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Possible involvement of K^+-conductance in the action of γ-aminobutyric acid in the guinea-pig hippocampus

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1 The mechanism underlying the action of γ-aminobutyric acid (GABA) in the hippocampus was investigated using guinea-pig brain slices.

2 GABA either superfused or applied directly by microiontophoresis produced a biphasic response in pyramidal cells, comprising hyperpolarizing and depolarizing components.

3 When different concentrations of GABA were applied to the same neurone, the lower concentrations generally produced a hyperpolarization-predominant response, while higher concentrations resulted in a depolarization-predominant response.

4 The depolarizing component of the response to GABA was augmented in a medium containing a low concentration of Cl^-, relatively unaffected by a change in external K^+ concentration, and blocked by picrotoxin (2×10^{-5} M). The depolarizing response to GABA persisted in a Ca^{2+}-free medium in which the concentration of Na^+ was reduced to 13 mM.

5 Combined application of low doses of picrotoxin and bicuculline eliminated the major part of the depolarizing component of the biphasic response to GABA and produced a relatively pure hyperpolarizing response. The reversal potential of this pharmacologically 'isolated' hyperpolarizing response to GABA was estimated, from the current-voltage relationships, to be about − 90 mV and was the same as that of the hyperpolarization induced by baclofen.

6 When the membrane was successively hyperpolarized by inward direct current (d.c.) injections, the reversal point of the 'pharmacologically isolated' hyperpolarizing response to GABA coincided with that of the post-burst hyperpolarization.

7 Low concentrations of Cl^- in the bathing medium had no noticeable effect on the hyperpolarizing component of the response to GABA, whereas it markedly increased the amplitude of the depolarizing component.

8 These results suggest that the action of GABA in the hippocampus may involve an activation of K^+ conductance.

Introduction

Although it appears to be established that γ-aminobutyric acid (GABA) is an inhibitory neurotransmitter in the hippocampus (Storm-Mathisen, 1977), the mode of action of GABA in this tissue is not fully understood. Electrophysiological investigations have revealed that GABA exerts a biphasic action in the hippocampus, comprising hyperpolarizing and depolarizing responses (Anderson *et al.*, 1980; Alger & Nicoll, 1982; Wong & Watkins, 1982).

The ionic basis of the biphasic action of GABA is still not fully understood (Alger & Nicoll, 1982). It has been suggested that the reversal of polarity of the response to GABA is due to rapid redistribution of Cl^- which results in a reversed electrochemical gradient for Cl^- ions (Barker & Ransom, 1978). However, Newberry & Nicoll (1985) have recently shown that GABA directly activates K^+ channels in rat hippocampal pyramidal cells. In addition, we found that baclofen, a β-chlorophenyl derivative of GABA and an agonist at bicuculline-insensitive $GABA_B$-receptors (Bowery *et al.*, 1980), directly hyperpolarizes the membrane of guinea-pig hippocampal pyramidal cells through activation of K^+ channels (Inoue *et al.*, 1985a,b). These findings warrant a review of the action of GABA in this tissue. Therefore, we have investigated the ionic mechanism underlying the

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biphasic action of GABA using slices of guinea-pig hippocampus.

Methods

The experiments were performed on transverse slices (400–600 μm thick) of the guinea-pig hippocampus. The procedures for incubation and recording were fundamentally the same as described previously (Abe & Ogata, 1982). The standard bathing medium was of the following composition (mM): NaCl 124, $NaHCO_3$ 13, KCl 5, $CaCl_2$ 2.6, KH_2PO_4 1.24, $MgSO_4$ 1.3, glucose 10. The slices were continuously perfused with the standard medium equilibrated with 97% O_2 and 3% CO_2 at 30–32°C. Pyramidal cells were impaled with a microelectrode filled with 2 M potassium acetate. Unless otherwise stated, recordings were made from cells in the CA3 pyramidal layer.

Drugs were either superfused or applied directly onto the cell by microiontophoresis through three-barrelled pipettes. The tip of the microiontophoretic electrode was positioned as close as possible to the recording electrode inserted into the pyramidal layer. Membrane input resistance was routinely measured by passing hyperpolarizing current pulses (0.3 Hz, 0.6 s pulse duration) of known intensities through the recording electrode using a conventional bridge circuit.

The experimental data are presented as means ± s.e.mean.

Results

The results presented here are based on about 400 stable intracellular recordings from pyramidal cells. The resting membrane potential and the input resistance were − 62.3 ± 1.0 mV and 20.9 ± 1.5 MΩ, respectively, when measured in 57 cells on which an intracellular recording could be made for more than 3 h.

Dose-response relationships

GABA had a biphasic effect on the hippocampal pyramidal cells comprising hyperpolarizing and depolarizing phases as has been noted by others (Andersen *et al.*, 1980; Alger & Nicoll, 1982; Wong & Watkins, 1982). Both phases were associated with a large increase in the membrane conductance and persisted during perfusion of Ca^{2+}-free, Mg^{2+}-rich (12 mM) and tetrodotoxin (0.1 μg ml^{-1})-containing medium in all of 7 cells tested, thus confirming their postsynaptic nature.

Figure 1 shows responses to GABA at different concentrations either superfused (a) or applied iontophoretically to the somatic region (b). In both cases, an application of low concentrations of GABA generally produced hyperpolarization while a higher concentration of GABA induced depolarization in addition to hyperpolarization. The depolarization became progressively larger when the concentration of GABA was successively increased.

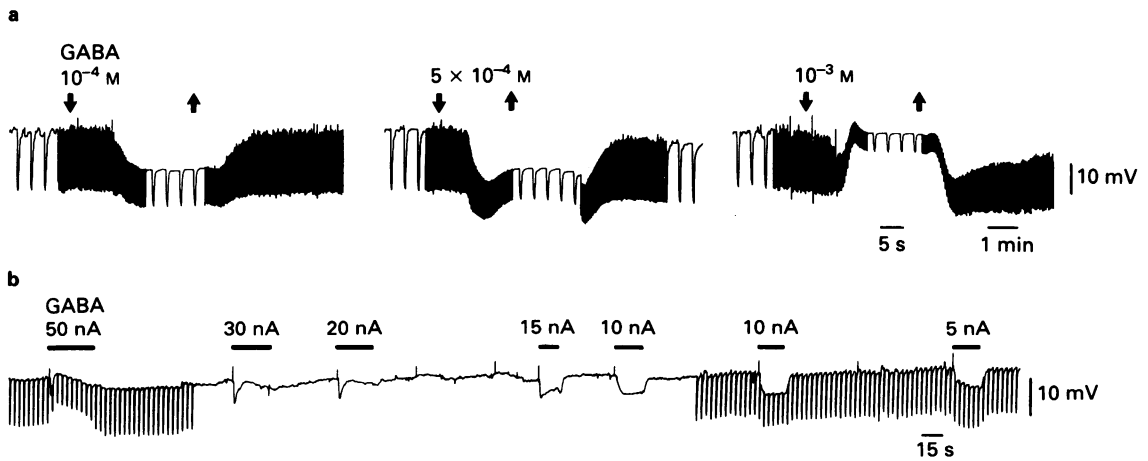

Figure 1 Effects of various concentrations of GABA, either superfused (a) or applied iontophoretically to the somatic region (b), on the electrical activity of hippocampal pyramidal cells. In this and subsequent figures: (a) downward and upward arrows represent the duration of superfusion with test solution; (b) the duration of the iontophoretic application of the drug is indicated by solid lines; repetitive negative deflections are electrotonic potentials to inward current injections (0.3 Hz, 0.6 s pulse duration) of constant intensity for measurement of input resistance; the chart of the pen recordings was intermittently run at a faster speed; time shown under gaps in the trace indicates an omitted period; upward deflection represents positive polarity; and spikes were almost entirely abolished in the traces due to limited frequency band width of pen recordings.

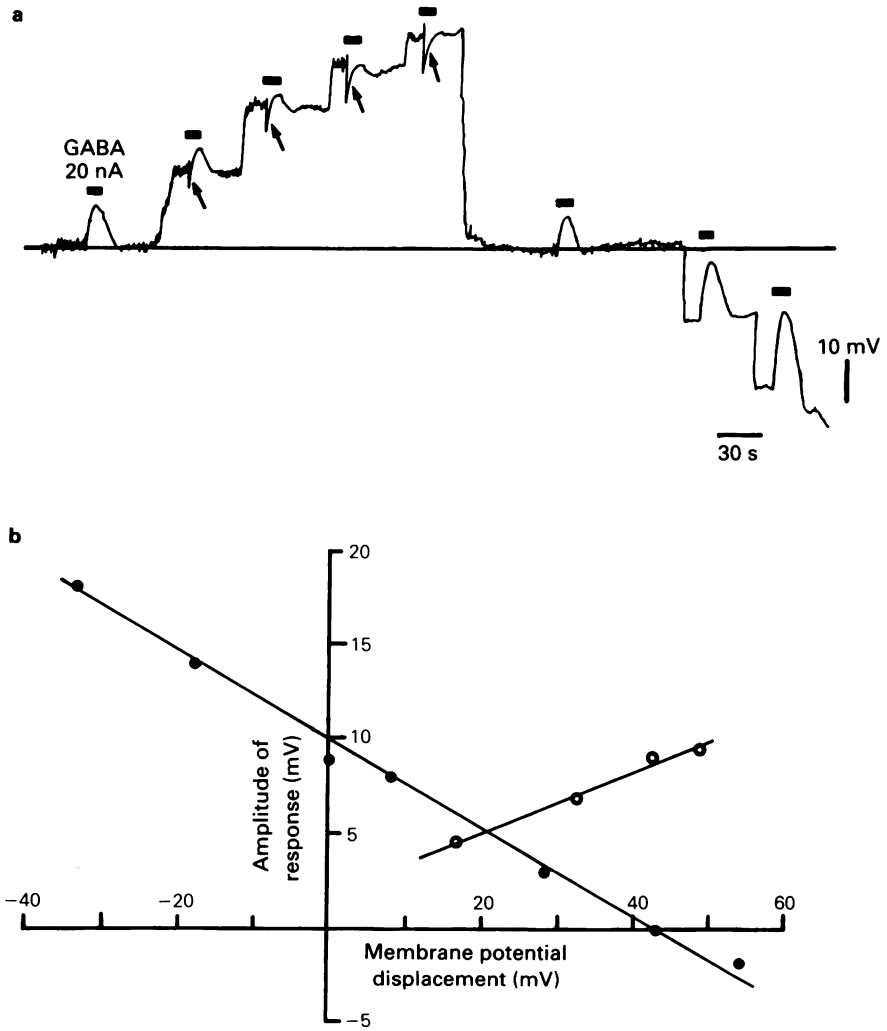

Figure 2 Voltage-dependence of the response to GABA. Effects of GABA were examined at various membrane potentials produced by inward and outward direct current (d.c.) injections. (b) The amplitudes of the depolarization (●) and hyperpolarization (○) (arrows in (a)) induced by GABA were plotted as a function of the membrane potential displacement produced by inward or outward d.c. injection.

Ionic mechanism of the depolarizing response to GABA

Figure 2 illustrates the voltage dependence of the depolarization-predominant response induced by iontophoretically-applied GABA. When the membrane was depolarized by direct current (d.c.) injections, the depolarizing response was progressively reduced and all but disappeared at the potential level about 40 mV positive to the resting membrane potential (− 67mV). In contrast, when the membrane was hyperpolarized, the depolarizing response to GABA became progressively larger. The reversal point for the depolarizing response to GABA estimated in 4 cells was 35.5 ± 4.8 mV more depolarized than the resting membrane potential. It should be noted that a transient hyperpolarization (arrows, Figure 2a) developed when the membrane was depolarized.

In all the cells tested ($n = 46$), the depolarizing component of the response to GABA was augmented in the medium in which the concentration of Na^+ was reduced from 137 to 13 mM by replacing total NaCl with equimolar choline-Cl thus converting the hyperpolarization-predominant response to a depolarization-predominant response. The augmented response

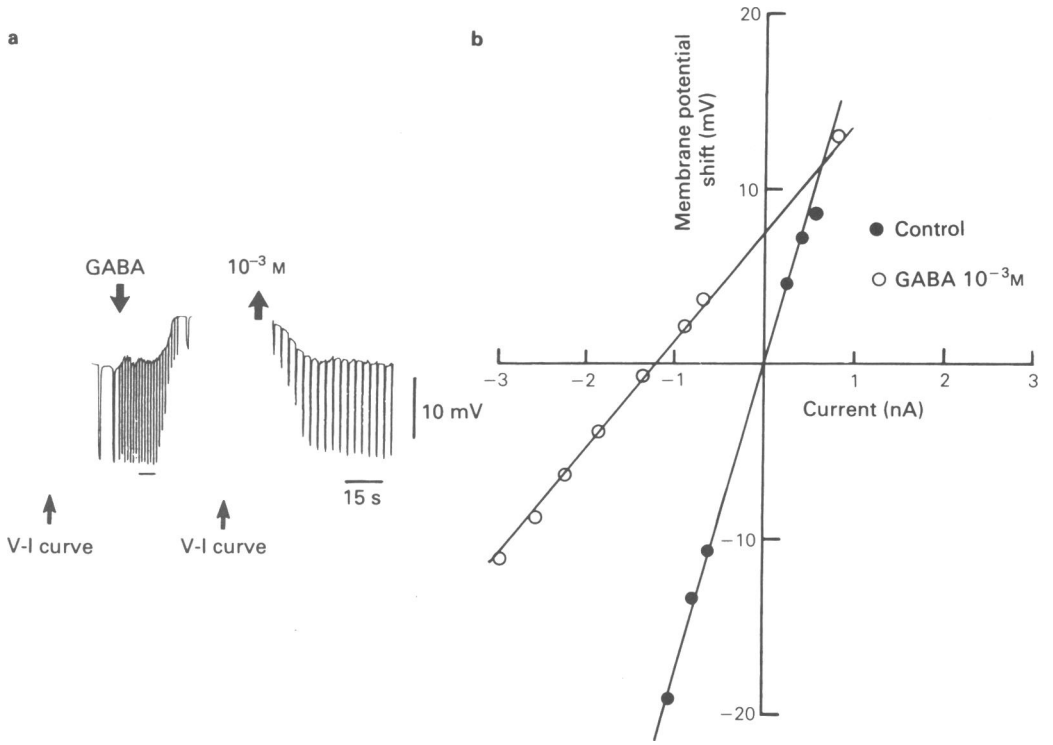

Figure 3 The effect of GABA on the current-voltage relationship. The measurements were made in the Na^+-deficient medium in which all the NaCl was replaced by an equimolar amount of choline-Cl, in order to minimize the involvement of the hyperpolarizing component (see text). In (a) recordings of the membrane potential (lower traces) and the current intensity (upper traces) from which the current-voltage curves were compiled are shown. In (b) the abscissa scale represents transmembrane current (nA; duration, 600ms); ordinate scale, steady-state membrane potential displacement (mV) produced by the current pulse. Solid and open circles represent the measurements in the control and GABA-containing solutions, respectively.

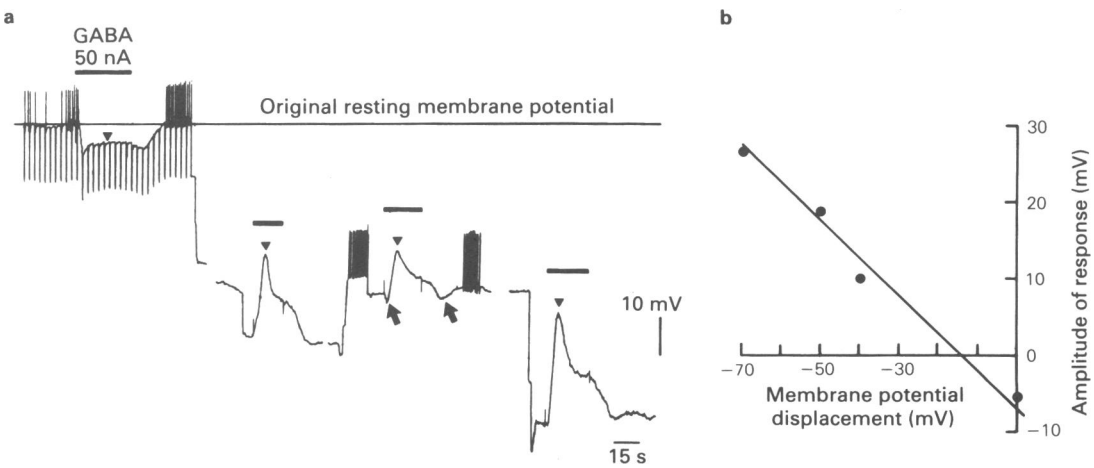

Figure 4 Voltage-dependence of the hyperpolarization-predominant response to GABA. (b) The graph was plotted at points indicated by triangles in (a).

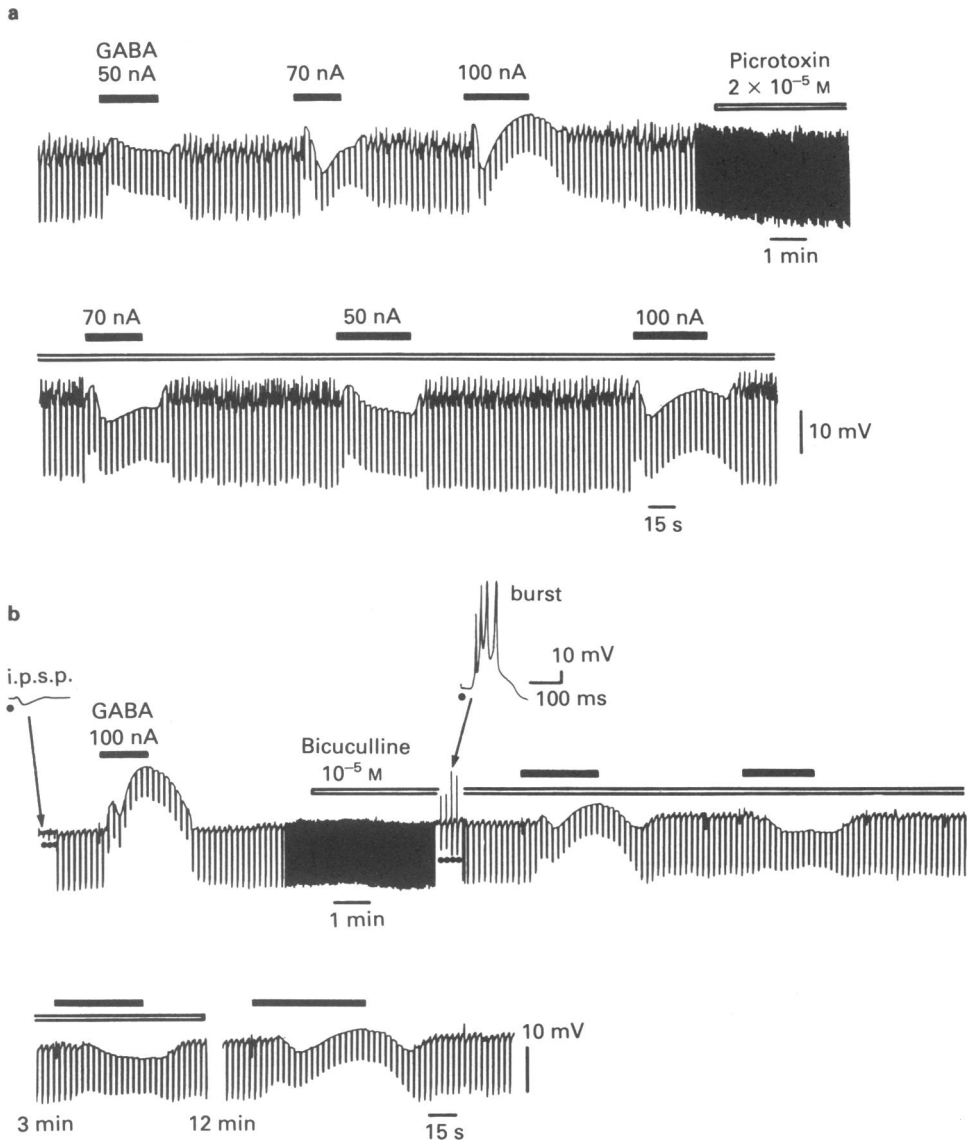

Figure 5 Effects of picrotoxin (a) and bicuculline (b) on the biphasic response to GABA. Picrotoxin or bicuculline was perfused at periods indicated by open bars. Inset traces in (b) illustrate the evoked responses to the mossy fibre stimulation (●).

to GABA in the Na^+-deficient medium remained almost unaffected when external Ca^{2+} was completely removed by replacing $CaCl_2$ in the Na^+-deficient medium with 10.7 mM $MgCl_2$ ($n = 20$).

Figure 3 shows the current-voltage relationships obtained during superfusions of Na^+-deficient medium in the absence (control) and presence of GABA. As can be seen, the current-voltage curve obtained in the control solution intersected with that obtained during the GABA-induced depolarization at about 12 mV above the resting membrane potential (− 59 mV). The mean potential at which the two curves intersected was 13.9 ± 1.0 mV ($n = 5$) positive to the resting membrane potential.

The depolarizing component of the response to GABA was markedly depressed by a low concentration of picrotoxin (10^{-5}M) in all of the 6 cells examined (see Figure 5a), whereas it was relatively insensitive to

changes in external K^+ concentration (1.24, 12.4 and 25 mM) in all 5 cells examined.

Pharmacological isolation of the hyperpolarizing component of the biphasic response to GABA

The hyperpolarization-predominant response to GABA reversed its polarity at membrane potentials 5–25 mV negative to the resting level in most cells examined (Figure 4). The reversal potential measured in 5 cells was 12.0 ± 2.5 mV ($n = 5$) negative to the resting membrane potential. However, it appeared that the measured value of the reversal level was distorted by the co-existing depolarizing component (see arrows in Figure 4).

In order to 'isolate' the relatively pure hyperpolarizing component, we manipulated the response to GABA with picrotoxin and bicuculline. As shown in Figure 5a, picrotoxin (2×10^{-5} M) eliminated the major part of the depolarizing component of the biphasic response to GABA and consequently revealed the hyperpolarizing component. Likewise, bicuculline at a concentration of 10^{-5} M initially depressed the depolarizing component of the biphasic response to GABA (Figure 5b). Thus, we could 'isolate' and maintain the relatively pure hyperpolarizing response by superfusing the slice continuously with low concentrations of picrotoxin and bicuculline. As there was a considerable variation in the effectiveness of bicuculline depending on the cell impaled, the critical concentration of bicuculline had to be determined by trial and error.

Ionic mechanism of the hyperpolarizing response to GABA

Figure 6 illustrates the voltage-dependence of the pharmacologically manipulated hyperpolarizing response. The measurement was compared with that of the post-burst afterhyperpolarization (arrow) triggered by the mossy fibre stimulation. An almost pure hyperpolarizing response to GABA was obtained by

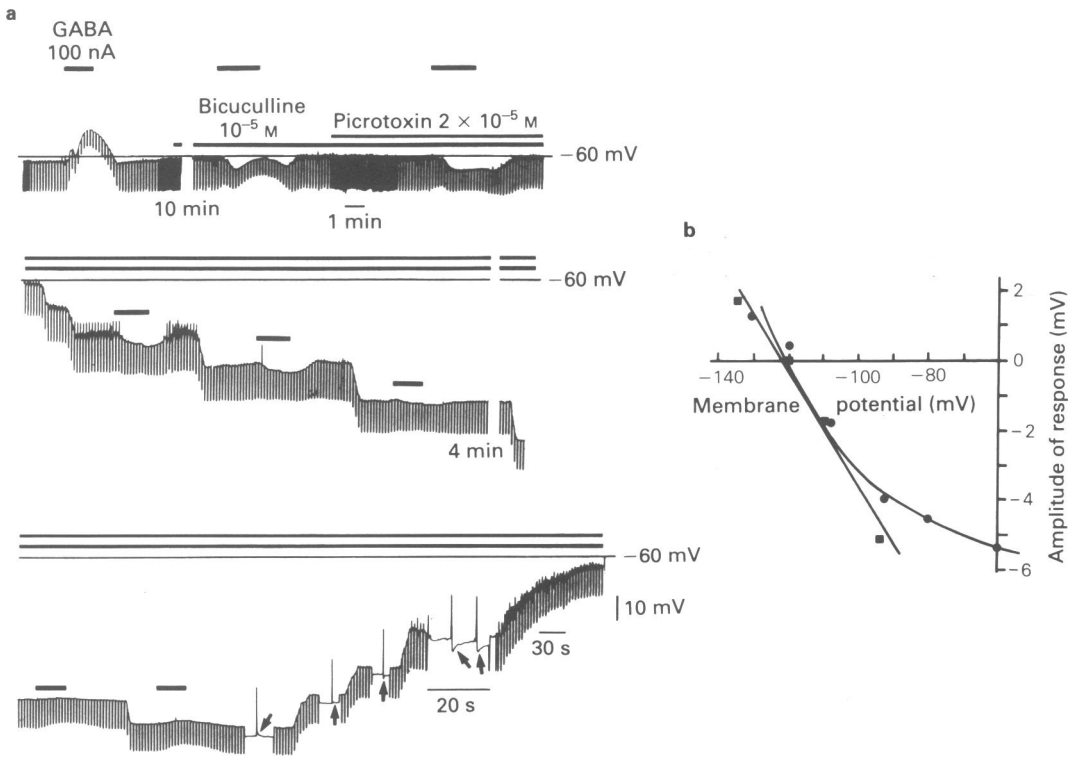

Figure 6 Voltage-dependence of the hyperpolarization-predominant response to GABA examined during superfusion of bicuculline and picrotoxin. (a) Bicuculline and picrotoxin were superfused at periods indicated by bars. Arrows represent the post-burst hyperpolarization triggered by mossy fibre stimulation. The burst discharges are seen on the pen-recording as large positive deflections due to their slow time course. In (b), (●) represents GABA-induced hyperpolarization and (■) post-burst hyperpolarization.

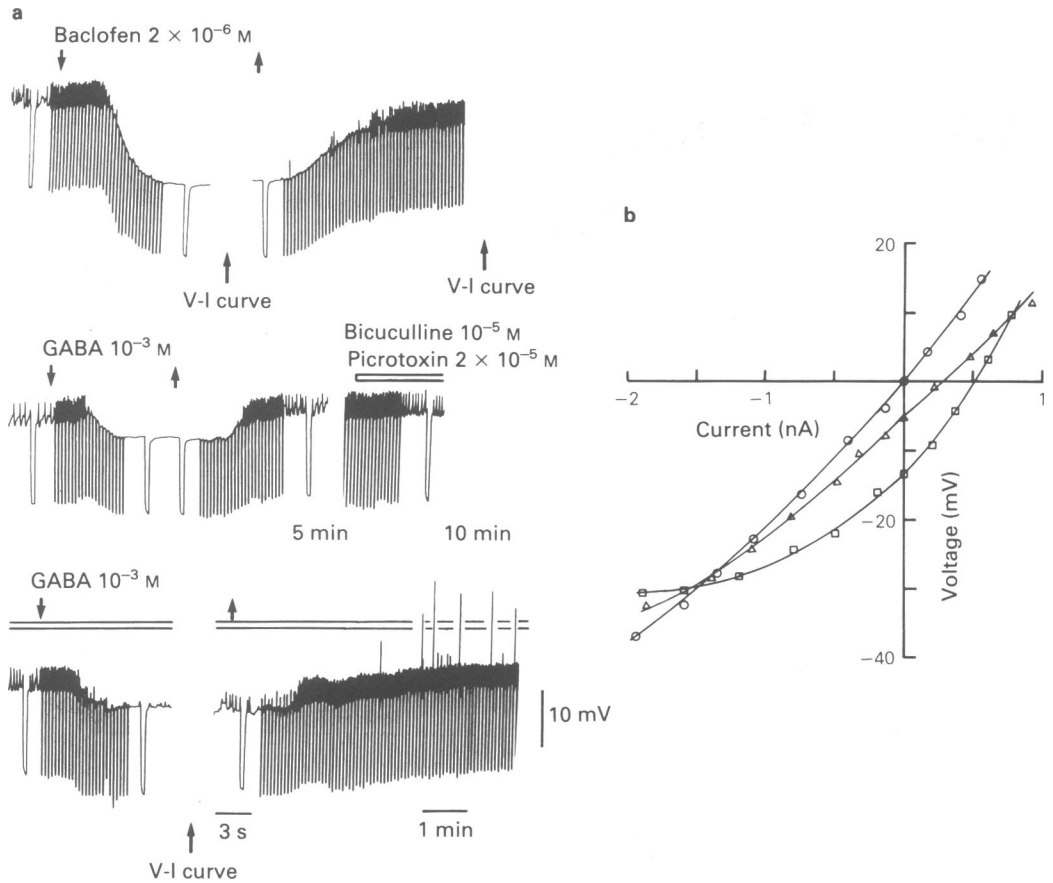

Figure 7 The current-voltage relationships measured during applications of GABA and baclofen. (a) All traces were recorded in the same neurone. At periods indicated by bars, bicuculline (10^{-5} M) and picrotoxin (2×10^{-5} M) were added to the medium. The current-voltage relationship was measured at three points indicated by arrows labelled 'V-I curve' in the traces. In (b), abscissa scale represents transmembrane current (nA; duration, 600ms); ordinate scale, steady-state membrane potential displacement (mV) produced by the current pulse; (○) Control medium, (△) GABA 10^{-3} M and (□) baclofen 2×10^{-6} M.

the combined application of bicuculline and picrotoxin. The hyperpolarization became larger with membrane depolarization and smaller with membrane hyperpolarization.

The reversal point of the hyperpolarizing response approximated that of the post-burst afterhyperpolarization (see graph in Figure 6). The reversal potential of the pharmacologically manipulated hyperpolarization was 58.3 ± 2.5 mV ($n = 5$) negative to the resting membrane potential, while that of the post-burst afterhyperpolarization was 56.8 ± 2.6 mV ($n = 5$) negative to the resting membrane potential. There was no statistically significant difference between these two values (Student's t test).

Figure 7 shows the current-voltage relationships obtained in the same neurone during superfusions of control, GABA-containing and baclofen-containing media. The current voltage curve obtained in the control solution intersected those obtained during the respective drug applications at about 30 mV below the resting membrane potential (− 63 mV). The membrane potential at which the two current-voltage curves intersect represents the potential at which the voltage effect of the drug is cancelled, i.e., the reversal potential for the drug action. The reversal potential for GABA thus estimated was -92.2 ± 2.2 mV ($n = 5$).

As shown in Figure 8a, the replacement of all the NaCl in the bathing medium with equimolar Na-isethionate induced an epileptiform burst which was shown as a large spiky deflection in the pen-recording. Introduction of the low Cl^- medium caused a d.c. potential shift. However, this d.c. potential shift was

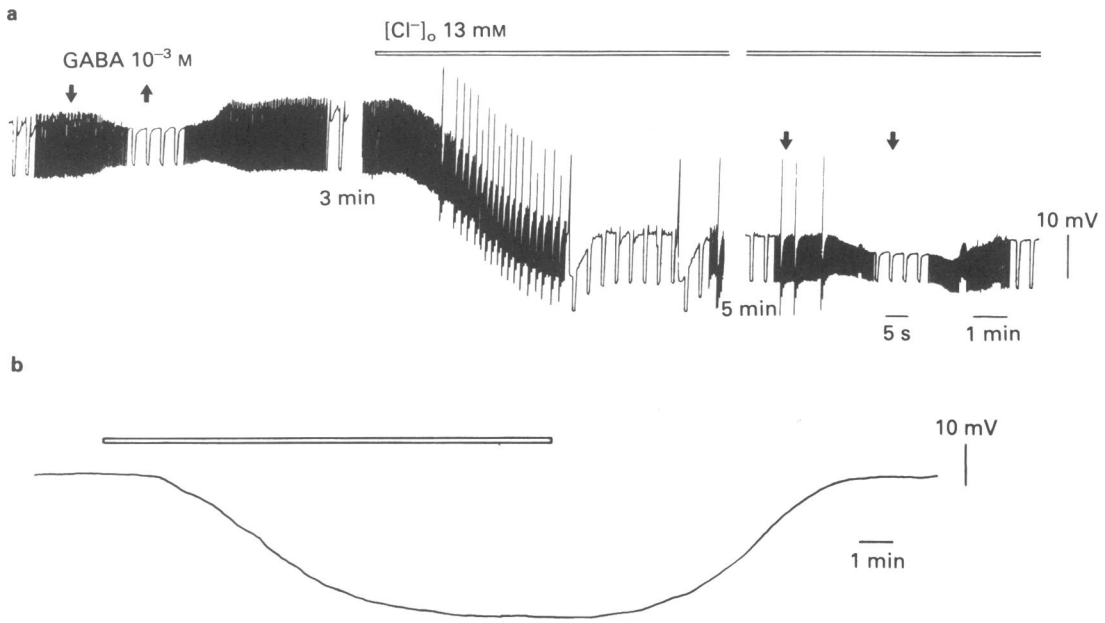

Figure 8 (a) The effect of low external Cl^- concentration on the hyperpolarization-predominant response to GABA. The concentration of Cl^- was decreased to 13 mM by replacing NaCl with equimolar Na-isethionate, at periods indicated by open bars. (b) Shows a d.c. potential shift due to the replacement of Cl^- with isethionate ion (for details see text).

mostly an artifact, since the tip of the electrode that was positioned outside the cell membrane picked up the identical d.c. potential shift (see Figure 8b). This was confirmed in 6 additional experiments. The d.c. shift was not due to a property of the isethionate ion, because replacement with propionate ion also resulted in a similar potential shift. The amplitude of the depolarizing component of the response to GABA was increased to 1.74 ± 0.22 times that of the control response ($n = 5$) by reducing external Cl^-. In contrast, hyperpolarization produced by GABA was not appreciably affected by the reduction of external Cl^-. Such a relative insensitivity of the hyperpolarizing response to GABA was reproducible in all 5 cells in which the effect of low Cl^- on the hyperpolarizing response to GABA was examined during superfusions with picrotoxin and bicuculline.

Discussion

Ionic mechanism for the depolarizing response to GABA

The biphasic character of the response to GABA has been shown in a number of vertebrate neurones besides hippocampal pyramidal cells (e.g. Nicoll *et al.*, 1976; Barker & Ransom, 1978; Obata *et al.*, 1978). It has been disputed whether the components of this biphasic response are generated by the same ionic channel or by separate ionic channels. Our finding that the depolarizing response to GABA was apparent in Na^+-deficient and Ca^{2+}-free media argues against the involvement of an increased cation permeability in the depolarizing action of GABA in the hippocampus (Djørup *et al.*, 1981). The augmentation of this depolarizing response to GABA in a Na^+-deficient medium may be attributed to an increased agonist concentration due to inhibition of the Na^+-sensitive GABA uptake mechanism (Brown & Galvan, 1977; Iversen & Kelly, 1975).

The depolarizing response was selectively blocked by picrotoxin, which blocks GABA-mediated Cl^- conductance in vertebrate neurones (Gallagher *et al.*, 1978), whereas it was augmented by a reduction of external Cl^-. These results strongly suggest that the depolarizing response to GABA in the hippocampus results from a specific increase in Cl^- conductance.

Ionic mechanism for the hyperpolarizing response to GABA

The hyperpolarization induced by GABA was in most cases associated with a concurrent depolarization.

Furthermore, even when the response to GABA was ostensibly 'hyperpolarizing', a possible contamination by the depolarizing component could not be excluded (Figure 4). In view of these observations, our attempt to eliminate a larger part of the depolarizing component from the biphasic response to GABA through combined application of picrotoxin and bicuculline (Figure 5) has made it feasible to analyse more precisely the ionic mechanism of the hyperpolarizing response.

The reversal potential of the 'pharmacologically isolated' hyperpolarizing response to GABA was deduced from the current-voltage relationships to be about -90 mV (Figure 7); this value agrees with the equilibrium potential for K^+ measured in this (Alger & Nicoll, 1980; Brown & Griffith, 1983) and other preparations (e.g. Bührle & Sonnhof, 1983). In addition, the reversal potential of the 'pharmacologically isolated' hyperpolarizing response to GABA was the same as that of the hyperpolarization induced by baclofen, a $GABA_B$ receptor agonist (Bowery *et al.*, 1980). Since the hyperpolarization induced by baclofen has been demonstrated to be due to an activation of K^+ conductance (Newberry & Nicoll, 1984; Inoue *et al.*, 1985a,b), it is likely that the hyperpolarization induced by GABA also involves activation of K^+ conductance. This notion is further supported by the observation that the reversal point of the 'pharmacologically isolated' hyperpolarizing response to GABA approximated that of the post-burst afterhyperpolarization (Figure 6; Newberry & Nicoll, 1985) which mainly reflects the Ca^{2+}-activated K^+ current (e.g. Alger & Nicoll, 1980; Hotson & Prince, 1980; Brown & Griffith, 1983).

Newberry & Nicoll (1985) have shown two types of GABA-induced hyperpolarization. One is an initial fast hyperpolarization induced by GABA ionophoresed to the somata of pyramidal cells at a weak ejection current intensity. The other is a late slow hyperpolarization induced by an application of GABA to the dendritic field, which may reflect a slow activation of K^+ conductance. The former hyperpolarization was attributed to a rapid activation of somatic Cl^- conductance on the basis of the following observations: (1) this potential was highly sensitive to GABA antagonists; (2) its reversal potential was apparently less negative than the equilibrium potential for K^+ and was dependent on the Cl^- gradient across the cell membrane. Thus, GABA ionophoresed to the somata in larger doses, or to the dendrites, typically causes a triphasic response comprising a Cl^--mediated fast hyperpolarization, a Cl^--mediated dendritic depolarization, and a K^+-mediated slow hyperpolarization.

In our experiments, GABA was applied in many cases by superfusion which would result in the direct activation of the dendritic GABA receptors. In addition, our ionophoretic application of GABA to the pyramidal layer usually provoked a depolarizing component as well, suggesting diffusion of GABA to the dendrites. Therefore, it is conceivable that the hyperpolarization observed in our experiments reflects the 'late hyperpolarization'. This notion is supported by the finding that the hyperpolarizing component of the response to GABA was insensitive to a reduction in external Cl^- concentration (Figure 8).

In conclusion, our results suggest that, in the guinea-pig hippocampus, GABA activates at least two separate receptor-ionophore complexes, both of which contribute to the complex action of GABA observed in this tissue: one is linked to K^+ channels and is responsible for at least part of the hyperpolarizing action of GABA; the other is associated with the picrotoxin-sensitive Cl^- channels and mediates the depolarizing effect.

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