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γ-AMINOBUTYRIC ACID HYPERPOLARIZES RAT HIPPOCAMPAL PYRAMIDAL CELLS THROUGH A CALCIUM-DEPENDENT POTASSIUM CONDUCTANCE

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SUMMARY

1. Application of γ-aminobutyric acid (GABA) to the dendrites of CA1 pyramidal cells in hippocampal slices produced depolarizing and hyperpolarizing responses.

2. Picrotoxin (50 μM) blocked the depolarizing response of the dendrites to GABA but not the hyperpolarizing responses of the dendrites. The hyperpolarizing response of the cell body to GABA was reduced but not blocked by picrotoxin, suggesting the presence of a complex response at the cell body.

3. The depolarizing response of the dendrites and the hyperpolarizing response of the cell body appeared to be at least partly Cl^- dependent as they were respectively increased and decreased in size in low-Cl^- artificial cerebrospinal fluid (ACSF), while the hyperpolarizing response of the dendrites was unaffected.

4. The hyperpolarizing response of the dendrites was increased in amplitude in low-K^+ ACSF and the extrapolated reversal potential of the response became more negative, suggesting that the response was K^+ dependent.

5. The hyperpolarizing response of the dendrites was decreased in size in high-K^+ ACSF and could be readily inverted by current injection. The reversal potential became less negative in high-K^+ ACSF in a similar manner to that of the slow after-hyperpolarization following a train of spikes, indicating that the response was a K^+ conductance.

6. Perfusion of the slice with normal or 0-Ca^{2+} ACSF containing Cd^{2+} or Mn^{2+} blocked synaptic transmission, increased spike duration and blocked the slow phase of the spike after-hyperpolarization (a.h.p.). This latter potential is thought to be mediated by a Ca^{2+}-dependent K^+ conductance. Later, the hyperpolarizing response of the dendrites to GABA was blocked without an effect on the other GABA responses.

7. Pressure application of Cd^{2+} (0·2–2 mM) onto the surface of the slice rapidly reduced or blocked the slow a.h.p. and the dendritic hyperpolarizing response to GABA.

8. Intracellular injection of EGTA rapidly blocked the slow phase of the a.h.p. and then later blocked or reduced the dendritic hyperpolarizing response to GABA.

9. We conclude that the hyperpolarizing response of the dendrites to GABA is mediated by a Ca^{2+}-dependent K^+ conductance.

INTRODUCTION

Hippocampal CA1 pyramidal cells respond in various ways to γ-aminobutyric acid (GABA). The dendrites are depolarized and the cell body is hyperpolarized (Alger & Nicoll, 1979; Andersen, Dingledine, Gjerstad, Langmoen & Mosfeldt-Larsen, 1980). Recently, a third response has been seen: a hyperpolarization of the dendrites that is pharmacologically distinct from the hyperpolarizing response of the cell body (Alger & Nicoll, 1982). The response can be seen most clearly when GABA or GABA agonists such as ethylenediamine are applied in the presence of picrotoxin which blocks the depolarizing response of the dendrites but to which the hyperpolarizing response of the dendrites is relatively insensitive. Blaxter & Cottrell (1985) reported that the response was resistant to bicuculline as well as picrotoxin and was not Cl^- dependent but was increased in size in low-K^+ artificial cerebrospinal fluid (ACSF), suggesting K^+ dependence.

There are many K^+ conductances in hippocampal (Halliwell & Adams, 1982; Segal & Barker, 1984) and other neurones. In sympathetic neurones, a Ca^{2+}-activated K^+ conductance is thought to contribute to spike repolarization (Adams, Constanti, Brown & Clark, 1982; MacDermott & Weight, 1982) and is also present in hippocampal pyramidal cells, contributing to the burst after-hyperpolarization (a.h.p.) (Alger & Nicoll, 1980) and the slow phase of the spike a.h.p. (slow a.h.p.) (Hotson & Prince, 1980; Johnston, Hablitz & Wilson, 1980; Gustafsson & Wigstrom, 1981). These slow a.h.p.s are blocked by the intracellular injection of ethyleneglycol-bis-(β-aminoethyl ether)*N*,*N*′-tetraacetic acid (EGTA) (Alger & Nicoll, 1980; Schwartzkroin & Stafstrom, 1980; Lancaster & Wheal, 1982). This conductance is presumably activated by Ca^{2+} that enters during the action potential.

We report here experiments using different extracellular K^+ concentrations, Ca^{2+}-channel blockers and intracellular EGTA showing that the dendritic hyperpolarizing response to GABA is mediated by a Ca^{2+}-dependent K^+ conductance.

METHODS

Transverse slices 300 μm thick were cut using a Vibratome from the hippocampus of male Wistar rats (140–160 g). Slices were stored for an hour before being transferred to the recording chamber of a modified Haas bath. Intracellular recordings were made at 32–35 °C from pyramidal cell bodies in the CA1a region of the slice. ACSF had the following composition (mM): Na^+, 154; K^+, 3·25; Ca^{2+}, 2; Mg^{2+}, 2; Cl^-, 131·5; HCO_3^-, 26; $H_2PO_4^-$, 1·25; SO_4^{2-}, 2 and dextrose 10 and was equilibrated with 95 % O_2, 5 % CO_2 giving a pH of 7·4. Low-K^+ ACSF was 0·63 mM-K^+ with 2·62 mM-NaCl substituted for the ions removed. High-K^+ ACSF was 6·5 mM or 13 mM-K^+ with the appropriate reduction in NaCl. 0-Ca^{2+} ACSF consisted of 0 $CaCl_2$ with 0·5 mM-$MnCl_2$ or 0·2 mM-$CdCl_2$ added. Sometimes 2 mM-$MgCl_2$ was added to substitute for the $CaCl_2$. For application of Cd^{2+} onto the slice, $CdCl_2$ was dissolved in ACSF containing no PO_4^{2-}, SO_4^{2-} or HCO_3^-. Sodium isethionate was substituted for part of the NaCl to reduce [Cl^-] to 88 or 70 mM. This represents positive shifts in the Cl^- equilibrium potential of 10 and 16 mV, respectively. Changes in the junction potential of the reference electrode were compensated for in determining the membrane potential. Lower [Cl^-] was not used since it has been reported that the binding of GABA is Cl^- dependent in ligand-binding experiments (Fujimoto & Okabayashi, 1981). Concentrations of Cl^- below 60 mM produced large

paroxysmal depolarizations of the neurone. Picrotoxin (usually 50 μM) was dissolved directly into the ACSF. Micro-electrodes (60–200 MΩ) were filled with 3 M-KCl, 3 M-K acetate or 2 M-KCl with 1 M-EGTA-K.

Recordings were accepted if the spike height was more than 70 mV, the input resistance was greater than 20 MΩ and the resting potential was more negative than −55 mV. Hyperpolarizing current pulses (0·1–0·2 nA, 100 ms, about 1 Hz) were passed into the cell to estimate changes in input resistance. The a.h.p. was elicited by depolarizing current pulses (0·1–0·6 nA, 100 ms, 0·1 Hz) producing three to eight spikes. Since the slowest part of the a.h.p. is mediated by a Ca^{2+}-activated K^+ conductance, the a.h.p. duration may be a good measure of the activity of this conductance. Spike durations were measured across the base line.

GABA (0·01 M, pH 7) was applied by pressure ejection (150 kPa, 3–240 ms) to the apical or basal dendrites of the impaled cell at 1 min intervals. In eleven cells, GABA was applied to the cell body and the stratum radiatum dendrites alternately from two pressure-ejection pipettes. In twenty cells, $CdCl_2$ (0·2–2 mM) in ACSF was applied directly to the surface of the slice. Picrotoxin (50 μM) was often included in the ACSF to block the depolarizing response of the dendrites to GABA.

RESULTS

A biphasic response of the stratum radiatum (apical) or stratum oriens (basal) dendrites was obtained in 116 of 118 cells (Fig. 1*A*). The depolarizing response became smaller with repeated applications of GABA at intervals of 30 s or less; the hyperpolarizing response was unaffected. Picrotoxin (50 μM) always blocked the depolarizing response (Fig. 1*B*) leaving a hyperpolarization ($n = 96$).

A hyperpolarizing response of the soma was present in eleven of eleven cells tested, but in three cells a depolarizing component was also present. In the other eight cells, a depolarizing component could be elicited with longer applications of GABA (Fig. 2*A*). Picrotoxin (50 μM) reduced but did not abolish the cell body hyperpolarizing response in six of six cells. In all cells the depolarizing phase of the biphasic response of the stratum radiatum dendrites was affected by picrotoxin first (Fig. 2*B* and *C*). The effect of picrotoxin was reversible after prolonged washing (up to 2 h), the hyperpolarizing response of the cell body recovering first (Fig. 2*D*). Occasionally picrotoxin, especially in 0-Ca^{2+} ACSF produced large (up to 50 mV), long-lasting (up to 5 min) depolarizations that were accompanied by large increases in conductance.

In order to test the effect of Cl^- on the response, about 25 % of the cells were impaled with electrodes containing 3 M-KCl. The somatic response was then only depolarizing, either because of an increase in size of the depolarizing response of the proximal dendrites or because the normally hyperpolarizing somatic response had been inverted. The hyperpolarizing responses of the stratum radiatum dendrites was unaffected.

Using electrodes containing 3 M-K acetate, perfusion of the slice with low-Cl^- ACSF increased the size of the depolarizing response of the dendrites without affecting the hyperpolarizing response of the dendrites (Fig. 3*A*) in twelve of twelve cells, suggesting that the depolarizing response alone was Cl^- dependent. The effect of low Cl^- appeared 8–15 min (mean 10 min) after beginning perfusion with low-Cl^- ACSF. The size of the hyperpolarizing response of the dendrites in picrotoxin (50 μM) was unaffected by low-Cl^- ACSF after 30 min (Fig. 3*B*). Low-Cl^- ACSF moderately reduced the size of the hyperpolarizing response of the cell body (Fig. 3*C*) in five of five cells. Depolarizing components were present in the somatic responses of two cells and were increased in size (Fig. 3*C*), which complicated the interpretation of the

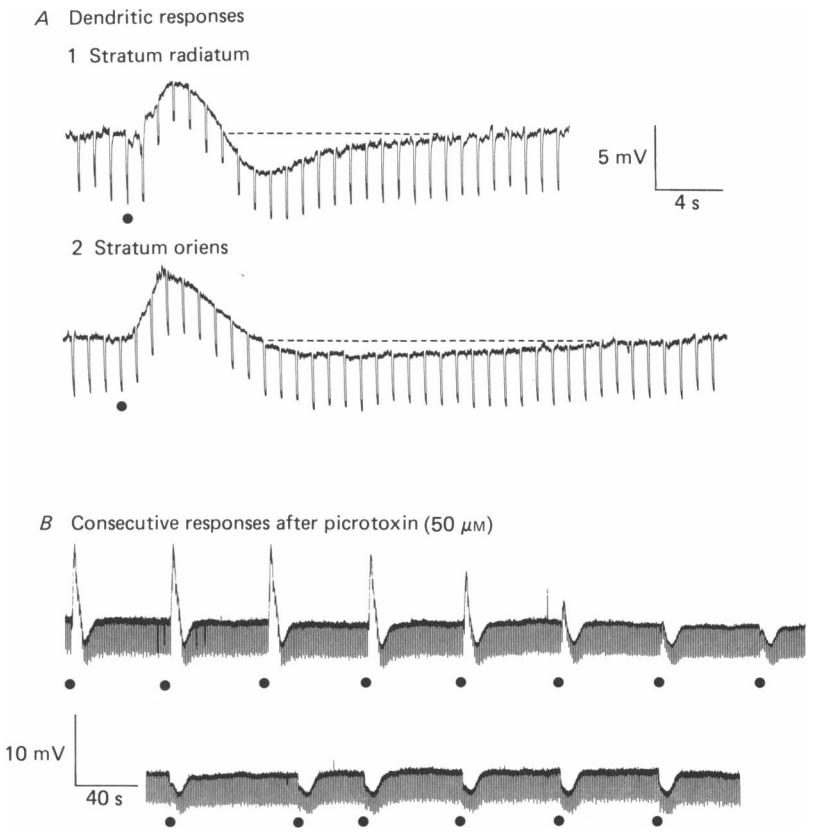

Fig. 1. Responses of the stratum radiatum (apical) and stratum oriens (basal) dendrites to GABA and the effect of picrotoxin (50 μM). In this and subsequent Figures, the downward deflexions are the electrotonic potentials in response to hyperpolarizing current injection (usually 0·2 nA). As shown in *A*, dendrites in both areas respond biphasically, with the depolarizing phase preceding the hyperpolarization phase, as was most common. The hyperpolarizing response of the stratum oriens dendrites was the longer in this neurone. The duration and relative size of the phases of the response were determined by the exact position of the pressure pipette and the extent of penetration into the slice. Membrane potential (V_m) = −56 mV. *B* is a continuous chart record of fourteen responses of the stratum radiatum dendrites showing the onset of the action of picrotoxin (50 μM) and the gradual smoothing of the hyperpolarizing response as the depolarizing phase is blocked. The ACSF containing picrotoxin was switched immediately before the first response. V_m = −62 mV. GABA was applied to the dendrites at ●.

results. In three cells, however, pure hyperpolarizing responses of the cell body were reduced but not inverted by low Cl^-.

Since the hyperpolarizing response of the dendrites appeared not to be Cl^- dependent, the effect of changes in the extracellular concentration of K^+ were tested. Low-K^+ ACSF reversibly increased the size of the response (Fig. 4*A*) in all cells tested (n = 7) despite the accompanying tonic hyperpolarization of up to 2 mV, and also made the extrapolated reversal potential more negative by 8 mV on average (Fig. 4*B*).

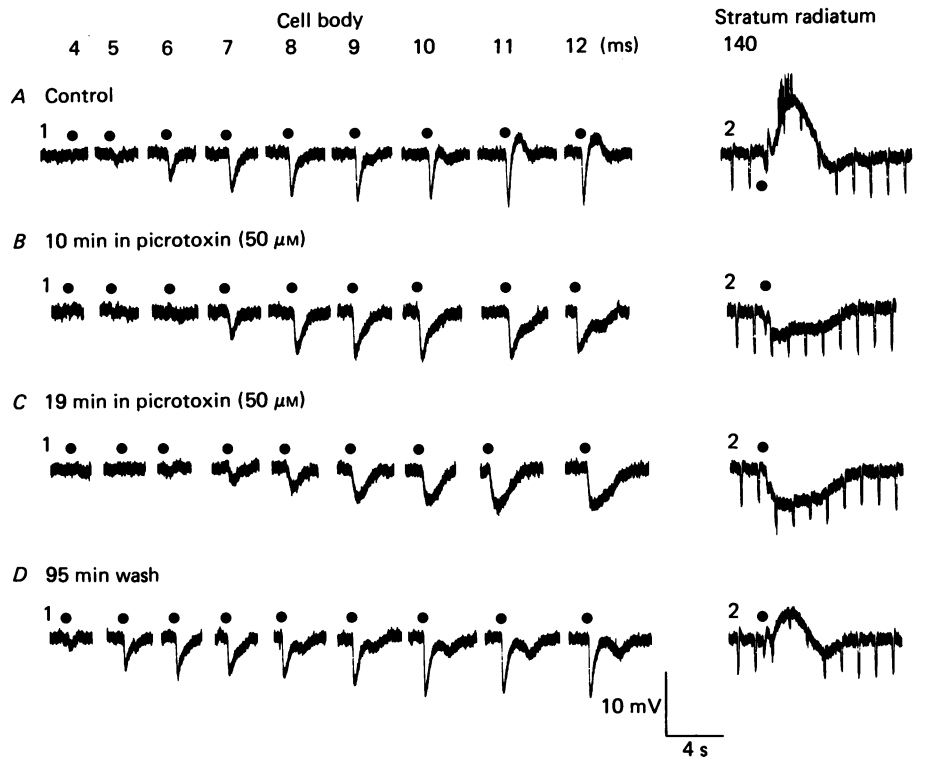

Fig. 2. Effect of picrotoxin (50 μM) on the somatic (left) and the stratum radiatum (right) response in the same neurone. Numbers above the columns of responses give the duration of the pressure application of GABA in milliseconds. *A* shows the control 'dose' – response relation for the somatic response (1). At short application times, the response appears to be purely hyperpolarizing. A depolarizing component is revealed at longer application times. In 2, the control response of the stratum radiatum dendrites is shown. *B*, after 10 min exposure to picrotoxin, the depolarizing phase has started to disappear and the responses to short applications of GABA are reduced. 2, the depolarizing phase of the stratum radiatum response is almost completely blocked, revealing an underlying hyperpolarization. *C*, after 19 min exposure to picrotoxin the larger hyperpolarizing responses are still resistant to picrotoxin although the smaller responses are blocked. The stratum radiatum response is now a smooth hyperpolarization and appears to be unaffected by picrotoxin (2). *D*, after 95 min wash, the somatic responses have recovered to their control size (1) and the stratum radiatum response has almost recovered (2). $V_m = -66$ mV. GABA applied to dendrites or cell body at ●.

Owing to the difficulty of obtaining clear reversal potentials in low-K^+ ACSF, the effects of two high concentrations of K^+ were examined ($n = 10$). In both 6·5 and 13 mM-K^+, the reversal potential of the response could be determined directly. Responses decreased in amplitude (Fig. 5 *B*) and the reversal potential became less negative by about 11 mV (3·25 to 6·5 mM) and 23 mV (3·25 to 13 mM). The reversal potential of the slow a.h.p. was also determined directly, and very similar shifts were obtained, although the reversal potentials obtained were several millivolts more negative than those of the hyperpolarizing response (Fig. 5*A*). The difference was significant in 3·25 mM-K^+ (Mann–Whitney *U* test, $P < 0{\cdot}05$), but not in 6·5 or 13 mM-K^+. These observations suggest that GABA activated a K^+ conductance.

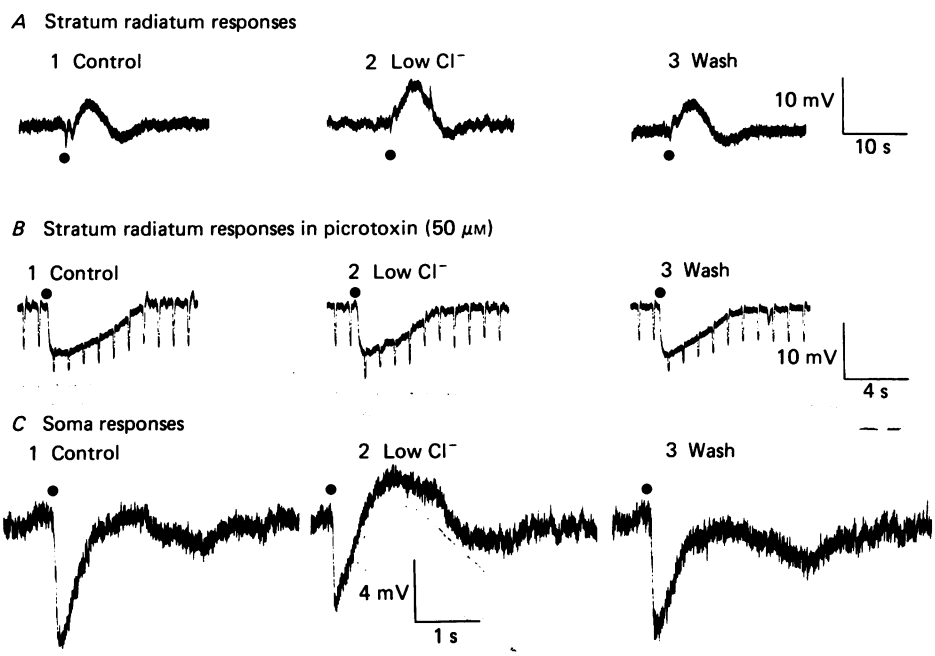

Fig. 3. Effect of low-Cl^- ACSF on the somatic response and the stratum radiatum response. *A* and *C* are from the same neurone. Stratum radiatum responses are shown in *A*. The size of the control response in normal ACSF (1) increased in size after 15 min in low-Cl^- (88 mM) ACSF (2). The depolarizing phase doubled in size but the hyperpolarizing phase was unaffected. The response recovered after 11 min in normal ACSF (3). $V_m = -65$ mV. Responses of the dendrites in the presence of picrotoxin (50 μM) are shown in *B*. The size of the control response (1) was largely unaffected after 30 min in low-Cl^- (70 mM) ACSF (2). The wash-out is shown in 3. $V_m = -63$ mV. Somatic responses are shown in *C*. In this example, the response has a small depolarizing phase. After 15 min in low-Cl^- (88 mM) ACSF (2), the depolarizing phase had increased in size compared to control (1), but the hyperpolarization is relatively unaffected. The response recovered after 11 min in normal ACSF (3). $V_m = -65$ mV. GABA applied to stratum radiatum dendrites or soma at ●.

The Ca^{2+} dependence of the dendritic hyperpolarizing response was tested by perfusing the slice with 0-Ca^{2+} ACSF containing either Mn^{2+} (0·5 mM) or Cd^{2+} (0·2 mM) and with ($n = 8$) or without ($n = 12$) addition of $MgCl_2$ to maintain the divalent ion concentration. In twenty-one of twenty-two cells where the slow a.h.p. was reduced or blocked and/or synaptic transmission was blocked, the hyperpolarizing response of the dendrites to GABA was reduced or blocked (Fig. 6), although some time (5–40 min) after the effects on synaptic transmission and the slow a.h.p. had been seen. The times taken to block synaptic activity, the slow a.h.p. and the response are shown in Fig. 7*A*. Block of the GABA response required about twice the time in 0-Ca^{2+} ACSF as block of the slow a.h.p. (Fig. 7*B*). This rough relation held true for any of the manipulations. In four cells, Cd^{2+} (0·2 mM) was added directly to the normal ACSF, with the consistent effect of blocking the slow a.h.p. and then the dendritic hyperpolarizing response to GABA. 0-Ca^{2+} ACSF increased the rate of firing in cells or initiated firing in silent cells and reversibly increased spike duration

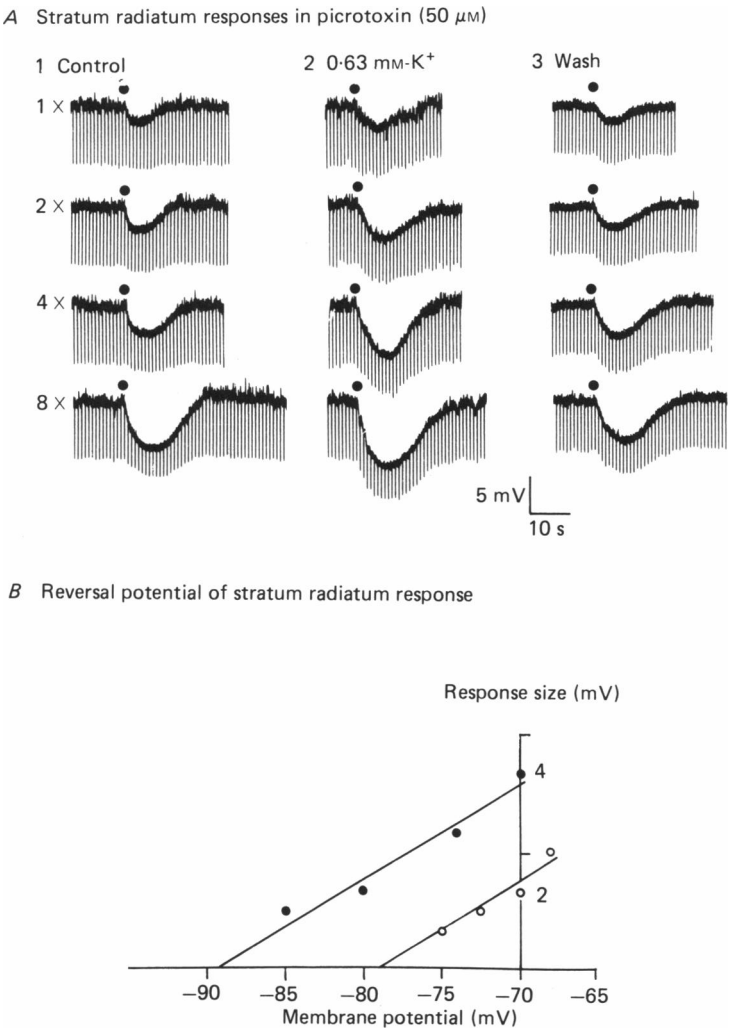

Fig. 4. Effect of low-K^+ ACSF on the hyperpolarizing response of the dendrites to GABA. Picrotoxin (50 μM) was present throughout. *A*, responses to multiple doses (e.g. 8 × was eight times the amount of GABA as applied for the 1 × response). 1, responses to multiple doses in normal ACSF (3·25 mM-K^+). 2, responses after 26 min in low K^+ (0·63 mM) ACSF. 3, responses after 20 min wash-out. GABA was applied by pressure to stratum radiatum at ●. *B*, reversal potential of the response in the same cell. Current was injected into the cell to give the membrane potentials indicated. GABA was applied, and the response size measured. The extrapolated reversal potential (best linear fit by eye) was −79 mV in normal ACSF (○) and −89 mV in low-K^+ (0·63 mM) ACSF (●). $V_m = -70$ mV.

(Fig. 6*B*, inset). There was sometimes a small reduction in input resistance, but no consistent effect on spike amplitude. In an additional twenty cells, Cd^{2+} (0·2, 0·5, 1·0 or 2·0 mM) was applied to the surface of the slice by pressure ejection. At all concentrations, the slow a.h.p. and the hyperpolarizing response were reduced or blocked by Cd^{2+}, but at 2 mM ($n = 1$), the depolarizing response was also reduced.

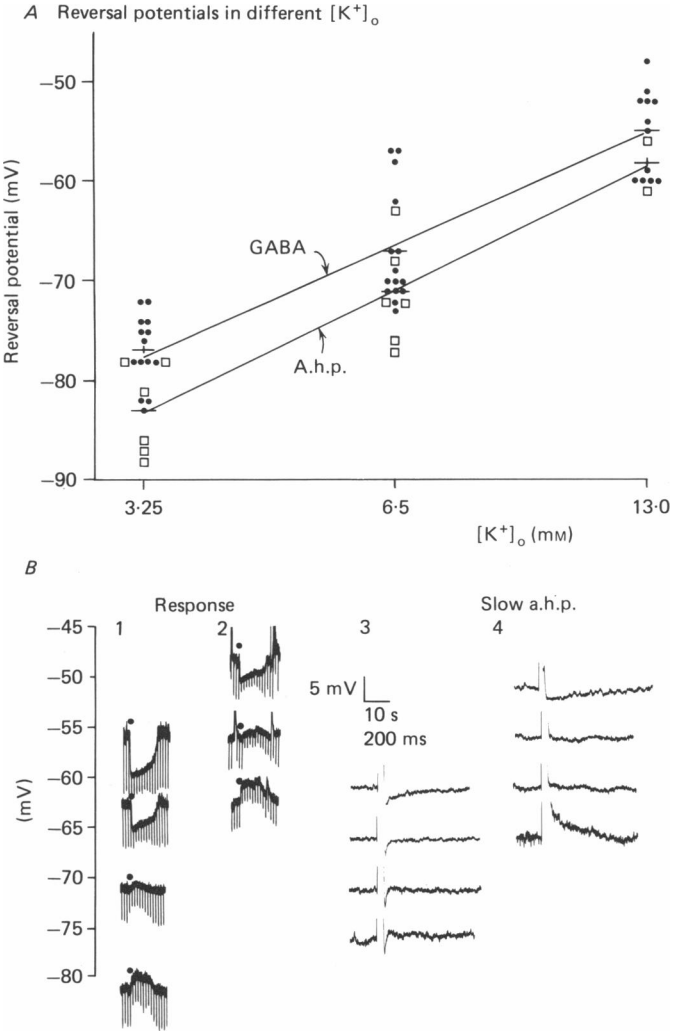

Fig. 5. Effects of high-K^+ ACSF on the slow a.h.p. (□) and the hyperpolarizing response of the dendrites to GABA (●). Picrotoxin (50 μM) present throughout. The reversal potential of the responses and the a.h.p. in normal K^+ (3·25 mM) and high K^+ (6·5 mM or 13 mM) are plotted in *A*. A best fit by eye through the mean of the reversal potential (——) at each $[K^+]$ is also shown. Means (±S.E. of means) are: 3·25 mM: $-77 \pm 3{\cdot}4$ mV (GABA); $-83 \pm 4{\cdot}5$ mV (slow a.h.p.). 6·5 mM: $-67 \pm 5{\cdot}6$ mV (GABA), $-71 \pm 5{\cdot}2$ mV (slow a.h.p.). 13 mM: $-55 \pm 4{\cdot}3$ mV (GABA), −58 mV (slow a.h.p.). There is a significant difference between the reversal potentials of the response and the slow a.h.p. for the values obtained in 3·25 mM-K^+ only (Mann–Whitney *U* test, $P < 0{\cdot}05$). All cells were exposed to at least two concentrations of K^+. The slopes of the lines are 38 mV (GABA response) and 40 mV (slow a.h.p.) per tenfold change in $[K^+]$. *B*, traces showing the reversal potential of the GABA response in 6·5 mM-K^+ (1) and 13·0 mM-K^+ (2) and the slow a.h.p. in 6·5 mM-K^+ (3) and 13·0 mM-K^+ (4). The responses and the a.h.p. are aligned at their holding potentials with the scale at left. Note that the voltage calibration bar has a different scale. Calibration bar is 200 ms for the slow a.h.p. and 10 s for the GABA response. GABA applied by pressure to stratum radiatum at ●.

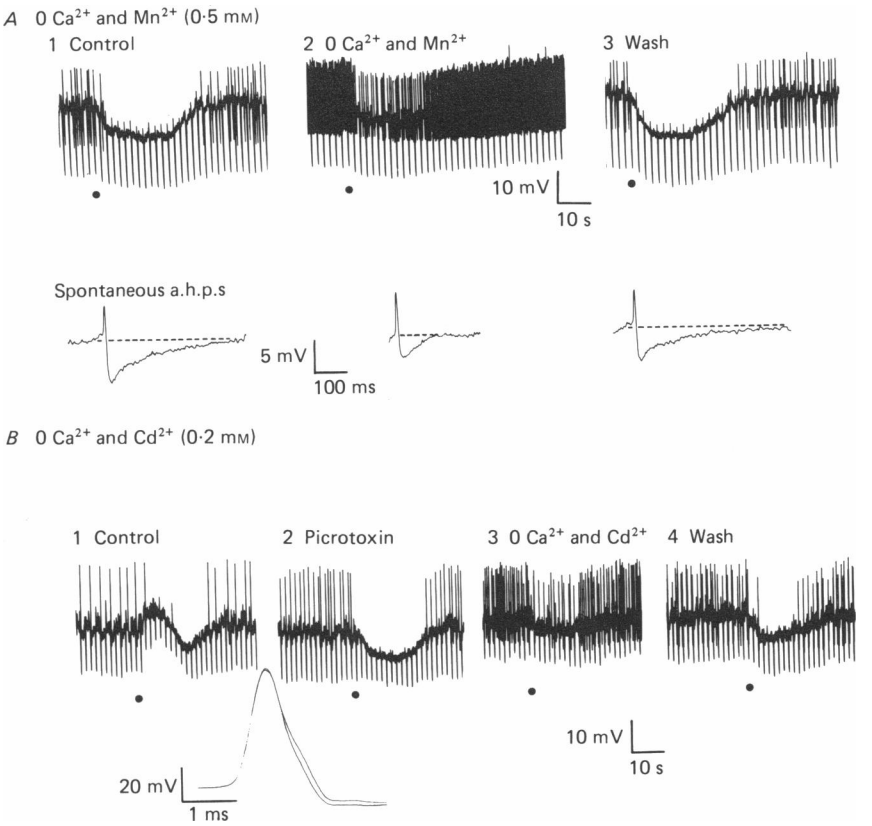

Fig. 6. Reduction of the hyperpolarizing response to GABA by Mn^{2+} and Cd^{2+}. *A*, picrotoxin (50 μM) present throughout. Response to GABA and a spontaneous a.h.p. in normal ACSF are shown (1). After 25 min in 0-Ca^{2+} ACSF containing 0·5 mM-Mn^{2+}, the GABA response is greatly diminished, the slow phase of the a.h.p. is blocked and there are more spontaneous spikes (2). After 24 min wash in normal ACSF, the response and the slow a.h.p. have recovered (3). $V_m = -65$ mV. In *B*, the effect of picrotoxin and 0-Ca^{2+}/Cd^{2+} ACSF on the response is shown. The depolarizing phase of the control response (1) is blocked by picrotoxin (50 μM), revealing a hyperpolarizing response (2). This response was greatly reduced after 11 min in 0-Ca^{2+} ACSF containing 0·2 mM-Cd^{2+} (3). The hyperpolarizing response recovered after 9 min in normal ACSF containing picrotoxin (4). Note the increase in firing rate and the reduction in input resistance. The inset shows the spike duration in normal and 0-Ca^{2+} ACSF. The inside rising phase and falling phase comprise the control spike. $V_m = -66$ mV. GABA applied by pressure to stratum radiatum at ●.

At 1 mM ($n = 4$), the depolarizing response was reduced temporarily. At 0·2 mM, the slow a.h.p. and the GABA response were reduced in three of four cells, but no complete block was seen. At 0·5 mM, however, the slow a.h.p. and the hyperpolarizing response to GABA was reduced or blocked in ten of eleven cells. The depolarizing response was not reduced by this concentration of Cd^{2+}. As shown in Fig. 8*A*, the depolarizing response increased in size following Cd^{2+} as the underlying hyperpolarization was blocked. The rapid effect of Cd^{2+} is shown in Fig. 8*B*. Here, GABA was applied just before and after the application of Cd^{2+}.

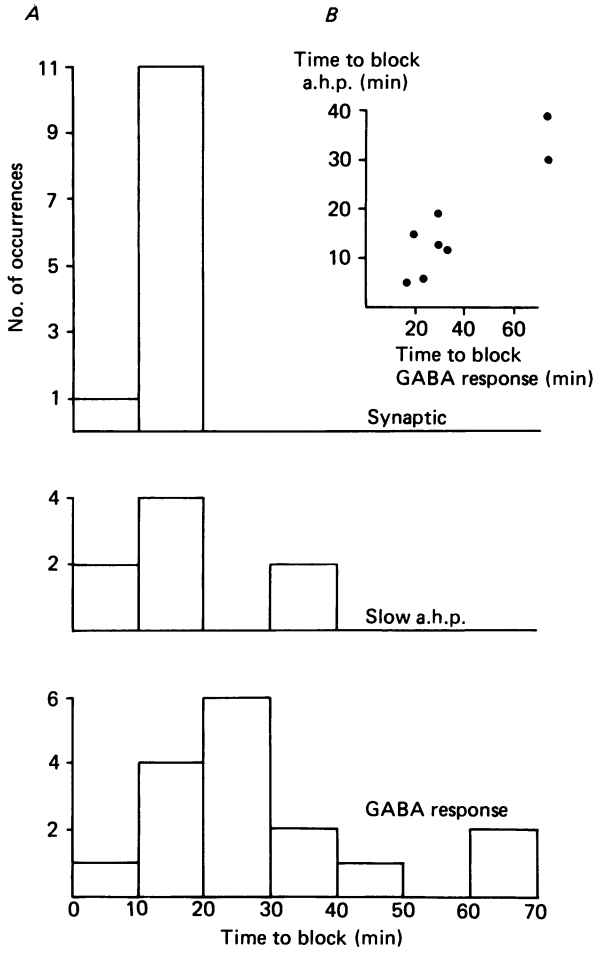

Fig. 7. 0-Ca^{2+}/Cd^{2+} ACSF in the presence of picrotoxin (50 μM) blocks the hyperpolarizing response of the dendrites. *A*, the time taken after switching solutions to block synaptic transmission (top), the slow a.h.p. (middle) and the GABA response (bottom). The relation between the times for the slow a.h.p. and the response is shown in *B*. Note that roughly twice as long was required to block the response.

As a more direct test of the Ca^{2+} dependence of the response, EGTA was injected into the cell body through the intracellular electrode. EGTA blocked or reduced the hyperpolarizing response (Fig. 9) in the dendrites in all cells tested ($n = 6$) without an effect on the depolarizing response. This took up to 25 min after the rapid block of the slow a.h.p.

DISCUSSION

The biphasic response found in the dendrites consists of a largely Cl^--dependent depolarization and a hyperpolarization, which cannot also be Cl^- dependent as E_{Cl} in the dendrites is less negative than the resting potential (Blaxter & Cottrell, 1985).

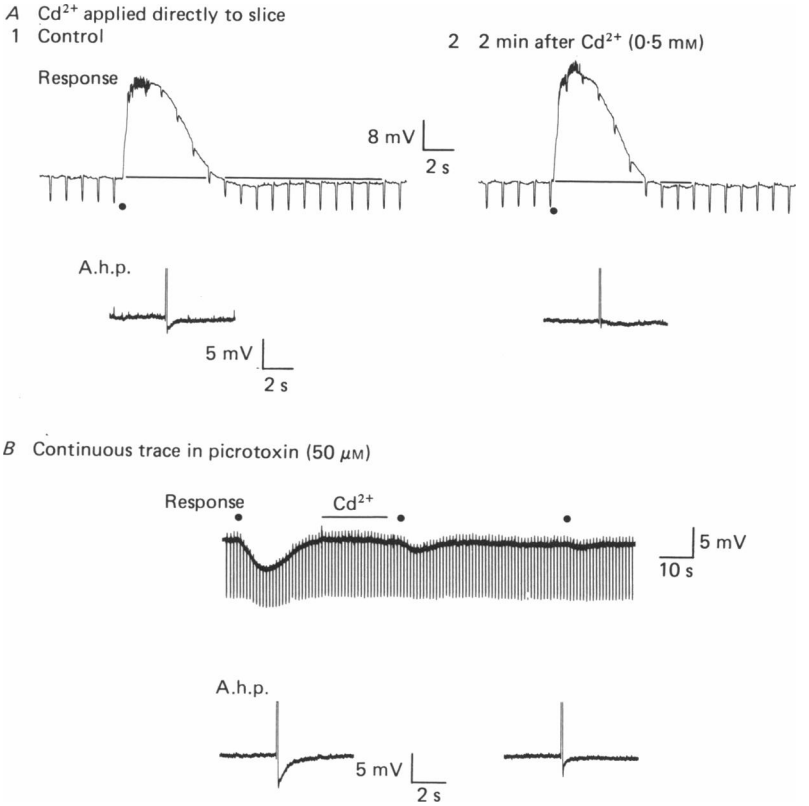

Fig. 8. Application of Cd^{2+} to the surface of the slice reduced or blocked the slow a.h.p. and the hyperpolarizing response to GABA. *A*1, biphasic response to GABA and the a.h.p. in normal ACSF. 2, 2 min after the application of Cd^{2+} (0·5 mM) to the surface of the slice. Note that the depolarizing response is larger and that the fast a.h.p. is still visible. $V_m = -70$ mV. *B*, continuous trace showing the onset of the block produced by Cd^{2+} (0·5 mM). Picrotoxin (50 μM) present throughout. The a.h.p. before and after Cd^{2+} (0·5 mM) is shown below. $V_m = -67$ mV. GABA applied by pressure to stratum radiatum at ●.

It is unlikely that the hyperpolarizing response obtained in the dendrites was actually the response of the cell body, since the response was found more than 200 μm from the cell body and was clearly present when the cell body response was reduced by picrotoxin. The hyperpolarizing response of the dendrities could be found in the absence of the depolarizing response and was not, therefore, a product of it.

While the depolarizing response was clearly Cl^- dependent, the hyperpolarizing response of the cell body was affected less by the low-Cl^- ACSF than would be expected for a completely Cl^--mediated response. This, and the lack of complete blockade by picrotoxin suggest that the response of the cell body to GABA may be partly a Cl^--dependent response and partly the response observed more clearly in the dendrites.

At the concentration used, picrotoxin had no effect on the dendritic hyperpolarization. This provides circumstantial evidence against the involvement of Cl^- in this response. The direct experiments with low-Cl^- ACSF and the use of electrodes

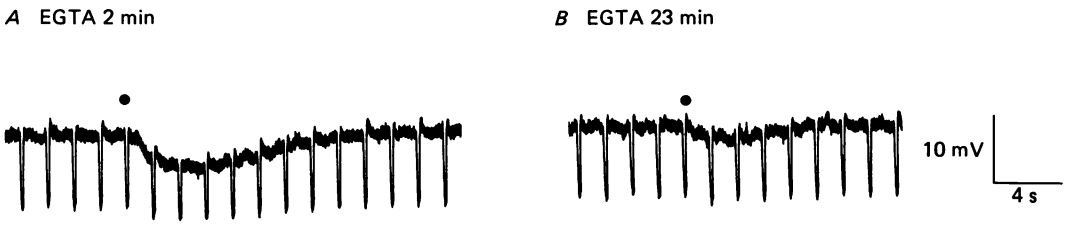

Fig. 9. Effect of intracellular injection of EGTA on the hyperpolarizing response of the dendrites. Picrotoxin (50 μM) present throughout. In *A*, GABA was applied 2 min after the impalement stabilized and in *B*, GABA was applied 21 min later. There was no change in the resting membrane potential. Electrode filled with 1 M-EGTA–K and 2 M-KCl. $V_m = -62$ mV. GABA applied to dendrites at ●.

filled with KCl support this conclusion. The dendritic hyperpolarizing response was first identified by Alger & Nicoll (1982). The initial suggestion of its ionic mechanism being an increase in K^+ conductance was provided by the experiment showing that the response increased in size in low K^+ ACSF (Blaxter & Cottrell, 1985). The results obtained here with changes in the reversal potential of the GABA hyperpolarizing response related to changes in extracellular $[K^+]$ also suggest that the response is mediated by a K^+ conductance. The shifts in reversal potential are less than those expected from the Nernst equation for K^+. The slopes of the relation between the reversal potential and the extracellular $[K^+]$ for the a.h.p. and the response are 40 and 38 mV, respectively, per tenfold change, as opposed to the ideal value of 61 mV. As the a.h.p. is considered to be a K^+ conductance, the similarity in slope and the parallel behaviour of the a.h.p. and the GABA response strongly suggest that the response also is mediated by a K^+ conductance. The sub-Nernstian slope may be due to the distant site of generation of both the GABA response and the a.h.p. relative to the site of current injection. Alger & Nicoll (1980) reported a similar sub-Nernstian slope for the burst a.h.p. It is possible that another ion may be involved in the response. The most likely candidate is Cl^-, but, since the GABA hyperpolarizing response was unaffected by picrotoxin or changes in the extracellular $[Cl^-]$, Cl^- can be excluded.

The dendritic hyperpolarizing response was reduced or blocked by Ca^{2+}-channel antagonists or 0 Ca^{2+} combined with Ca^{2+}-channel antagonists. This suggests either that the response was indirect (i.e. the result of the release of transmitter from some presynaptic site) or that the post-synaptic response to GABA was Ca^{2+} dependent. Intracellular injection of EGTA, which diminished the hyperpolarizing response of the dendrites, showed that the latter was most likely, since EGTA inside the dendrites would reduce $[Ca^{2+}]_i$ presumably without affecting $[Ca^{2+}]_o$. Also, in 0 Ca^{2+}, synaptic transmission was blocked before the response was affected, suggesting a direct post-synaptic effect of GABA. GABA reduces the evoked release of several transmitters, usually without affecting basal release. In intact tissue, these include noradrenaline (Bowery & Hudson, 1979), dopamine and serotonin (Bowery, Hill, Hudson, Doble, Middlemiss, Shaw & Turnbull, 1980). In hippocampal synaptosomes, Fillenz & Fung (1983) report an increase in the basal release of noradrenaline. A discussion of the effects of GABA on transmitter release is provided in Bowery *et al.*

(1980). The increase in firing rate produced by 0-Ca^{2+} ACSF may be due to the blockade of the slow a.h.p. and an effect on Na^+-channel activation.

The hyperpolarizing response resembles the hyperpolarization produced in these cells by baclofen acting on $GABA_B$ receptors in its resistance to picrotoxin and bicuculline and its K^+ dependence (Newberry & Nicoll, 1984). However, in that study the response to baclofen was unaffected by Cd^{2+} (0·1 mM) at least at a time when the a.h.p. was blocked. In agreement with this, in the present study the a.h.p. was blocked by 0 Ca^{2+} as much as 40 min before the response was affected. We have found that the response to baclofen was blocked by intracellular EGTA and by Cd^{2+} perfused outside the cell; these effects also took consistently longer to appear than the block of the a.h.p. (Blaxter & Carlen, 1985). It is of interest that Gahwiler & Brown (1985) have recently reported a GABA-activated K^+ current in hippocampal neurones in culture.

The hyperpolarizing response of the dendrites was dependent on intracellular and extracellular Ca^{2+} and on extracellular K^+. We suggest that the response is mediated by a Ca^{2+}-dependent K^+ conductance. Such a conductance is known to be present in these neurones (see Introduction), but the conductance described here is different in some respects, since the Ca^{2+} required for the response is in some way provided by GABA and not by the action potential depolarization as with the slow a.h.p.

There are two possible sources of increased intracellular Ca^{2+}. First, Ca^{2+} could enter from the outside through Ca^{2+} channels. Ca^{2+} could enter the cell if the depolarizing phase of the biphasic dendritic response to GABA were large enough, but this could not account for the hyperpolarizing response found in the absence of the depolarizing response (i.e. in picrotoxin). Also, it has been reported that Ca^{2+} entry which activates the outward current of the slow a.h.p. in these cells only occurs at potentials more positive than −45 mV (Brown & Griffith, 1983). Responses to GABA never reached these potentials. It is unlikely, therefore, that the Ca^{2+} enters the cell through voltage-dependent Ca^{2+} channels.

Secondly, GABA could somehow increase the intracellular concentration of Ca^{2+} from an internal source. Although the response took longer to block than the slow a.h.p. in 0-Ca^{2+} ACSF, suggesting that depletion of intracellular Ca^{2+} was occurring and was responsible for the block, this explanation seems unlikely as the response was rapidly blocked when Cd^{2+} was applied directly to the surface of the slice. Also, depletion of intracellular Ca^{2+} would be less likely to occur when the extracellular Ca^{2+} was maintained, yet under those conditions the responses still took longer to block than the a.h.p. We suggest, therefore, that GABA increases the influx of Ca^{2+} from the outside by a Cd^{2+}-sensitive mechanism. The prolonged time needed to block this hyperpolarizing response of the dendrites to GABA may be because the hyperpolarizing response requires less Ca^{2+} than the a.h.p. or because the Ca^{2+}-channel blockers may be less effective on the GABA mechanism than the a.h.p. mechanism. We conclude that the dendritic hyperpolarizing response to GABA is mediated by a Ca^{2+}-dependent K^+ conductance.

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