Journal of Physiology (1993), **465**, *pp.* 595–614
With 8 figures
Printed in Great Britain

SYNAPTIC INHIBITION IN PRIMARY AND SECONDARY CHRONIC EPILEPTIC FOCI INDUCED BY INTRAHIPPOCAMPAL TETANUS TOXIN IN THE RAT

BY R. M. EMPSON AND J. G. R. JEFFERYS

From the Department of Physiology and Biophysics, St Mary's Hospital Medical School, Imperial College, Norfolk Place, London W2 1PG

(*Received 25 August 1992*)

SUMMARY

1. Injecting twelve mouse minimum lethal doses of tetanus toxin into one hippocampus of a rat leads to the development of chronic epileptic foci in both hippocampi. These generate intermittent epileptic discharges for 6–8 weeks. Here we compare GABAergic inhibition, 10–18 days after injection, in slices prepared from the injected and contralateral hippocampi (respectively the primary and the secondary or 'mirror' foci), using both neurochemical and electrophysiological methods.

2. Epileptic activity was recorded from slices of both hippocampi from all tetanus toxin-injected rats. Evoked epileptic discharges were similar on the two sides, but spontaneous epileptic discharges were more common contralaterally.

3. Ca^{2+}-dependent, K^+-stimulated (synaptic) release of radiolabelled GABA was depressed in slices from the injected hippocampus, compared with vehicle-injected controls. In contrast, slices from the contralateral hippocampus had normal levels of Ca^{2+}-dependent, K^+-stimulated GABA release, even though adjacent slices were epileptogenic.

4. Intracellular recordings revealed that both fast and slow stimulus-evoked inhibitory postsynaptic potentials (IPSPs) were abolished in CA3 pyramidal cells in the primary focus. In the secondary focus, however, fast IPSPs were seen in seven of twenty-five cells, and slow IPSPs were seen in all cells if the stimulus was strong enough.

5. Monosynaptic IPSPs were isolated pharmacologically by blocking glutamatergic excitatory postsynaptic potentials (EPSPs) with 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX) and D(−)-2-amino-5-phosphopentanoic acid (AP-5). No monosynaptic IPSPs were uncovered in cells from the primary focus at any stimulus strength. Monosynaptic IPSPs were evoked in all cells from both the secondary focus and control slices. The estimated conductances of monosynaptic fast IPSPs were similar in cells from the secondary focus and from the controls, although the former required twice the stimulus strength.

6. Slow IPSPs were found in the secondary focus and in controls, but not in the primary focus. They were sensitive to 3-amino-2-(4-chlorophenyl)-2-hydroxy-propylsulphonic acid (2-OH saclofen). The estimated conductances of slow IPSPs

evoked by weak stimuli in the secondary focus were much smaller than in the controls. However, stimuli that could trigger epileptic discharges in the secondary focus, evoked 2-OH saclofen-sensitive slow IPSPs with estimated conductances approaching the controls. This marked increase in the slow IPSP did not occur when EPSPs, and epileptic bursts, were blocked with CNQX and AP-5, suggesting that a strong barrage of excitation is needed to generate full-sized slow IPSPs in the secondary focus.

7. The chronic epileptic foci induced by tetanus toxin in the injected and contralateral hippocampi have distinct mechanisms. The ipsilateral focus behaved as expected from the acute actions of tetanus toxin; the synaptic release of GABA was disrupted and IPSPs were abolished, providing a probable mechanism for epileptogenesis. In the secondary focus, however, the synaptic release of GABA was unimpaired. Here the cellular deficit appears to be in the synaptic excitation and intrinsic excitability of the inhibitory interneurones.

INTRODUCTION

Epileptic discharges represent a pathological extreme of neuronal synchronization; understanding the origins of this synchronization provides insights into both the clinical condition and the ways in which populations of neurones interact (Wong, Traub & Miles, 1986; Jefferys, 1990). The acute effects of convulsant drugs have revealed much about the cellular mechanisms of epilepsy. Chronic models not only provide closer parallels with clinical epilepsies but also provide insights into long-term 'plastic' changes in neural function induced by recurrent episodes of excessive and hypersynchronous neuronal activity (Jefferys, 1990; Brener, Chagnac-Amitai, Jefferys & Gutnick, 1991; Najlerahim, Williams, Pearson & Jefferys, 1992).

Intracerebral tetanus toxin has long been known to be epileptogenic (Roux & Borrel, 1898). Low doses injected into the hippocampus lead to epileptic discharges, which recur spontaneously for 6–8 weeks (Mellanby, George, Robinson & Thompson, 1977; Brace, Jefferys & Mellanby, 1985; Hawkins & Mellanby, 1987), and cause minimal mortality and tissue damage (Mellanby *et al.* 1977; Jefferys, Evans, Hughes & Williams, 1992). This time course contrasts with the clearance of the toxin from neural tissue. It has a half-life of the order of days in tissue culture (Habig, Bigalke, Bergey, Neale, Hardegree & Nelson, 1986), and only 1·5% is detectable 9 days after intrahippocampal injection (Mellanby, 1989). Tetanus toxin is an exotoxin secreted by *Clostridium tetani* which has very potent and specific actions on the nervous system. The toxin blocks exocytosis in a variety of preparations acutely (Bevan & Wendon, 1984; Penner, Neher & Dryer, 1986; Bittner & Holz, 1988), and has partial specificity for inhibitory synapses (Bigalke, Heller, Bizzini & Habermann, 1981; Collingridge, Thompson, Davies & Mellanby, 1981; Bergey, Macdonald, Habig, Hardegree & Nelson, 1983). Studies of epileptogenic hippocampi 2 weeks after bilateral injection of low doses of tetanus toxin have shown that both GABA exocytosis and inhibitory postsynaptic potentials (IPSPs) were depressed (Jefferys *et al.* 1991; Jordan & Jefferys, 1992). Recently we have reported the development of an independent secondary 'mirror' focus in the uninjected hippocampus following unilateral injection of toxin (Jefferys & Empson, 1990). While the toxin is known to be transported along axons (Schwab,

Agid, Glowinski & Thoenen, 1977; Habermann & Erdmann, 1978), the amount that does so after such intrahippocampal injections is below the threshold for detection (Mellanby, 1989). Here we compare inhibitory function in the primary and secondary epileptic foci to determine whether they share common basic epileptic mechanisms.

METHODS

Stereotaxic injections

Tetanus toxin was a gift from Wellcome Biotech (Beckenham, UK), it was dissolved under sterile conditions in 0·1 M sodium phosphate buffer containing 0·85 % NaCl and 0·2 % bovine serum albumin (Sigma, UK). For control injections the identical sterile buffer was used without the addition of the tetanus toxin.

Male Sprague–Dawley rats (Harlen Olac, Bicester, UK) were anaesthetized with a mixture of Hypnoval and Hypnorm (Roche, UK and Janssen, UK respectively) to final doses of 4·1 mg kg^{-1} midazolam HCl (Hypnoval), 8·2 mg kg^{-1} fluanesone and 0·26 mg kg^{-1} fentanyl citrate. Twelve mouse minimum lethal doses (MLD) of toxin were injected into the hippocampus using a Hamilton 1 μl 7101N syringe. Stereotaxic co-ordinates were 2·8 mm caudal to bregma, 3·5 mm lateral of the midline and 3·5 mm below the cortical surface. This corresponds to a site in the dorsal hippocampus near the rostral end of the hippocampal fissure, equidistant from an arc described by the rostral end of the CA3 pyramidal layer. The needle was always positioned with its bevelled orifice pointing caudally. The toxin was injected continuously over a period of 2 min and the needle was left in position for 6 min to prevent leakage of toxin up the needle track. Control animals were injected in an identical manner with sterile vehicle buffer only. After injection the wound was cleaned and sutured, and the animal allowed to recover. The animals were housed three to four to a cage, with toxin- and control-injected animals kept separately.

Observation of animals

The animals were checked daily; out of a total of fifty-nine animals, full-blown motor seizures were witnessed in fourteen. These consisted of rearing and falling with forelimb myoclonus. 'Epileptic behaviours', such as absence behaviour, teeth gnashing and aggression were usually seen during a 10 min period of observation before slicing.

Preparation of slices

Rats were stunned and killed by cervical dislocation, the brain was then rapidly removed from the skull and the two hemispheres separated by cutting down the midline. The hemispheres were placed cut side down on to the stage of a Vibroslice (Campden Instruments, Loughborough, UK), secured with cyanoacrylate glue and immersed in ice-cold, oxygenated artificial cerebrospinal fluid (ACSF) containing (mM): 135 NaCl, 16 $NaHCO_3$, 3 KCl, 2 $CaCl_2$, 1·25 NaH_2PO_4, 1 $MgCl_2$, and 10 glucose. Hippocampal slices 400 μm thick were used for electrophysiology; these were alternated with 300 μm slices when GABA release was also measured.

Release of radiolabelled GABA

Two 300 μm slices were taken from each hemisphere (i.e. injected and contralateral) of each rat. Each experiment used two matched rats, one injected with toxin, the other with vehicle; the order of preparation alternated between successive experiments. The slices from each rat were placed immediately in 2 ml of ACSF at 34 °C containing 0·4 μM tetrodotoxin and 100 μM amino-oxyacetate, respectively to prevent spontaneous activity and GABA metabolism to glutamate (Szerb, Ross & Gurevich, 1981). When all eight slices were ready, 2·5 μCi of [^{3}H]GABA (Amersham, UK) was added per 2 ml ACSF, and the slices were incubated for 15 min at 34 °C. Then each slice was placed in a separate 1 ml perfusion chamber maintained at 34 °C. The order in which the slices were placed in the chambers was randomized between experiments.

Each chamber was perfused at 0·5 ml min^{-1} with a succession of ACSFs which contained the following: 3 mM K^+ with 0 mM Ca^{2+}; 30 mM K^+ with 0 mM Ca^{2+}; 30 mM K^+ with 2 mM Ca^{2+}; and all contained 100 μM amino-oxyacetic acid and 0·4 μM tetrodotoxin. The perfusate was discarded for the first 20 min in order to wash off excess radiolabel. Samples of perfusate were then collected over consecutive 2 min periods. The first 10 min following the wash measured the basal release prior to stimulation. From 10 to 24 min the ACSF contained 30 mM K^+ and 0 mM Ca^{2+}. From 24

to 46 min the ACSF contained 30 mM K^+ and 2 mM Ca^{2+}. After a total of 46 min the ACSF was returned to its original composition (with 3 mM K^+ and 0 mM Ca^{2+}) for a further 10 min to check that release returned to basal levels. At the end of the experiment the slices were collected and disrupted mechanically using a Whirlimixer to allow remaining radiolabel to be counted. All samples were then treated with 4 ml of Ecoscint (Canberra Packard, Pangbourne, UK) scintillation fluid and counted on a Tricarb scintillation counter (Canberra Packard) incorporating a quench curve for tritium at 37–40 % efficiency. The raw counts were transformed to express the release of the radiolabel over each 2 min sample period as a fraction of the amount of label left in the tissue at that time using a spreadsheet running under RS/1 (BBN Inc, Cambridge, MA, USA). This was termed the fractional release.

Electrophysiology

The 400 μm slices were transferred to a recording chamber where the slices were maintained at 34 °C on a double layer of lens tissue and perfused with warm oxygenated ACSF at a flow rate of approximately 0·2 ml min^{-1}. The upper surface of the slices was kept moist and oxygenated by passing warm, water-saturated 95 % O_2–5 % CO_2 over the surface of the slices. Slices were placed in the chamber so that their position along the dorsal hippocampus could be identified within the range 0·7 mm lateral to 1·2 mm medial of the injection track, or its homotopic point. Beyond this range parasagital slices ceased to be transverse to the hippocampus. At least 1 h elapsed before recording started. Extracellular recordings were made from the CA1 and CA3 pyramidal layers of the hippocampus. Intracellular recordings were made from the CA3b/c sub-area of the pyramidal layer. Extracellular recording electrodes were filled with 3 M NaCl and had a tip resistance of 2–10 MΩ. Intracellular electrodes were filled with 4 M potassium acetate buffered to pH 7·1 and had tip resistances of 70–150 MΩ. Glass-coated sharpened monopolar tungsten electrodes positioned in stratum radiatum were used to stimulate the slices. Recordings were made using an Axoprobe (Axon Instruments, Burlingham, CA, USA), Digitimer amplifiers and filters, Racal Store 4 FM tape recorder, and a 1401 laboratory computer system running SIGAVG (Cambridge Electronic Design, UK).

Evoked responses were recorded from the pyramidal layer in the subregions of CA3, following stimulation of afferents in stratum radiatum at less than once every 15 s. Stimuli varied between 2 and 15 V amplitude and were 0·02 ms in duration. A few slices were excluded from both toxin and control groups because they failed to produce population spikes greater than 1·5 mV, and were considered not to be healthy.

Cells were only used if their resting potential was greater than −50 mV, if their input resistance was greater than 30 MΩ, if action potentials were of an amplitude greater than 70 mV and if they were impaled long enough to record a full voltage relationship using both positive and negative current injection. Following impalement, cells were left for at least 15 min before recording. A standard protocol was observed for each cell. The electrical stimulus required to generate action potentials was determined. A stimulus of 90–95 % of this threshold value was used to evoke synaptic responses. Current pulses were injected into the cell during the period from 200 ms before the stimulus to 500–600 ms after it, in order to measure the relationship of both fast and slow IPSPs with membrane potential.

Monosynaptic IPSPs were isolated by the application of CNQX and AP-5 (Tocris Neuramin, UK) in the bath (20 and 40 μM respectively) or by pressure ejection from a blunt-ended glass pipette placed in stratum radiatum (200 and 400 μM respectively; 45–60 kPa for 0·5 s; Medical Systems Corp., Greenvale, NY, USA). The stimulating electrode remained in stratum radiatum while the stimulus strength was increased until maximal IPSPs were elicited. In control cells this turned out to be the same as the 90–95 % of threshold stimulus used when excitation was intact ($P = 0{\cdot}15$). Bicuculline methiodide (Sigma) and 2-OH saclofen (Tocris Neuramin) were also applied to cells from blunt pressure-ejection pipettes at concentrations of 2 and 5 mM respectively.

Estimation of conductance of postsynaptic potentials

Conductances were estimated from the individual gradient of the voltage dependence of the synaptic potential amplitude and the input resistance of the cell (Miles, 1990), using the following equation derived from Ginsborg (1973):

$$g = \frac{-M}{(1+M)R_m},$$

where g is the conductance of the postsynaptic potential, M is the gradient of synaptic potential amplitude against membrane potential and R_m is input resistance.

Statistics

Populations were tested for a normal distribution using a Wilk–Shapiro test of normality. Where the distribution of the population was normal the means were compared using Student's t test; where it was not a normal distribution, medians were compared using the Mann–Witney test. Comparisons of more than two groups were made by one-way analysis of variance (expressed as the variance ratio, F (with degrees of freedom as subscript), and the significance level, P), and group means were subsequently compared by Bonferroni simultaneous comparisons at a 0·05 significance level. Regression lines were fitted to the voltage dependence of fast and slow IPSPs. All tests were carried out with the RS/1 statistics package.

No significant differences were found between cells from hippocampi ipsilateral and contralateral to the buffer injection in any of the measurements made. Thus data from cells ipsilateral and contralateral to the buffer injection have been pooled.

RESULTS

Extracellular recordings

Epileptic responses were recorded from the CA3 pyramidal layers of all healthy slices prepared from tetanus toxin-injected rats. These consisted of positive potentials with superimposed negative population spikes, ranging in duration from 30 to 800 ms. They were found in slices from both injected and contralateral hippocampi (Fig. 1), and were an all-or-none component of the evoked responses in CA3. Epileptic responses were evoked from CA1 in twenty-one of thirty-nine slices from toxin-injected hippocampi, and four of thirty from contralateral hippocampi (not shown). They differed from those in CA3 in that they required much stronger stimulation.

The shapes of these epileptic potentials and their amplitudes and durations were similar in the different subregions of CA3, although they could vary within any one slice (Fig. 2*A* and *B*). They did not change with distance along the dorsal hippocampus (Fig. 2*C* and *D*). Furthermore they were similar between the injected and contralateral hippocampi (Fig. 2*A* and *C vs.* *B* and *D*; $P > 0{\cdot}05$ for all comparisons, t tests).

Spontaneous epileptic activity was seen in slices from sixteen out of fifty-five toxin-injected rats. In five rats both hippocampi generated spontaneous epileptic discharges. Of the remainder, spontaneous epileptic activity was found in slices from the injected hippocampus only in one rat, and from the contralateral in ten rats. The incidence of spontaneous activity was not uniform ($\chi^2 = 4{\cdot}8$ with 1 degree of freedom, $P = 0{\cdot}03$), with the uninjected hippocampus more likely to generate spontaneous epileptic discharges.

Neurochemical release

Intact hippocampal slices were preloaded with [^{3}H]GABA as a marker for estimating the release of this inhibitory transmitter. The radiolabel provided a reliable measure of GABA release because previous studies have shown that metabolism of radiolabelled GABA was negligible under the conditions used here (López-Colomé, Tapia, Salceda & Pasantes-Morales, 1978). The experiment provides estimates of: the basal release, the Ca^{2+}-independent part of K^+-stimulated release, and the Ca^{2+}-dependent part of K^+-stimulated release. Individual time courses from four different slices in Fig. 3 show the initial basal release followed by the increases in release due to the successive addition of 30 mM K^+ and 2 mM Ca^{2+} to the ACSF. The basal release was very low, which indicated

that the slices were healthy. Basal release did not differ between the four experimental conditions, ipsi- and contralateral to the control and toxin injections, in the fifteen experiments with two duplicates for each condition ($F_{3,106} = 0{\cdot}51$, $P = 0{\cdot}68$, one-way analysis of variance).

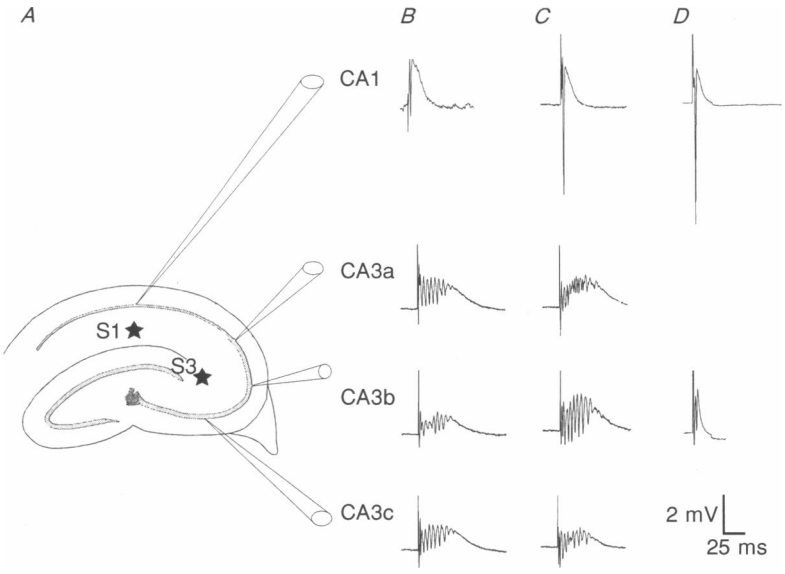

Fig. 1. *A*, evoked field potentials from CA3 and CA1 regions were recorded from slices taken from tetanus toxin-injected animals following stimulation at S3 and S1 respectively. *B*, the slice from the injected 'ipsilateral' hippocampus was 0·3 mm from the injection track. *C*, the slice from the 'contralateral' hippocampus contained the site homotopic to the injection. Note the apparently normal responses in CA1 and the prolonged epileptiform responses in the subregions of CA3. *D*, normal evoked responses from the CA1 and CA3b regions of a slice prepared from the hippocampus contralateral to a buffer injection made 15 days earlier.

Raising $[K^+]$ to 30 mM led to a marked increase in release from 18 to 28 min, which corresponds to the Ca^{2+}-independent K^+-stimulated component of release. One-way analysis of variance revealed no significant difference between the groups ($F_{3,106} = 2{\cdot}24$, $P = 0{\cdot}09$).

The addition of 2 mM Ca^{2+} caused a further marked increase in release from 28 to 40 min. This appeared as a peak after which there was a decline as the perfusate returned to one containing 3 mM K^+ and 0 mM Ca^{2+} alone. The Ca^{2+}-dependent K^+-stimulated release was estimated from the mean increase during the period 28–40 min over and above the Ca^{2+}-independent K^+-stimulated component (Fig. 4*A* and *B*). The Ca^{2+}-dependent K^+-stimulated release was about twice as large as the Ca^{2+}-independent component. One-way analysis of variance showed that the Ca^{2+}-dependent K^+-stimulated release was significantly different between the groups ($F_{3,106} = 9{\cdot}52$, $P < 0{\cdot}001$). An analysis of simultaneous multiple comparisons between the group means (Fig. 4*D*) showed that this difference was due to a

reduction of the Ca^{2+}-dependent K^+-stimulated release from slices ipsilateral to the tetanus toxin injection. Furthermore it showed that the Ca^{2+}-dependent K^+-stimulated release from slices contralateral to the toxin injection was at normal levels, despite the presence of epileptic activity in adjacent slices.

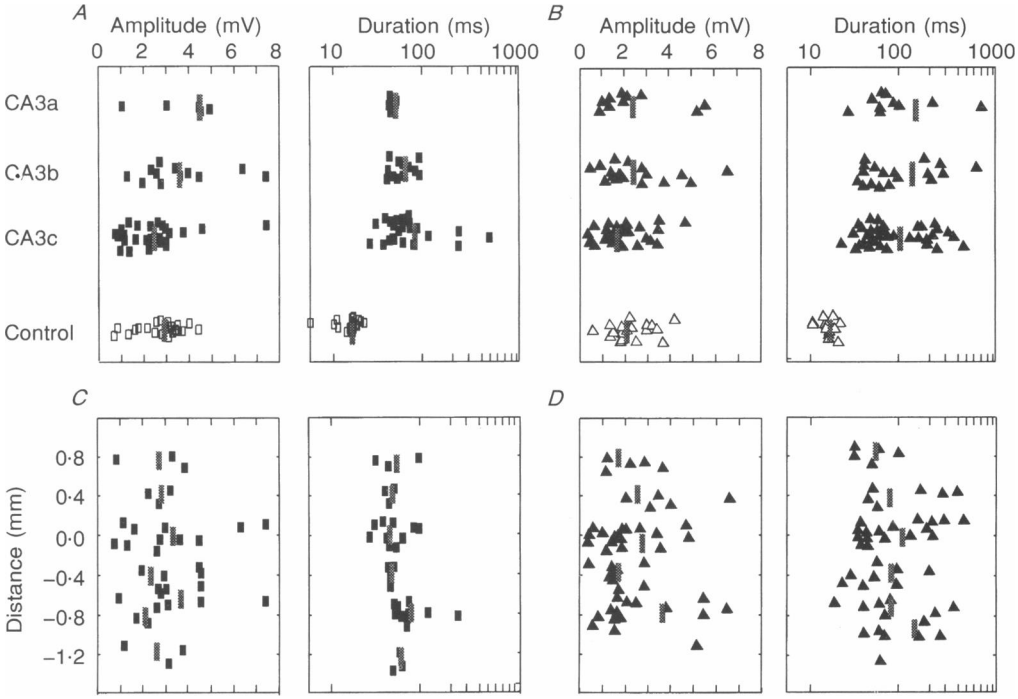

Fig. 2. The amplitude and duration of the epileptic bursts in the different regions of CA3 were measured from 94 slices, from 33 toxin-treated animals, both ipsilateral (*A*) and contralateral (*B*) to the injection. Control data were from 25 slices from 10 animals with CA3b and CA3c pooled. The peak amplitude of the burst and its duration (on a log scale) were measured. Similar measurements were made from CA3b and c in slices at various distances along the dorsal hippocampus both ipsilateral and contralateral to the toxin injection (*C* and *D* respectively). Filled symbols represent measurements from slices ipsi- and contralateral to the site of tetanus toxin injection respectively. Open symbols represent measurements from slices ipsilateral and contralateral to the buffer injection respectively. Positive distances are medial, and negative are lateral, to the injection site and its homotopic point. The cross-hatched bars represent mean values.

Intracellular recordings

The preservation of GABA release from the slices contralateral to the tetanus toxin injection together with reliable epileptic activity in adjacent slices prompted a second series of experiments in which intracellular recordings were made from pyramidal cells in CA3b and c. The objective was to measure fast and slow inhibitory postsynaptic potentials (IPSPs) in the primary and secondary epileptic foci.

Cells were recorded in slices: ipsilateral to the tetanus toxin injection, contralateral to the toxin injection, and controls, pooling data for slices ipsilateral

and contralateral to the buffer injection. There was no difference between cells from these groups in either their resting membrane potential or input resistance. (Resting potentials: control $-65{\cdot}6 \pm 1{\cdot}1$ mV, $n = 15$; contralateral $-65{\cdot}7 \pm 0{\cdot}6$ mV, $n = 27$; ipsilateral $-67{\cdot}5 \pm 1{\cdot}4$ mV, $n = 10$. Input resistances: control $50{\cdot}9 \pm 5{\cdot}6$ MΩ; contralateral $52{\cdot}4 \pm 2{\cdot}7$ MΩ, ipsilateral $48{\cdot}1 \pm 5{\cdot}8$ MΩ. All comparisons $P > 0{\cdot}05$.).

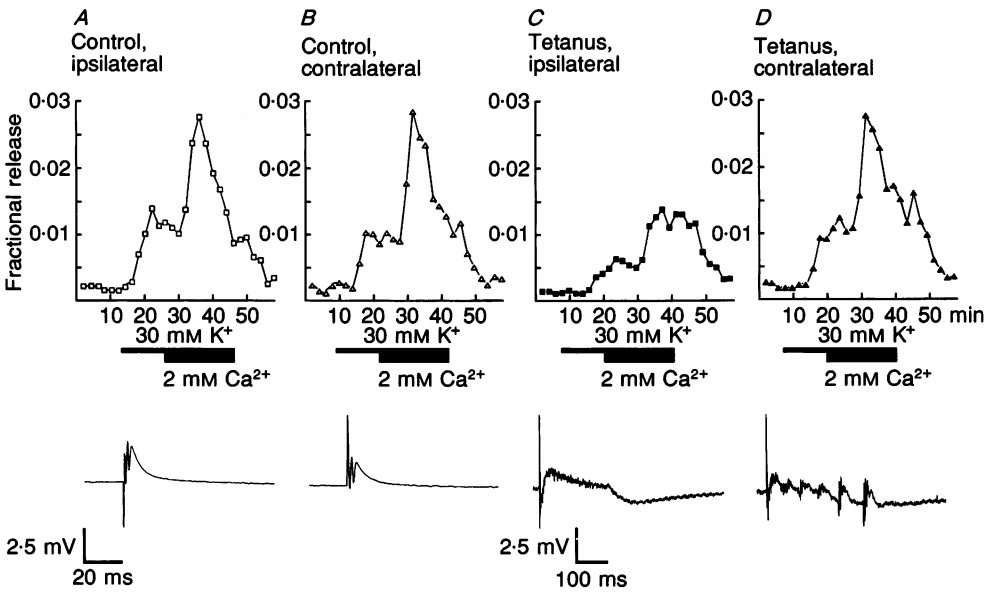

Fig. 3. The fractional release (the release of radiolabel over a 2 min sample period as a fraction of the amount of label left in the tissue) of [^{3}H]GABA is plotted against time for individual slices from control (*A* and *B*; □ and △) and toxin-treated animals (*C* and *D*; ■ and ▲). All slices underwent the same stimulation protocol (marked by filled bars): a baseline period using ACSF containing 3 mM K^+ and 0 mM Ca^{2+}, followed successively by 30 mM K^+ and 0 mM Ca^{2+}, 30 mM K^+ and 2 mM Ca^{2+}, and return to baseline. Field potential recordings from the CA3b/c regions of slices from the toxin-treated animal revealed stimulus-evoked epileptic activity (lower panels *C* and *D*) while those from control revealed normal evoked potentials (lower panels *A* and *B*; note faster time scale). Field potentials were recorded from a slice 0·4 mm away from that used for the release data shown in *C* and the directly medial slice in *D*.

Postsynaptic potentials

The excitatory postsynaptic potential (EPSP) evoked by stimulation of stratum radiatum in control slices was followed by fast and slow IPSPs (respectively marked by open and filled circles above the family of traces in the lower panel, Fig. 5*A*) which were hyperpolarizing at rest and during depolarizing current injections. Cells from slices taken from the hippocampus ipsilateral to the toxin injection (the primary focus) clearly had an EPSP which was followed by neither a fast nor a slow IPSP (Fig. 5*B*). The EPSP was prolonged compared with the controls (130 ± 15 compared with 16 ± 2 ms). Cells from the contralateral secondary focus were much more variable, many showed little sign of inhibition and resembled cells from the primary focus, but others clearly had IPSPs (Fig. 5*C*, upper and lower families of traces, respectively).

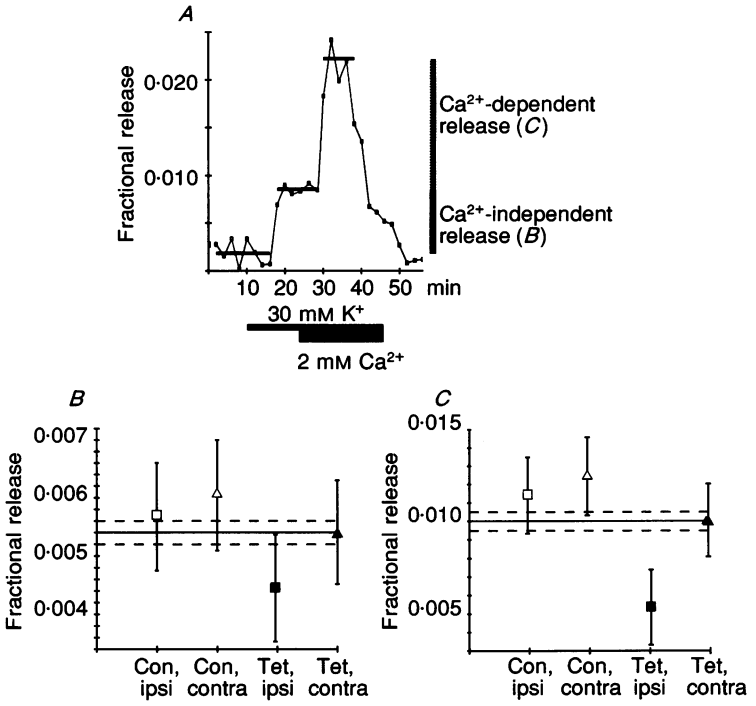

Fig. 4. *A*, the fractional release of [^{3}H]GABA is plotted against time for a typical slice. Superimposed upon this are the measurements made to estimate the Ca^{2+}-independent K^+-stimulated release (filled vertical bar) and the Ca^{2+}-dependent K^+-stimulated release (cross-hatched vertical bar). The filled horizontal bar represents the application of the different ACSFs containing 30 mM K^+ and 2 mM Ca^{2+}. One-way analysis of variance was conducted on the mean values obtained from these measurements from 15 toxin-treated and 15 buffer-injected rats. There was no significant difference between the experimental groups in the Ca^{2+}-independent K^+-stimulated release, but there was a significant effect of the experimental groups on the Ca^{2+}-dependent K^+-stimulated release. *B* and *C* summarize Bonferroni simultaneous comparisons between the experimental groups (ipsi, ipsilateral; contra, contralateral; con, control). Means, with error bars, are presented for each experimental group. The continuous horizontal line represents the grand mean and the dashed lines its error bounds. If error bars and/or error bounds do not overlap then they differ at the 5 % significance level. *B*, no significant difference was found in the Ca^{2+}-independent K^+-stimulated release as expected from the analysis of variance. *C*, the Ca^{2+}-dependent K^+-stimulated release from the slices ipsilateral to the tetanus toxin injection was significantly different to all the other groups and also to the grand mean.

The amplitudes of the EPSPs and fast and slow IPSPs were measured from a baseline just before the stimulus to their respective peaks 4–10, 20 and 150–175 ms after the stimulus. These amplitudes were plotted against membrane potential (IPSP data are shown in upper panels of Fig. 5), and used, with the input resistance, to estimate conductances for all cells from the three experimental groups (see Methods). There was no significant difference between the groups in the estimated EPSP conductance ($F_{2,46} = 0{\cdot}76$, $P = 0{\cdot}47$; data not shown).

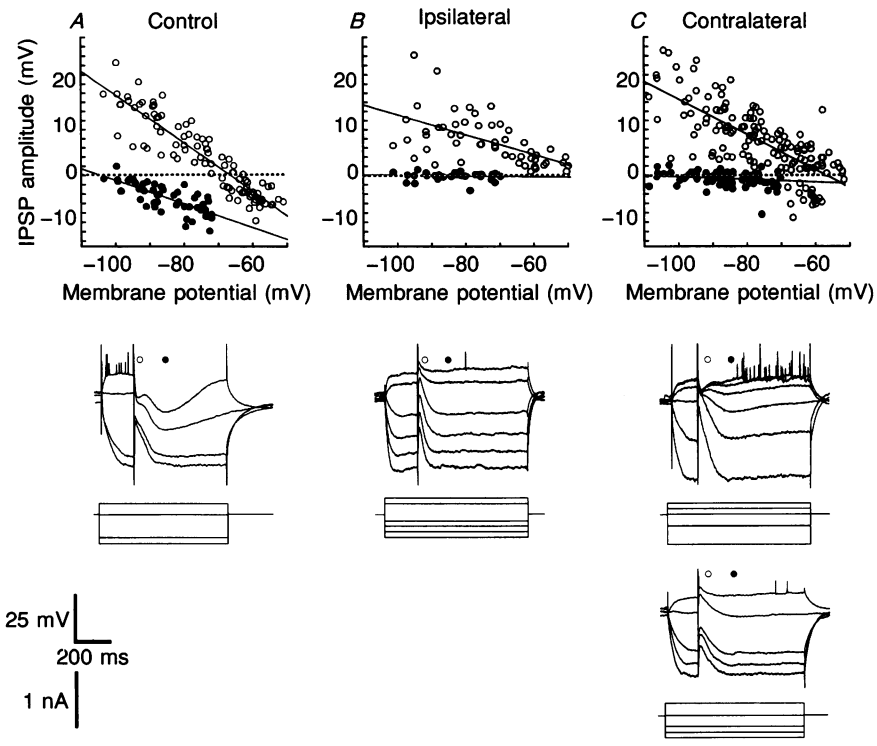

Fig. 5. The relationship of the amplitude of the fast and slow IPSPs with the membrane potential of cells from CA3b/c was revealed by current injection. Fast (○) and slow (●) IPSPs have been measured at their peaks, 20–25 and 150–175 ms after the stimulus respectively. Each point represents a single measurement from an averaged trace for a single value of injected current in a single cell. The currents injected are shown below the potentials. *A*, the control group contains 15 cells from either hippocampus following injection of buffer. Fast and slow IPSPs are both clearly visible in the family of traces below the graph. Both decrease in amplitude with hyperpolarization, and the fast IPSP reverses at −67 mV (action potentials are attenuated by averaging). *B*, 10 cells from the toxin-injected hippocampus revealed no slow IPSP, and a potential at 25 ms which appeared to reverse at −44 mV, and which was attributed to the tail of the prolonged EPSP. *C*, cells in slices contralateral to the toxin injection were more variable. Of the 25 cells, 7 had a clear reversable fast IPSP (upper family of traces), while the remainder did not (lower family). The average reversal of the fast IPSP in the cells from the secondary focus was −51·4 mV.

Fast IPSPs

In control cells the average reversal potential for the fast IPSPs was −66·6 mV (○, Fig. 5*A*). Although the cells for the toxin-injected hippocampus had measurable potentials at 20 ms, they were not hyperpolarizing within the range of membrane potentials used here and corresponded to the prolonged tail of the EPSP (Fig. 5*B*). Fast IPSPs from cells from the contralateral hippocampus were much more variable. On average they reversed at −51·4 mV, but they ranged from −77 to > −50 mV.

Estimated fast IPSP conductances differed significantly between the experimental groups ($F_{2,46} = 7{\cdot}54$, $P < 0{\cdot}001$, one-way analysis of variance). The two groups from the toxin-injected rats had significantly lower conductances than the controls, but did not differ from each other (at 0·05 significance, Bonferroni simultaneous multiple comparisons). The mean conductance for the controls was $29{\cdot}9 \pm 4{\cdot}9$ nS; for the ipsilateral toxin group, $7{\cdot}8 \pm 1{\cdot}7$ nS; and for the contralateral group, $15{\cdot}8 \pm 2{\cdot}7$ nS. The interpretation of these measures at 20 ms was complicated by the tails of EPSPs. The essential difference between EPSPs and IPSPs in these recordings was in their reversal potentials, which have been measured directly where possible, and by extrapolation in all cells from the injected hippocampus, and some from the contralateral, because they reversed beyond the reliable range of the recording system (−50 mV). There were significant differences in reversal potentials between the experimental groups ($F_{2,46} = 14{\cdot}65$, $P < 0{\cdot}001$). Cells from the toxin-injected hippocampus had reversal potentials significantly depolarized to the controls ($-43{\cdot}3 \pm 9{\cdot}7$ mV compared with $-66{\cdot}2 \pm 4{\cdot}6$ mV, $P < 0{\cdot}05$, Bonferroni multiple comparisons). Cells from the secondary focus had an intermediate mean reversal potential of $-51{\cdot}0 \pm 12{\cdot}9$ mV, which differed from the controls but not from the ipsilateral group. However, individual measurements from these cells from the secondary focus straddle the entire range of the control and ipsilateral groups, and seven out of twenty-five had reversal potentials and conductances within the normal range for fast IPSPs.

Slow IPSPs

Slow IPSPs peaked some 150–175 ms after the stimulus (●, Fig. 5). In control cells the slow IPSP reversed at a mean of −103·9 mV, much more negative than the fast IPSP, and entirely consistent with their well-known physiological properties (Fig. 5*A*). In the toxin-injected animals, slow IPSPs were clearly smaller or absent (Fig. 5*B* and *C*).

The estimated conductances of the slow IPSPs differed significantly between the experimental groups ($F_{3,54} = 31{\cdot}8$, $P < 0{\cdot}001$). Cells from both sides of the toxin-injected rats had conductances significantly smaller than the control cells, but did not differ from each other (controls, $10{\cdot}1 \pm 1{\cdot}2$ nS; primary focus, $0{\cdot}7 \pm 0{\cdot}3$ nS; and secondary focus, $1{\cdot}8 \pm 0{\cdot}2$ nS). However, the slices contralateral to the toxin injection did have discernable, if very small, slow IPSPs. Increasing the stimulus to such slices to levels that triggered a population burst increased the slow IPSP conductance to $5{\cdot}7 \pm 1{\cdot}3$ nS, significantly greater than the lower stimuli, but still less than the controls. Reversal potentials were ill-defined in the primary focus because the potentials at 150 ms were so small. The reversal potentials in the secondary focus were $-90{\cdot}1 \pm 3{\cdot}9$ and $-83{\cdot}4 \pm 4{\cdot}7$ mV with the stronger stimuli, compared with $-103{\cdot}9 \pm 2{\cdot}9$ mV in the controls. These group means were not significantly different ($P > 0{\cdot}05$, Bonferroni simultaneous multiple comparisons). The identity of the small slow IPSPs seen in cells from the secondary focus was confirmed by their being blocked by 2-OH saclofen, a potent $GABA_B$ antagonist (4 cells, Fig. 6*A* and *B*, lower panels). Slow IPSPs in cells from control slices were also blocked by 2-OH saclofen (3 cells not shown). The larger slow IPSPs, elicited in cells from the secondary focus when population bursts were evoked (Fig. 6*C*), were also blocked by 2- OH saclofen (Fig. 6*D*).

Monosynaptic IPSPs

The discrepancy between the normal levels of Ca^{2+}-dependent K^+-stimulated GABA release and the variable preservation of IPSPs in slices contralateral to the toxin injection site suggested that inhibitory interneurones were not being sufficiently activated by the afferent stimulation. It is possible to block EPSPs with

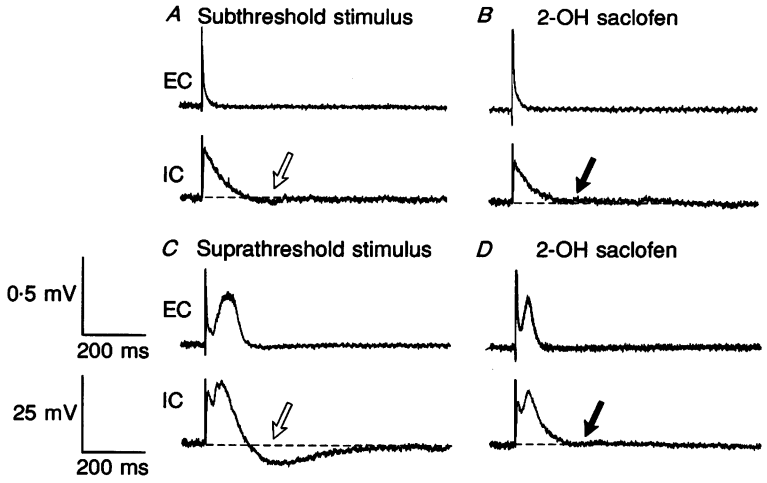

Fig. 6. Postsynaptic responses in a cell (resting potential = −71 mV, input resistance 52 MΩ) from a slice contralateral to the injection of tetanus toxin have putative slow IPSPs (open arrows) in response to stimuli that were below (*A*) and above (*C*) the threshold for an evoked epileptic burst. Application of 2-OH saclofen (5 mM in a pressure-ejection pipette) abolished the hyperpolarizing potentials in both cases, (*B* and *D*, filled arrows). This was also the case in 6 other cells. Extracellular (EC) recordings are shown to illustrate the appearance of the synchronous burst at suprathreshold stimuli. IC, intracellular recording.

excitatory amino acid antagonists so that electrical stimulation directly excites interneurones and elicits monosynaptic IPSPs in pyramidal cells (Davies & Collingridge, 1989; Davies, Davies & Collingridge, 1990).

Monosynaptic fast IPSPs

When CNQX and AP-5 were applied to cells in control slices, the EPSP was blocked, leaving fast and slow IPSPs (Fig. 7*A*). There was no evidence of a fast monosynaptic IPSP following stimulation of the cells in slices from the hippocampus that had previously received an injection of tetanus toxin (Fig. 7*B*). However, cells in slices from the contralateral hippocampus did generate monosynaptic IPSPs under CNQX and AP-5 (Fig. 7*C*). Moreover, the estimated conductance of the monosynaptic fast IPSP from contralateral cells (8·7 nS) did not differ significantly from the estimated conductance seen in control cells (14·8 nS; respective medians, 7·1 and 14·5 nS, $P > 0{\cdot}05$, Mann–Witney non-parametric test; Fig. 8*A*).

Adding the glutamate antagonists changed the reversal potentials of monosynaptic fast IPSPs in cells in slices both from control tissue and from secondary foci ($P = 0{\cdot}04$ and $< 0{\cdot}001$ respectively, *t* tests). However, there was no

significant difference in reversal potentials between the monosynaptic fast IPSPs recorded in the control slices (−73·7 ± 2·3 mV) and in the contralateral secondary focus (−68·0 ± 2·4 mV; $P = 0{\cdot}09$, t test; Fig. 8*B*). Bicuculline methiodide blocked the monosynaptic fast IPSPs both in two control cells and in two cells from the secondary focus, confirming their mediation by $GABA_A$ receptors.

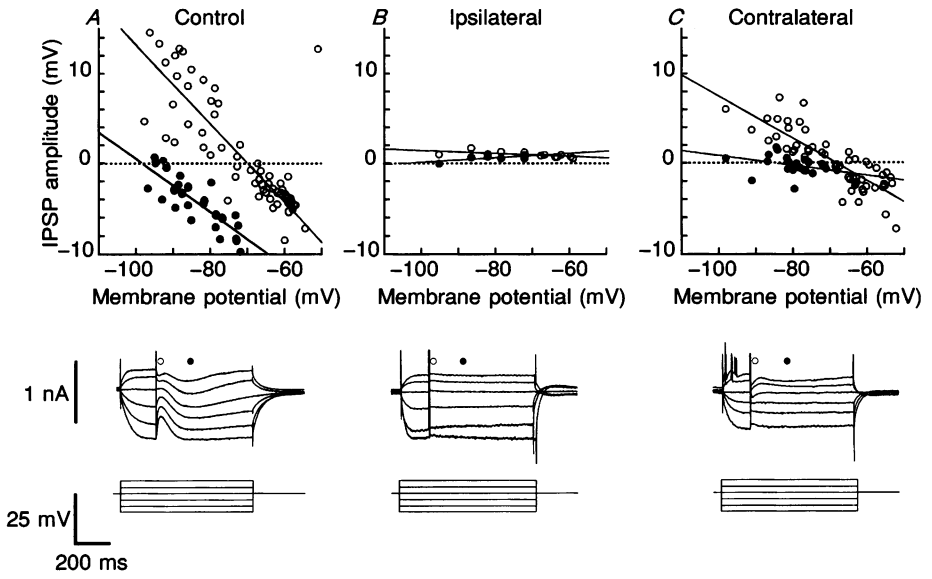

Fig. 7. Monosynaptic fast and slow IPSPs were revealed by direct stimulation of the interneurones when synaptic excitation was blocked by CNQX and AP-5. The relation of the amplitude of the fast (○) and slow (●) IPSPs to the membrane potential of the cells was revealed by current injection. *A*, data from 9 control cells reveal monosynaptic IPSPs in all cases. *B*, no IPSPs were seen in 2 cells from the hippocampus ipsilateral to the toxin injection. *C*, fast IPSPs were seen in all 12 cells from the hippocampus contralateral to the tetanus toxin injection.

Stronger stimuli were needed to elicit monosynaptic fast IPSPs in cells from the secondary focus than in controls (6·7 ± 0·6 V compared with 3·8 ± 0·3 V; $P < 0{\cdot}001$, t test). The application of CNQX and AP-5 to control cells did not change the stimulus required to elicit a fast IPSP ($P = 0{\cdot}15$, t test). The same stimulus range was used on the secondary focus before applying CNQX and AP-5 and did not reliably elicit fast IPSPs; after applying these antagonists larger stimuli could be used because they did not trigger epileptic bursts, and they did evoke monosynaptic fast IPSPs.

Monosynaptic slow IPSPs

Slow IPSPs could also be evoked by electrical stimulation after the application of CNQX and AP-5 in slices from control tissue and from the secondary focus. The reversal potentials of these slow IPSPs were not changed significantly by the addition of glutamate antagonists ($P > 0{\cdot}3$ in each case, t tests), nor were their conductances ($P > 0{\cdot}15$ in each case, t tests). Monosynaptic slow IPSPs were not seen in slices ipsilateral to the tetanus toxin injection.

There was a significant difference in the reversal of the monosynaptic slow IPSP in the cells from the secondary focus, $-82{\cdot}0 \pm 4{\cdot}3$ mV, compared with the monosynaptic slow IPSP from control cells, $-104{\cdot}5 \pm 6{\cdot}1$ mV ($P = 0{\cdot}008$, t test;

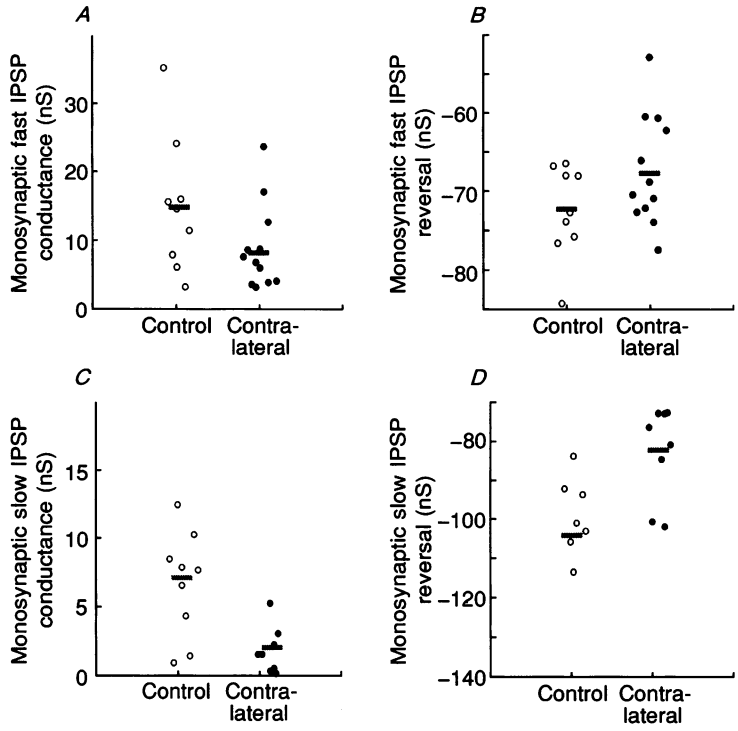

Fig. 8. Conductances (*A*) and reversal potentials (*B*) were estimated for the monosynaptic fast IPSPs from control cells (○) and from cells from the hippocampus contralateral to the tetanus toxin injection (●). The same measurements were made for the slow IPSPs (*C* and *D* respectively).

Fig. 8*D*). Furthermore the conductance of the slow IPSP for the contralateral hippocampus was $2{\cdot}1 \pm 0{\cdot}6$ nS, significantly lower than the controls $6{\cdot}6 \pm 1{\cdot}3$ nS, ($P < 0{\cdot}001$, t test; Fig. 8*C*).

DISCUSSION

Injection of a small amount of tetanus toxin into one hippocampus of a rat leads to the development of independent epileptic foci of similar intensity and extent in both hippocampi. However, a combination of neurochemical studies of GABA release and intracellularly recorded IPSPs showed that the two foci possess radically different underlying epileptogenic mechanisms.

The key result was the preservation, 2 weeks after injecting the toxin, of normal levels of Ca^{2+}-dependent K^+-stimulated, 'synaptic' (Sihra & Nicholls, 1987), GABA release in slices from the secondary focus, in contrast with its considerable reduction in the primary focus (Fig. 4*C*) and following bilateral intrahippocampal

injections (Jefferys *et al.* 1991). In contrast, Ca^{2+}-independent K^+-stimulated GABA release did not differ between slices from either side of the tetanus toxin-injected rats, nor from the control slices. The Ca^{2+}-independent K^+-stimulated release is attributed to the reversal of the GABA uptake system at both neurones and glia (Nicholls, 1989). We can exclude the actual loss of GABAergic neurones, or a failure of expression of the gene for glutamic acid decarboxylase (GAD), as being responsible for the impaired GABA release on the basis of a parallel study where cells containing GAD mRNA were counted (Najlerahim *et al.* 1992).

Intracellular recordings confirmed that neither fast nor slow IPSPs could be evoked in pyramidal cells from slices of the primary focus in the injected hippocampus, which thus resembled slices after bilateral injections of tetanus toxin (Jordan & Jefferys, 1992). Furthermore no monosynaptic fast or slow IPSPs could be evoked using strong stimuli when EPSPs were blocked by CNQX and AP-5. Activation of $GABA_A$ receptors by the application of exogenous GABA is possible in such cells (M.A. Whittington, personal communication) also suggesting that the impairment was presynaptic. The discrepancy between the complete loss of fast and slow IPSPs in pyramidal cells of the primary focus with the partial (50 %) block of 'synaptic' GABA release probably arises from contributions to the release from non-epileptic regions of the slice (e.g. CA1 in Fig. 1), which may be boosted by the possible hypertrophy of GABAergic synapses (Najlerahim *et al.* 1992).

The contralateral, secondary, 'mirror' focus showed normal levels of Ca^{2+}-dependent K^+-stimulated release which might suggest that inhibition was intact. In seven out of twenty-five CA3 cells, fast IPSPs were evoked by afferent stimulation, and had reversal potentials and estimated conductances within the range of the controls. However, the remaining cells from the secondary focus did not possess fast IPSPs that could be detected by conductance and reversal potential. Directly activating the inhibitory interneurones, by electrical stimulation when the EPSPs were blocked pharmacologically, showed that the inhibitory interneurones in the secondary focus were still functional and capable of producing $GABA_A$-mediated monosynaptic fast IPSPs in all pyramidal cells studied. The reversal potentials and estimated conductances of these fast IPSPs were not significantly different to values obtained from control cells under the same conditions. This might suggest that the absence of IPSPs in eighteen of twenty-five cells when EPSPs were not blocked was due to a reduction of the excitatory synaptic input to interneurones. However, when EPSPs were blocked, the stimuli required to generate monosynaptic fast IPSPs in cells in the mirror focus were twice those for control cells, and could suggest that interneurones were intrinsically less excitable than normal. (Alternatively, it could suggest that while interneurones were able to release GABA, they were less efficient than normal, and that more needed to be recruited to produce a detectable IPSP.)

There are precedents for the functional loss of inhibition in the hippocampus. Intense stimulation of the perforant path leads to hyperexcitability of both the dentate area and CA1 (Sloviter, 1987, 1991). This has been attributed to losses of inhibition resulting from the death of neurones which excite inhibitory cells, hilar mossy and somatostatin-positive cells in the case of the dentate area and of CA3

pyramidal cells in the case of CA1. While there is no direct evidence in the CA3 region for this 'dormant basket cell' hypothesis, the present data suggest that it should be explored further, particularly given that hilar interneurones inhibit CA3 pyramidal cells in addition to dentate granule cells (Müller & Misgeld, 1990; Michelson & Wong, 1991). At this stage reduced excitation of inhibitory neurones onto CA3 pyramidal cells provides a plausible hypothesis for the patchy failure of IPSPs in the secondary focus. If at least part of this excitation were not glutamatergic, then this hypothesis could also apply to the increased threshold for monosynaptic IPSPs in the presence of CNQX and AP-5.

In all cells from the secondary focus there was a small slow IPSP identified by time course, reversal potential and sensitivity to 2-OH saclofen. The estimated conductance of the slow IPSP was very much less than seen in controls. The reversal potentials of the slow IPSPs in the mirror focus were more positive than those in the controls; while it is not clear whether this is due to changes in potassium gradients or in the ion channels activated, it would certainly reduce the efficacy of the slow IPSPs. Stronger stimuli could evoke slow IPSPs, approaching the size of the controls, only if they triggered an epileptic discharge. This suggests that strong sustained excitation could overcome the impairments of the slow IPSP. Monosynaptic slow IPSPs remained when EPSPs had been blocked pharmacologically. However, they were much smaller than in the control slices, and, unlike the corresponding fast IPSPs, did not increase significantly with increasing stimuli. This would lend support to suggestions that different interneurones mediate fast and slow IPSPs (Müller & Misgeld, 1990; Segal, 1990), and would further suggest that they were subject to different impairments in the tetanus toxin secondary focus.

The epileptic activity preserved in the slices included the briefer forms of the discharges recorded *in vivo* (Hawkins & Mellanby, 1987; Jefferys, 1989; Jefferys & Empson, 1990). The secondary focus generated spontaneous epileptic bursts significantly more often than the primary. The bursts from the secondary focus also were shorter and more frequent, and it may be that the better preserved synaptic inhibition stops them before they can trigger longer-lasting inhibitory mechanisms, and resets the CA3 network to a state where it is susceptible to the next wave of excitation. A more speculative possibility is that extra connections may form between CA3 pyramidal cells during the time course of this chronic epilepsy, and that this occurs more effectively at sites which have not been exposed directly to the toxin. Epileptic activity did not spread into CA1 in slices from either hippocampus. This contrasts with many acute models where bursts spread from CA3 to CA1 very reliably (reviewed in Jefferys, 1990). This discrepancy is probably due to the parasagital orientation of our slices not being optimal for the rather oblique (and divergent) course of the Schaffer collaterals between CA3 and CA1 (Ishizuka, Weber & Amaral, 1990; Finnerty & Jefferys, 1993). Furthermore, slices bathed in convulsant media have hyperexcitability in CA1 which makes the area excessively responsive to the small subset of Schaffer collaterals preserved in 400 μm transverse hippocampal slices, but this does not occur following a focal injection of tetanus toxin into CA3.

The best-documented action of tetanus toxin is to block exocytosis, with some selectivity for inhibitory synapses (Mellanby & Green, 1981). The preservation of apparently normal EPSPs, the loss of IPSPs and the marked reduction in GABA release in the primary focus are entirely consistent with this mechanism, even though only a very small amount of toxin remains 2 weeks after injection (Habig *et al.* 1986; Mellanby, 1989). However, the secondary focus does not easily fit this scheme because release of GABA remained normal, and because IPSPs could be evoked by electrical stimulation. If the undetectably small amount of toxin that probably is transported across the midline (Mellanby, 1989) were sufficient to block synaptic transmission, it could block axon collaterals of the CA3 pyramidal cells which excite the interneurones (Miles, 1990). This possibility receives some support from the reliable presence of monosynaptic IPSPs in the secondary focus when EPSPs were blocked. However, CA3 pyramidal cell synapses could not have been extensively blocked, or we would not then have been able to record EPSPs nor indeed to recruit epileptic discharges (Wong *et al.* 1986; Traub, Knowles, Miles & Wong, 1987). Furthermore we found no differences between the groups in estimated EPSP conductances. Moreover, the stronger than normal stimulus required to evoke monosynaptic IPSPs in the secondary focus suggested intrinsic changes in the excitability of the interneurones themselves. More than one change may contribute to epileptic discharges in the secondary focus, and these could arise from both the direct action of the toxin and plastic changes of the kind known to be set in train by recurrent epileptic activity (Brace *et al.* 1985; Cain, 1989; Najlerahim *et al.* 1992).

Previous studies on chronic experimental epilepsies have implicated disruption of inhibition at several stages. A relatively greater loss of inhibitory neurones and synapses, compared with excitatory, has been reported around the lesions established by alumina foci in the neocortex (Ribak, Joubran, Kesslak & Bakay, 1989). Inhibitory neurones are preserved in other models. Kainic acid causes lesions of CA3 which lead to the adjacent CA1 and dentate areas becoming epileptic, and has been associated with a persistent loss of inhibition (Cornish & Wheal, 1989), which was not due to losses either of interneurones or of GABA receptors (Ashwood & Wheal, 1986; Franck, Kunkel, Baskin & Schwartzkroin, 1988). Kindling has been extensively studied as an experimental epilepsy, as well as a model of learning (Cain, 1989), and resembles the tetanus toxin model in not causing gross lesions. It has different effects in different areas, but in CA1 inhibition was disrupted at the postsynaptic receptor level, while GABA release increased, in marked contrast to tetanus toxin foci (Kamphuis, Gorter & Lopes da Silva, 1991*a*; Kamphuis, Huisman, Veerman & Lopes da Silva, 1991 *b*).

The role of inhibition in clinical and some chronic experimental epileptic foci has been contentious; in many cases measures such as the tissue level or release of GABA and the numbers of neurones containing GABA or GAD did not change when epileptic activity was clearly present (e.g. Lloyd, Bossi, Morselli, Munari, Rougier & Loiseau, 1986; Babb, Pretorius, Kupfer & Crandall, 1989; Houser, 1991; Tasker & Dudek, 1991). The secondary focus in hippocampi contralateral to the tetanus toxin injection site has now revealed that inhibition was significantly

weakened in the face of normal GABA release and normal numbers of inhibitory interneurones. This reinforces the importance of assessing inhibition directly by electrophysiological recording. The nature of the impairment found here remains to be fully resolved, but reductions in the excitability and/or the excitation of inhibitory interneurones are prime, and novel, candidates.

This work was supported by the SERC, the MRC and the Wellcome Trust. R.M. Empson was an SERC Research Student, J. G. R. Jefferys is a Wellcome Trust Senior Lecturer. We thank Drs G. T. Finnerty and M. A. Whittington for helpful comments on this work and manuscript.

REFERENCES

Ashwood, T. J. & Wheal, H. V. (1986). Loss of inhibition in the CA1 region of the kainic acid lesioned hippocampus is not associated with changes in postsynaptic responses to GABA. *Brain Research* **367**, 390–394.

Babb, T. L., Pretorius, J. K., Kupfer, W. R. & Crandall, P. H. (1989). Glutamate decarboxylase-immunoreactive neurons are preserved in human epileptic hippocampus. *Journal of Neuroscience* **9**, 2562–2574.

Bergey, G. K., Macdonald, R. L., Habig, W. H., Hardegree, M. C. & Nelson, P. G. (1983). Tetanus toxin: convulsant action on mouse spinal cord neurons in culture. *Journal of Neuroscience* **3**, 2310–2323.

Bevan, S. & Wendon, L. M. B. (1984). A study of the action of tetanus toxin at rat soleus neuromuscular junctions. *Journal of Physiology* **348**, 1–17.

Bigalke, H., Heller, I., Bizzini, B. & Habermann, E. (1981). Tetanus toxin and botulinum A toxin inhibit release and uptake of various transmitters, as studied with particulate preparations from rat brain and spinal cord. *Naunyn-Schmiedebergs Archives of Pharmacology* **316**, 244–251.

Bittner, M. A. & Holz, R. W. (1988). Effects of tetanus toxin on catecholamine release from intact and digitonin-permeabilized chromaffin cells. *Journal of Neurochemistry* **51**, 451–456.

Brace, H. M., Jefferys, J. G. R. & Mellanby, J. (1985). Long-term changes in hippocampal physiology and in learning ability of rats after intrahippocampal tetanus toxin. *Journal of Physiology* **368**, 343–357.

Brener, K., Chagnac-Amitai, Y., Jefferys, J. G. R. & Gutnick, M. J. (1991). Chronic epileptic foci in neocortex: *in vivo* and *in vitro* effects of tetanus toxin. *European Journal of Neuroscience* **3**, 47–54.

Cain, D. P. (1989). Long-term potentiation and kindling: How similar are the mechanisms. *Trends in Neurosciences* **12**, 6–10.

Collingridge, G. L., Thompson, P. A., Davies, J. & Mellanby, J. (1981). In vitro effect of tetanus toxin on GABA release from rat hippocampal slices. *Journal of Neurochemistry* **37**, 1039–1041.

Cornish, S. M. & Wheal, H. V. (1989). Long-term loss of paired pulse inhibition in the kainic acid-lesioned hippocampus of the rat. *Neuroscience* **28**, 563–571.

Davies, C. H., Davies, S. N. & Collingridge, G. L. (1990). Paired-pulse depression of monosynaptic GABA-mediated inhibitory postsynaptic responses in rat hippocampus. *Journal of Physiology* **424**, 513–531.

Davies, S. N. & Collingridge, G. L. (1989). Role of excitatory amino acid receptors in synaptic transmission in area CA1 of rat hippocampus. *Proceedings of the Royal Society of London* B **236**, 373–384.

Finnerty, G. T. & Jefferys, J. G. R. (1993). Functional connectivity from CA3 to the ipsilateral and contralateral CA1 in the rat dorsal hippocampus. *Neuroscience* (in the Press).

Franck, J. E., Kunkel, D. D., Baskin, D. G. & Schwartzkroin, P. A. (1988). Inhibition in kainate-lesioned hyperexcitable hippocampi: Physiologic, autoradiographic, and immunocytochemical observations. *Journal of Neuroscience* **8**, 1991–2002.

Ginsborg, B. L. (1973). Electrical changes in the membrane in junctional transmission. *Biochimica et Biophysica Acta* **300**, 289–317.

HABERMANN, E. & ERDMANN, G. (1978). Pharmacokinetic and histoautoradiographic evidence for the intraaxonal movement of toxin in the pathogenesis of tetanus. *Toxicon* **16**, 611–623.

HABIG, W. H., BIGALKE, H., BERGEY, G. K., NEALE, E. A., HARDEGREE, M. C. & NELSON, P. G. (1986). Tetanus toxin in dissociated spinal cord cultures: long-term characterization of form and action. *Journal of Neurochemistry* **47**, 930–937.

HAWKINS, C. A. & MELLANBY, J. H. (1987). Limbic epilepsy induced by tetanus toxin: a longitudinal electroencephalographic study. *Epilepsia* **28**, 431–444.

HOUSER, C. R. (1991). GABA neurons in seizure disorders: A review of immunocytochemical studies. *Neurochemical Research* **16**, 295–308.

ISHIZUKA, N., WEBER, J. & AMARAL, D. G. (1990). Organization of intrahippocampal projections originating from CA3 pyramidal cells in the rat. *Journal of Comparative Neurology* **295**, 580–623.

JEFFERYS, J. G. R. (1989). Chronic epileptic foci *in vitro* in hippocampal slices from rats with the tetanus toxin epileptic syndrome. *Journal of Neurophysiology* **62**, 458–468.

JEFFERYS, J. G. R. (1990). Basic mechanisms of focal epilepsies. *Experimental Physiology* **75**, 127–162.

JEFFERYS, J. G. R. & EMPSON, R. M. (1990). Development of chronic secondary epileptic foci following intrahippocampal injection of tetanus toxin. *Experimental Physiology* **75**, 733–736.

JEFFERYS, J. G. R., EVANS, B. J., HUGHES, S. A. & WILLIAMS, S. F. (1992). Neuropathology of the chronic epileptic syndrome induced by intrahippocampal tetanus toxin in the rat: preservation of pyramidal cells and incidence of dark cells. *Neuropathology and Applied Neurobiology* **18**, 53–70.

JEFFERYS, J. G. R., MITCHELL, P., O'HARA, L., TILEY, C., HARDY, J., JORDAN, S. J., LYNCH, M. & WADSWORTH, J. (1991). *Ex vivo* release of GABA from tetanus toxin-induced chronic epileptic foci decreased during the active seizure phase. *Neurochemistry International* **18**, 373–379.

JORDAN, S. J. & JEFFERYS, J. G. R. (1992). Sustained and selective block of IPSPs in brain slices from rats made epileptic by intrahippocampal tetanus toxin. *Epilepsy Research* **11**, 119–129.

KAMPHUIS, W., GORTER, J. A. & LOPES DA SILVA, F. (1991 *a*). A long-lasting decrease in the inhibitory effect of GABA on glutamate responses of hippocampal pyramidal neurons induced by kindling epileptogenesis. *Neuroscience* **41**, 425–431.

KAMPHUIS, W., HUISMAN, E., VEERMAN, M. J. & LOPES DA SILVA, F. H. (1991 *b*). Development of changes in endogenous GABA release during kindling epileptogenesis in rat hippocampus. *Brain Research* **545**, 33–40.

LLOYD, K. G., BOSSI, L., MORSELLI, P. L., MUNARI, C., ROUGIER, M. & LOISEAU, H. (1986). Alterations of GABA-mediated synaptic transmission in human epilepsy. *Advances in Neurology* **44**, 1033–1044.

LOPEZ-COLOMÉ, A. M., TAPIA, R., SALCEDA, R. & PASANTES-MORALES, H. (1978). K^+-stimulated release of labeled γ-aminobutyrate, glycine and taurine in slices of several regions of rat central nervous system. *Neuroscience* **3**, 1069–1074.

MELLANBY, J., GEORGE, G., ROBINSON, A. & THOMPSON, P. (1977). Epileptiform syndrome in rats produced by injecting tetanus toxin into the hippocampus. *Journal of Neurology, Neurosurgery and Psychiatry* **40**, 404–414.

MELLANBY, J. & GREEN, J. (1981). How does tetanus toxin act? *Neuroscience* **6**, 281–300.

MELLANBY, J. H. (1989). Elimination of ^{125}I from rat brain after injection of small doses of ^{125}I-labelled tetanus toxin into the hippocampus. *Neuroscience Letters Supplement* **36**, S55.

MICHELSON, H. B. & WONG, R. K. S. (1991). Excitatory synaptic responses mediated by $GABA_A$ receptors in the hippocampus. *Science* **253**, 1420–1423.

MILES, R. (1990). Synaptic excitation of inhibitory cells by single CA3 hippocampal pyramidal cells of the guinea-pig *in vitro*. *Journal of Physiology* **428**, 61–77.

MÜLLER, W. & MISGELD, U. (1990). Inhibitory role of dentate hilus neurons in guinea pig hippocampal slice. *Journal of Neurophysiology* **64**, 46–56.

NAJLERAHIM, A., WILLIAMS, S. F., PEARSON, R. C. A. & JEFFERYS, J. G. R. (1992). Increased expression of GAD mRNA during the chronic epileptic syndrome due to intrahippocampal tetanus toxin. *Experimental Brain Research* **90**, 332–342.

NICHOLLS, D. G. (1989). Release of glutamate, aspartate, and γ-aminobutyric acid from isolated nerve terminals. *Journal of Neurochemistry* **52**, 331–341.

PENNER, R., NEHER, E. & DREYER, F. (1986). Intracellularly injected tetanus toxin inhibits exocytosis in bovine adrenal chromaffin cells. *Nature* **324**, 76–78.

RIBAK, C. E., JOUBRAN, C., KESSLAK, J. P. & BAKAY, R. A. E. (1989). A selective decrease in the number of GABAergic somata occurs in pre-seizing monkeys with alumina gel granuloma. *Epilepsy Research* **4**, 126–138.

ROUX, E. & BORREL, A. (1898). Tétanos cérébral et immunité contre le tétanos. *Annals de l'Institut Pasteur* **4**, 225–239.

SCHWAB, M. E., AGID, Y., GLOWINSKI, J. & THOENEN, H. (1977). Retrograde axonal transport of ^{125}I-tetanus toxin as a tool for tracing fibre connections in the central nervous system: connections of the rostral part of the rat neostriatum. *Brain Research* **126**, 211–224.

SEGAL, M. (1990). A subset of local interneurons generate slow inhibitory postsynaptic potentials in hippocampal neurons. *Brain Research* **511**, 163–164.

SIHRA, T. S. & NICHOLLS, D. G. (1987). 4-Aminobutyrate can be released exocytotically from guinea-pig cerebral cortical synaptosomes. *Journal of Neurochemistry* **49**, 261–267.

SLOVITER, R. S. (1987). Decreased hippocampal inhibition and a selective loss of interneurons in experimental epilepsy. *Science* **235**, 73–76.

SLOVITER, R. S. (1991). Permanently altered hippocampal structure, excitability, and inhibition after experimental status epilepticus in the rat: The "dormant basket cell" hypothesis and its possible relevance to temporal lobe epilepsy. *Hippocampus* **1**, 41–66.

SZERB, J. C., ROSS, T. E. & GUREVICH, L. (1981). Compartments of labeled and endogenous γ-aminobutyric acid giving rise to release evoked by potassium or veratridine in rat cortical slices. *Journal of Neurochemistry* **37**, 1186–1192.

TASKER, J. G. & DUDEK, F. E. (1991). Electrophysiology of GABA-mediated synaptic transmission and possible roles in epilepsy? *Neurochemical Research* **16**, 251–262.

TRAUB, R. D., KNOWLES, W. D., MILES, R. & WONG, R. K. S. (1987). Models of the cellular mechanism underlying propagation of epileptiform activity in the CA2–CA3 region of the hippocampal slice. *Neuroscience* **21**, 457–470.

WONG, R. K. S., TRAUB, R. D. & MILES, R. (1986). Cellular basis of neuronal synchrony in epilepsy. *Advances in Neurology* **44**, 583–592.