Journal of Physiology (1993), **460**, *pp.* 503–523
With 10 *figures*
Printed in Great Britain

PROPERTIES OF GABA-MEDIATED SYNAPTIC POTENTIALS INDUCED BY ZINC IN ADULT RAT HIPPOCAMPAL PYRAMIDAL NEURONES

BY XINMIN XIE AND TREVOR G. SMART

From The School of Pharmacy, Department of Pharmacology, 29–39 Brunswick Square, London WC1N 1AX

(*Received 13 January 1992*)

SUMMARY

1. Intracellular recording techniques were used to study the actions of the transition ion, zinc, on CA1 and CA3 pyramidal neurones in adult rat hippocampal slices.

2. Zinc (300 μM) hyperpolarized pyramidal neurones, increased the membrane excitability and also induced periodic, spontaneous giant depolarizing potentials associated with a conductance increase mechanism.

3. The occurrence of spontaneous giant depolarizations was dependent on the zinc concentration (10 μM–1 mM) with an apparent dissociation constant of 98 μM. The frequency of zinc-induced depolarizations was unaffected by the membrane potential from -50 to -100 mV.

4. Stimulation of the Schaffer collaterals or mossy fibre pathways evoked an excitatory and inhibitory synaptic potential complex. In the presence of zinc, nerve fibre stimulation evoked, in an all-or-none fashion, a giant depolarizing potential with an increased membrane conductance. Both spontaneous and evoked depolarizations were inhibited by 1 μM tetrodotoxin.

5. Evoked giant depolarizations were labile with too frequent stimulation resulting in a failure of generation. A minimum time of 140 s was required between stimuli to ensure successive giant depolarizations.

6. Spontaneous and evoked zinc-induced depolarizing potentials were inhibited by bicuculline (10 μM) or picrotoxin (40 μM) and enhanced by pentobarbitone (100 μM) or flurazepam (10 μM), suggesting that these potentials are mediated by activation of γ-aminobutyric acid$_A$ (GABA$_A$) receptors.

7. Ionophoretic application of GABA produced biphasic responses at -60 mV membrane potential. The reversal potentials for the depolarizing and hyperpolarizing GABA responses were -56 ± 5 and -66 ± 8 mV respectively. The giant depolarizations induced by zinc reversed at -57 ± 4 mV. This suggests a dendritic location for the generation of these potentials.

8. Excitatory amino acid antagonists, 2-amino-5-phosphonovalerate (APV, 40 μM) or 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX, 10 μM) did not affect the amplitude but slightly reduced the frequency of the giant depolarizations.

9. It is concluded that zinc induces a synchronized release of GABA, quite independent of intact excitatory synaptic transmission, which acts on GABA$_A$

MS 1034

receptors producing large depolarizing synaptic potentials. This increased level of GABA release may be of physiological and pathological importance since zinc is a naturally occurring metal ion endogenous to the central nervous system.

INTRODUCTION

A considerable number of studies have identified the presence of zinc in the mammalian central nervous system (CNS) using a variety of histochemical techniques, including silver amplification (Danscher, 1984), chelation with dithizone (Danscher, Howell, Perez-Clausell & Hertel, 1985) and quinoline fluorescence (Frederickson, Kasarskis, Ringo & Frederickson, 1987). These procedures can, in principle, detect the presence of metals other than just zinc, but only zinc can be detected by all three methods (see Frederickson, 1989, for comprehensive review). Using these techniques, various areas of the CNS have been identified containing 'histochemically reactive zinc'. Overall, this pool of zinc represents only a small percentage of the total stores in the CNS, but interestingly it appears to be closely associated with nerve terminals (Frederickson, 1989).

Zinc is concentrated within neurones, defined by Frederickson (1989) as 'zinc-containing neurones', in selected areas of the CNS forming an extensive network throughout the cortex, hippocampus and cerebellum (Haug, 1973; Crawford & Connor, 1972; Faber, Braun, Zuschratter & Scheich, 1989). The hippocampus exhibits one of the largest concentrations of zinc which is mostly localized to the mossy fibre pathway running from the dentate gyrus granule cell bodies and synapsing with the apical dendrites of large pyramidal neurones in the CA3 region (see Haug, 1973, for review). Zinc can be actively taken up into nerve terminals (Wolf, Schutte & Romhild, 1984; Wensink, Molenaar, Woroniecka & Van Den Hamer, 1988) and stored within structures resembling synaptic vesicles (Ibata & Otsuka, 1969; Friedman & Price, 1984; Perez-Clausell & Danscher, 1985; Holm, Andreasen, Danscher, Perez-Clausell & Nielsen, 1988). Moreover, other investigators have established that zinc can also be released in a calcium-dependent manner by applying electrical or chemical stimulation to the mossy fibre pathway (Assaf & Chung, 1984; Howell, Welch & Frederickson, 1984; Charlton, Rovira, Ben-Ari & Leviel, 1985).

An indication of possible physiological or neuromodulatory roles for zinc was prompted when this divalent cation was observed to interact with both inhibitory and excitatory amino acid receptors (Smart & Constanti, 1983; Peters, Koh & Choi, 1987; Westbrook & Mayer, 1987; Mayer, Vyklicky & Westbrook, 1989; Smart & Constanti, 1990; Smart, 1990). Many of these studies utilized tissue cultured neurones which allowed greater access for study by electrophysiological methods, but such preparations are devoid of their *in vivo* synaptic connections and native neuronal circuitry. Very few studies have examined the effect of zinc on neurones in brain slices. Recently, an intracellular study using hippocampal slices from immature animals indicated that a large depolarizing synaptic potential in CA3 neurones, mediated by activation of γ-aminobutyric acid$_A$ ($GABA_A$) receptors, may be caused by endogenous zinc (Xie & Smart, 1991*a*). These depolarizing potentials are not present in older, more mature hippocampal neurones, but similar potentials can be induced by the application of zinc.

In the present study we have characterized in detail the effects of zinc on the properties of pyramidal neurones in both CA1 and CA3 regions, and examined the actions of zinc on synaptic transmission in the adult hippocampal brain slice preparation. Some of our preliminary results have been presented in abstract form (Xie & Smart, 1991*b*).

METHODS

Brain slice preparation

CA1 and CA3 pyramidal neurones were studied in transverse hippocampal slices prepared from male Wistar rats using a McIlwain tissue chopper. Adult rats (200–300 g mass) were anaesthetized by ether inhalation and guillotined prior to the rapid removal of the brain. Hippocampal slices were cut to 400 μm thickness and preincubated for 1 h in a Krebs solution containing (mM): NaCl, 118; KCl, 3; $CaCl_2$, 2·5; $MgCl_2$, 2; $NaHCO_3$, 25; D-glucose, 11, bubbled with 95% O_2–5% CO_2, pH 7·4. A single slice was placed into the recording chamber and fully submerged between two pieces of nylon mesh and then superfused continuously at 6 ml/min (bath volume 1 ml) with oxygenated Krebs solution at 30 °C. All the other slices were maintained in the incubation chamber at room temperature (20–25 °C) until required. To enhance the action of NMDA, nominally zero magnesium Krebs solution was used to which 10 μM glycine was added (Johnson & Ascher, 1987). To inhibit activation of NMDA receptors, the extracellular concentration of magnesium was raised to 4 mM, with nominally zero glycine concentration. Most drugs were dissolved in Krebs solution and applied via superfusion at known concentrations. GABA (1 M, pH 4, dissolved in distilled water) was also applied using ionophoresis from single-barrelled ionophoretic electrodes. All ionophoretic pipettes were positioned in the apical dendritic field for both CA1 and CA3 neurones.

Maintenance of brain slices in vitro for 24 h

Occasionally, some slices were used after 24 h following dissection. Slices were kept overnight at room temperature and suspended on a nylon mesh from a 'tea strainer' submerged in a large volume of Krebs solution (250 ml) bubbled with 95% O_2–5% CO_2. These '24 h old' slices were viable, with the neurones exhibiting virtually identical membrane and synaptic properties when compared with cells in 'fresh' slices. Data were therefore pooled from slices of either age. Increasing the incubation time for brain slices *in vitro* in normal Krebs solution from 36 to 48 h proved unsuccessful with very few viable neurones.

Electrophysiology

Intracellular recordings were performed on pyramidal neurones in CA1 and CA3 subfields using a single microelectrode current–voltage clamp preamplifier (Dagan 8100). The switching frequency was 2–3 kHz (25% duty cycle) after adjustment of the capacity neutralization in switch clamp operating mode. Sampled membrane currents and voltages were recorded on a Brush-Gould ink pen chart recorder (2200) and also stored on a Racal store 4D FM tape recorder (DC to 5 kHz) for analysis using a Tandon or Mission 286-based computer system. Intracellular microelectrodes were filled with either 3 M KCl or 4 M potassium acetate (resistances 40–70 MΩ) and buffered to pH 7·1. Orthodromic stimulation (6–40 V, 0·1 ms) was applied to the slices using bipolar stainless-steel stimulating electrodes with a separation of < 50 μm. These electrodes were located in stratum lucidum for stimulating the mossy fibre input to CA3 and also placed in stratum radiatum for stimulating the Schaffer collateral afferents to the CA1 subfield. The data reported in this study were taken from recordings in 245 neurones including both CA1 and CA3 cells. Neurones were selected for study if the resting membrane potential was at least -60 mV with action potential amplitudes of 90–100 mV. Intracellular recordings were stable for 1–6 h. Data are presented as the mean $\pm$ standard deviation (S.D.) and statistical significance was assessed where appropriate using an unpaired t test.

RESULTS

Induction of spontaneous depolarizing potentials by zinc in pyramidal neurones

Intracellular recordings from adult rat pyramidal neurones in either CA1 or CA3 regions using KCl-filled microelectrodes are characterized by a background activity of small amplitude (2–9 mV) synaptic potentials, mediated largely by $GABA_A$

receptor activation. After bath application of zinc (50–300 μM), the membrane was gradually hyperpolarized by 3–8 mV from the resting membrane potential (-68 ± 5 mV, $n = 69$) with a concurrent increase (20%) in the cell input resistance (42 ± 12 MΩ in control Krebs solution, 51 ± 15 MΩ in zinc, $P < 0{\cdot}01$, Fig. 1*A*). Zinc

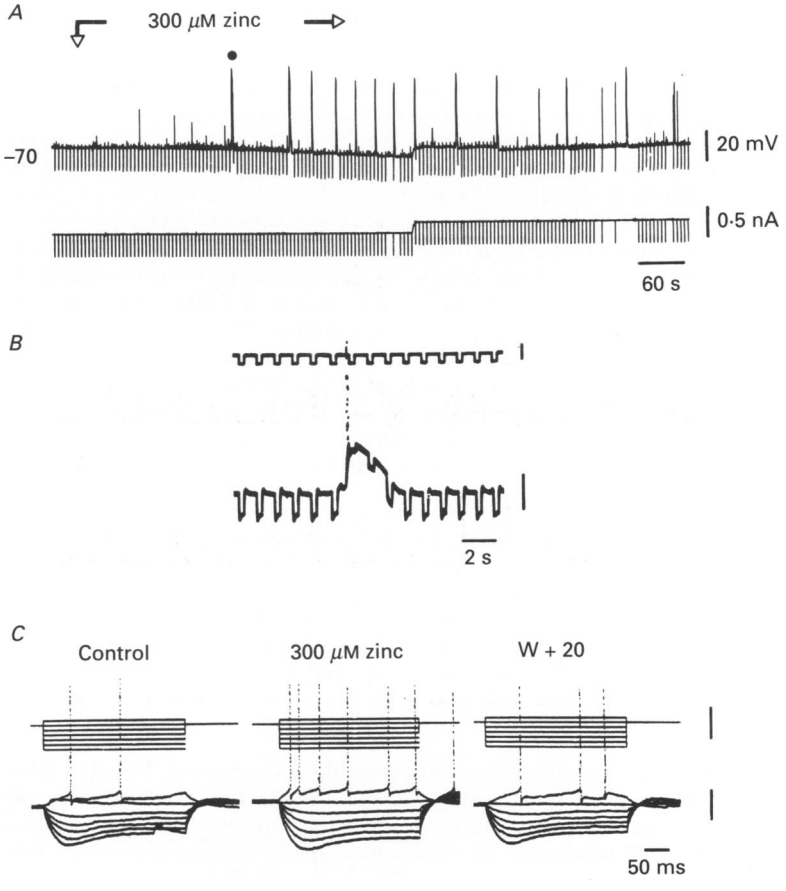

Fig. 1. Effect of zinc on CA1 pyramidal cell properties. *A*, chart recording of membrane potential and superimposed hyperpolarizing electrotonic potentials (upper trace) evoked by $-0{\cdot}3$ nA, 300 ms, 0·2 Hz current pulses (lower trace). Bath-application of zinc (300 μM; open triangles) induced a slow hyperpolarization and the appearance of giant depolarizing potentials (●) after 4 min incubation. Repolarizing the cell to -70 mV with DC current injection did not affect the spontaneous depolarizations. In this and all other figures unless otherwise specified, recordings were made using 3 M KCl-filled microelectrodes. *B*, in a different CA1 neurone using a potassium acetate-filled microelectrode, a single zinc-induced depolarization is associated with a large increase in the input conductance (lower trace) monitored by repetitive injection of current pulses (upper trace, $-0{\cdot}3$ nA, 300 ms, 1 Hz). Associated action potential firing was usually restricted to the early rising phase of the depolarizing potential. Membrane potential -72 mV. *C*, superimposed electrotonic potentials (lower traces) produced by depolarizing or hyperpolarizing current pulses (upper traces; 0·1 nA steps, 300 ms) in the absence and presence of zinc (300 μM) and following a 20 min recovery from zinc. Membrane potential -70 mV, maintained with current injection. Voltage and current calibration in *A* also applies to *B* and *C*.

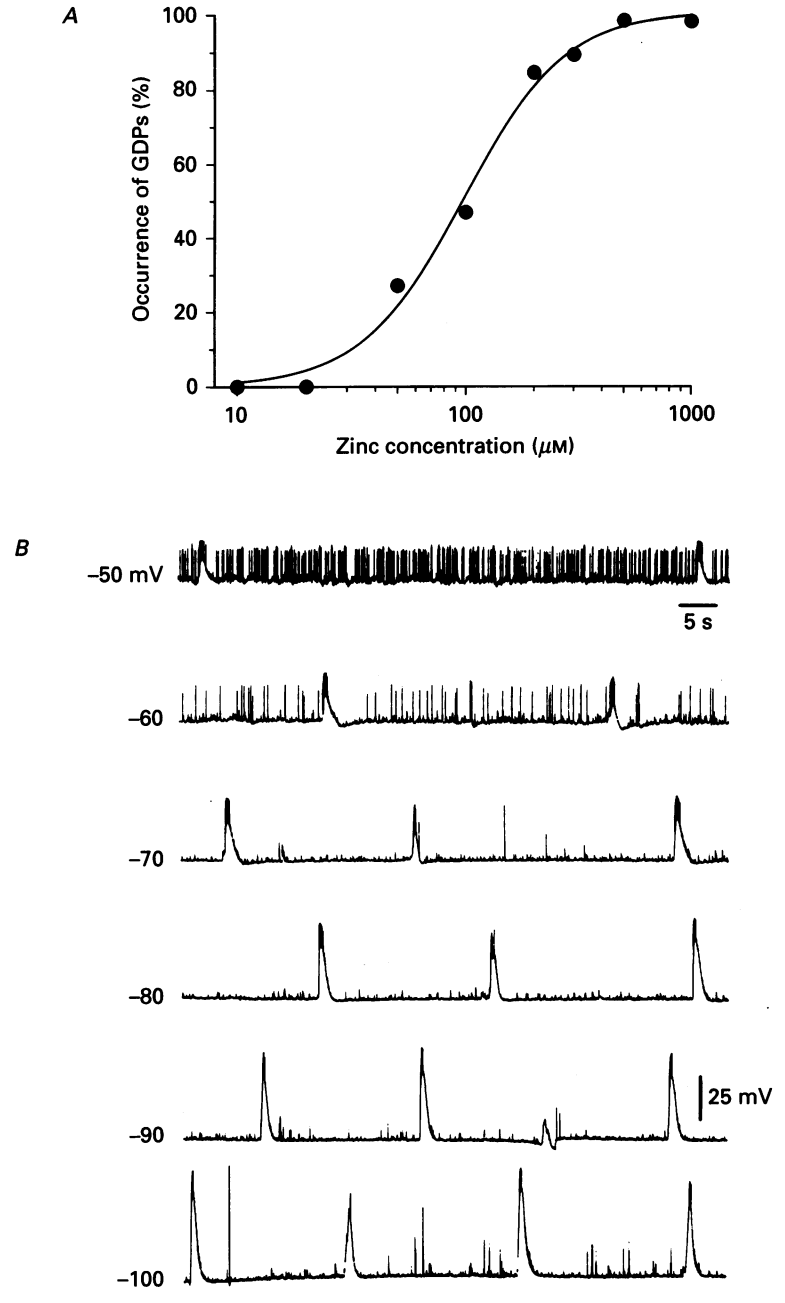

Fig. 2. *A*, zinc induced the appearance of giant depolarizing potentials (GDPs) in a concentration-dependent manner. The percentage of neurones exhibiting spontaneous GDPs is plotted against the zinc concentration (μM). Percentages were determined at each concentration from 8 to 127 separate applications of zinc to 205 CA1 and CA3 neurones. The data were fitted by a curve generated from a state equation of the form; $y/y_{max} = 1/\{1+(K/A)^n\}$, where y/y_{max} represents the relative proportion of neurones exhibiting GDPs, A is the zinc concentration, n the Hill coefficient and K the apparent dissociation constant for zinc. K and n were estimated using a Marquardt non-linear least squares fitting routine as $98{\cdot}3 \pm 8{\cdot}7$ μM and $1{\cdot}9 \pm 0{\cdot}3$ (mean ± S.E.M.) respectively. *B*, the

reduced the action potential accommodation causing multiple spike firing after injection of depolarizing current into the neurone. The after-hyperpolarization following each action potential was also reduced (Sim & Cherubini, 1990); however, the inward rectifying properties of these cells (Halliwell & Adams, 1982), typified by the 'sag' in the hyperpolarizing electrotonic potentials, were unaffected (Fig. 1*C*).

In the presence of zinc, spontaneous depolarizing potentials also appeared with characteristically large amplitudes of 39 ± 9 mV ($n = 52$) and durations of 2–4 s. These values are 5- to 20- and at least 100-fold greater than the respective mean amplitudes and durations determined for the spontaneous 'background' synaptic potentials. The large depolarizing events invariably terminated with a small hyperpolarization of 2–6 mV and duration of 1–3 s (Fig. 1*A*).

Similar depolarizing events were also recorded at the resting membrane potential (approximately -70 mV) in neurones using 4 M potassium acetate-filled microelectrodes. However, the depolarizations were smaller in amplitude and produced a less intense firing of action potentials (mean amplitude 15 ± 6 mV, $n = 27$). Each depolarizing potential, recorded with either electrolyte, was associated with an increased membrane conductance which abolished the firing of action potentials often evoked during the early phase of the depolarization (Fig. 1*B*).

The ability of neurones to support the zinc-induced depolarizing events was quite robust, since they also occurred in slices maintained *in vitro* for over 24 h and are therefore not due to acute damage following the preparation of 'fresh' brain slices.

Spontaneous depolarizations are dependent on zinc concentration

The threshold concentration of zinc which induced the spontaneous depolarizing events varied between different hippocampal neurones, even within the same brain slice. If no large depolarizing events were observed after incubation with zinc for 10–15 min, then this concentration was deemed ineffective. In some neurones, up to three different concentrations of zinc were applied before any depolarizing events occurred. A recovery from the effects of the preceding dose of zinc was gauged by monitoring the resting membrane properties. The relationship between zinc concentration and the induction of the synaptic events can be described by a sigmoidal curve, with a threshold concentration of 20–30 μM and an apparent dissociation constant for zinc of 98 μM. Increasing the zinc concentration to 300 μM, resulted in most cells ($> 90\%$; $n = 121$) exhibiting depolarizing events (Fig. 2*A*).

In any one cell, the spontaneous depolarizations appeared with a consistent periodicity. The mean time interval between successive events was 117 ± 62 s ($n = 52$), but there was considerable variation in the mean intervals between different cells (20–200 s). The frequency was apparently unaffected by the membrane

frequency of zinc-induced depolarizations is independent of membrane potential. Chart records of spontaneous giant depolarizations and IPSPs in the presence of 300 μM zinc in a CA1 neurone at membrane potentials of -50 to -100 mV maintained by constant current injection. The amplitude of the depolarizations increased as the membrane potential was hyperpolarized away from the chloride equilibrium potential. At more depolarized potentials (< -60 mV), spontaneous action potential firing occasionally occluded the resolution of the depolarizing events. The frequency of spontaneous depolarizations in this cell varied between 0·035 Hz (-50 mV) to 0·06 Hz (-100 mV). Resting membrane potential -67 mV.

potential from −50 to −100 mV, but using a KCl-filled microelectrode and depolarizing the cell above −50 mV in 300 μM zinc, the amplitude of the spontaneous depolarizations decreased as the reversal potential for these events was approached. Moreover, the increased level of action potential firing combined to make accurate

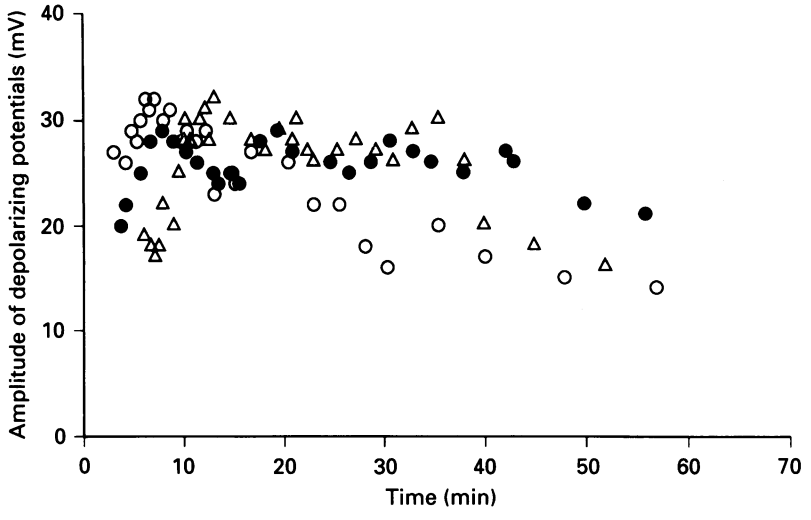

Fig. 3. Time dependence of the zinc-induced depolarizing potentials. The representative data were accrued from three separate CA1 neurones in different brain slices following addition of 300 μM zinc. The appearance of spontaneous depolarizations at the appropriate times after exposure of the slices to zinc (defined as time zero) are plotted according to their amplitude. The amplitudes are usually well maintained over 40–50 min, but the frequency of appearance decreases. After a very long exposure to zinc (60 min), it was rare to record any more giant depolarizing events. These three cells were monitored for 70 min. Membrane potentials adjusted with DC current to −70 mV.

measurements of frequency difficult (Fig. 2*B*). In Fig. 2*B*, the depolarizing events occurred at a frequency of 0·05 Hz at −100 mV, marginally decreasing to 0·03 Hz at −50 mV. At more depolarized membrane potentials (< −70 mV), spontaneous depolarizations were often associated with after-hyperpolarizations which were reduced in amplitude when approaching the potassium equilibrium potential (Fig. 2*B*).

If zinc application was continued for 40–80 min, the spontaneous depolarizations would eventually wane and disappear. The first indication of waning was a decrease in the event frequency followed by the duration and finally the amplitude (Fig. 3). Following a prolonged wash with control Krebs solution after long exposures to zinc, a second application of zinc would either not induce any further spontaneous depolarizing potentials in the same cell, or depolarizing events would reappear but at much lower frequencies (< 0·005 Hz). In addition, a second application of zinc could also induce giant depolarizations in other neurones impaled in the same slice, but the frequency of occurrence was again lower than that measured in the first recording.

Zinc-induced depolarizations are synaptic events

The periodic appearance of the giant depolarizations induced by zinc, suggested that they may be of synaptic origin. Injecting depolarizing current pulses with variable widths and amplitudes into pyramidal neurones in control Krebs solution

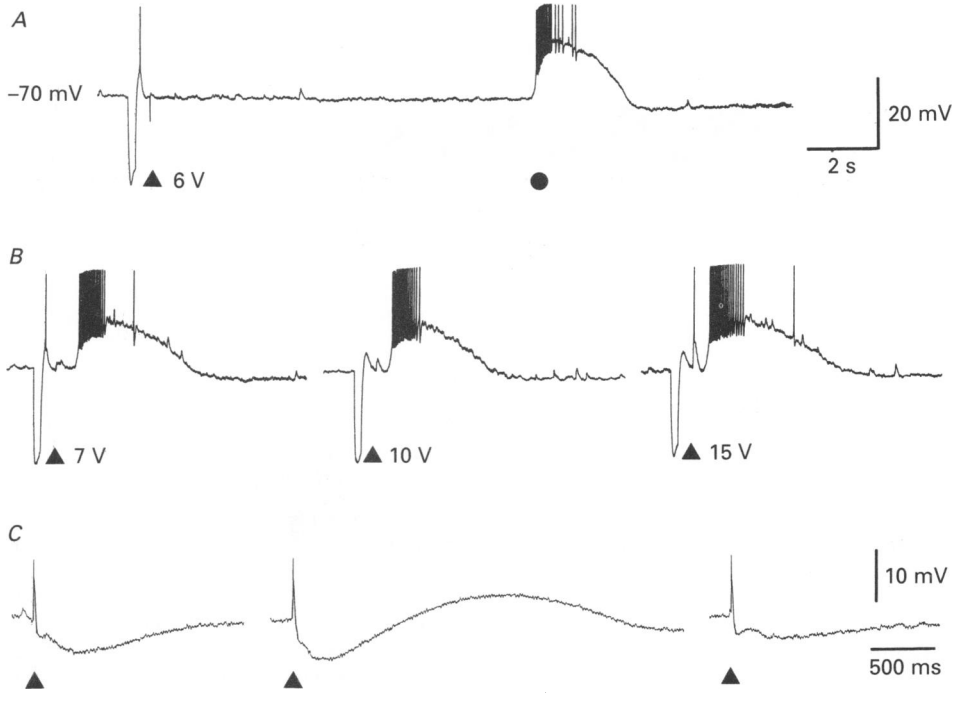

Fig. 4. Stimulus-evoked giant depolarizing potentials in the presence of zinc occurred in an all-or-none fashion. *A*, in the presence of zinc (300 μM) which induced the appearance of spontaneous giant depolarizations (●), low-intensity stimulation (▲, 6 V, 0·1 s) of the Schaffer collaterals evoked only a small EPSP–IPSP complex. *B*, increasing the stimulus intensity to 7–15 V produced a non-incrementing giant depolarizing synaptic potential with a measurable latency from the initial EPSP/IPSPs. Membrane input conductance was monitored with a hyperpolarizing electrotonic potential evoked by constant current pulse injection (−0·5 nA, 300 ms) prior to each stimulus. *C*, in a different CA1 neurone and using a potassium acetate-filled microelectrode, stimulation of the Schaffer collateral pathway (▲; 38 V, 0·1 ms) evoked an EPSP followed by fast and slow IPSPs (lefthand trace). In 300 μM zinc, the slow IPSP was occluded by a large depolarizing potential (middle trace) which disappeared on washing with control Krebs solution for 10 min (righthand trace). Membrane potential −72 mV.

failed to initiate any large depolarizations similar to those induced by zinc. In the presence of zinc (300 μM), orthodromic low-intensity stimulation (6 V) of the Schaffer collaterals when recording with a KCl-filled microelectrode in CA1 cells, only evoked consistent excitatory (EPSP) and inhibitory (IPSP) depolarizing postsynaptic potential complexes, despite the appearance of spontaneous giant depolarizing potentials (Fig. 4*A*). However, subsequent higher intensity stimulation of these

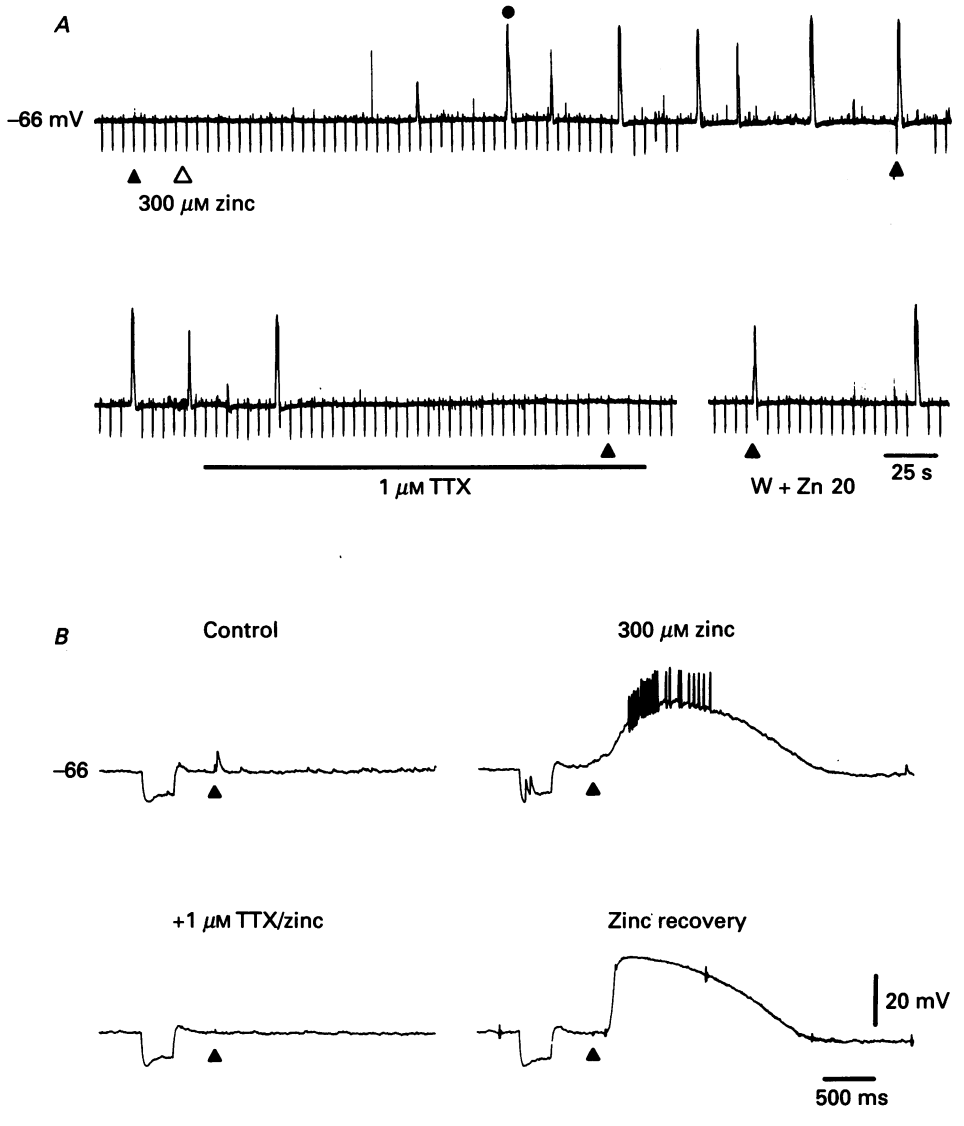

Fig. 5. Zinc-induced spontaneous and stimulus-evoked giant depolarizing potentials are blocked by tetrodotoxin (TTX). *A*, intracellular recording from a CA1 neurone at the resting potential of −66 mV. Bath-application of 300 μM zinc (△) induces spontaneous (●) and stimulus-evoked depolarizations (▲). Co-application of 1 μM TTX abolishes both spontaneous and evoked depolarizations. A recovery from TTX was obtained after 20 min in the zinc-containing Krebs solution (W + Zn 20). *B*, in the same neurone, stimulation (▲; 10 V, 0·1 s) of the Schaffer collateral pathway induced an EPSP–IPSP complex in control. A hyperpolarizing electrotonic potential was used to monitor membrane input conductance (−0·3 nA, 300 ms). Further stimulation in 300 μM zinc evoked a giant depolarization which is blocked by TTX and recovered on washing in zinc-containing Krebs solution.

pathways (7–15 V) induced a giant depolarizing potential following the EPSP in an all-or-none fashion (Fig. 4*B*).

The polarity of the giant synaptic depolarizations generated at the resting potential (approximately −70 mV) was also established with potassium acetate-

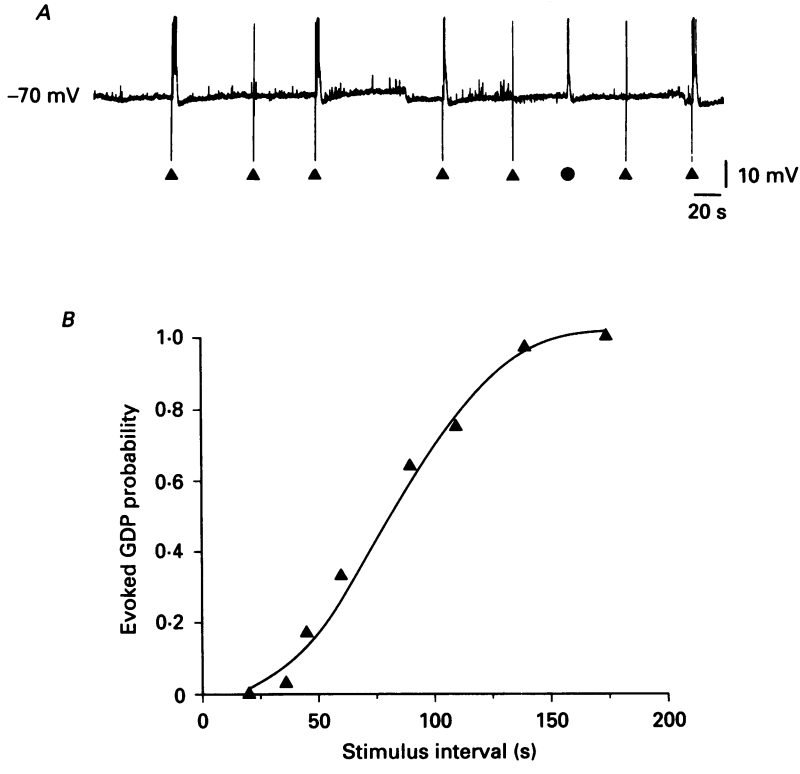

Fig. 6. Determination of the minimum time interval required between two evoked giant depolarizations in 300 μM zinc. *A*, chart recording of giant depolarizations in a CA1 neurone evoked by stimulating the Schaffer collaterals (▲; 15 V; 0·1 ms). If stimuli were applied too frequently, or if a spontaneous giant depolarization (●) occurred, further stimuli failed to evoke any giant depolarizations. Hyperpolarizing electrotonic potentials were evoked by current injection (−0·5 nA, 300 ms) and monitored the membrane conductance prior to each stimulus. *B*, the probability of evoking a giant depolarizing potential is plotted against the interstimulus interval. The minimum interval required between two successive stimuli was determined in each of twenty-six CA1 and CA3 neurones. The interstimulus interval was increased systematically until the second stimulus consistently evoked a giant depolarization on three occasions. When the interstimulus interval was sufficiently long, all neurones supported the production of successive GDPs which was then defined as a probability of 1. Up to four different stimulus intervals were tested in each neurone.

filled microelectrodes. Orthodromic stimulation of the Schaffer collaterals resulted in an EPSP followed now by hyperpolarizing fast and slow IPSPs (Fig. 4*C*). Zinc did not apparently affect the EPSP or the fast IPSP, but the slow IPSP was occluded by a giant depolarizing potential with a similar time course to the spontaneous

potentials induced by zinc (Fig. 4*C*). These giant potentials disappeared within 2 min following superfusion with control Krebs solution.

The zinc-induced potentials probably resulted from activity in mono- and/or polysynaptic pathways, since the addition of tetrodotoxin (TTX; 1 μM) caused the

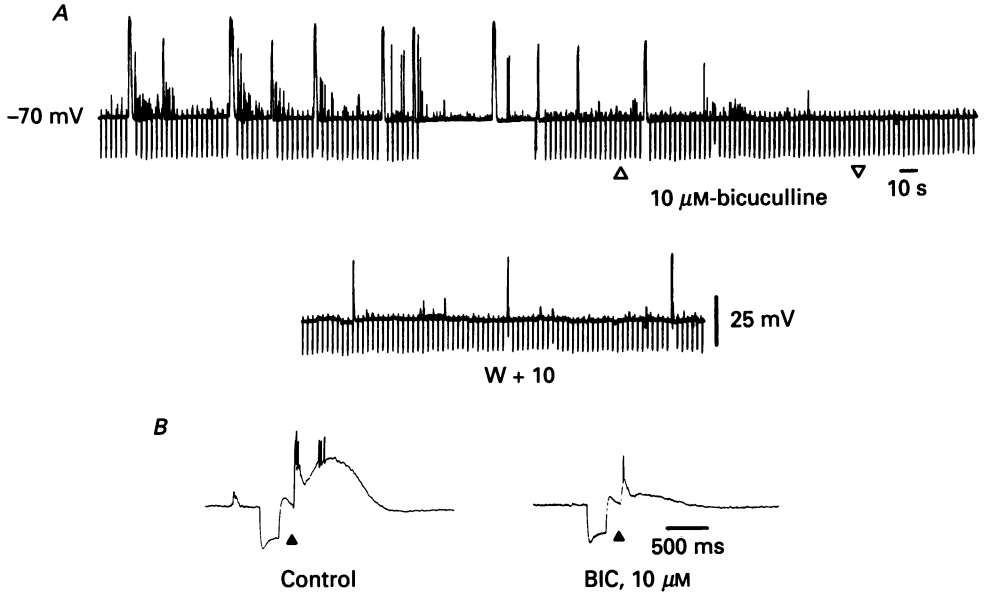

Fig. 7. Giant depolarizations induced by zinc are blocked by bicuculline. *A* shows a chart recording of membrane potential and superimposed hyperpolarizing electrotonic potentials evoked by current injection (−0·3 nA, 300 ms, 0·2 Hz) in the presence of 300 μM zinc. The periodic appearance of the GDPs was abolished by bath-applied bicuculline (BIC, 10 μM). A partial recovery (lower trace) was obtained after washing with zinc-containing Krebs solution for 10 min (W+10). *B* illustrates two sample traces following stimulation of the Schaffer collateral pathway (10 V, 0·1 ms) evoking a giant depolarization which was also substantially reduced by bicuculline. Membrane potential −70 mV.

cessation of spontaneous depolarizations and also abolished the evoked response following nerve fibre stimulation (Fig. 5).

Paired pulse depression of the zinc-induced synaptically evoked depolarizing potentials

Paired pulse stimulation was applied to the Schaffer collaterals in 300 μM zinc to assess the degree of facilitation or inhibition of the second evoked depolarization caused by the first response. For each neurone, following the first stimulation, a well-defined time interval could be measured during which repeated stimulation, even at increased stimulus strengths, would not evoke a second response (Fig. 6*A*). If a minimum time of approximately 90 s was allowed between successive stimuli, the probability of obtaining a second response equal in amplitude and duration to the first was increased; however, any spontaneous depolarizing potential intervening

between the two stimuli was capable of inhibiting further evoked potentials for the next 60–90 s (Fig. 6*A*). The time required between successive stimuli to ensure a high probability of producing a second evoked response in twenty-six neurones superfused with 300 μM zinc was approximately 140 ± 92 s (eighty-eight stimuli; Fig. 6*B*).

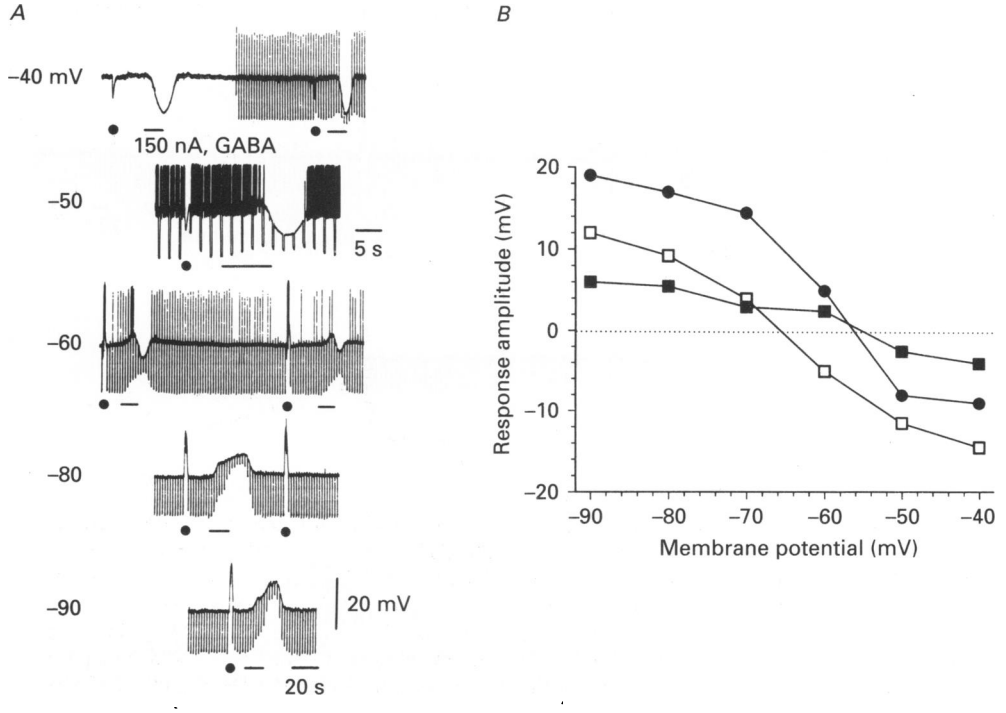

Fig. 8. Reversal potential for $GABA_A$ responses and the zinc-induced giant depolarizing potentials recorded using a potassium acetate-filled microelectrode. *A* illustrates chart records of GABA responses and spontaneous giant depolarizing potentials (●) in the presence of 300 μM zinc. GABA was applied from an ionophoretic pipette positioned in the apical dendrites of a CA1 neurone (resting potential −64 mV). GABA was ejected (continuous lines) using 150 nA currents (10 s; −10 nA holding current) and the membrane potential was varied from −40 to −90 mV using constant current injection. Spontaneous action potential firing occurred at membrane potentials more positive than −60 mV (brief upward deflections). The GABA response was largely depolarizing at −90 mV, hyperpolarizing at −40 mV and biphasic at −60 mV. To increase clarity the constant current pulses were temporarily stopped at −40 mV and the chart speed was increased at −50 mV. *B*, determination of the reversal potentials for the GABA response and zinc-induced GDP (data taken from *A*). The GABA response amplitudes were measured at two latencies, following ejection of GABA: at 10 (short, ■) and 15 s (long latency, □). The GDP amplitude (●) was measured at the peak response. These amplitudes are plotted against the membrane potential. In this neurone, the short and long latency GABA responses reversed at −55 and −65 mV respectively. The GDP reversed at −56 mV.

Pharmacology of the zinc-induced depolarizing potentials

The increased amplitude of depolarizing events recorded using 3 M KCl-filled microelectrodes suggested that underlying these depolarizations was a chloride-mediated current. As the events were synaptically generated relying on the release

of neurotransmitter, and since γ-aminobutyric acid$_A$ (GABA$_A$) receptors directly gate chloride channels, this was a candidate membrane protein to mediate the zinc-induced depolarizations. Bath application of the GABA$_A$ antagonists, (+)-bicuculline (10 μM; $n = 16$) or picrotoxin (40 μM; $n = 3$, not shown), inhibited, in a

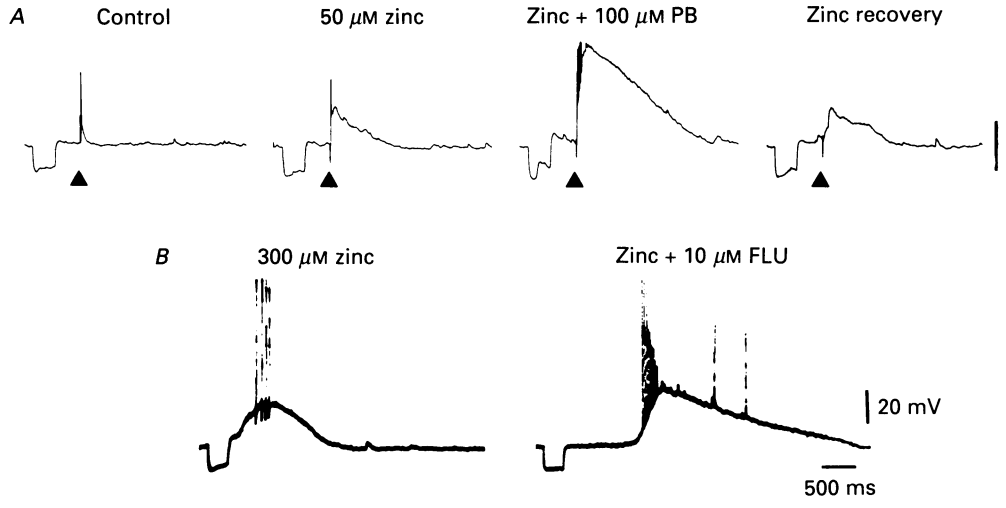

Fig. 9. Effect of a barbiturate and benzodiazepine on zinc-induced giant depolarizing potentials. *A*, stimulation of the Schaffer collaterals (▲; 8 V, 0·1 ms) evoked giant depolarizing potentials in 50 μM zinc. Co-application of 100 μM pentobarbitone (PB) caused an enhancement in the evoked GDP which recovered after washing with zinc-containing Krebs solution. *B*, in another CA1 neurone, spontaneous giant depolarizing potentials were induced by 300 μM zinc. Subsequent co-application of 10 μM flurazepam (FLU) slightly enhanced the amplitude and more clearly prolonged the duration of the spontaneous GDPs. Hyperpolarizing electrotonic potentials were evoked by current pulses (−0·3 nA, 300 ms, 0·2 Hz). Membrane potentials −60 (*A*) and −73 mV (*B*). The peak amplitudes were measured and the durations of the potentials were determined at the baseline.

partly reversible manner, both the spontaneous and evoked depolarizing potentials induced by 300 μM zinc (Fig. 7). The spontaneous 'background' depolarizing IPSPs were also inhibited by this low concentration of bicuculline, producing a quiescent neurone.

The dependence of these giant potentials on the GABA$_A$ receptor was further demonstrated by measuring the reversal potential for the GABA-evoked responses and the zinc-induced depolarizations using potassium acetate-filled microelectrodes. Ionophoretically applied GABA to the apical dendrites of a CA1 neurone superfused with 300 μM zinc induced a biphasic GABA response at −60 mV with an initial short latency depolarization followed by a long latency hyperpolarization (Fig. 8*A*). Both responses were associated with an increase in the membrane conductance. Previous studies suggested that the *depolarizing* response may represent the activation of dendritic GABA$_A$ receptors, whereas the *hyperpolarizing* response occurs following activation of somatic GABA$_A$ receptors (Alger & Nicoll, 1982*b*; Scharfman & Sarvey, 1987). The biphasic nature of these responses was presumed to be dependent on different transmembrane chloride gradients maintained between the soma and

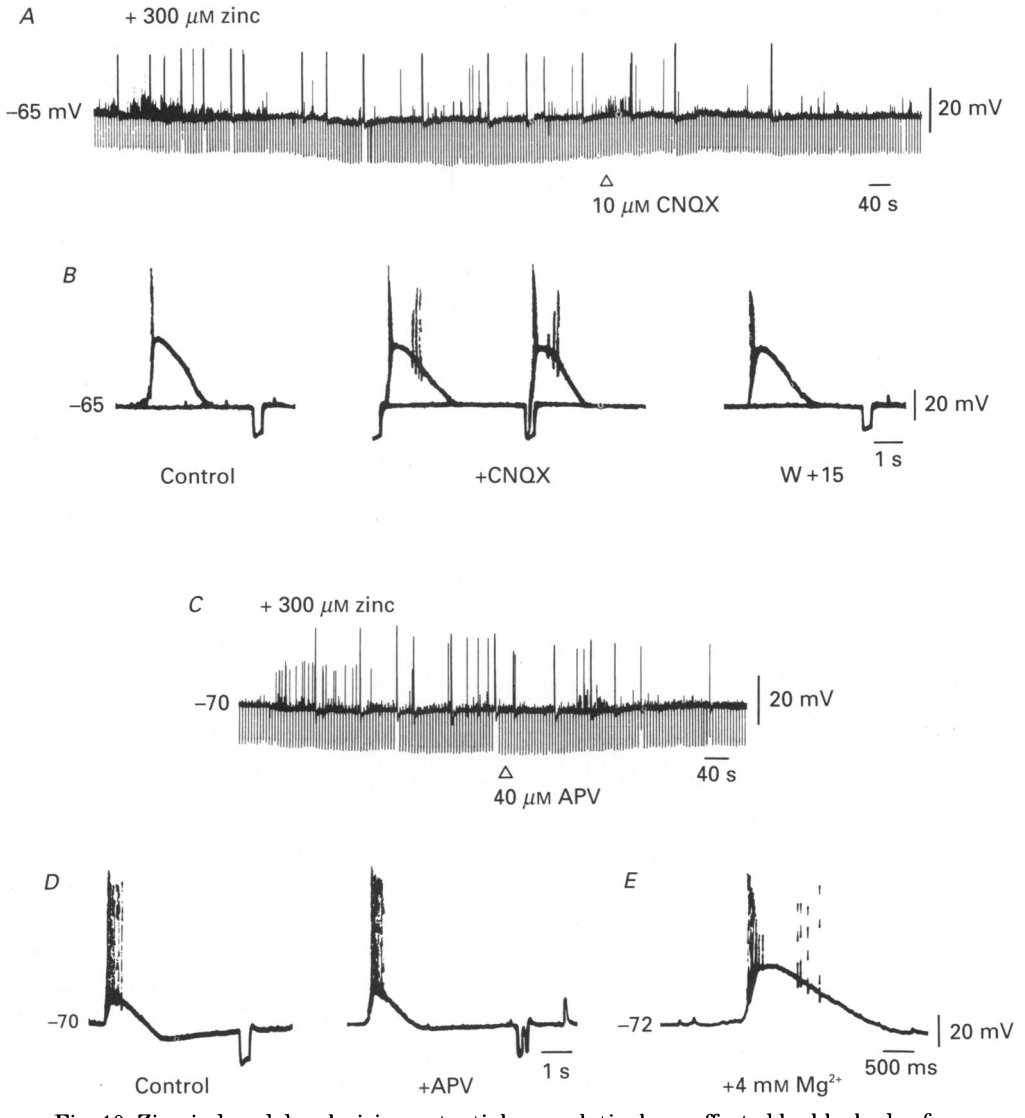

Fig. 10. Zinc-induced depolarizing potentials are relatively unaffected by blockade of non-NMDA or NMDA receptors. *A*, chart record of membrane potential in the presence of 300 μM zinc showing spontaneous giant depolarizations, interposed action potentials and depolarizing IPSPs. Application of 10 μM 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX) did not block the GDPs but slightly reduced their frequency. *B*, selected zinc-induced depolarizations in control, +10 μM CNQX, and following wash-out of CNQX for 15 min (W+15). Hyperpolarizing electrotonic potentials were applied throughout (−0·3 nA, 300 ms, 0·3 Hz). Resting potential −65 mV. *C*, in a different neurone, spontaneous giant depolarizations induced by 300 μM zinc were minimally affected by co-application of 40 μM 2-amino-5-phosphonovalerate (APV) which caused only a small reduction in frequency, but did not block the GDPs. *D*, selected traces of zinc-induced spontaneous potentials in control Krebs solution and 40 μM APV. Membrane potential −70 mV maintained with DC current injection. Hyperpolarizing electrotonic potentials were evoked with negative current pulses (−0·3 nA; 300 ms, 0·2 Hz). *E*, in another CA1 neurone, raising the external Mg^{2+} concentration to 4 mM also failed to block the zinc-induced depolarizing potentials. Membrane potential −72 mV.

dendrites (Misgeld, Deisz, Dodt & Lux, 1986; Thompson, Deisz & Prince, 1988; cf. Alger & Nicoll, 1982*b*, and Lambert, Borroni, Grover & Teyler, 1991). The current–voltage relationships for the short and long latency GABA responses and the zinc-induced depolarizing events were determined. The reversal potentials for the short and long latency GABA responses were -56 ± 5 and -66 ± 8 mV respectively ($n = 4$). Similarly, the reversal potential for the zinc-induced depolarizations was -57 ± 4 mV ($n = 8$) which was not significantly different from the reversal of the short latency GABA response (Fig. 8*B*; $P > 0{\cdot}1$). This suggested that the depolarizing events may be largely mediated by $GABA_A$ receptors located primarily on the dendrites of the pyramidal neurones. Using KCl-filled microelectrodes, the reversal potentials for both the zinc-induced events and the totally monophasic depolarizing GABA responses were more depolarized at -30 ± 3 mV ($n = 3$).

The pharmacological similarity between GABA responses and the zinc-induced depolarizing events was emphasized by using allosteric modulators represented by the barbiturates or benzodiazepines. Both classes of compound have discrete binding sites on the allosteric $GABA_A$ receptor complex. In a CA1 neurone, following stimulation of the Schaffer collateral pathway, pentobarbitone (100 μM) substantially enhanced the amplitude ($62 \pm 36\%$) and prolonged the duration ($40 \pm 28\%$; $n = 4$) of the zinc-induced depolarization in a reversible manner (Fig. 9*A*). Flurazepam (1–10 μM) also prolonged the duration ($130 \pm 56\%$) of the depolarizations in 300 μM zinc, but unlike pentobarbitone, had only a slight enhancing effect on the amplitude ($24 \pm 8\%$; $n = 3$; Fig. 9*B*). These features of the zinc-induced depolarizations are entirely consistent with such events being mediated by $GABA_A$ receptors.

Are zinc-induced depolarizations dependent on excitatory synaptic transmission?

Inhibitory synaptic potentials mediated by $GABA_A$ receptor activation and generated in the hippocampus, can participate in either feedback or feedforward inhibition requiring functional excitatory synaptic transmission. The involvement of *N*-methyl-D-aspartate (NMDA) or non-NMDA receptors in the generation of zinc-induced depolarizing events was studied using excitatory amino acid (EAA) antagonists. Neither 2-amino-5-phosphonovalerate (APV; 40 μM) nor 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX; 10 μM), nor 2 mM kynurenic acid were able to block spontaneous or evoked synaptic events in the presence of 300 μM zinc. At most, in some cells ($n = 3$), a small reduction in the frequency of the spontaneous events was evident (Fig. 10). These concentrations of NMDA and non-NMDA antagonists routinely inhibited directly evoked responses using bath or ionophoretically applied EAA agonists. A role for NMDA receptors in controlling, or initiating, the zinc-induced events was also largely discounted following the lack of effect of a 4 mM Mg^{2+} and nominally zero glycine-containing Krebs solution (Fig. 10*E*; $n = 12$).

DISCUSSION

Zinc induces a large depolarizing synaptic potential mediated by GABA$_A$ receptors

These results describe a profound effect of zinc on adult hippocampal neurones in brain slices, manifest by the induction of large synaptic potentials mediated by $GABA_A$ receptors. Previous studies on cultured *embryonic* neurones indicated that

zinc is an effective non-competitive blocker of $GABA_A$-induced responses and $GABA_A$-mediated IPSPs with little effect on the composite EPSP (Westbrook & Mayer, 1987; Mayer & Vyklicky, 1989; Smart & Constanti, 1990). However, $GABA_A$-mediated responses and IPSPs evoked on mature adult neurones are far less sensitive to inhibition by zinc (Smart & Constanti, 1990; Xie & Smart, 1991*a*, *b*; Smart, 1992). This insensitivity may be dependent on different populations of $GABA_A$ receptors. Molecular cloning studies have established that there are multiple types of $GABA_A$ receptor protein subunit (α, β, γ, δ and ρ), and also various subtypes of individual subunits (Olsen & Tobin, 1990; Burt & Kamatchi, 1991). Expression studies in cell lines, using recombinant cDNAs for individual $GABA_A$ receptor subunits, has enabled the construction of relatively homogenous $GABA_A$ receptor populations. Interestingly, $GABA_A$ receptors containing the γ subunit mediated responses which are considerably less sensitive to inhibition by zinc (Draguhn, Verdoorn, Ewert, Seeburg & Sakmann, 1990; Smart, Moss, Xie & Huganir, 1991), suggesting that zinc-induced depolarizations may be mediated by $GABA_A$ receptors containing one or more γ-subunits.

Intracellular recordings from hippocampal pyramidal neurones at -60 to -70 mV with 3 M KCl-filled microelectrodes revealed a continuous barrage of depolarizing synaptic activity largely composed of IPSPs mediated by $GABA_A$ receptor activation which were unaffected by zinc. The zinc-induced spontaneous giant potentials are generated with a large conductance increase and can be inhibited by TTX or bicuculline, and enhanced by pentobarbitone or flurazepam. This indicated that an intact neuronal circuitry is required for generating these potentials which are mediated by activation of $GABA_A$ receptors following the presynaptic release of GABA. The ubiquity of these potentials is so far unknown, but similar events do not appear when recording intracellularly from olfactory cortical neurones (surface slice; Smart & Constanti, 1983, 1990). The lack of organized synaptic circuitry as found in the intact hippocampus and also the use of embryonic cultured neurones, may be why such events have eluded previous investigators (Mayer & Vyklicky, 1989; Smart & Constanti, 1990).

The intervals between successive spontaneous depolarizations were usually quite regular in a single neurone. Paired pulse stimulation revealed that if the interval between two stimuli was insufficient, the second depolarizing event was labile and subject to failure. This apparent 'activity-dependent disinhibition' was not followed by any excitatory or epileptic-like discharges and the postsynaptic membrane was still responsive to exogenously applied GABA, suggesting that the failure of transmission was a presynaptic phenomenon. There are many possible reasons for transmission failure, including: an increased level of released GABA activating presynaptic $GABA_B$ receptors and inhibiting further GABA release (Deisz & Prince, 1989; Thompson & Gahwiler, 1989*c*); alternatively, $GABA_A$ receptor desensitization or a shift in the GABA response reversal potential (E_{GABA}) could reduce the synaptic response. Repetitive stimulation can also lead to a use-dependent depression of IPSPs (Ben-Ari, Krnjevic & Reinhardt, 1979; McCarren & Alger, 1985; Thompson & Gahwiler, 1989*a*), possibly by raising extracellular potassium concentration (McCarren & Alger, 1985; Korn, Giacchino, Chamberlin & Dingledine, 1987) which may affect E_{GABA} (Thompson & Gahwiler, 1989*b*). These possibilities are considered

unlikely, since there is no small decrement in the amplitude of the large depolarizing potentials but an abrupt failure of transmission and this phenomenon was observed with KCl-filled microelectrodes rendering any subsequent large shift in E_{GABA} unlikely. Moreover, in cortical or sympathetic neurones, zinc did not affect E_{GABA} (Smart & Constanti, 1990). We cannot yet discount a prolonged inactivation of presynaptic calcium channels, or an acute depletion of neurotransmission, but spontaneous IPSP activity was very often unaffected when the transmission failed.

Origin of GABA-mediated synaptic potentials

The insensitivity of zinc-induced depolarizations to APV or CNQX suggests that excitatory synaptic transmission is not involved in the underlying release of GABA. The correlation of the reversal potentials for the depolarizing (dendritic) ionophoretic GABA response and the zinc-induced depolarizations indicated the most likely site(s) for GABA release mediating the zinc-induced events is in the pyramidal cell dendrites. This location could be explained if zinc induced GABA release from nearby inhibitory neurones, for example, basket cells, oriens/alveus and stratum lacunosum-moleculare interneurones (Lacaille & Schwartzkroin, 1988). Mossy fibre projections are known to arborize into the dendritic field of basket neurones as well as forming large proximal synapses with pyramidal neurones (Frotscher, 1985). Interestingly, immunohistochemical evidence suggests that in addition to glutamate, mossy fibre terminals may also contain GABA which if co-released, might modulate the activity of a hitherto considered pure excitatory nerve pathway (Sandler & Smith, 1991). Furthermore, some hilar neurones which receive axon collaterals from the zinc-containing mossy fibres (Claiborne, Amaral & Cowan, 1986) may project their axons *directly* to pyramidal neurones, providing a source of GABA to underlie the giant IPSPs without a requirement for excitatory synaptic transmission (Muller & Misgeld, 1991).

How are zinc-induced depolarizations generated?

The large amplitude of the GABA-mediated depolarizations may result from a synchronous release of transmitter. Zinc can inhibit a variety of potassium channels, including calcium-activated or transient A-type potassium channels (Constanti & Smart, 1987; Sim & Cherubini, 1990; Spigelman & Carlen, 1991) which if present in nerve terminals and partly responsible for spike repolarization, could broaden the duration of the presynaptic action potential and increase transmitter release. Zinc inhibition of some potassium currents may also account for the reduced action potential accommodation and spike after-hyperpolarization which are features also seen with embryonic neuronal cultures (Mayer & Vyklicky, 1989). However, other divalent cations such as cadmium and copper, which have similar effects on the pyramidal cell membrane properties, do not induce giant GABA-mediated potentials (X. Xie & T. G. Smart, unpublished observations). Furthermore, whether zinc has any effect on presynaptic neuronal calcium channels remains to be established, although on myotubes, zinc blocked dihydropyridine-sensitive calcium channels (Winegar & Lansman, 1990). A similar effect on neurones (cf. Sim & Cherubini, 1990) may *decrease* transmitter release which would be broadly incompatible with our results.

In addition to a likely presynaptic locus of action, zinc may also exert an effect postsynaptically. On cortical neurones, zinc enhanced $GABA_A$-mediated responses which may have resulted from a decreased input conductance making the cell electrotonically more compact. Thus depolarizations induced in more distal parts of the cell (e.g. dendrites), could now be passively transmitted over a longer distance (i.e. to the recording site at the cell soma) augmenting the somatic response (Smart & Constanti, 1983, 1990). Similarly, in hippocampal neurones, the large amplitude depolarizations induced by zinc could therefore travel from their presumed dendritic site of generation and also be resolved in the soma. This would explain why large GABA-mediated depolarizations, and also hyperpolarizing chloride-mediated responses to direct somatic applications of GABA, can co-exist in the soma at the resting potential.

Comparison with previous work

Large depolarizing synaptic potentials have been reported previously; including an innate occurrence in immature hippocampal neurones (Ben-Ari, Cherubini, Corradetti & Gaiarsa, 1989; Xie & Smart, 1991*a*), or induced following either high intensity afferent stimuli (Perreault & Avoli, 1988), or after pharmacological manipulation with either pentobarbitone (Alger & Nicoll, 1982*a*), 4-aminopyridine (4-AP; Buckle & Haas, 1982; Perreault & Avoli, 1989; Avoli, 1990; Muller & Misgeld, 1990, 1991), or guanosine-5′-O-(3-thio)-triphosphate (Thalmann, 1988). The large depolarizing potentials induced by 4-AP are similar to the potentials induced by zinc. 4-AP-induced $GABA_A$-mediated potentials are insensitive to excitatory amino acid antagonists, suggesting that these potentials may also be mediated by bursting in interneurones evoking synchronized potentials in the hilus and CA3/CA4 neurones (Avoli, 1990; Muller & Misgeld, 1990, 1991; Aram, Michelson & Wong, 1991; Michelson & Wong, 1991). Also, the GABA-mediated potentials are probably generated dendritically since ionophoresis of bicuculline methiodide in stratum radiatum abolished the giant IPSPs (Perreault & Avoli, 1989). However, 4-AP induces a large increase in both excitatory and inhibitory transmitter release (Buckle & Haas, 1982; Rutecki, Lebeda & Johnston, 1987), whereas zinc apparently induces only GABA release. The amplitudes of the GABA-mediated potentials induced by zinc in our study, are much larger than the mean amplitude of the smaller 'background' IPSPs, suggesting that either a distinct inhibitory cell population is responsible for their generation, or that many interneurones are synchronously discharging to produce giant potentials.

The significance of the present work is that until now giant $GABA_A$-mediated synaptic potentials have only been resolved using compounds not usually found in the CNS. Zinc is a naturally occurring trace element in neural tissues and is particularly concentrated in neurones within the hippocampus. The observation that zinc appears to have uncovered a novel $GABA_A$-mediated synaptic potential to modulate cell excitability adds to the established feedback and feedforward inhibitory networks with which we are familiar. Whether this potential has any physiological or pathological relevance remains to be seen.

This work was supported by the MRC and Wellcome Trust.

REFERENCES

ALGER, B. E. & NICOLL, R. A. (1982*a*). Feed-forward dendritic inhibition in rat hippocampal pyramidal cells studied *in vitro*. *Journal of Physiology* **328**, 105–123.

ALGER, B. E. & NICOLL, R. A. (1982*b*). Pharmacological evidence for two kinds of GABA receptor on rat hippocampal pyramidal cells studied *in vitro*. *Journal of Physiology* **328**, 125–141.

ARAM, J. A., MICHELSON, H. B. & WONG, R. K. S. (1991). Synchronized GABAergic IPSPs recorded in the neocortex after blockade of synaptic transmission mediated by excitatory amino acids. *Journal of Neurophysiology* **65**, 1034–1041.

ASSAF, S. Y. & CHUNG, S. H. (1984). Release of endogenous Zn^{2+} from brain tissue during activity. *Nature* **308**, 734–736.

AVOLI, M. (1990). Epileptiform discharges and a synchronous GABAergic potential induced by 4-aminopyridine in the rat immature hippocampus. *Neuroscience Letters* **117**, 93–98.

BEN-ARI, Y., CHERUBINI, E., CORRADETTI, R. & GAIARSA, J.-L. (1989). Giant synaptic potentials in immature rat CA3 hippocampal neurones. *Journal of Physiology* **416**, 303–325.

BEN-ARI, Y., KRNJEVIC, K. & REINHARDT, W. (1979). Hippocampal seizures and failure of inhibition. *Canadian Journal of Physiology and Pharmacology* **57**, 1462–1466.

BUCKLE, P. J. & HAAS, H. L. (1982). Enhancement of synaptic transmission by 4-aminopyridine in hippocampal slices of the rat. *Journal of Physiology* **326**, 109–122.

BURT, D. R. & KAMATCHI, G. L. (1991). $GABA_A$ receptor subtypes: from pharmacology to molecular biology. *FASEB Journal* **5**, 2916–2923.

CHARLTON, G., ROVIRA, C., BEN-ARI, Y. & LEVIEL, V. (1985). Spontaneous and evoked release of endogenous Zn^{2+} in the hippocampal mossy fiber zone of the rat in situ. *Experimental Brain Research* **58**, 202–205.

CLAIBORNE, B. J., AMARAL, D. G. & COWAN, W. M. (1986). A light and electron microscopic analysis of the mossy fibers of the rat dentate gyrus. *Journal of Comparative Neurology* **246**, 435–458.

CONSTANTI, A. & SMART, T. G. (1987). Zinc blocks the A-current in cultured rat sympathetic neurones. *Journal of Physiology* **396**, 159*P*.

CRAWFORD, I. L. & CONNOR, J. D. (1972). Zinc in maturing rat brain: Hippocampal concentration and localization. *Journal of Neurochemistry* **19**, 1451–1458.

DANSCHER, G. (1984). Do the Timm sulphide silver method and the selenium method demonstrate zinc in the brain? In *The Neurobiology of Zinc*, part A, *Physiochemistry, Anatomy and Techniques*, ed. FREDERICKSON, C. J., HOWELL, G. A. & KASARSKIS, E. J., pp. 273–287. Alan R. Liss, New York.

DANSCHER, G., HOWELL, G. A., PEREZ-CLAUSELL, J. & HERTEL, N. (1985). The dithizone, Timm's sulphide silver and the selenium methods demonstrate a chelatable pool of zinc in the CNS. *Histochemistry* **83**, 419–422.

DEISZ, R. A. & PRINCE, D. A. (1989). Frequency-dependent depression of inhibition in guinea-pig neocortex *in vitro* by $GABA_B$ receptor feed-back on GABA release. *Journal of Physiology* **412**, 513–541.

DRAGUHN, A., VERDOORN, T. A., EWERT, M., SEEBURG, P. H. & SAKMANN, B. (1990). Functional and molecular distinction between recombinant rat $GABA_A$ receptor subtypes by Zn^{2+}. *Neuron* **5**, 781–788.

FABER, H., BRAUN, K., ZUSCHRATTER, W. & SCHEICH, H. (1989). System specific distribution of zinc in the chick brain. A light- and electron-microscopic study using the Timm method. *Cell and Tissue Research* **258**, 247–257.

FREDERICKSON, C. J. (1989). Neurobiology of zinc and zinc-containing neurons. *International Review of Neurobiology* **31**, 145–238.

FREDERICKSON, C. J., KASARSKIS, E. J., RINGO, D. & FREDERICKSON, R. E. (1987). A quinoline fluorescence method for visualizing and assaying the histochemically reactive zinc (bouton zinc) in the brain. *Journal of Neuroscience Methods* **20**, 91–103.

FRIEDMAN, B. & PRICE, J. L. (1984). Fiber systems in the olfactory bulb and cortex: A study in adult and developing rats, using the Timm method with light and electron microscope. *Journal of Comparative Neurology* **223**, 88–109.

FROTSCHER, M. (1985). Mossy fibres form synapses with identified pyramidal basket cells in the

CA3 region of the guinea-pig hippocampus: a combined Golgi–electron microscope study. *Journal of Neurocytology* **14**, 245–259.

HALLIWELL, J. V. & ADAMS, P. R. (1982). Voltage-clamp analysis of muscarinic excitation in hippocampal neurons. *Brain Research* **250**, 71–92.

HAUG, F. M. (1973). Heavy metals in the brain. A light microscopic study of the rat with Timm's sulphide silver method. Methodological considerations and cytological and regional staining patterns. *Advances in Anatomy, Embryology and Cell Biology* **47**, 1–71.

HOLM, I. E., ANDREASEN, A., DANSCHER, G., PEREZ-CLAUSELL, J. & NIELSEN, H. (1988). Quantification of vesicular zinc in the rat brain. *Histochemistry* **89**, 289–293.

HOWELL, G. A., WELCH, M. G. & FREDERICKSON, C. J. (1984). Stimulation-induced uptake and release of zinc in hippocampal slices. *Nature* **308**, 736–738.

IBATA, Y. & OTSUKA, N. (1969). Electron microscopic demonstration of zinc in the hippocampal formation using Timm's sulphide silver technique. *Journal of Histochemistry Cytochemistry* **17**, 171–175.

JOHNSON, J. W. & ASCHER, P. (1987). Glycine potentiates the NMDA response in cultured mouse brain neurons. *Nature* **325**, 529–531.

KORN, S. J., GIACCHINO, J. L., CHAMBERLIN, N. L. & DINGLEDINE, R. (1987). Epileptiform burst activity induced by potassium in the hippocampus and its regulation by GABA-mediated inhibition. *Journal of Neurophysiology* **57**, 325–340.

LACAILLE, J.-C. & SCHWARTZKROIN, P. A. (1988). Stratum lacunosum-moleculare interneurons of hippocampal CA1 region. II. Intrasomatic and intradendritic recordings of local circuit synaptic interactions. *Journal of Neuroscience* **8**, 1411–1424.

LAMBERT, N. A., BORRONI, A. M., GROVER, L. M. & TEYLER, T. J. (1991). Hyperpolarizing and depolarizing $GABA_A$ receptor-mediated dendritic inhibition in area CA1 of the rat hippocampus. *Journal of Neurophysiology* **66**, 1538–1548.

MCCARREN, M. & ALGER, B. E. (1985). Use-dependent depression of IPSPs in rat hippocampal pyramidal cells in vitro. *Journal of Neurophysiology* **53**, 557–571.

MAYER, M. L. & VYKLICKY, L. JR (1989). The action of zinc on synaptic transmission and neuronal excitability in cultures of mouse hippocampus. *Journal of Physiology* **415**, 351–365.

MAYER, M. L., VYKLICKY, L. JR & WESTBROOK, G. L. (1989). Modulation of excitatory amino acid receptors by group IIB metal cations in cultured mouse hippocampal neurons. *Journal of Physiology* **415**, 329–350.

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

MISGELD, U., DEISZ, R. A., DODT, H. U. & LUX, H. D. (1986). The role of chloride transport in postsynaptic inhibition of hippocampal neurons. *Science* **232**, 1413–1415.

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

MULLER, W. & MISGELD, U. (1991). Picrotoxin- and 4-aminopyridine-induced activity in hilar neurons in the guinea-pig hippocampal slice. *Journal of Neurophysiology* **65**, 141–147.

OLSEN, R. W. & TOBIN, A. J. (1990). Molecular biology of the $GABA_A$ receptors. *FASEB Journal* **4**, 1469–1480.

PEREZ-CLAUSELL, H. & DANSCHER, G. (1985). Intravesicular localization of zinc in rat telencephalic boutons. A histochemical study. *Brain Research* **337**, 91–98.

PERREAULT, P. & AVOLI, M. (1988). A depolarizing inhibitory postsynaptic potential activated by synaptically released γ-aminobutyric acid under physiological conditions in rat hippocampal pyramidal cells. *Canadian Journal of Physiology and Pharmacology* **66**, 1100–1102.

PERREAULT, P. & AVOLI, M. (1989). Effects of low concentrations of 4-aminopyridine on CA1 pyramidal cells of the hippocampus. *Journal of Neurophysiology* **61**, 953–970.

PETERS, S., KOH, D. W. & CHOI, D. W. (1987). Zinc selectively blocks the action of N-methyl-D-aspartate on cortical neurons. *Science* **236**, 589–593.

RUTECKI, P. A., LEBEDA, F. T. & JOHNSTON, D. (1987). 4-Aminopyridine produces epileptiform activity in hippocampus and enhances synaptic excitation and inhibition. *Journal of Neurophysiology* **57**, 1911–1924.

SANDLER, R. & SMITH, D. A. (1991). Coexistence of GABA and glutamate in mossy fiber terminals of the primate hippocampus: An ultrastructural study. *Journal of Comparative Neurology* **303**, 177–192.

SCHARFMAN, H. E. & SARVEY, J. M. (1987). Responses to GABA recorded from identified rat visual cortical neurons. *Neuroscience* **23**, 407–422.

SIM, J. A. & CHERUBINI, E. (1990). Submicromolar concentrations of zinc irreversibly reduce a calcium-dependent potassium current in rat hippocampal neurons *in vitro*. *Neuroscience* **36**, 623–629.

SMART, T. G. (1990). Uncultured lobster muscle, cultured neurons and brain slices: The neurophysiology of zinc. *Journal of Pharmacy and Pharmacology* **42**, 377–387.

SMART, T. G. (1992). A novel modulatory binding site for zinc on the $GABA_A$ receptor complex in cultured rat neurones. *Journal of Physiology* **447**, 587–625.

SMART, T. G. & CONSTANTI, A. (1983). Pre- and postsynaptic effects of zinc on in vitro prepyriform neurones. *Neuroscience Letters* **40**, 205–211.

SMART, T. G. & CONSTANTI, A. (1990). Differential effect of zinc on the vertebrate $GABA_A$ receptor complex. *British Journal of Pharmacology* **99**, 643–654.

SMART, T. G., MOSS, S. J., XIE, X. & HUGANIR, R. L. (1991). $GABA_A$ receptors are differentially sensitive to zinc: dependence on subunit composition. *British Journal of Pharmacology* **103**, 1837–1839.

SPIGELMAN, I. & CARLEN, P. L. (1991). Zinc reduces the transient outward current in immature rat hippocampal neurons. *Society for Neuroscience Abstracts* **17**, 608.8.

THALMANN, R. H. (1988). Blockade of a late inhibitory postsynaptic potential in hippocampal CA3 neurons in vitro revels a late depolarizing potential that is augmented by pentobarbital. *Neuroscience Letters* **95**, 155–160.

THOMPSON, S. M., DEISZ, R. A. & PRINCE, D. A. (1988). Relative contributions of passive equilibrium and active transport to the distribution of chloride in mammalian cortical neurons. *Journal of Neurophysiology* **60**, 105–124.

THOMPSON, S. M. & GAHWILER, B. H. (1989*a*). Activity-dependent disinhibition. I. Repetitive stimulation reduces IPSP driving force and conductance in the hippocampus in vitro. *Journal of Neurophysiology* **61**, 501–511.

THOMPSON, S. M. & GAHWILER, B. H. (1989*b*). Activity-dependent disinhibition. II. Effects of extracellular potassium, furosemide, and membrane potential on E_{Cl} in hippocampal CA3 neurons. *Journal of Neurophysiology* **61**, 512–523.

THOMPSON, S. M. & GAHWILER, B. H. (1989*c*). Activity-dependent disinhibition. III. Desensitization and $GABA_B$ receptor-mediated presynaptic inhibition in the hippocampus in vitro. *Journal of Neurophysiology* **61**, 524–533.

WENSINK, J., MOLENAAR, A. J., WORONIECKA, U. D. & VAN DEN HAMER, C. J. A. (1988). Zinc uptake into synaptosomes. *Journal of Neurochemistry* **50**, 783–789.

WESTBROOK, G. L. & MAYER, M. L. (1987). Micromolar concentrations of Zn^{2+} antagonise NMDA and GABA responses in hippocampal neurons. *Nature* **328**, 640–643.

WINEGAR, B. D. & LANSMAN, J. B. (1990). Voltage-dependent block by zinc of single calcium channels in mouse myotubes. *Journal of Physiology* **425**, 563–578.

WOLF, G., SCHUTTE, M. & ROMHILD, W. (1984). Uptake and subcellular distribution of ^{65}zinc in brain structures during the post-natal development of the rat. *Neuroscience Letters* **51**, 277–280.

XIE, X. & SMART, T. G. (1991*a*). A physiological role for endogenous zinc in rat hippocampal synaptic neurotransmission. *Nature* **349**, 521–524.

XIE, X. & SMART, T. G. (1991*b*). GABA-mediated giant depolarizing synaptic potentials induced by zinc in adult rat hippocampal pyramidal neurones. *British Journal of Pharmacology* **102**, 300*P*.