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The role of excitatory amino acid receptors in the propagation of epileptiform discharges from the entorhinal cortex to the dentate gyrus in vitro

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Summary. The relationship between epileptiform events in the medial entorhinal cortex (MEC) and the dentate gyrus was investigated using a slice preparation from rat brain. Simultaneous intracellular recordings were made from neurones in layer II of the MEC and neurones in the granule cell layer of the dentate gyrus (DGC). Epileptiform activity was induced by perfusion with Mg^{++}-free medium or $GABA_A$-receptor blockers. Epileptiform discharges in MEC cells were reflected on a one-to-one basis and at a latency of 1–3 ms by depolarizing events in DGC. The latter rarely gave rise to action potentials. Bath perfusion of the N-methyl-D-aspartate (NMDA) receptor blocker, 2-aminophosphonovalerate (2-AP5) abolished the Mg^{++}-free induced events in MEC cells and the corresponding depolarizations in the DGC but local application of 2-AP5 to the dentate gyrus only reduced the depolarizations. The non-NMDA-receptor antagonist 6-cyano-7-nitro-quinoxaline-2,3-dione (CNQX), whether bath applied or applied locally to the DG, had little effect on the cortical events but strongly reduced the depolarizations of the DGC. The discharges induced in MEC cells by GABA-blockers were reduced by bath applied 2-AP5 but abolished by CNQX. These effects were mirrored in the dentate gyrus by a reduction in the depolarizing events by 2-AP5 and their abolition by CNQX. Local application of either antagonist to the dentate gyrus reduced but did not abolish the depolarizations. Thus, Mg^{++}-free induced events in MEC depend mainly on enhanced NMDA-receptor activity, while events induced by bicuculline are primarily dependant on non-NMDA receptors. The depolarizing events in the DGC which reflect the activity in the EC are mediated by both types of receptor, although non-NMDA receptors play a much greater role.

Key words: Entorhinal cortex – Dentate gyrus – Excitatory amino acids – Epileptiform activity – Simultaneous intracellular recording – Rats

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Introduction

Blockade of synaptic inhibition mediated by $GABA_A$-receptors (Schwartzkroin and Prince 1978; Hablitz 1984; Miles et al. 1984; Dingledine et al. 1986) or depletion of extracellular Mg^{++} (Anderson et al. 1986; Walther et al. 1986; Mody et al. 1987; Schneiderman and MacDonald 1987) induces interictal-like epileptiform discharges in hippocampal pyramidal cells. In slices consisting only of dentate gyrus and Ammons horn, neither treatment elicits paroxysmal activity in granule cells of the dentate gyrus (DGC; Schwartzkroin and Prince 1978; Fricke and Prince 1984; Misgeld et al. 1982; Mody et al. 1987; Mody et al. 1988). Although some studies have shown an increase in the amplitude of synaptically evoked EPSPs and the number of action potentials associated with them in DGC (Schwartzkroin and Prince 1978; Misgeld et al. 1982; Fricke and Prince 1984; Coan et al. 1987; Melchers and Pennartz 1987), others have not (Mody et al. 1987; Mody et al. 1988). However, all these studies are in agreement that synchronous, spontaneous events do not occur.

Spontaneous events can be induced in the dentate gyrus by $[Mg^{++}]_0$-depletion when the adjacent entorhinal cortex (EC) remains as an integral part of the slice (Walther et al. 1986; Stanton et al. 1987; Jones and Heinemann 1988). Recorded extracellularly in the granule cell layer, these events are small (0.2–0.5 mV) but long lasting (10's of seconds) negative-going deflections (Jones and Heinemann 1988). They can be prevented by severing the connections between the dentate gyrus and the EC and are clearly driven by long-lasting ictal-like discharges which arise in the EC and propagate via the perforant path (Jones and Heinemann 1988).

In the present study we have used intracellular recording in combined EC-hippocampus slices to examine the Mg^{++}-free induced events in DGC. In addition, we have investigated DGC responses recorded in the presence of $GABA_A$-blockers since these agents elicit entirely different epileptiform activity in the EC to that caused by Mg^{++}-depletion (Jones 1988). We have made simultaneous intracellular recordings from DGC and from neurones of origin of the medial perforant path in layer II of the medial EC (MEC) (Steward and Scoville 1976; Schwartz and

Coleman 1981; Ruth et al. 1982) to examine the temporal relationships between epileptiform discharges in the two areas. Finally, we have determined the relative contributions of N-methyl-D-aspartate (NMDA) and non-NMDA (i.e. quisqualate/kainate) subtypes of excitatory amino acid receptors to the genesis of events in the MEC and their propagation to the dentate gyrus since we have recently shown that both NMDA and non-NMDA receptors normally contribute to low frequency excitatory transmission in the dentate gyrus in this preparation (Lambert and Jones 1989; 1990). These results have appeared in abstracts (Jones and Lambert 1989; 1990).

Methods

Experiments were performed on slices (400–450 μ thick) prepared from the brains of male, adult Wistar rats. The slices, whose form and preparation have been described in detail previously (Jones and Heinemann 1988) consisted of ventral hippocampus, DG, the subicular complex and the EC. The brain was submerged in chilled (2–4 °C) artificial CSF (ACSF) during dissection and the slices were cut using a Campden Vibroslice. They were transferred directly to the recording chamber and maintained at the interface between a continuous stream of ACSF and warm, moist Carbogen gas (95% O_2 – 5% CO_2). The ACSF had the following composition (mM): NaCl (126); KCl (3.25); $CaCl_2$ (2.0); $MgSO_4$ (2.0); NaH_2PO_4 (1.25); $NaHCO_3$ (24.0); D-glucose (10). This had a pH of 7.4 at the recording temperature of 34.0 +/− 0.2 °C.

Intracellular recordings were made from neurones in the MEC and DGC using electrodes filled with potassium acetate (4M; resistances 60–120 MΩ). Voltage records were obtained using an Axoclamp 2 amplifier in the bridge mode. Membrane potential was continuously displayed on a Gould chart recorder and all data were stored on magnetic tape using an Hewlett-Packard 3960 FM tape recorder. Transient signals were digitized using a Gould digital oscilloscope or an IBM-PC with an Unkelscope data acquisition program (Unkel Software Inc.). These were plotted on a Hewlett-Packard 7475 plotter. Epileptiform discharges were often written directly on the chart recorder from the magnetic tape run at 25% of the recording speed.

In many experiments separate electrodes and amplifiers were used to make simultaneous intracellular recordings from pairs of neurones. One of the neurones was always located in the DG, while the other was usually located in layer II of the MEC. Crosstalk between the two recording headstages resulted in action potentials occuring in one cell appearing as small deflections in the voltage recording of the other. These artefacts of the recording situation can be seen in many of the figures in this paper. Bipolar, glass insulated, platinum electrodes were used to activate synaptic pathways with square wave pulses (100 us duration, 1–20 V). The stimulating electrode was usually positioned to stimulate the perforant path fibres where they traverse the subiculum. Stimulation would therefore activate both lateral and medial perforant paths as well as excitatory afferents from subiculum to the deep layers of the MEC (Jones and Heinemann 1988).

Epileptiform activity was induced by perfusion with bicuculline methochloride (5–10 μM), picrotoxin (10–20 μM) or a medium from which $MgSO_4$ had been omitted. The NMDA-antagonist, D,L-2-amino-5-phosphonovalerate (2-AP5, Sigma, 10–50 μM) and the non-NMDA-antagonist, 6-cyano-7-nitro-quinoxaline-2,3-dione (CNQX, a gift from Dr Tage Honore, Ferrosan Research Division, 0.5–10 μM) were delivered by bath perfusion or applied directly to the surface of the slice as a droplet from a broken micropipette in a volume of approximately 0.2 μl. In the latter experiments, higher concentrations of the antagonists were used (20 μM for CNQX, 80 μM for 2-AP5) since lower concentrations will be present at the recording site following dilution by diffusion into the tissue.

Obviously it is not possible to accurately assess the concentrations reaching the recorded cell using this local application approach. However, since the purpose of these experiments was to qualitatively assess a contribution of the receptor subtypes in mediating responses in the dentate gyrus while bypassing any effects on ongoing activity in the cortex, the absolute concentrations were not important. Control experiments using a dye solution (Brilliant Blue, Sigma) indicated that using a volume of about 0.2 μl, the application could be largely restricted to the dentate gyrus.

The possibility of synaptic connections between simultaneously recorded cells was tested for by directly depolarizing each cell in turn to evoke action potentials and looking for synaptic potentials in the other cell. On no occasion was any evidence of direct synaptic connections between neurones seen. However, in this study we were concerned with epileptiform discharges. By definition epileptiform responses are synchronous within a given neuronal population so we feel justified in using the responses of single cells to look at the temporal relationships between events occurring in different populations which have pronounced monosynaptic connections.

Results

Neurones studied

The morphological and electrophysiological properties of layer II neurones will be described elsewhere (R.S.G. Jones in preparation). The dentate gyrus neurones were all recorded in the granule cell layer and exhibited properties similar to those described previously for DGC (Fricke and Prince 1984). Stimulation in the perforant path evoked a monosynaptic EPSP in the DGC and, sometimes, small depolarizing potentials in the layer II neurones. Stimulation at high intensities could evoke antidromic spikes in most layer II cells. Initiation of epileptiform activity in layer II cells in either bicuculline or Mg^{++}-free medium never required stimulation at intensities sufficient to evoke antidromic spikes. Previous studies (Jones and Lambert 1990) have shown that epileptiform events in MEC following block of inhibition arise first in the deeper layers and propagate to the superficial layers and a similar situation applies to Mg^{++}-free induced events (see below). Thus, the epileptiform responses arising in layer II cells following stimulation in the perforant path or at other sites in the slice are evoked indirectly as a result of the afferent activation and the subsequent build up of synchronous activity in neurones of the deeper layers of the MEC. This largely accounts for the delays in eliciting events in layer II cells following stimulation.

Epileptiform events in Mg^{++}-free medium

Perfusion with Mg^{++}-free medium induced long-lasting ictal-like events in layer II neurones of the MEC similar to those seen in layer IV/V neurones (Jones and Heinemann 1988; Jones 1989) consisting of a prolonged plateau of depolarization superimposed with a series of smaller "afterdischarges" (Fig. 1). Simultaneous intracellular recordings from neurones in layer IV/V and layer II of the MEC (n = 3) showed that the ictal-like activity occurred first in the neurones of the deep layers and was followed 3–8 ms later by the start of activity in the superficial cells.

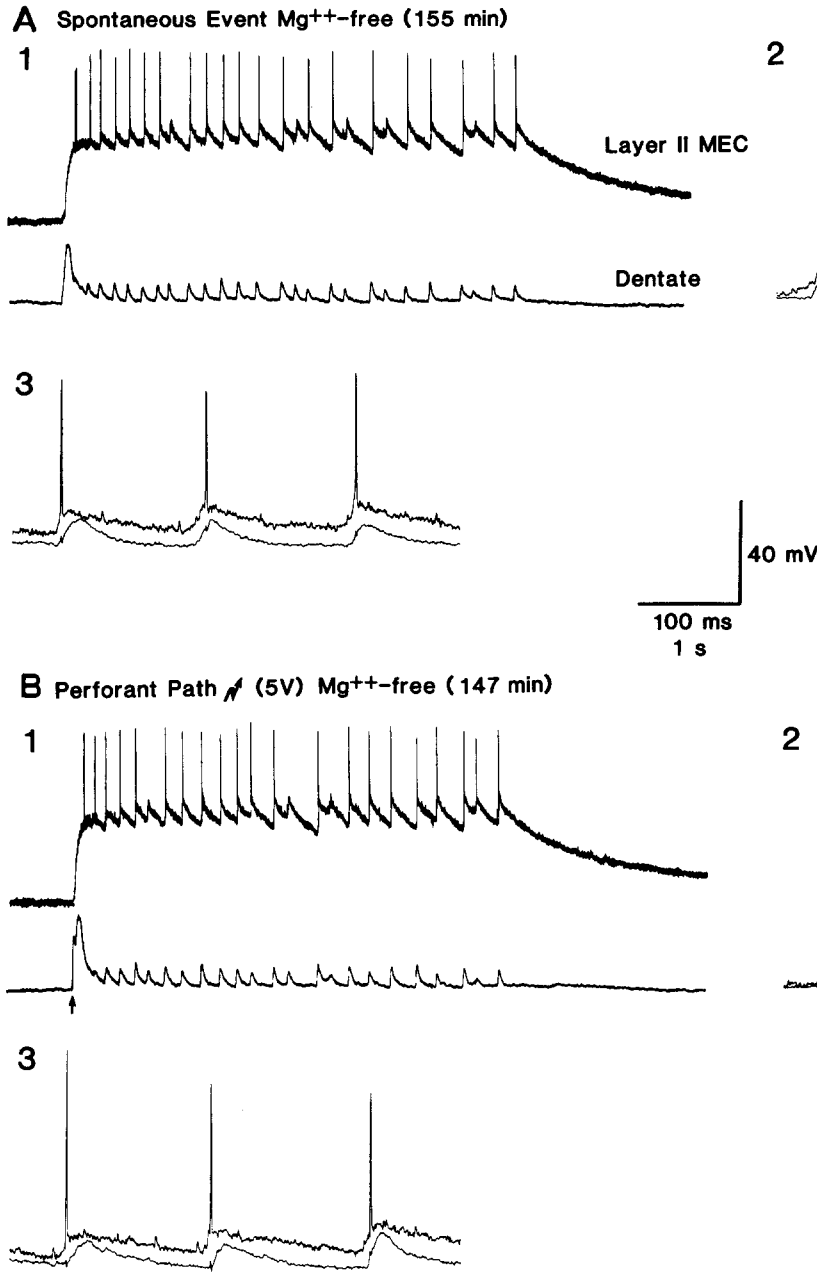

Fig. 1A, B. Simultaneous recordings from a layer II cell and a DGC in Mg^{++}-free medium. The records in **A** show a spontaneously occurring ictal-like event in the layer II cell with small depolarizations and spiking superimposed on the plateau. These are reflected as a series of corresponding depolarizations in the DGC **A2** Fast time base records showing that initiation of the event in II clearly precedes the first depolarization in the DGC. The records in **A3** are taken from about half way through the ictal-like event and show that each "afterdischarge" in the layer II cell is followed by a small depolarization in the DGC. **B** Same cell pair as **A**. An ictal-like event was evoked by a single shock in the perforant path. This event is similar in all respects to that arising spontaneously with the exception that a small EPSP occurs just before the initiation of the event in II and a large EPSP is evoked in the DGC. The DGC EPSP precedes the initiation of the event in II and the depolarization corresponding to the latter arises on the falling phase of the synaptic EPSP. The calibration bar is 100 ms for the all fast time base records and 1 s for the slow events. Resting potentials were −76 mV (II) and −85 mV (DGC)

A similar relationship existed between the afterdischarges associated with the events.

Simultaneous recordings from DGC and MEC neurones in Mg^{++}-free medium

Simultaneous intracellular recordings were made from DGC and layer II cells in the MEC in Mg^{++}-free medium on 5 occasions. Records from one of these pairs are illustrated in Fig. 1. The rise of the spontaneous event in the layer II cell ($1A_1$) preceded a large depolarization in the DGC. The series of afterdischarges superimposed on the ictal-like event in the layer II cell were reflected by smaller depolarizations in the DGC, on a one-to-one basis. All the events in the cortical neurone preceded those occurring in the DGC. Identical events to those occurring spontaneously were evoked following stimulation in the perforant path (Fig. 1B). However, the first response observed was a large, mono-synaptic EPSP in the DGC. The EPSP clearly occurred before the initiation of the discharge in layer II such that the large depolarization corresponding to the layer II event appeared on the falling phase of the EPSP. Thereafter, the events occurring in the two cells showed the same relationship as those occurring spontaneously. Similar results were obtained from the other four DGC-layer II cell pairs.

There was a clear dissociation between the EPSP evoked by perforant path stimulation and the depolarizations being driven by epileptiform activity from layer II. Stimulation at intensities which did not initiate an epileptiform discharge in the MEC could evoke an EPSP in

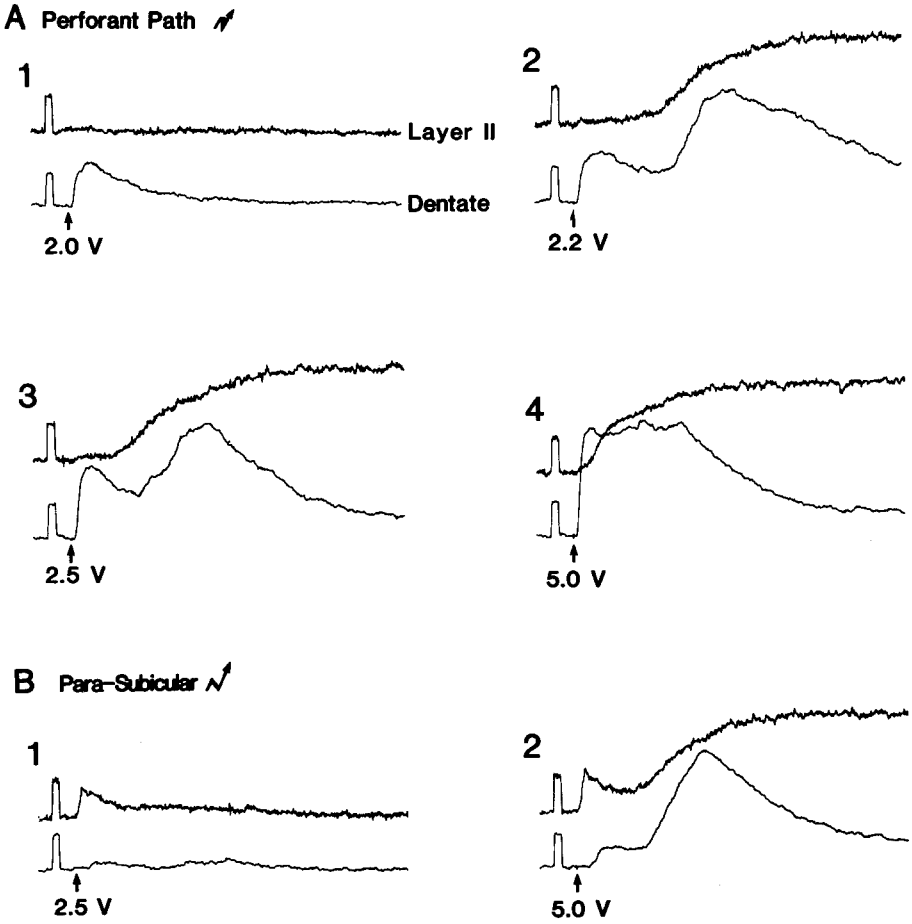

Fig. 2. **A** Simultaneous intracellular recording from a DGC and layer II cell in Mg^{++}-free medium. **A1** Stimulation in the perforant path at low intensity evoked and EPSP in the granule cell but no response in the MEC neurone. **A2** Increasing the intensity slightly increased the EPSP amplitude and elicited an all-or-none discharge in the cortical cell at a long latency. This was reflected by a large depolarization in the DGC. **A3** Further increasing the intenstiy evoked a larger EPSP and decreased the latency to the ictal-like discharge in the Layer II cell and its reflected depolarization in the DGC. **B** Shows records from the same cell pair as **A.** Stimulation in the para-subiculum could evoke an EPSP in the cortical cell without an epileptiform discharge and no response in the DGC. An increase in intensity initiated the ictal-like event and the reflected event in the DGC. The calibration pulses at the start of each trace represent 10 mV and 5 ms. Resting potentials as for Fig. 1

isolation in DGC. This is illustrated in Fig. 2 (A_1). Increasing the stimulation intensity increased the amplitude of the EPSP and evoked an all-or-none, ictal-like event in the cortical cell. This was associated with the appearance of the reflected depolarizing events in the DGC. Further increases in stimulation intensity increased the EPSP amplitude in the DGC and decreased the latency to the initiation of the discharge in the layer II cell with a corresponding decrease in latency to the large depolarizing wave in the DGC, such that it merged with the EPSP. The depolarizations in the DGC which reflected the epileptiform discharges in the cortex were all-or-none in nature and varied little in amplitude or duration with changes in stimulation intensity. Figure 2B shows that it was also possible to elicit an EPSP in the layer II neurone in the absence of an epileptiform discharge by stimulating in the para-subiculum. No reflected event was recorded in the DGC until the stimulation intensity was increased to a level sufficient to evoke the ictal-like event (Fig. 2B).

Effect of 2-AP5 in Mg^{++}-free medium

Ictal-like activity in layer II neurones, as in layer IV/V neurones (Walther et al. 1986; Stanton et al. 1987; Jones and Heinemann 1988) was rapidly abolished by bath application of 2-AP5 in an all-or-none fashion (n = 7). The abolition of ictal-like activity in layer II cells was associated with a simultaneous disappearance of the initial large depolarization and all the subsequent smaller events in the DGC (Fig. 3). 2-AP5 also consistently reduced the perforant path evoked EPSP in the DGC to a small extent (Fig. 3A, 3B). Recovery of ictal activity in the cortical neurones during washout of 2-AP5 was associated with an immediate reappearance of the full sequence of depolarizing events in the DGC.

Application of 2-AP5 as a droplet directly onto the dentate gyrus during recording from a DGC reduced the amplitude of all the depolarizing events, including the perforant path evoked EPSP (n = 4, Fig. $5A_2, 5B_2$). In contrast to the effect of bath applied 2-AP5, the events driven from the cortex were not abolished. The ictal-like discharges recorded from the layer II cells were completely unaffected by the local application of 2-AP5 to the dentate gyrus. This was tested during a simultaneous DGC-layer II cell recording on one occasion and in 2 further unpaired layer II cells. However, when 2-AP5 was applied directly onto the recording site in layer II it rapidly and reversibly abolished the ictal-like discharges (n = 3). In addition, ictal-like events in layer IV/V cells in the MEC were unaffected by 2-AP5 applied to the dentate gyrus but abolished when the antagonist was applied to the cortex (n = 3).

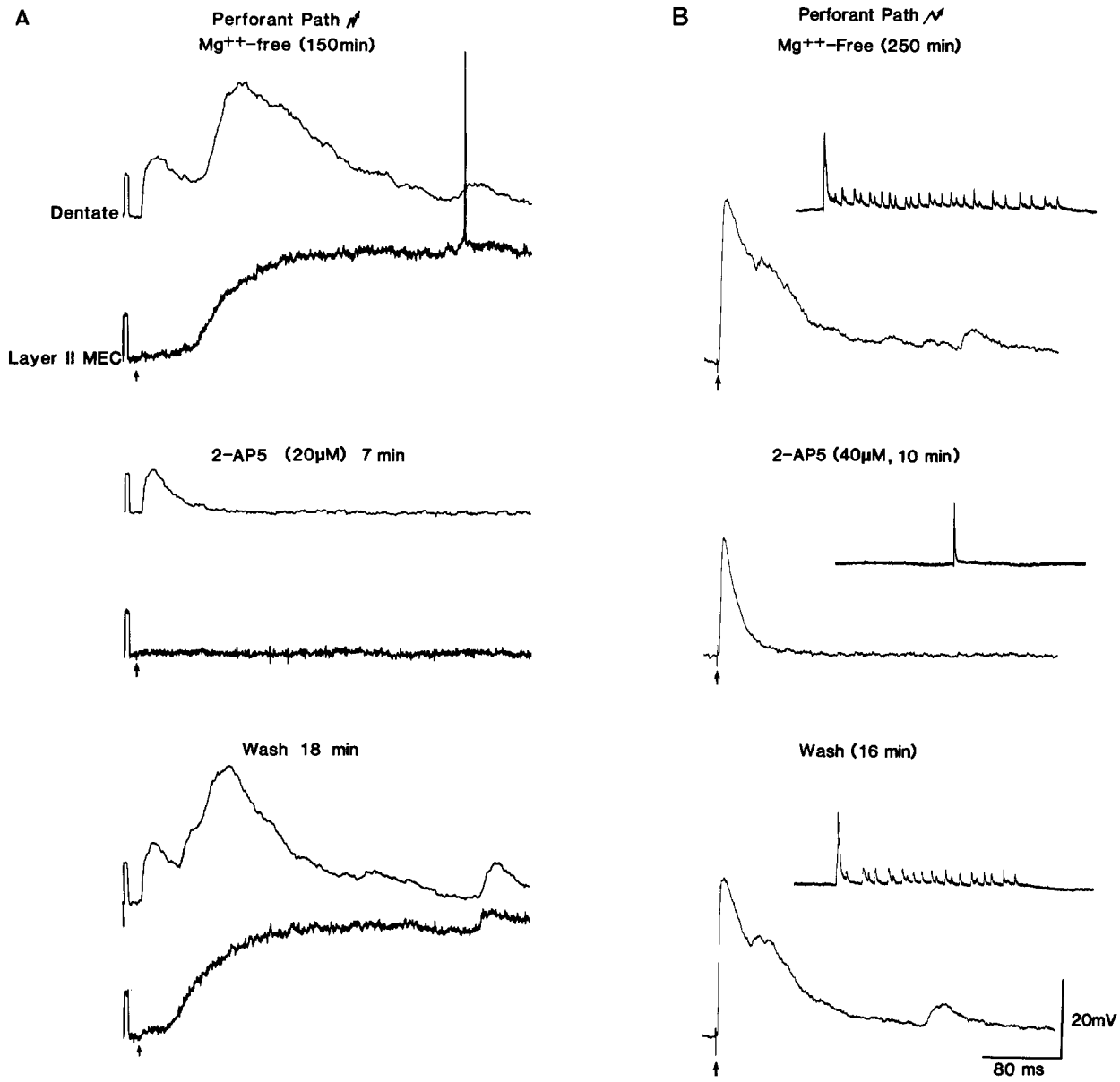

Fig. 3. **A** The effects of 2-AP5 on simultaneously recorded events evoked by perforant path stimulation in a layer II cell and a DGC in Mg^{++}-free medium. 2-AP5 rapidly abolished the ictal-like discharge in the cortical cell and all the corresponding depolarizing events in the DGC. The perforant path evoked monosynaptic EPSP remained, but was reduced to some extent. **B** Effects of 2-AP5 on repetitive depolarizations recorded in a DGC in isolation. The insets show chart records of the whole series of events evoked after perforant path stimulation while the fast time base records show the monosynaptic EPSP and the early part of the discharge more clearly. 2-AP5 blocked all the late depolarizations and reduced the perforant path evoked EPSP. Calibration mark in **A** is 10 mV, 5 ms. The calibration mark in **B** represents 1.5 s for the inset records. Resting potentials in **A** are −74 mV (II) and −84 mV (DGC) and **B**, −87 mV

Effects of CNQX in Mg^{++}-free medium

The effects of CNQX were determined on 6 DGC (3 paired with layer II cells, 3 recorded alone). CNQX (1–5 μM) greatly reduced the amplitude of the perforant path evoked EPSP, the initial large wave of depolarization and the subsequent smaller depolarizations (Fig. 4) in all 6 cells. In contrast, the ictal-like events in the layer II cells were relatively unaffected. However, with increasing concentrations of CNQX, there was a progressive increase in the latency to the initiation of the event following perforant path stimulation. This was reflected by an increase in the latency to the initial depolarizing wave in the DGC, concurrent with the reduction in its amplitude (Fig. 4). A small depolarization was apparent prior to the epileptiform event (Fig. $4A_1$, single arrow) and this was reduced by CNQX. As the latency to the ictal discharge was increased a second peak of depolarization preceding it became apparent (Fig. $4B_1$, double arrow) and this was also reduced by CNQX (Fig. 4C). The rise time of the

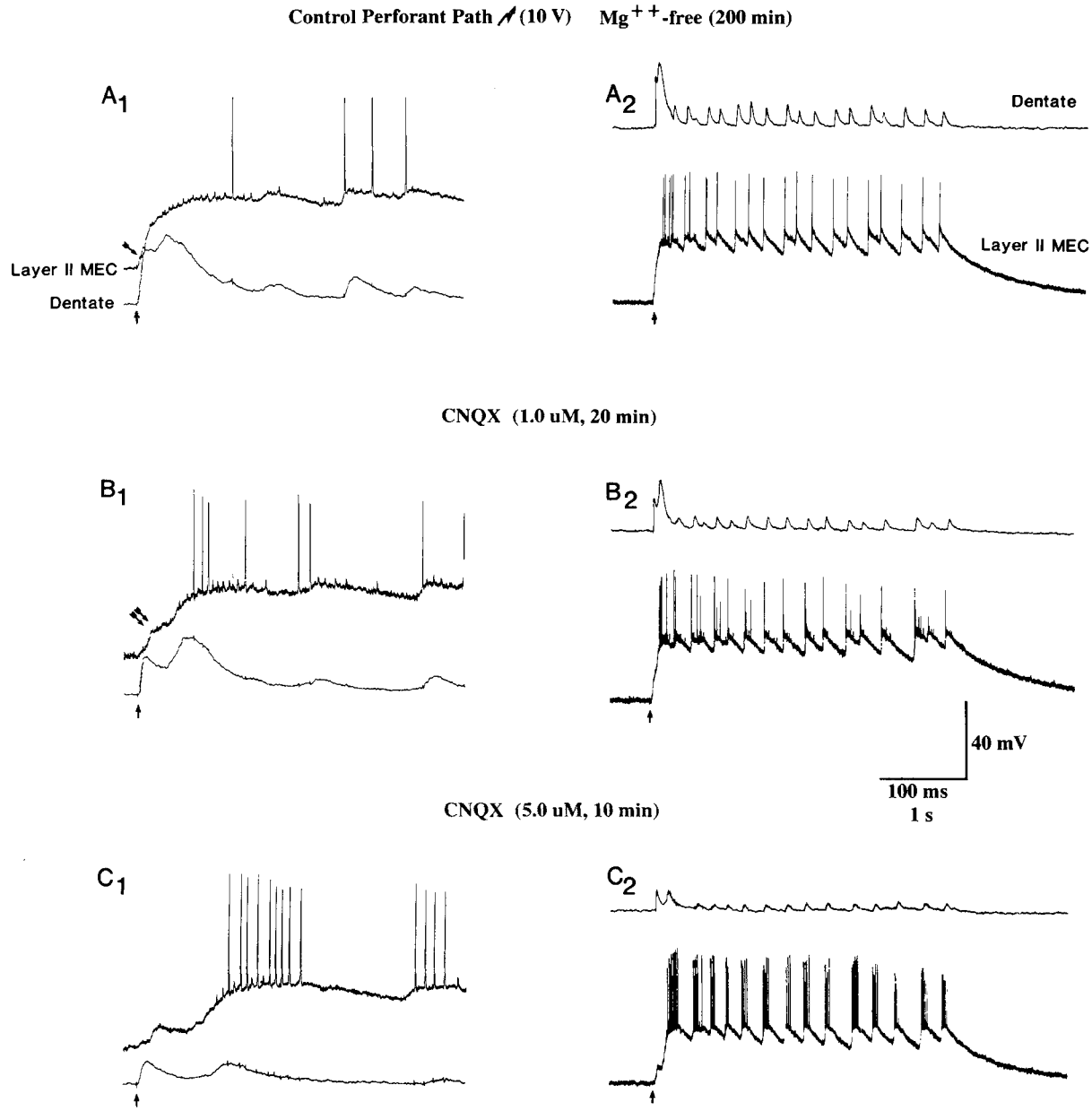

Fig. 4. A1, A2 Ictal-like events recorded simultaneously in a layer II cell and a DGC in Mg^{++}-free medium following stimulation in the perforant path. Slow and fast time base records of the same responses are shown on the right and left respectively. The single arrow indicates a small synaptic potential prior to the epileptiform event in the layer II cell. **B1, B2** 20 min after start of perfusion with CNQX (1.0 μM) the amplitude of all the depolarizing events including the perforant path evoked EPSP were reduced by 40–50% while there was little change in the ictal-like discharge in the cortical cell other than an increase in latency to its initiation. The double arrow in **B1** indicates a second depolarization in the layer II cell revealed by this increase in latency. **C1, C2** The events in the DGC were greatly reduced when the CNQX concentration was increased to 5 μM. Although there were some changes in the form of the event in layer II, CNQX did not prevent its occurrence. However, the depolarizing potentials prior to the epileptiform event were reduced by CNQX. Note that the increase in latency to the discharge in Layer II is accompanied by an increase in latency to the depolarizing wave in the DGC. The calibration bar represents 100 ms for the fast and 1 s for the slow records respectively. Resting potentials: −75 mV (II), −86 mV (DGC)

event was slowed and there was a slight reduction in the maximum plateau of depolarization attained (about 10% at 5 μM). Similar effects of CNQX on ictal-like activity were seen in a further 3 layer II cells and also in 3 cells in layer IV/V of the MEC. With prolonged periods (>30 min) of perfusion with CNQX at 10 μM (n = 3) there was a further reduction in the amplitude and also the duration of ictal-like events. However, they were never abolished by CNQX. A further 6 cells (2 in layer II, 4 in layer IV/V) were impaled in slices already bathed in CNQX (10 μM). All 6 cells displayed both spontaneous and evoked ictal-like activity.

Application of CNQX (20 μM) directly onto the dentate gyrus greatly reduced the monosynaptic EPSP, the

initial large depolarization and subsequent smaller events over a period of 20–25 min (Fig. 5). As described above, a droplet application of 2-AP5 reduced the amplitude of the spontaneous events by about 25% (Fig. $5A_2$). Perforant path evoked responses of the same cell are shown in Fig. 5B. After the 2-AP5 application the peak of the EPSP was reduced and a greater separation between the EPSP and the slow phase of depolarization occurring on its falling phase was seen. Following recovery, CNQX (20 μM) reduced the spontaneous depolarizations by over 50% and also reduced the amplitude, duration and rise time of the perforant path evoked potential. These effects showed little recovery over the subsequent 45 min during which the impalement was maintained.

Epileptiform events in bicuculline and picrotoxin

The interictal-like events elicited by blockade of inhibition have been described in detail elsewhere (Jones 1988; Jones and Lambert 1990) and examples can be seen in Fig. 6 and 7. Simultaneous intracellular recording from cells in layers IV/V and layer II (Jones and Lambert 1990) have

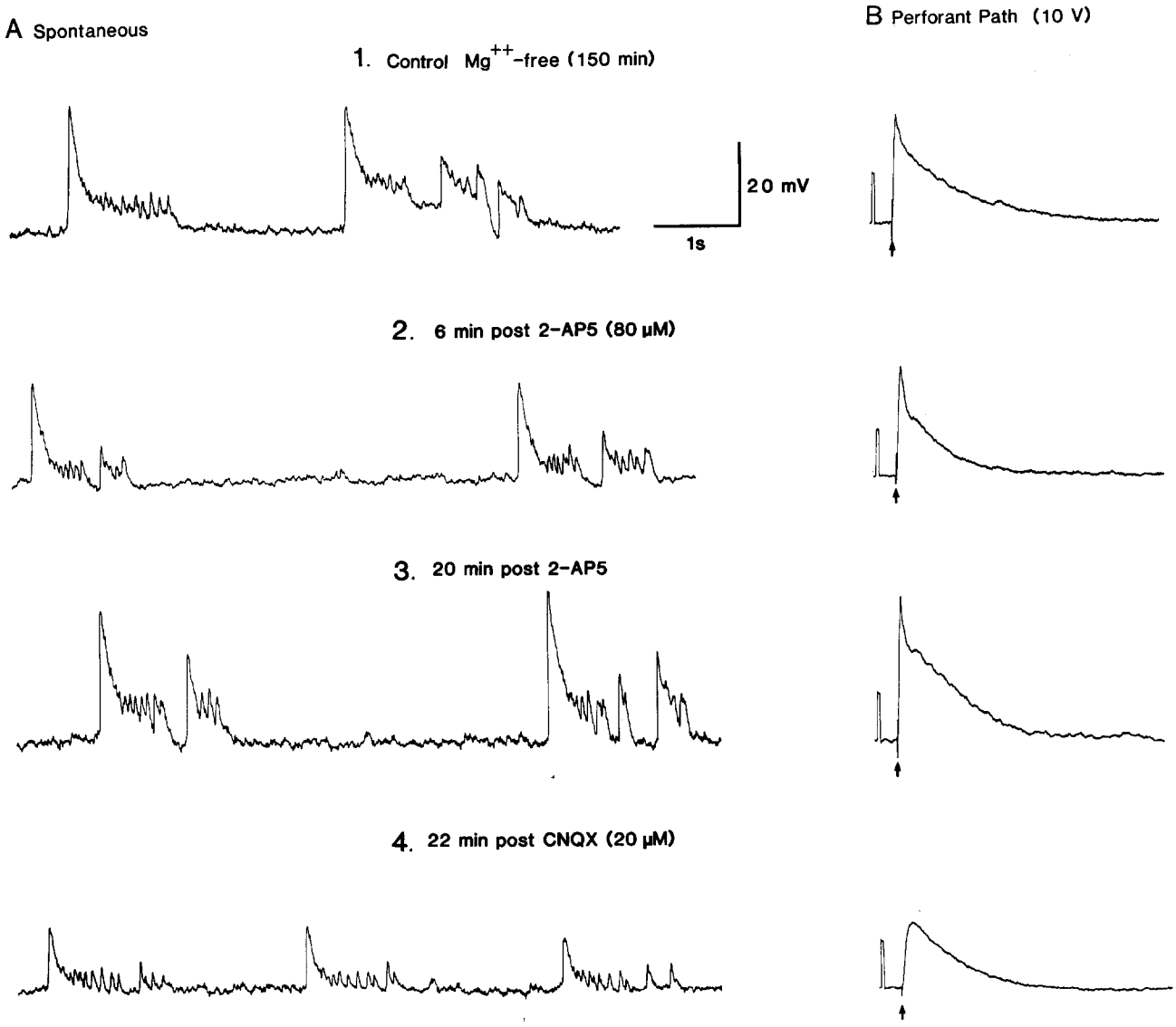

Fig. 5A, B. Effect of local applications of 2-AP5 and CNQX on Mg^{++}-free induced events in a DGC. Spontaneous events are shown in **A** and the early part of evoked events in the same cell **B**. 2-AP5 reduced the amplitude of initial large spontaneous depolarization and the subsequent smaller events **A2.** In the control situation **B1**, perforant path simulation evoked a large EPSP with a prolonged falling phase, but no clear late depolarization. After 2-AP5 the amplitude of the EPSP was practically unaltered but the duration was decreased and a hump was apparent on the decay phase. Following recovery local application of CNQX greatly reduced the amplitude of the spontaneous depolarizations (**A4**) and the peak, duration and rise time of the perforant path evoked EPSP(**B4**). Calibration mark in B represents 10 mV and 5 ms. Resting potential was −84 mV

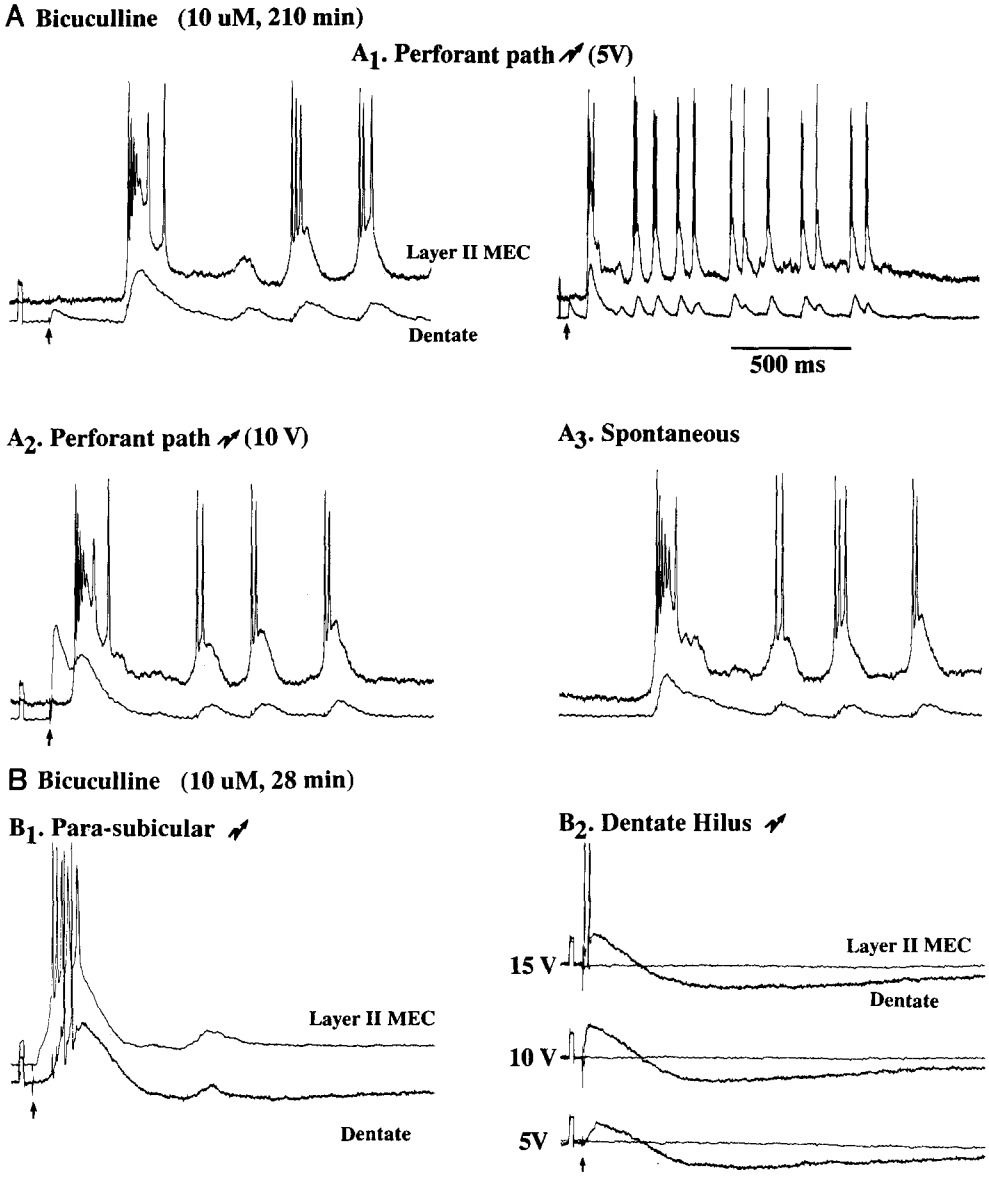

Fig. 6. **A** Simultaneous recordings from a layer II neurone and a DGC in bicuculline. **A1** Stimulation at low intensity in the perforant path evoked a small EPSP in the granule cell and initiated a PDS and a series of afterdischarges in the cortical cell (fast and slow time bases of the same responses on the left and right respectively). The discharges in the cortical cell were followed by depolarizing events in the DGC on a one-to-one basis. **A2** Increasing the stimulation intensity increased the amplitude of the monosynaptic EPSP in the DGC and shortened the latency to the initial PDS in the cortical neurone. The reflected depolarization in the DGC now appeared on the falling phase of the EPSP. **A3** Identical events also occurred spontaneously. Resting potentials were −82 (II) and −81 (DGC). **B** Simultaneous recordings in a different cell pair to **A**. **B1** Parasubicular stimulation evoked a PDS in the layer II neurone which was reflected as a large depolarization giving rise to action potentials in the DGC. A slow hyperpolarization followed the latter event. **B2** Stimulation in the hilus of the dentate gyrus evoked a graded EPSP in the DGC followed by a slow hyperpolarization but no response in the layer II cell. Resting potentials were −80 (II) and −73 (DGC). Calibration mark in **A1, A2, B1, B2** represent 10 mV, 5 ms, and also applies to **A3**

shown that the discharges in layer II are probably driven by those arising in the deeper layers.

Simultaneous recordings from DGC and MEC neurones in bicuculline or picrotoxin

Simultaneous recordings from cells in layer II of MEC and DGC (n = 11) are shown for two different pairs in Fig. 6. Epileptiform discharges occurred both spontaneously (Fig. $6A_3$) and in response to perforant path stimulation (Figs. $6A_1$ and $6A_2$). The PDS in the layer II cell was reflected at a latency of 1–3 ms by a large depolarization in the DGC. As with the Mg^{++}-free induced events, each afterdischarge was followed at a similar latency by a smaller amplitude depolarization in the DGC. Stimulation in the perforant path evoked an EPSP in the DGC and a PDS in the layer II cell at a long latency (Fig. $6A_1$). This PDS was identical to that occurring spontaneously and the events thereafter were also identical to those occurring spontaneously (Fig. $6A_3$). Increasing the stimulation intensity evoked a larger EPSP in the DGC and shortened the latency to the PDS in the layer II neurone (Fig. $6A_2$). The reflected depolarization now occurred on the falling phase of the EPSP in the DGC.

Figure $6B_1$ shows a PDS evoked in a layer II neurone following parasubicular stimulation. This was followed by a large depolarization in the simultaneously recorded DGC in the absence of a preceding synaptic EPSP. In contrast, stimulation in the hilus of the dentate gyrus evoked a synaptic EPSP in the DGC, but no response in the cortical neurone ($6B_2$)

Inhibitory responses in DGC

Depolarizing responses of DGC rarely generated action potentials. In several cells strong stimulation in the perforant path could evoke one or sometimes two spikes on the

monosynaptic EPSP but only after prolonged periods of perfusion with the $GABA_A$-blockers. In three cells the large depolarization which reflected the PDS in layer II neurones gave rise to multiple (2–7) spikes (Fig. $6B_1$).

The failure to generate action potentials may be due to the very negative resting potentials of the cells (typically −80–−90 mV) but could also be associated with bicuculline insensitive inhibition. Long duration, slow hyperpolarizing responses were occasionally recorded in DGC in the presence of either bicuculline or picrotoxin. An example is shown in Fig. 6B. The resting potential of the DGC was rather more positive (−73 mV) than that usually recorded. The PDS evoked in the layer II neurone by para-subicular stimulation was followed by a large depolarization in the DGC which gave rise to two spikes. This was succeeded by a slow hyperpolarization. Stimulation in the hilus of the dentate gyrus (Fig. $6B_2$) elicited a graded EPSP followed by a prolonged slow hyperpolarization in the DGC with no detectable response in the layer II cell. Stimulation at a high enough intensity gave rise to spikes on the EPSP, the first of which was almost certainly antidromic. However, the presence of action potentials was not a prerequisite for eliciting the slow hyperpolarization which was already maximal at the lowest stimulation intensity (Fig. $6B_2$).

Effect of 2-AP5 in bicuculline

2-AP5 readily blocks afterdischarges and reduces the amplitude and duration of the PDS in layers II and IV/V of the MEC (Jones 1988; Jones and Lambert 1990). However, it does not abolish the events entirely nor does it prevent the occurrence of spontaneous events. Figure 7A shows

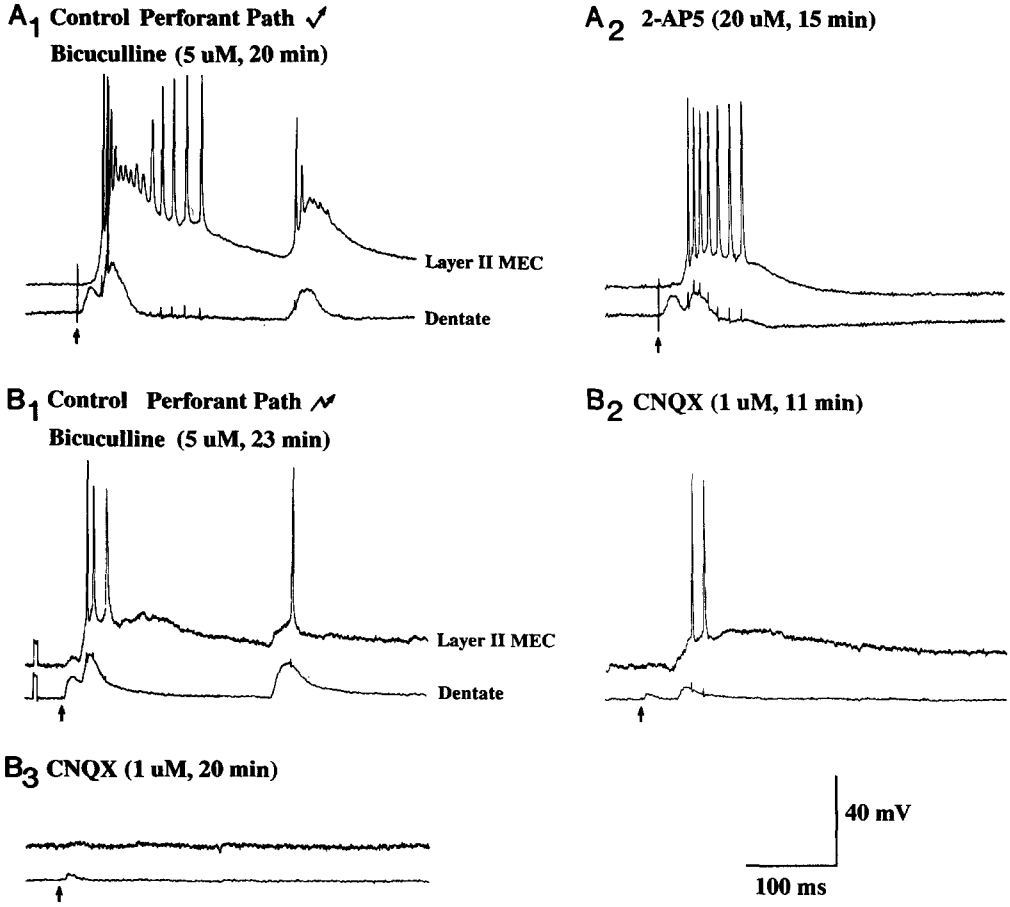

Fig. 7. **A** Effects of 2-AP5 on simultaneously recorded events in a layer II neurone and a DGC during perfusion with bicuculline. **A1** Stimulation in the perforant path elicited a PDS and afterdischarge in the cortical cell. Both events were reflected in the DGC with a single spike riding on the inital depolarization. **A2** 2-AP5 blocked the afterdischarge in the layer II cell and the corresponding event in the DGC disappeared at the same time. 2-AP5 also reduced the amplitude of the PDS and the corresponding depolarization in the DGC. There was also some reduction in the amplitude of the monosynaptic EPSP in the DGC. Resting potentials were −73 (II) and −78 (DGC). **B** Effect of CNQX on simultaneously recorded events in a layer II cortical cell and a DGC. **B1** Control responses were similar to those seen in the cell pair shown in **A**. **B2** As with 2-AP5 CNQX caused an all-or-none disappearance of the afterdischarge in layer II and the depolarization in the DGC. There was a progressive reduction in the PDS and increase in the latency to its initiation. At the same time the EPSP and the large depolarization in the DGC were reduced in amplitude and the latency to the latter also increased. **B3** Eventually, the remaining PDS in the cortical cell and the corresponding event in the DGC disappeared in an all-or-none fashion. A small EPSP in the DGC remained at this time. Resting potentials were −73 (II) and −85 (DGC). Calibration in B_1 applies to all records

these effects of 2-AP5 in a layer II cell, and its effects on concurrently recorded events in a DGC. The abolition of the afterdischarge in the layer II cell was accompanied by the disappearance of the reflected depolarization in the DGC. Although the PDS in the layer II cell and the reflected depolarization in the DGC were greatly reduced neither were abolished. Note also that the perforant path evoked EPSP was reduced in amplitude but to a lesser degree than the reflected depolarization. Similar results to those described were recorded in two other cell pairs and from a further 3 DGC recorded alone.

Effects of CNQX in bicuculline

The effects of CNQX were tested during simultaneous impalements of DGC and layer II cells on 4 occasions (Fig. 7B). As with 2-AP5, afterdischarges in layer II were rapidly abolished by CNQX in an all-or-nothing fashion and the corresponding depolarizations in the DGC disappeared simultaneously (Fig. $7B_2$). There was a progressive reduction in the PDS and a concurrent reduction in the reflected depolarization in the DGC until both events were abolished (Fig. $7B_3$). The latency to the PDS in the layer II cell was progressively increased. These effects were paralleled by a pronounced reduction in the perforant path evoked EPSP (Fig. 7B), although a small EPSP usually remained. This EPSP could be blocked by 2-AP5 (Lambert and Jones 1989; 1990). Similar results were obtained on a further 2 DGC recorded alone.

Local applications of 2-AP5 and CNQX in bicuculline

The effects of local applications of 2-AP5 (80 μM, n = 3) and CNQX (20 μM, n = 3) depolarizing events in DGC were essentially the same as described for Mg^{++}-free induced events (see Fig. 5). Thus, 2-AP5 reduced the monosynaptic EPSP, the initial depolarization driven by the cortical PDS and all the subsequent smaller depolarizing events (15–25%). CNQX had similar, but more pronounced effects (50–60%).

Discussion

Epileptogenesis in MEC

The generation of ictal-like events in MEC neurones following depletion of $[Mg^{++}]_0$ has been shown to depend primarily on enhanced NMDA-receptor activation since it is abolished by 2-AP5 (present study; Walther et al. 1986; Stanton et al. 1987; Jones and Heinemann 1988). The failure CNQX to cause marked changes in the ictal-like events in either layer II or layer IV/V cells emphasizes again the dominant role of enhanced NMDA-receptor activation. However, the increased latency to the initiation of the events, small reduction in plateau amplitude and inconsistent reduction in duration do indicate that non-NMDA receptors are involved to some extent in the generation of ictal activity in Mg^{++}-free medium. The reverse situation applies to the interictal-like discharges elicited by $GABA_A$-receptor blockade since CNQX can abolish discharges but 2-AP5 only reduces their amplitude and duration (present study; Jones 1988; Jones and Lambert, 1990). Simultaneous intracellular recording of bicuculline-induced events in layer II and layer IV/V cells in MEC has indicated that events in the superficial layer are driven by those occurring in the deeper layer (Jones and Lambert, 1990) and a similar situation pertains to ictal-like events recorded in Mg^{++}-free medium. However, the ictal-like activity in layer II neurones was blocked by 2-AP5 but not by CNQX, so its generation must involve NMDA-receptor mediated synapses on the layer II neurones. Indeed, droplet applications of 2-AP5 restricted to the superficial layers can abolish ictal-like events in layer II cells without affecting those occurring concurrently in the deeper layers (R.S.G. Jones, unpublished observations).

NMDA vs. non-NMDA receptors in the dentate gyrus

Until recently NMDA-receptors were not considered to participate in normal, low frequency synaptic transmission in the dentate gyrus. Crunelli et al. (1983) found that iontophoretically applied 2-AP5 generally had little effect on the intracellularly recorded EPSP in DGC evoked by medial perforant path stimulation. Mody and colleagues (Mody and Heinemann 1987; Mody et al.1988) reported that the lateral perforant path evoked EPSP in normal DGC was little affected by either 2-AP5 or Mg^{++}-free medium. However, in DGC in slices from electrically kindled animals, a component of the EPSP was respectively blocked or enhanced by these treatments suggesting that NMDA-receptors became active in transmission during the kindling procedure (Mody and Heinemann 1987; Mody et al. 1988).

We have recently made a detailed re-evaluation of the role of non-NMDA versus NMDA receptors in perforant path transmission (Lambert and Jones 1989; 1990). We found that the EPSP in DGC, recorded in the absence of convulsants, had a component which was resistant to blockade by CNQX, but could be blocked by 2-AP5. This component was dramatically enhanced in Mg^{++}-free medium. This is similar to the situation at Schaffer-collateral synapses on CA1 cells and mossy fibre synapses in CA3 (Andreasen et al. 1989). Our conclusion is that both NMDA and non-NMDA receptors are involved in normal, low frequency transmission at perforant path synapses although a much greater role is played by the latter (Lambert and Jones 1989, 1990).

What are the relative roles of these receptor subtypes in the propagation of epileptiform activity from cortex to DG? The repetitive depolarizing events in DGC in Mg++-free medium were abolished by bath-applied 2-AP5. Clearly this could have resulted secondarily to the abolition of the ictal-like events occurring in the MEC and need not necessarily have implied any involvement of NMDA-receptors located on the DGC. However, when 2-AP5 was directly applied to the dentate gyrus, although the depolarizations were not prevented they were reduced

to some extent indicating that they were mediated in part by NMDA receptors activated by transmitter released from perforant path synapses. It is unlikely that the reduction in DGC responses could result from spread of the antagonist to the EC since the ictal-like events in the cortex were unaffected. It is a little more likely that the locally applied antagonist could reach the adjacent subiculum. However, any effect here would be unlikely to influence events in the dentate gyrus. The subiculum has little in the way of direct projections to dentate gyrus (Kohler 1985). Although subicular projections to the EC are strongly excitatory (Jones and Heinemann 1988) it has been shown that blocking synaptic transmission by application of xylocaine to the subiculum does not affect epileptiform activity in the MEC (Jones and Lambert 1990). Therefore, we feel confident in ascribing the effects of locally applied 2-AP5 to an interaction with NMDA receptors located on DGC.

The large reduction in the depolarizations of DGC in Mg^{++}-free medium by CNQX, despite the virtually unchanged ictal-like activity in MEC, indicates that non-NMDA receptors are primarily responsible for transmission of these epileptiform events at perforant path synapses. This was largely confirmed by local applications of CNQX which again left the activity in the cortex unchanged but drastically reduced all the events in the DGC. The degree of this reduction with CNQX was much greater than that noted with 2-AP5, so although the depolarizations arise from concurrent activation of both NMDA and non-NMDA receptors on DGC, the latter play by far the greater role.

Both types of receptors were also involved when epileptiform activity was evoked by $GABA_A$-blockers. The depolarizing events in the DGC were similarly reduced, but not abolished, by local applications of either 2-AP5 or CNQX. However, when the antagonists were bath applied the effects differed from those seen in Mg^{++}-free medium and could be correlated with different effects on the epileptiform events in the MEC. Thus, 2-AP5 blocked the afterdischarges but had much less effect on the initial PDS (see also Jones 1988; Jones and Lambert 1990). Consequently, it blocked the small depolarizing events in the DGC but had much less effect on the initial large event. Note that the reduction in the latter event was greater than that of the perforant path evoked EPSP. This would result from a combination of a reduced input from the cortex and blockade of NMDA receptors on the DGC. CNQX, on the other hand, blocked the afterdischarge and also blocked the PDS. This was accompanied by a rapid disappearance of the small depolarizations in the DGC and a gradual abolition of the initial large depolarization.

Thus, the cortical epileptiform activity which propagates to the dentate gyrus via the perforant path clearly activates both NMDA and non-NMDA receptors on the DGC. The differing effects seen in the DGC with the various convulsant/antagonist combinations are a more a consequence of the different roles of the receptor subtypes in generating the cortical activity, rather than their relative roles in mediating depolarization on the DGC.

Although NMDA-receptors do normally contribute to low frequency transmission at perforant path synapses (Lambert and Jones 1989; 1990) it is possible that the NMDA-component of the DGC depolarizations driven by paroxysmal activity propagating from the cortex may be enhanced as a consequence of that activity. Stelzer et al (1987) showed that repeated activation of Schaffer-commisural axons in slices eventually enhances the sensitivity of CA1 cells to NMDA. It could be speculated that the NMDA-component of the DGC responses in the present study could be enhanced the repeated synaptic activation resulting from spontaneous synchronous discharges in the EC.

In the present study we were not particularly concerned with the relative contributions made by the medial and lateral perforant path synapses to the NMDA component of the DGC depolarizations since the epileptiform activity arriving in the dentate gyrus will essentially choose its own pathways. The experiments of Mody and colleagues (Mody and Heinemann 1987; Mody et al. 1988) which did not detect an NMDA component of the perforant path EPSP specifically stimulated the lateral perforant path. There is some evidence that NMDA receptors may play a greater role in transmission at medial perforant path synapses (Coan et al. 1987). Both MEC and LEC are an integral part of our slices, but the orientation of cutting and the greater distance over which the fibres would have to travel mean that less of the lateral perforant path will remain intact form LEC to dentate gyrus. Although some of the input arriving at DGC will result from epileptiform discharges in LEC neurones, the majority will come from MEC neurones and this could contribute to the easily detectable NMDA-component. However, it should be noted that Crunelli et al (1984) failed to detect any NMDA-component of the EPSP when specifically stimulating the medial perforant path so the situation is clearly a complex one.

Relationships between dentate gyrus and MEC

The decline in the amplitude of the reflected depolarizations in the DGC after the initial large event is may be explained by changes in intensity of presynaptic activity. The initial PDS in the MEC cells in bicuculline was always larger and more intense than the afterdischarges. Although not illustrated in this paper, there was often an intense initial discharge associated with the ictal-like events in Mg^{++}-free medium. It is also possible that activation of bicuculline-resistant inhibition in the dentate gyrus could contribute to the decline in amplitude of the depolarizing events. There is a slow component of synaptic inhibition in the dentate gyrus which is K^+-dependent and resistant to picrotoxin (Thalmann and Ayala 1982). We occasionally observed slow hyperpolarizations in DGC. Such inhibition, activated following the initial hyperactivity in the perforant path, although not obvious due to the very negative resting potentials in the cells, could have contributed to the reduction in amplitude of the later depolarizations by short-circuiting the membrane to some extent. Of course, in Mg^{++}-free medium, bicuculline-sensitive inhibition should also be intact in the dentate gyrus. However, such inhibition is often difficult to detect even under normal conditions (see Lambert and Jones

1990). This may not be surprising in view of the reluctance of the DGC to fire action potentials and thus activate recurrent interneurones. Therefore, it is unlikely that feedback $GABA_A$-mediated inhibition could contribute to the decline in amplitude of the events in DGC seen in Mg^{++}-free medium. A contribution of slowly-inactivating, intrinsic, inhibitory currents to the decline in the depolarizations could also be speculated upon.

The reflected depolarizations in DGC rarely gave rise to action potentials. This clearly suggests that the epileptiform activity arising in the MEC does not propagate beyond the dentate gyrus via the mossy fibres to CA3 and thence to CA1. Thus, the dentate gyrus may act as a barrier to the spread of epileptiform activity to the rest of the hippocampus. A similar situation may exist *in vivo*. Collins et al (1983) studied [^{14}C]-deoxyglucose metabolism in rat brain after activation of seizures in the MEC. Moderate seizures lasting less than 10 sec increased metabolism in the dentate gyrus but failed to affect it in other areas of the hippocampus. Very strong seizure activity in the MEC was required to elicit changes in metabolism in the hippocampus. Of course, it is possible that the propagation of epileptiform activity can occur via afferents other than the perforant path, such as the direct projections from the entorhinal area to both CA1 and CA3 (Witter et al. 1988; Steward and Scoville 1976; Wyss 1981).

Finally, whether these pharmacological observations have any relevance to the clinical picture in epilepsy is a matter of pure conjecture. If we assume that they do, then it might be speculated that a combination of NMDA and non-NMDA antagonists, or perhaps a more non-specific excitatory amino acid antagonist might be the most useful form of anticonvulsant.

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