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PROLONGED EPILEPTIFORM BURSTING INDUCED BY 0-Mg^{2+} IN RAT HIPPOCAMPAL SLICES DEPENDS ON GAP JUNCTIONAL COUPLING

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Abstract—The transition from brief interictal to prolonged seizure, or 'ictal', activity is a crucial event in epilepsy. *In vitro* slice models can mimic many phenomena observed in the electroencephalogram of patients, including transition from interictal to ictaform or seizure-like activity. In field potential recordings, three discharge types can be distinguished: (1) primary discharges making up the typical interictal burst, (2) secondary bursts, lasting several hundred milliseconds, and (3) tertiary discharges lasting for seconds, constituting the ictal series of bursts. The roles of chemical synapses in these classes of burst have been explored in detail. Here we test the hypothesis that gap junctions are necessary for the generation of secondary bursts.

In rat hippocampal slices, epileptiform activity was induced by exposure to 0-Mg^{2+}. Epileptiform discharges started in the CA3 subfield, and generally consisted of primary discharges followed by 4–13 secondary bursts. Three drugs that block gap junctions, halothane (5–10 mM), carbenoxolone (100 μM) and octanol (0.2–1.0 mM), abolished the secondary discharges, but left the primary bursts intact. The gap junction opener trimethylamine (10 mM) reversibly induced secondary and tertiary discharges. None of these agents altered intrinsic or synaptic properties of CA3 pyramidal cells at the doses used. Surgically isolating the CA3 subfield made secondary discharges disappear, and trimethylamine under these conditions was able to restore them.

We conclude that gap junctions can contribute to the prolongation of epileptiform discharges.

Key words: gap junctions, seizure, ictogenesis, hippocampus, magnesium.

The transition from brief primary to longer secondary and tertiary epileptic bursts is thought to be critical for ictogenesis (Swann et al., 1993; Traub et al., 1996). These three classes of burst discharge have most thoroughly been investigated and modelled in hippocampal slice preparations (Swann et al., 1993; Traub et al., 1994, 1995, 1996, 1999a; Borck and Jefferys, 1999). The mechanisms proposed for these three types of epileptiform bursting differ. The primary burst depends on the intrinsic properties of CA3 neurons (the interplay of non-uniformly distributed Na^+, Ca^{2+} and K^+ currents) and the recurrent excitatory synaptic connections between CA3 pyramidal cells (Miles and Wong, 1986; Traub et al., 1994, 1996). Secondary bursts, in turn, appear to require prolonged depolarizing dendritic currents, again interacting with intrinsic neuronal properties (Traub et al., 1993, 1996; Swann et al., 1993). The details of secondary epileptiform bursts depend on the model used. Secondary bursts have been attributed to *N*-methyl-D-aspartate (NMDA) receptor-mediated currents in the case of the 0-Mg^{2+} and picrotoxin models (Traub et al., 1993, 1994); local α-amino-3-hydroxy-5-methyl-4-isoxazole propionate (AMPA) receptor-mediated excitatory circuits in the neonatal penicillin model (Swann et al., 1993); and AMPA and depolarizing GABA-mediated recurrent excitation in the 4-aminopyridine model (Perreault and Avoli, 1992; Traub et al., 1995). Further candidate mechanisms for secondary and tertiary bursts include unspecific Ca^{2+}-activated inward currents (Caeser et al., 1993), metabotropic glutamate receptors (Taylor et al., 1995), local rises of the extracellular potassium concentration (Prince et al., 1973; Heinemann et al., 1977; Borck and Jefferys, 1999), and/or the occurrence of spontaneous ectopic spikes (Traub et al., 1996; Borck and Jefferys, 1999), which are thought to be of axonal origin in the case of epileptiform activity induced electrically or by 4-aminopyridine (Stasheff et al., 1993a,b; Traub et al., 1995).

Another intriguing mechanism facilitating fast synchronization involves electrotonic, i.e. gap junctional coupling of neurons. In the 0-Ca^{2+} model chemical synapses do not function, and epileptiform bursts are synchronized by non-synaptic mechanisms (Jefferys and Haas, 1982; Dudek et al., 1986; Jefferys, 1995). Gap junctional coupling plays a significant role in the syn-

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Abbreviations: ACSF, artificial cerebrospinal fluid; AMPA, α-amino-3-hydroxy-5-methyl-4-isoxazole propionate; DMSO, dimethylsulfoxide; NMDA, *N*-methyl-D-aspartate; PDS, paroxysmal depolarization shift; TriMA, trimethylamine.

chronization of 0-Ca^{2+} field bursts, most likely by facilitating ephaptic (or field effect) interactions (Perez-Velazquez et al., 1994; Valiante et al., 1995; Bikson et al., 1999). Computer simulations suggest that in models with intact synaptic transmission (e.g. 4-aminopyridine) a low density of gap junctions could lead to synchrony of hippocampal neuronal populations (Traub, 1995; Traub et al., 1999b). While gap junctions between neurons are generally thought to be rare in adults, there is histological and electrophysiological evidence for their existence in hippocampal pyramidal neurons, in both CA1 and CA3 in adult tissue (MacVicar and Dudek, 1981; Knowles et al., 1982; Dudek et al., 1986; Perez-Velazquez et al., 1994; Jefferys, 1995; Draguhn et al., 1998; Gladwell and Jefferys, 2001). There is some evidence, too, that chronic epileptic activity can promote functional gap junctions, at least in terms of increased dye coupling in chronic experimental models (Colling et al., 1996) and in clinical epilepsy (Cepeda et al., 1993). Furthermore, expression of connexin mRNA is increased in human neocortical tissue resected during epilepsy surgery (Naus et al., 1991). This raises the question whether electrotonic coupling among neurons can contribute to acute epileptiform activity. There is evidence in favor of such a role in epileptic bursts induced by low extracellular Ca^{2+} and perhaps also for repeated tetanic stimulation (Carlen et al., 2000). In the present paper, we address this issue by testing the influence of treatments that modulate gap junction on 0-Mg^{2+}-induced epileptiform activity in rat hippocampal slices, focussing in particular on the transition from primary to secondary bursts.

EXPERIMENTAL PROCEDURES

Transverse hippocampal slices (450 μm) were prepared from male Sprague–Dawley rats ($n = 27$, 90–350 g; Charles River, UK) under terminal anesthesia with 7.1 mg/kg ketamine and 0.4 mg/kg medetomidine. The minimum of rats was used, and all experiments conformed to the UK Animals (Scientific Procedures) Act of 1986. Slices were maintained in an interface-type chamber at 32–34°C in gassed (5% CO_2, 95% O_2) artificial cerebrospinal fluid (ACSF) containing (in mM): NaCl 125, $NaHCO_3$ 26, $CaCl_2$ 2, KCl 3, NaH_2PO_4 1.25, $MgCl_2$ 1, and glucose 10. In some experiments, the CA3 subfield was surgically dissected from the rest of the slice. A cut across the ends of the two blades of the dentate gyrus was made, using a shard of a scalpel, to leave the isolated crescent-shaped CA3 region, separated from the dentate area, the hilus, and the CA1 region. During the lesioning procedure, recording electrodes were lifted from the slices and then subsequently replaced at the same locations on the resulting minislices.

Electrophysiological recordings

Field and membrane potential recordings were obtained from stratum pyramidale of areas CA3 and CA1 using blunt glass micropipettes (1–2 MΩ) filled with ACSF placed onto the slice surface, and sharp microelectrodes (50–80 MΩ) filled with 2 M potassium methylsulfate. Intracellular recordings were accepted provided the resting membrane potential was more negative than −55 mV; mean resting levels were -65.4 ± 1.1 mV (CA3, $n = 22$) and -65.3 ± 1.6 mV (CA1, $n = 13$). A bipolar Nichrome wire stimulating electrode was used to stimulate Schaffer collaterals in stratum radiatum. Stimulation intensity was set at 2× the threshold intensity necessary to elicit population spikes.

Epileptiform activity and drug application

Epileptiform activity was elicited by omitting Mg^{2+} from the perfusate. In most cases, Mg^{2+} withdrawal lasted for at least 180 min before drug application. In experiments where only primary discharges were investigated, this period was reduced to ≤ 60 min.

Three drugs that block gap junctions were used: halothane (5 and 10 mM), octanol (200 μM and 1 mM), and carbenoxolone (100 μM; added from stock solution dissolved in dimethylsulfoxide, DMSO) (Perez-Velazquez et al., 1994; Ishimatsu and Williams, 1996; Draguhn et al., 1998). Halothane was dissolved in ACSF in the reservoir leading to the slice chamber, after which the gassing was stopped for the duration of the halothane application and the reservoir covered with Parafilm. The quoted dose of halothane is an upper limit because some of it will leave solution before the thin film of ACSF reaches the slice, but the conditions, and hence the losses, are equivalent in the experiments on epileptic and control tissues described below. The permeant weak base, trimethylamine (TriMA, 10 mM, substituting 10 mM NaCl in ACSF to keep osmolality constant), which increases intracellular pH, was used to open gap junctions (Perez-Velazquez et al., 1994). Drugs were added to the perfusate. Whenever DMSO was used as solvent, control experiments were made using the same concentration of DMSO, 0.1%, and revealed that the solvent had no effect on the activity recorded in this study.

Analysis and statistical evaluation

Potentials were evaluated regarding the appearance of secondary discharges as defined by Traub et al. (1996). All potentials appearing during the last 5 min under each experimental condition were quantified for statistical analysis. All values are expressed as means ± S.E.M., and differences were assessed using Student's *t*-test or Mann–Whitney rank sum test, as appropriate, with a significance criterion of $P < 0.05$.

RESULTS

Epileptiform activity

Omission of Mg^{2+} from the perfusate induced epileptiform potentials in all slices ($n = 37$) within 31 ± 2 min of perfusion. Initially the activity comprised primary bursts only; more complex, longer primary plus secondary bursts appeared progressively over the subsequent several hours (Fig. 1A). After 180 min the epileptiform discharges attained a stable repetition rate of 3.1 ± 0.3 min^{-1}. The percentage of slices that showed secondary bursts rose from 42% at 60 min to 77% after 180 min (these and other measures quantified over representative 5-min epochs). Over this period the proportion of bursts with secondary discharges in all slices rose from $21 \pm 10\%$ to $77 \pm 7\%$ ($P = 0.003$). Over the same period the mean number of secondary bursts averaged over all discharges changed significantly from 2.0 ± 0.8 to 6.6 ± 0.6 ($P = 0.004$; estimated from all discharges during a representative 5-min period in each of 37 slices). However, if slices did already generate secondary discharges at early stages, i.e. at 60 min, neither the percentage of discharges with secondary bursts ($50 \pm 17\%$) nor the shapes of the complex discharges (mean number of secondary bursts within complex discharges 5.5 ± 0.9) differed from later periods, i.e. at 180 min, indicating that once secondary discharges

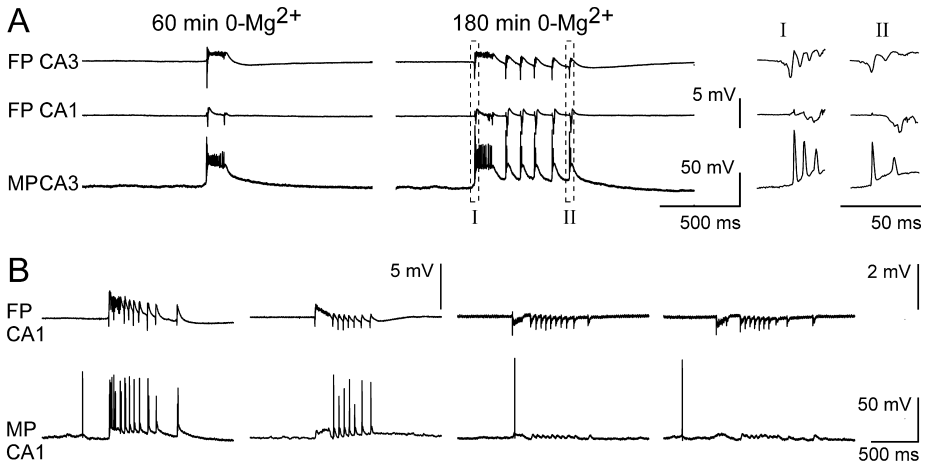

Fig. 1. Features of epileptiform discharges during progressive washout of Mg^{2+}. (A) Typical epileptiform bursts after 60 min (primary, interictal discharge) and 180 min (primary discharge followed by secondary afterdischarges or bursts) of Mg^{2+} withdrawal. Field (FP) and membrane potential (MP) recordings from CA1 and CA3 subfields of a rat hippocampal slice. Insets I, II (dotted lines): Onset of primary (I) and secondary (II) burst showing that CA3 initiates activity. (B) Typical membrane potential changes occurring in CA1 neurons during epileptiform field bursts. Field and membrane potential recordings from CA1 subfields of different rat hippocampal slices 120–180 min after Mg^{2+} withdrawal. CA1 neurons generate PDS, sequences of excitatory postsynaptic potentials (EPSP) and action potentials, single action potentials and EPSP, or EPSP alone.

are established, they change little over periods of at least 3 h.

CA3 initiates primary and secondary bursts

To determine which neuronal population was responsible for pacing epileptiform potentials, we first looked at the discharge patterns of CA3 and CA1 neurons. In CA3 neurons ($n = 22$), the typical discharge pattern, synchronous with the field epileptiform potential, was paroxysmal depolarization shift (PDS), with secondary bursts riding on a prolonged depolarization (Fig. 1). The same pattern occurred whether or not the neurons were intrinsically bursting (approximately 1/3) or regularly spiking (approximately 2/3) as judged from firing patterns produced by 0.3–1.0-nA, 200-ms current injections (Connors and Gutnick, 1990). Each of the discharges was initiated in the CA3 subfield (Fig. 1AI, II), with CA1 always following, confirming that CA3 represents the pacemaker region within the hippocampal formation (Mody et al., 1987; Colom and Saggau, 1994; Köhling et al., 1994). This is further supported by recordings from CA1 pyramidal layer neurons ($n = 13$) during population epileptic discharges recorded in CA3, which showed a variety of membrane potential fluctuations during the field epileptiform potentials (Fig. 1B). Of these CA1 cells, only one was an intrinsically bursting neuron. Typical PDS could be observed in seven neurons; the intrinsically bursting neuron belonged to this group (Fig. 1B). The rest showed either excitatory postsynaptic potentials followed or preceded by single action potentials, or excitatory postsynaptic potentials alone (Fig. 1B).

Table 1. Changes in intrinsic and synaptic properties of CA3 pyramidal cells caused by drugs affecting gap junctions

Drug	EPSP slope mV/ms	IPSP at 30 ms	V_m (mV)	R_{in} (MΩ)	Threshold (mV)	AP discharge†
Halothane 5 mM	−0.03 ± 0.05 (12)	0.162 ± 0.05	−1.1 ± 0.7 (16)	0.1 ± 0.2	1 ± 0.7	−1.0 [2] (5)
Halothane 10 mM	−0.05 ± 0.02 (6)	0.26 ± 0.07	−4.6 ± 3.7 (7)	3 ± 1.9	2 ± 1.2	0.5 [1] (2)
Octanol 1 mM	−0.08 ± 0.05 (3)	0 ± 0.05	−0.1 ± 0.05 (3)	0 ± 0.08	1 ± 1.1	−1.0 [0] (3)
Carbenoxolone 100 µM	−0.09 ± 0.05 (6)	−0.18 ± 0.12	3.6 ± 2.6 (6)	−2.6 ± 2.3	0 ± 0	0 [4] (4)
TriMA 10 mM	−0.07 ± 0.06 (5)	−0.22 ± 0.16	1.2 ± 0.9 (6)	−3.2 ± 2.6	0 ± 0	−1.0 [3] (5)

All the gap junction blockers were effective as judged by their ability to reverse alkalinization enhanced antidromic activation of CA3 (Gladwell and Jefferys, 2001). Number of replicates is given in parentheses: for the excitatory postsynaptic potential (EPSP) column also applies to the inhibitory postsynaptic potential (IPSP); for the membrane potential column (V_m) also applies to input resistance (R_{in}) and action potential (AP) threshold; and for the AP discharge applies to that column only. Data are means ± standard error, except for AP discharge where they are median [range]. Change in EPSP slope was measured from a mean control value of 1.06 to 1.35 mV/ms; change in IPSP amplitude measured at 30 ms was from a mean control value of 3.7 to 4.45 mV. All the drugs tested here had no significant effect on EPSP slope, IPSP amplitude, V_m, R_{in} and threshold as tested with paired t-tests ($P > 0.05$). The change in 'AP discharge†' is for the number of action potentials evoked by a 100-ms, 1-nA depolarizing pulse; none of the drugs had a significant effect on action potential number (Mann–Whitney rank sum test, $P \geq 0.383$).

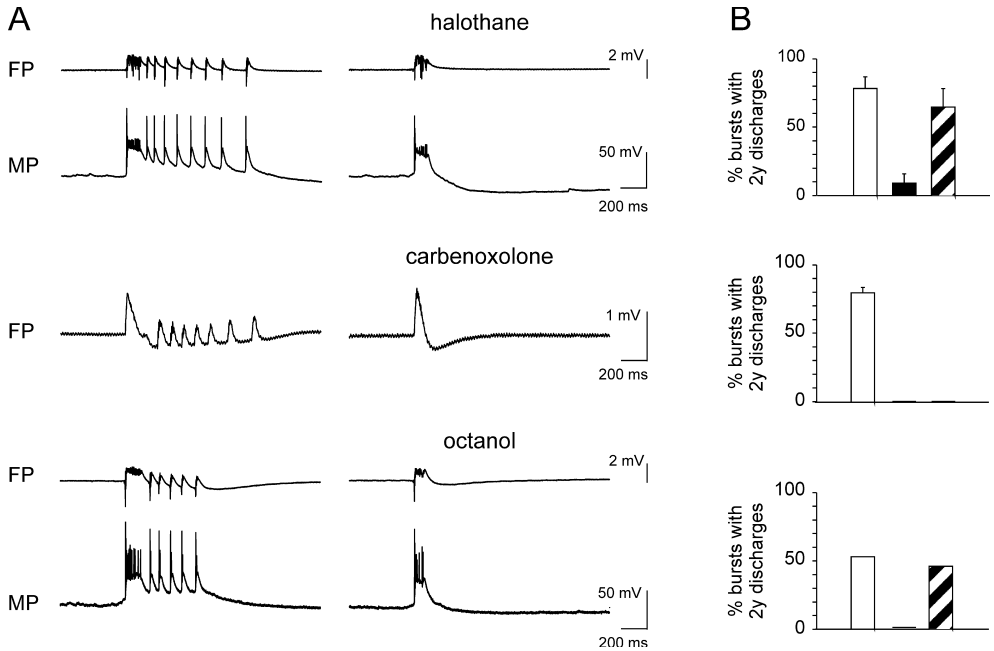

Fig. 2. Effect of gap junction blockade on secondary discharges. (A) Typical epileptiform potentials consisting of primary and secondary bursts after >120 min of Mg^{2+} withdrawal in hippocampal slice preparations (left panel), and during gap junction blockade by halothane (5 and 10 mM, applied for 15 min, $n=6$), carbenoxolone (100 μM, 60 min, $n=3$) and octanol (200 μM, 10 min, $n=3$) with loss of secondary bursts (right panel). Field (FP) and membrane potential (MP) recordings from CA3 stratum pyramidale. (B) Bar charts showing the mean relative number of bursts with afterdischarges before (white bars), during (black bars) and after (striped bars) application of the drugs. Data for both concentrations of halothane are pooled, because no differences were found between them.

Together these findings suggest that 0-Mg^{2+}-induced epileptiform activity is regularly initiated by neurons in the CA3 population, and that the synchronizing mechanisms responsible for primary and secondary epileptic bursts should operate in CA3 neuronal circuits.

Effect of gap junction blockade on epileptiform potentials

To elucidate the role of gap junctions in the generation of secondary discharges, the action of gap junction blockers halothane, carbenoxolone and octanol (Perez-Velazquez et al., 1994; Ishimatsu and Williams, 1996; Strata et al., 1997; Draguhn et al., 1998; Zhang et al., 1998) on epileptiform potentials was tested. None of these drugs is particularly selective. However, their only common, overlapping, effect is the block of gap junctional coupling (Draguhn et al., 1998). We therefore chose these three very different drugs, on the basis that a congruent effect would most likely be due to their one common mechanism.

Halothane (5 and 10 mM, $n=2$ and 4 respectively) substantially reduced, and in three of the four cases at 10 mM totally blocked, the secondary discharges within 15–20 min of application (Fig. 2). Pooling the data, the percentage of bursts with secondary discharges dropped from $78\pm9\%$ to $9\pm7\%$ ($P<0.001$), and the number of secondary discharges within each burst decreased from 6.5 ± 0.7 to 2.6 ± 1.6 ($P=0.05$). After wash, both measures recovered within 30–60 min to $65\pm14\%$ and 6 ± 0.9, respectively (Fig. 2). Primary discharges continued to be generated during halothane perfusion, usually at a 1.3–2 times increased rate (Fig. 2). Experiments where no secondary discharges had appeared, during the early stage of Mg^{2+} withdrawal, were unaffected by halothane ($n=2$, data not shown).

The actions of carbenoxolone (100 μM) and octanol (200 μM) were qualitatively similar to those observed with halothane. Carbenoxolone ($n=3$) specifically abolished secondary discharges within 60–100 min of application, without affecting primary bursts. In this experiment $80\pm4\%$ of the total bursts had secondary discharges, with a mean of 6.7 ± 1.1 per burst (Fig. 2). This effect was irreversible, even after prolonged periods of wash (>90 min), presumably due to the high lipophilicity of this drug. Octanol blocked all activity in two cases, most likely due to its action on NMDA receptors (McLarnon et al., 1991). In one slice, however, it resembled halothane in that it reversibly blocked secondary discharges while leaving primary bursts intact, but when the dose increased from 200 μM to 1 mM it abolished all activity in this case too.

The gap junction blockers currently available have poor selectivity. In order to determine whether those used here affected neuronal excitability or synaptic function in ways that could affect the generation of secondary bursts, we assessed their effects on the responses of individual CA3 neurons to current injection, and to synaptic responses evoked in CA3 by mossy fiber stimulation (Table 1). Halothane (5 mM), octanol (1 mM) and carbenoxolone (100 μM) had no effect on resting potentials, input resistance, action potential threshold, number of action potentials triggered by 1-nA depolarizing current pulses, or evoked synaptic responses (Table 1; the small hyperpolarization of the resting potential seen with the

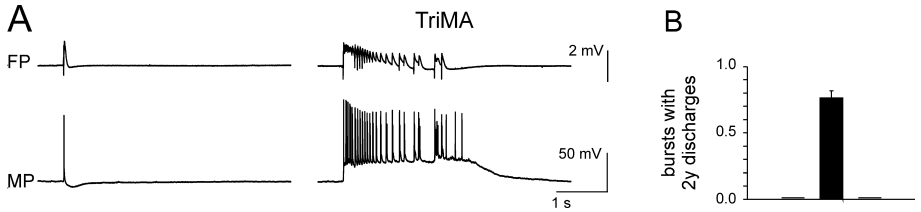

Fig. 3. Effect of gap junction opening on epileptiform discharges. (A) Typical epileptiform potentials consisting of primary bursts only after <60 min of Mg^{2+} withdrawal in hippocampal slice preparation (left panel), and during application of the gap junction opener TriMA (10 mM, 30–60 min, $n=4$) with appearance of secondary bursts (right panel). Field (FP) and membrane potential (MP) recordings from CA3 stratum pyramidale. (B) Bar charts showing the mean relative number of bursts with afterdischarges before (left hand bar, ≈ 0), during (middle black bar) and >60 min after washout (right hand bar, ≈ 0) application of the drug.

higher, 10 mM, dose of halothane was not significant). These results suggest that these drugs did not affect neuronal excitability at the doses we used here.

Effect of gap junction opening on epileptiform potentials

If blockade of gap junctions abolishes secondary discharges, increasing their open probability under conditions when only primary bursts are generated should facilitate the appearance of secondary bursts. Therefore, we tested the effect of TriMA, which opens gap junctions, on epileptiform potentials lacking secondary bursts, after <60 min of 0-Mg^{2+} perfusion. In all slices, TriMA (10 mM, $n=4$) reversibly induced secondary, and in some cases even full blown tertiary discharges, within 30–60 min (Fig. 3). Under these conditions, $76\pm6\%$ of the epileptiform potentials had secondary bursts, and the number of bursts within each epileptiform potential was 7.1 ± 3.2. This is comparable with the situation after prolonged Mg^{2+} withdrawal (Fig. 3), but significantly greater than expected at this time in the absence of TriMA (incidence of slices with secondary bursts 42%, and a mean incidence of secondary bursts of $21\pm10\%$). On washout the incidence of secondary bursts dropped to zero, below that expected for the length of time in 0-Mg^{2+}; this is most likely due to rebound acidification (Perez-Velazquez et al., 1994). When secondary discharges, in turn, were already established, TriMA had no effect or slightly prolonged the epileptiform potentials ($n=2$, data not shown).

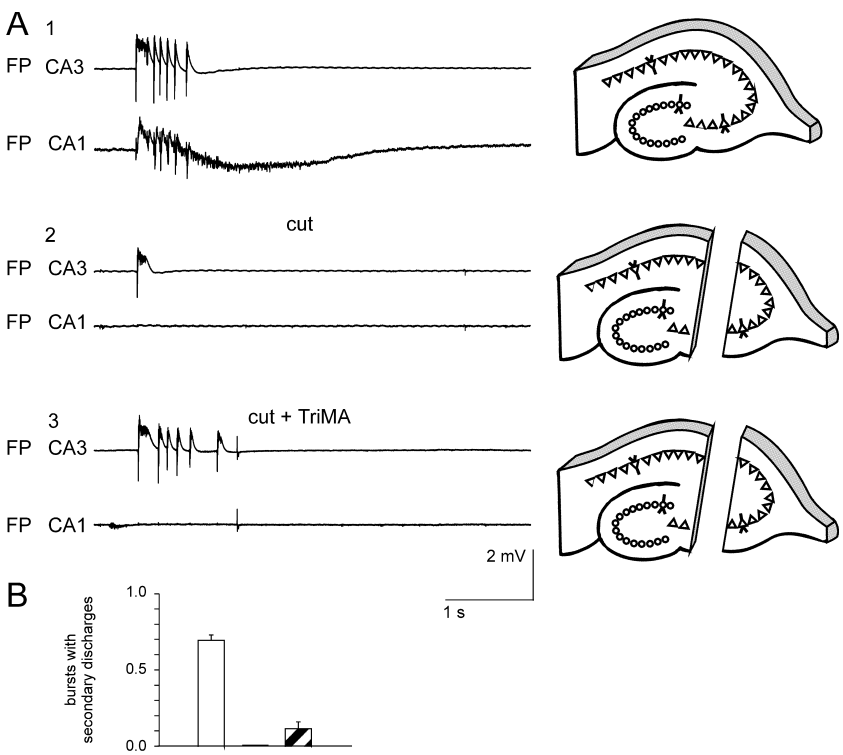

Fig. 4. CA3 minislices support secondary discharges if gap junctions are opened. (A) Typical epileptiform potentials consisting of primary and secondary bursts after >180 min of Mg^{2+} withdrawal in intact hippocampal slice preparation (a), with loss of secondary bursts in minislices obtained by separating the CA3 region from the rest of the slice (b), and with reappearance of secondary discharges during application of the gap junction opener TriMA (10 mM, 30 min, $n=3$) (c). Field potential recordings (FP) from CA3 and CA1 stratum pyramidale. (B) Bar chart showing the mean relative number of bursts with afterdischarges before the cut (white bar), after the cut (black bar, center, ≈ 0) and with application of TriMA after cut (striped bar).

Gap junctional coupling alters the minimal tissue volume needed to generate prolonged bursts

Whereas in juvenile hippocampal slices, CA3 minislices can support prolonged epileptiform potentials (Smith et al., 1995; Gomez-Di Cesare et al., 1996), adult tissue required larger minislices which include the hilus (Borck and Jefferys, 1999). The present paper extends to the 0-Mg^{2+} model the observations of Borck and Jefferys (1999) on the bicuculline model; CA3 minislices were not able to support prolonged discharges: after dividing the slice, only primary bursts continued to be generated in CA3, and activity ceased completely in CA1 (Fig. 4). Application of the gap junction opening agent, TriMA (10 mM, $n = 3$), to the CA3 minislices restored secondary discharges, although with lower probability. Before dissection, $70 \pm 3\%$ of the epileptiform potentials displayed secondary discharges, with an average number of 10.2 ± 2.6 afterdischarges per burst; after dissection and with TriMA, $12 \pm 4\%$ of epileptiform potentials were prolonged, with 6.5 ± 3.3 afterdischarges on average (Fig. 4).

DISCUSSION

In the present paper, we have shown that drugs which block gap junctions are able to convert prolonged discharges into single interictal bursts. Conversely, intracellular alkalinization by TriMA, a manipulation which opens gap junctions (Perez-Velazquez et al., 1994), elicits prolonged discharges under conditions when only primary bursts occur.

Different mechanisms generate primary and secondary discharges

The data presented here confirm the notion that primary and secondary discharges are governed by different mechanisms. Primary discharges are considered to be triggered by the spontaneous burst, shaped by the intrinsic neuronal properties (Traub and Wong, 1982; Traub et al., 1993, 1996). Indeed, single neurons in the CA3 subfield have been reported to entrain the population burst (Miles and Wong, 1983; Smith et al., 1995; Gomez-Di Cesare et al., 1996). The spread of bursting then occurs due to recurrent excitatory network connections dependent on synaptic transmission mediated by AMPA receptors (4-aminopyridine model) (Perreault and Avoli, 1992), or on NMDA receptors (0-Mg^{2+} model as used here) (Mody et al., 1987; Traub et al., 1994), or both (picrotoxin and bicuculline models) (Borck and Jefferys, 1999; Psarropoulou and Descombes, 1999). The primary burst thus arises spontaneously and is synchronized synaptically, apparently without any requirement for gap junctions, because gap junction blockers do not impede their generation, in marked contrast with their effects on secondary bursts reported here.

Currently available drugs that affect gap junctions lack selectivity. Their specific actions described here on secondary, and not primary, bursts argue that they did not affect synaptic interactions between CA3 pyramidal cells. We also showed explicitly that evoked synaptic responses and intrinsic neuronal properties were not affected by halothane, carbenoxolone or octanol at the doses used to modulate gap junctions. Another gap junction modulator, heptanol, has previously been shown not to affect similar measures of intrinsic neuronal properties (Bikson et al., 1999). Octanol produced no 'substantial decrease' in excitatory postsynaptic currents or inhibitory postsynaptic currents (Zhang et al., 1998), and carbenoxolone produced no change in field excitatory postsynaptic potentials (Ross et al., 2000). We have found effects of gap junction blockers on antidromic responses of CA3 pyramidal cells (Gladwell and Jefferys, 2001), but we interpret these results as evidence supporting the prediction of a low incidence of gap junctions between pyramidal cell axons rather than on the excitability of the neurons themselves (Draguhn et al., 1998; Traub et al., 1999b).

Secondary discharges are triggered by primary bursts, but appear more fragile. They depend on sustained depolarization activating additional mechanisms (Perreault and Avoli, 1992; Traub et al., 1993 1994, 1995, 1996; Swann et al., 1993). Up to now, evidence for electrotonic neuronal coupling as a synchronizing mechanism derives mainly from epilepsy models lacking synaptic transmission (Dudek et al., 1986; Perez-Velazquez et al., 1994; Jefferys, 1995). The evidence in the present paper suggests that gap junctions contribute to prolonging, but not initiating, synchronization in the 0-Mg^{2+} model. In contrast gap junctions appear necessary for the initiation of synchronous bursts in the non-synaptic 0-Ca^{2+} model (Carlen et al., 2000). Gap junctions may thus be complementary to the synchronization mediated by chemical synaptic transmission in the 0-Mg^{2+}, and perhaps other, models.

Functional significance of gap junctions for secondary discharges

The incidence of gap junctions in adult brain tissue is reported to be lower than in juvenile (Rörig et al., 1996). Nevertheless there is evidence for gap junctions between neurons in adult tissue (MacVicar and Dudek, 1981; Knowles et al., 1982; Dudek et al., 1986; Perez-Velazquez et al., 1994; Jefferys, 1995) and this can be functionally significant. Modelling studies indicate that neuronal synchronization can be mediated by low densities of gap junctions either between dendrites, as long as these dendrites are excitable (Traub, 1995), or between axons of pyramidal cells (Draguhn et al., 1998; Traub et al., 1999b; Traub and Bibbig, 2000). Furthermore, electrotonic coupling could be promoted during epileptogenesis (Perez-Velazquez et al., 1994; Colling et al., 1996). pH changes found during intense neuronal activity (Chesler and Kaila, 1992), neuronal damage (Murphy et al., 1983), and hyperventilation [which can be used to provoke seizures in some patients (Miley and Forster, 1977)] will promote electrotonic coupling, which may explain the increased risk of seizures.

The relatively slow development of the secondary bursts in the present paper, along with their sensitivity to gap junction blockers, could reflect the induction of gap junctions by such mechanisms, although other mechanisms, such as erosion of inhibition, have been implicated too (Whittington et al., 1995). The ability of TriMA to produce secondary bursts in minislices not normally capable of sustaining them lends further support to a role for gap junctions in epileptogenesis.

Localization of gap junctions

Where are the gap junctions located? They have been well documented between glia, where they have been implicated in spatial buffering of potassium ions (McKhann et al., 1997); but blocking them should [if anything (Gardner-Medwin, 1983)] weaken spatial buffering, not obviously an anti-epileptic action. However, in models dependent on K^+ fluxes, such as in low Ca^{2+}, an argument can be made that blocking gap junctions can be anti-epileptic (Bikson et al., 1999). Glial gap junctions are also implicated in the propagation of intracellular Ca^{2+} waves, which can lead to excitation of neighboring neurons (Parpura et al., 1994; Nedergaard, 1994). This effect is stronger than normal in brain tissue resected during epilepsy surgery (Lee et al., 1995). Ca^{2+} waves could be pro-epileptic, but their propagation is slow (Basarsky et al., 1998) compared with the time course of the secondary bursts.

The evidence that gap junctions occur between pyramidal cells is directly relevant to the issue of epileptic synchronization (MacVicar and Dudek, 1981; Knowles et al., 1982; Dudek et al., 1986; Jefferys, 1995). The presence of gap junctions between pyramidal cell axons has been proposed to explain certain fast collective oscillations in the hippocampus (Draguhn et al., 1998; Traub et al., 1999b; Gladwell and Jefferys, 2001). Simulations (Traub et al., 1999b), supported by some experimental evidence (Empson et al., 1998), suggest that such gap junctions have an impact on epileptic bursts synchronized by chemical synapses, but those studies were restricted to the primary burst, and not to the prolongation into secondary bursts that is the subject of the present paper.

There is good evidence that gap junctions occur between particular classes of interneurons (Katsumaru et al., 1988; Ribak et al., 1993; Freund and Buzsaki, 1996; Fukuda and Kosaka, 2000) and that such coupling might be functionally significant both in juvenile tissue (Gomez-Di Cesare et al., 1996; Strata et al., 1997) and also in adult slices (Zhang et al., 1998). They provide an alternative, if less obvious, means of prolonging epileptiform discharges. Dye-coupled interneurons have been reported to be responsible for slow (< 1 Hz) oscillations (Zhang et al., 1998) and even to entrain epileptiform discharges (Gomez-Di Cesare et al., 1996). In the latter case the authors speculate that the entrainment of activity results from a depolarizing action of GABA, which is also thought to underlie so-called giant depolarizing potentials mediated by an electrotonically coupled interneuronal network in the hilus of juvenile tissue (Strata et al., 1997) and in the adult hippocampus exposed to 4-aminopyridine (Michelson and Wong, 1991). Recently we demonstrated that $GABA_A$ receptor-mediated depolarization can prolong epileptiform bursts in 0-Mg^{2+} on the basis of sensitivity to ethoxyzolamide (Köhling et al., 2000); this carbonic anhydrase inhibitor blocks depolarizing GABA responses.

We conclude that gap junctions play a key role in prolonging epileptiform activity beyond the primary or interictal burst. Until more selective methods of blocking gap junctions become available, we cannot definitively identify the location of those responsible for the effects reported here.

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