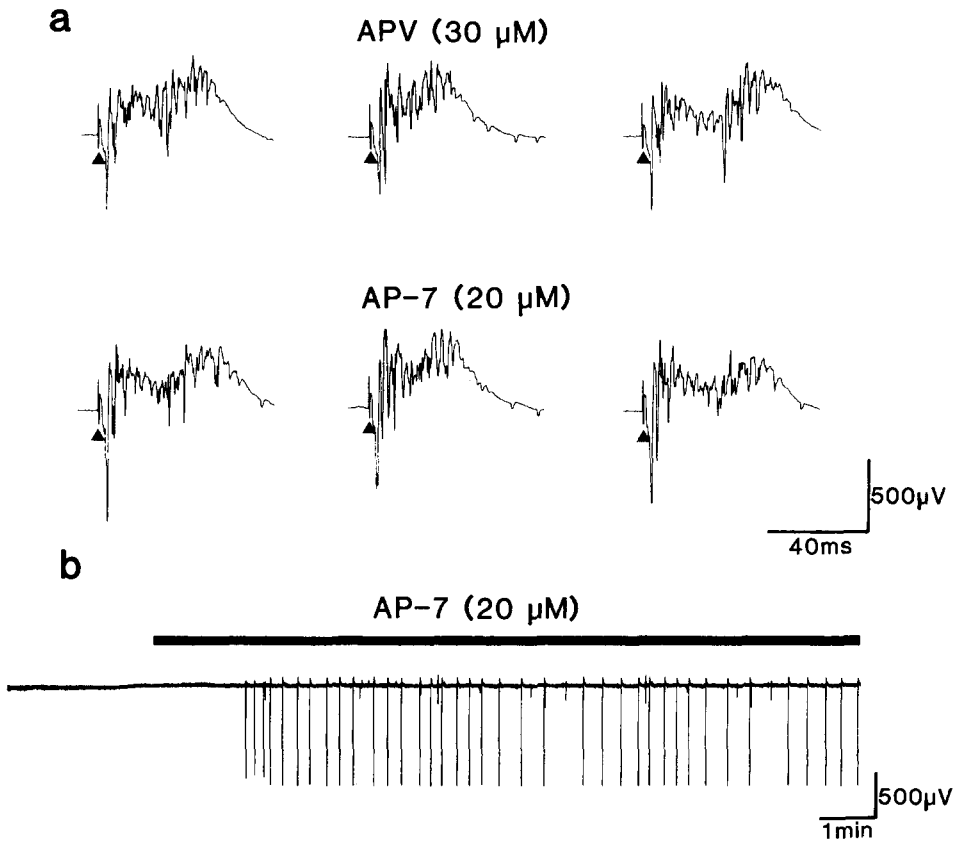

Fig. 7. APV and AP-7 do not block evoked bursts induced by anoxia. a: after repeated anoxic episodes (5 min perfusion of the slice with ACSF saturated with 95% N_2 5% CO_2) stimulation evoked bursts (left). Neither APV nor AP-7 (middle traces) blocked these bursts although in both cases the duration of the bursts were reduced. Full recovery was obtained after wash (right). b: following the long lasting change in excitability induced by anoxia in another slice, AP-7 reversibly induced spontaneous bursting.

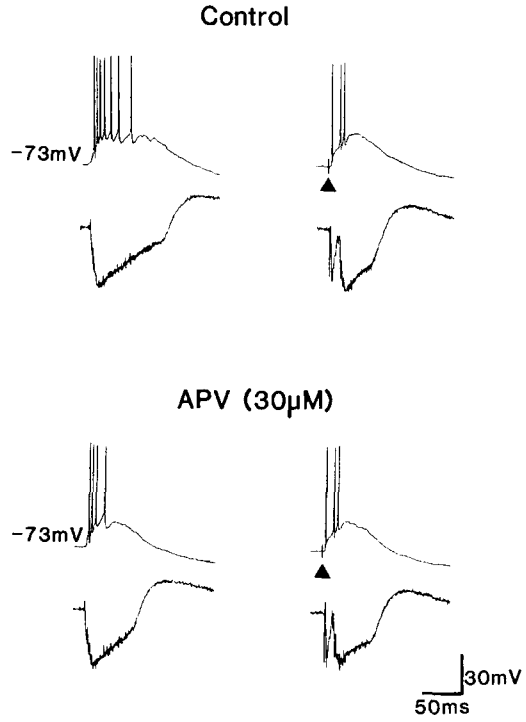

Fig. 8. APV fails to block spontaneous and evoked bursts of unknown origin. Upper and lower traces are intracellular and extracellular recordings, respectively. Spontaneous (left) and evoked bursts (triangles, right) were only slightly reduced by application of APV.

Activation of NMDA receptors may contribute to the bursting induced by Mg-free ACSF[1], but the failure of APV and AP-7 to prevent these bursts suggests that additional mechanisms must be operative. Our observations are thus consistent with those of Mody et al.[28] who found only a 50% reduction of the spontaneous bursts induced by Mg-free ACSF during application of NMDA antagonists. Other factors which likely contribute to burst generation include a decrease in membrane surface charge screening[15] and increased Ca^{2+} entry[28]. In these circumstances the enhancement of presynaptic calcium uptake[23] may lead to an increased release of other transmitters acting at non-NMDA receptors.

Our results with CPP and Mg-free ACSF are to some extent contradictory with those obtained with APV and AP-7. Although CPP acts more rapidly than AP-7, as shown by the time taken to block ongoing NMDA-induced bursts, this cannot reconcile the difference between CPP and AP-7. Superfusion of AP-7 for up to 30 min prior to the addition of Mg-free ACSF is more than sufficient to block the NMDA receptors. Alternatively, CPP may bind to an additional NMDA or non-NMDA site which contributes to burst suppression. Further to this point, recent binding studies have shown CPP, but not AP-7, displaces flunitrazepam from neuronal benzodiazepine binding sites with an IC_{50} of 430 pM[36]. Thus, CPP may act in part by an action on benzodiazepine receptors[36].

Other agents

In contrast to bursts induced by NMDA and Mg-free ACSF, those bursts induced by kainate, MCD, 7 mM K^+, or anoxic episodes were never prevented or blocked by NMDA antagonists. This implies that activation of NMDA receptors is not required for the generation of these bursts.

It bears stressing that different mechanisms may underlie the genesis of the bursts induced by NMDA, kainate, MCD, elevated K^+ or anoxia. Thus kainate reduces two voltage-dependent potassium conductances in hippocampal neurons which would enhance neural excitability[17]. In addition, kainate reduces the Cl^--dependent GABAergic inhibition in CA_1 pyramidal neurons[14]. In this respect the interictal paroxysmal activity induced by kainate closely resembles that produced by PTX or bicuculline[34,37]. Furthermore, as with PTX or bicuculline[3,34] bursts induced by kainate are insensitive to NMDA antagonists.

The mechanism of action of MCD is not presently understood but it is known that it does not involve the reduction of either $GABA_A$- or $GABA_B$-mediated inhibition[6]. Like apamin, a related peptide, there is an endogenous MCD-like peptide in the brain which both cross-reacts in immunoassays for MCD and competes with MCD for binding to synaptosomes[5]. MCD increases synaptic noise in the presence of TTX, suggesting that it may enhance transmitter release[6].

Elevated extracellular K^+ enhances transmitter release[8], induces large amplitude EPSPs and reduces the amplitude of IPSPs[24], all of which likely contribute to burst induction in CA_3 neurons.

The bursts induced by repeated anoxic episodes may reflect the loss of inhibition which occurs with anoxia[16] and/or partial blockade of the electrogenic sodium pump[25].

Despite the differences between these agents or procedures with respect to the underlying mecha-

nisms of burst induction, the bursts themselves are remarkably similar. Thus, the bursts are generated by polysynaptic circuitry[4,6,37]; have a reversal potential near zero mV[4,6,27]; the synaptic inputs generating the bursts in individual CA_3 neurons are located above the mossy fiber input[4,27]; the time course and amplitudes of the bursts are similar[4,6,24,31]. The anatomical and physiological substrate for these bursts may well be the recurrent excitatory collaterals between CA_3 neurons[27]. Whatever pathway is involved in generating these bursts, the transmissive processes cannot be entirely dependent on activation of NMDA receptors as bursts are readily generated in the presence of NMDA antagonists. However, a late component of this process is likely mediated by NMDA receptors as reflected by the shortened burst duration in the presence of the NMDA antagonists.

Our seemingly paradoxical observations that NMDA antagonists can enhance burst activity, a proconvulsant action, is not new. It has been reported[13] that APV potentiates burst activity induced by bicuculline or picrotoxin. Furthermore, APV enhances unit activity induced by quisqualate and kainate in CA_1 neurons[7]. A general increase in excitability is unlikely to account for this effect as it is apparently specific for amino acids[7]. In a number of cases the NMDA antagonists enhanced the frequency of the bursts but reduced the burst duration and the number of spikes in the discharge. Reducing the number of spikes would reduce the following afterhyperpolarization which in turn could account for the increased frequency, since the AHP is important in controlling burst duration and frequency[22].

In conclusion, our observations show that NMDA receptor activation is sufficient but is not necessary for burst generation in the CA_3 region of the hippocampus. As such, NMDA antagonists are effective in preventing or blocking bursts induced by activation of NMDA receptors but are not effective in blocking bursts induced by other mechanisms.

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