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DEPOLARIZATION OF NEURONES IN THE ISOLATED OLFACTORY CORTEX OF THE GUINEA-PIG BY γ-AMINOBUTYRIC ACID

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1 Effects of γ-aminobutyric acid (GABA) on single neurones in slices of guinea-pig olfactory cortex maintained *in vitro* were recorded with single intracellular microelectrodes. The average resting potential of 52 cells was −75 mV and apparent input resistance ranged from 20 to 200 MΩ.

2 Superfusions of GABA over the slice invariably depolarized the neurones and reduced their input resistance. The minimum effective concentration was 50 to 200 μM.

3 The reversal potential for the depolarization produced by 0.1 mM GABA (E_g) was -66 ± 2 mV. At concentrations >0.1 mM the reversal potential became progressively more positive (−55 to −50 mV).

4 Reduction of external chloride, with isethionate as the substitute anion, increased the amplitude of the depolarization.

5 GABA reduced the amplitude of the excitatory postsynaptic potential produced by lateral olfactory tract stimulation, and occluded or reversed the subsequent depolarizing recurrent inhibitory postsynaptic potential.

6 Action potentials elicited by injection of depolarizing current or by focal antidromic stimulation were slowed and reduced in amplitude by GABA.

7 The effects of GABA on membrane conductance (potency = 1) were duplicated by 3-aminopropanesulphonic acid (potency = 20), β-alanine (0.5), β-amino-*n*-butyric acid (0.5), glycine (0.3) and L-2,4-diaminobutyric acid (0.2). For a given conductance change, 3-aminopropanesulphonic acid, glycine and β-alanine produced less depolarization than did GABA.

8 It is concluded that the action of GABA on the neurones is compatible with a role in mediating recurrent postsynaptic inhibition.

Introduction

γ-Aminobutyric acid (GABA) depresses the discharge rate of neurones in the mammalian olfactory cortex (Legge, Randić & Straughan, 1966). This region of the brain is particularly well suited for *in vitro* studies (Yamamoto & McIlwain, 1966; Richards & Sercombe, 1968; Harvey, Scholfield & Brown, 1974). Single olfactory neurones can be impaled and show excitatory and recurrent inhibitory responses to lateral olfactory tract stimulation (Scholfield, 1978a, b). Accordingly, we have used an *in vitro* preparation of the guinea-pig olfactory cortex to examine the action of GABA on olfactory neurones in more detail. A preliminary account of this work has been published (Scholfield, 1978d).

Methods

Guinea-pig brains were removed after decapitation. Surface slices of the olfactory cortex containing the lateral olfactory tract (LOT), but devoid of the olfactory bulb, were cut to a thickness of around 700 μm (see Harvey *et al.*, 1974). Slices were maintained *in vitro* in Krebs solution at ambient temperature (normally about 25°C) bubbled with 95% O_2 and 5% CO_2. The composition of the solutions used is given below.

For recording, the slice was placed horizontally

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between two nylon mesh grids suspended in a bath and superfused on both surfaces with Krebs solution. Neurones in the prepyriform cortex were impaled with a single microelectrode filled with 4 M potassium acetate, connected to an amplifier coupled with a constant current source and facilities for electrode resistance and capacitance compensation (see Scholfield 1978a for details). Drugs were added to the superfusate in fixed concentrations, for up to 3 min; the bath exchange time was 5 to 10 s. Resting cell input resistance (R_i) was monitored by passing depolarizing current pulses through the electrode, to give small voltage deflections of ≤ 10 mV within the range where the current-voltage curve was fairly linear (Scholfield, 1978a).

Solutions

The composition of the Krebs solution was (mM): Na 144, K 5.9, Ca 2.5, Mg 1.3, Cl 128, HCO_3 25, SO_4 1.3, PO_4 1.2 and D-glucose, 11. Low [Cl] solution was made by replacing 90 mM NaCl by either 90 mM sodium proprionate or 90 mM sodium isethionate. All solutions were equilibrated with 95% O_2 and 5% CO_2 to a pH of 7.4.

Results

The present experiments were performed on a sample of 52 neurones with electrical properties comparable to those described previously (Scholfield, 1978a). Their average resting membrane potential was −75 mV; apparent input resistance ranged from 20 to 200 MΩ.

Superfusion of GABA over the slice invariably depolarized the neurones (Figure 1). The minimum effective concentration ranged from 50 μM to 200 μM; the maximal depolarization (at 1 to 5 mM) ranged from 15 to 30 mV (Table 1). Individual neurones varied in their sensitivity, rendering composite dose-response curves misleading.

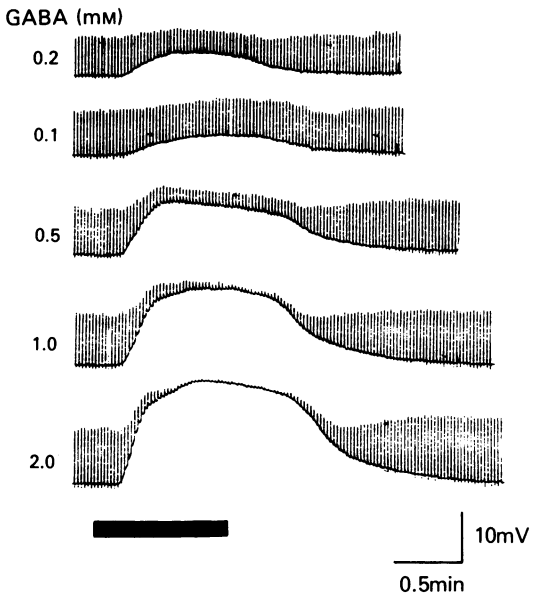

Figure 1 Responses of a single pyriform neurone to increasing concentrations of γ-aminobutyric acid (GABA) recorded at slow speed. Upward deflections from the baseline show peak depolarizing responses to injected current pulses (+0.3 nA, 360 ms). GABA was perfused over the slice for the periods indicated by the bar at about 7 min intervals: the lag of about 15 s before the response represents bath exchange time.

Depolarization was accompanied by a large increase in input conductances (fall in input resistance) (see also Figure 4). This was not simply due to the depolarization, since the current-voltage curve remained fairly linear between −90 and −60 mV and showed a reduced slope at the original membrane potential (Figure 2).

Table 1 Summary of the effects of γ-aminobutyric acid (GABA) on olfactory neurones

GABA concentration mM	n	*Depolarization* (ΔE_m) *mV*	*Conductance increase* ($\Delta G/G_m$)	**Reversal potential* ($E_g - E_m$) *mV*
0.05	2	1.9	0.09	12
0.1	7	2.7 ± 1.5	0.44 ± 0.12	10.4 ± 3.0
0.2	11	5.7 ± 3.2	0.76 ± 0.48	17.7 ± 6.0
0.5	12	10.3 ± 5.9	2.12 ± 1.11	20.8 ± 5.5
1.0	11	14.2 ± 4.5	2.81 ± 0.77	22.4 ± 3.3
2.0	9	17.6 ± 4.2	8.85 ± 3.41	22.7 ± 5.7

Mean values ± s.d. are given; n = number of cells tested at each concentration.
*Calculated from text-equation (1).

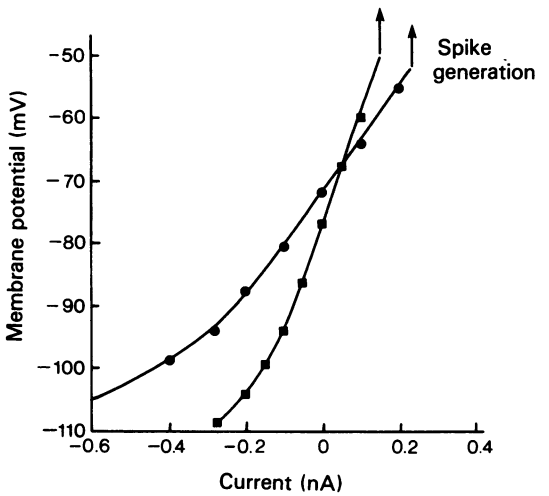

Figure 2 Current-voltage curves from a pyriform neurone in the absence (■) and presence (●) of 0.1 mM γ-aminobutyric acid (GABA). Points show the plateau membrane potential (ordinate scale) attained during each 180 ms current pulse (abscissa scale). The resting potential was −77 mV: GABA depolarized the cell by 5 mV.

Reversal potential

The intersection of the current-voltage curves in Figure 2 provides a reversal potential E_g for the GABA-induced depolarization of around −65 mV. This was confirmed in a number of neurones from constant-current pulse measurements of conductance changes, using the relationship (from Ginsborg, House & Silinsky, 1974):

$$E_g = E_m + \Delta E_m\left(1 + \frac{G_m}{\Delta G}\right) \qquad (1)$$

where E_m is the resting membrane potential measured before adding GABA, ΔE_m is the depolarization produced by GABA, G_m is the resting input conductance and ΔG is the apparent increase in input conductance during the depolarization. At low concentrations of GABA (0.1 mM), the calculated value for E_g was -66 ± 2 mV i.e., about 9 mV positive to E_m. However, at higher concentrations of GABA a more positive value for E_g of −55 to −50 mV (Table 1) resulted, as evidenced by Figure 1, where the depolarization produced by high GABA concentrations clearly exceeded 9 mV.

Ionic mechanism

Reduction of external [Cl] , from 128 to 38 mM, increased the depolarization produced by GABA without a corresponding increase in the conductance change, implying a more positive reversal potential (Figure 3). This accords with a contribution of Cl^- flux to the response (as in most other vertebrate neurones: cf. Krnjević, 1974). Further reduction in external [Cl] was impracticable because of resultant 'seizure' activity (see Yamamoto & Kawai, 1968).

Effects of replacing sodium ions were difficult to assess. When Na^+ alone was replaced with Li^+ the cell rapidly depolarized and its input resistance fell drastically, precluding accurate assessment of the effects of GABA. Replacement of Na^+ and Cl^- with mannitol (in the presence of 20 mM Mg^{2+}, to reduce seizure activity) increased the sensitivity of the preparation to GABA. This may reflect diminished uptake of GABA (Galvan & Scholfield, 1978; see also Brown & Galvan, 1977).

Effect on transmission

Stimulation of the lateral olfactory tract (LOT) produces (i) a short latency excitatory postsynaptic

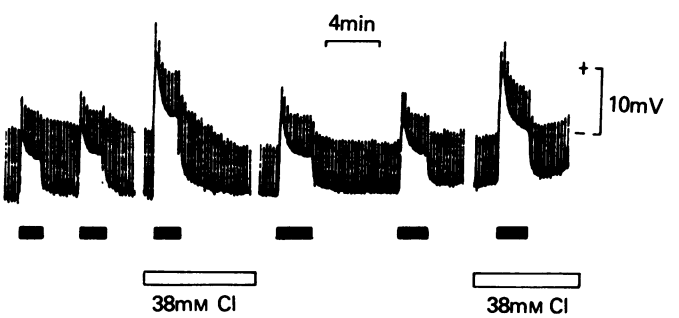

Figure 3 Effect of reducing [Cl] from 128 mM to 38 mM (open bars) on responses of a pyriform neurone to 0.1 mM γ-aminobutyric acid (GABA, solid bars), recorded as in Figure 1. Cl was substituted with isethionate, and [Mg] was raised to 20 mM to stabilize the membrane potential. (In normal solution 20 mM [Mg] did not affect the action of GABA.) Gaps in the record represent periods of 7 to 10 min for solution changes and membrane potential stabilization.

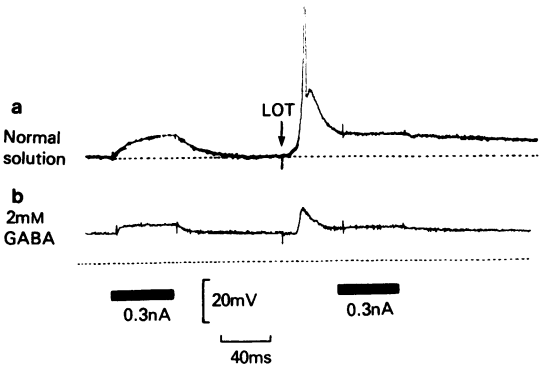

Figure 4 Effect of 2 mM γ-aminobutyric acid (GABA) on responses of an olfactory cortex neurone to injected positive current pulses (+0.3 nA, 45 ms; bars) and to a single lateral olfactory tract stimulus (LOT). (a) Shows the control response (initial resting membrane potential, $E_m = -76$ mV). The current pulse produced a 10 mV depolarization, indicating an input resistance of about 33 MΩ. The LOT stimulus evokes an e.p.s.p., with superimposed action potential (truncated in the record). This is followed by a prolonged after depolarization during which the input resistance is greatly reduced, as indicated by the absence of depolarization produced by the injected current pulse. This after-depolarization has the characteristics of an i.p.s.p. (see Scholfield, 1978b). In the presence of GABA (b) the membrane potential was reduced by 14 mV and the input resistance was reduced to 12 MΩ. The e.p.s.p. evoked by LOT stimulation was reduced and now failed to produce an action potential, and the subsequent i.p.s.p. was less depolarizing.

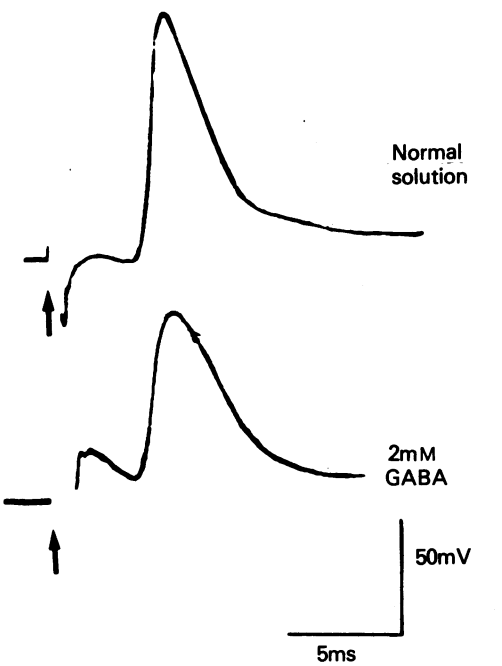

Figure 5 Effect of γ-aminobutyric acid (GABA, 2 mM) on neuronal action potentials evoked by focal antidromic stimulation (0.5 ms at arrows) using a tungsten microelectrode inserted in the region of efferent axons towards the cut surface of the slice. The stimulus voltage was selected to give intermittent failures. Under normal conditions (a) the antidromic spike amplitude (101 mV) was comparable to that of the orthodromic and direct spikes, and the rate of rise was 143 V/s. In the presence of GABA (which depolarized the membrane by 14 mV (b) the spike amplitude was 76 mV and rate of rise 81 V/s. Note: the stimulus voltage necessary to elicit a spike was increased 6 times in the presence of GABA.

potential (e.p.s.p.), generating a neuronal spike, which is followed by (ii) a prolonged depolarizing inhibitory postsynaptic potential (i.p.s.p.) reversing at −60 to −65 mV (Scholfield, 1978b; see Figure 4). In the presence of GABA the e.p.s.p. was reduced to below the threshold for spike generation (Figure 4b). The voltage change accompanying the subsequent i.p.s.p. was usually occluded, though some residuum of the underlying conductance increase persisted. Occasionally the i.p.s.p. became hyperpolarizing in direction when the cell was strongly depolarized by large doses of GABA.

Excitability changes

Notwithstanding the frequently substantial depolarization, which sometimes exceeded the voltage-threshold for spike initiation by injected positive current, superfused GABA did not normally initiate spikes (occasionally, spikes were initiated in low-Cl^- solution). On the contrary, GABA reduced excitability. This was manifest as a reduced spike amplitude, spike prolongation and elevated current threshold for direct or antidromic spike initiation (Figure 5).

Action of other agonists

GABA-like depolarizations were produced by the following amino acids: 3-aminopropanesulphonic acid (20), β-alanine (0.5), β-amino-*n*-butyric acid (0.5), glycine (0.3), and L-2,4-diaminobutyric acid (0.2). Numbers in brackets show approximate potencies (GABA = 1) measured from the relative concentrations required to double input conductance. However, there seemed to be some difference between GABA and 3-aminopropanesulphonic acid on the one hand and glycine and β-alanine on the other in that the former pair produced about twice as much depo-

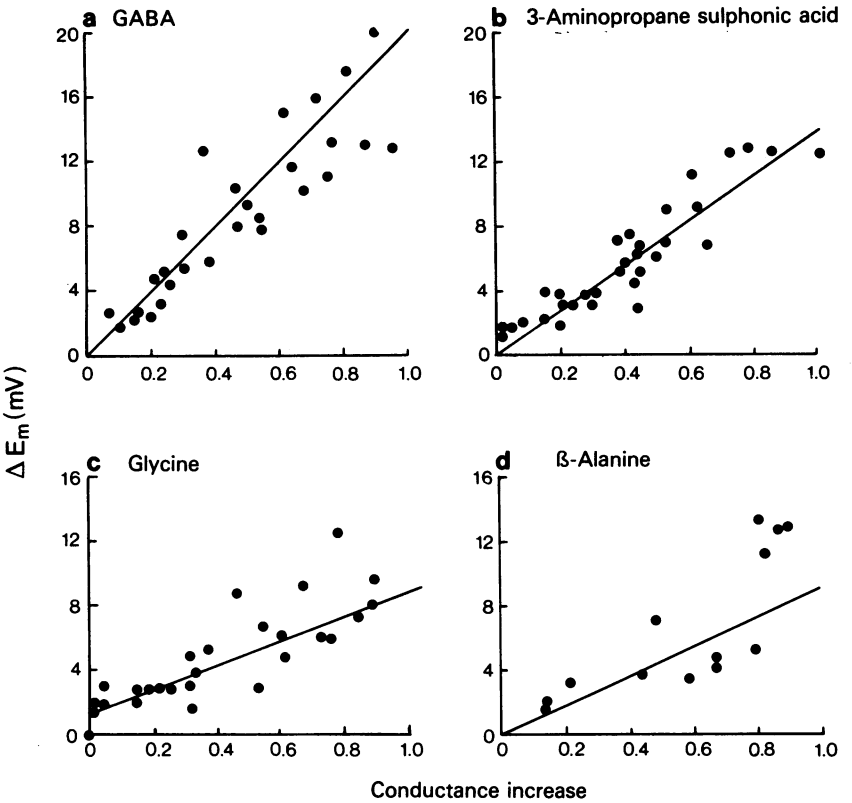

Figure 6 Relationship between recorded depolarization (ordinates, mV) and relative conductance increases (abscissae) produced by (a) γ-aminobutyric acid (GABA), (b) 3-aminopropanesulphonic acid, (c) glycine and (d) β-alanine. Each point represents the peak response to a single drug administration to 3–10 neurones. The conductance increase is expressed as $\Delta G/(G_m + \Delta G)$ (see text equation (2)). The lines are the slopes calculated from text equation (2) by least squares regression analysis. The values of E_g so calculated were 20.2 mV, 13.9 mV, 8.0 mV and 9.1 for GABA, 3-aminopropanesulphonic acid, glycine and β-alanine respectively.

larization for a given conductance increase. Figure 6 shows the relationship between the observed depolarization and conductance increase produced by these four agonists, plotted in the form (from text-equation 1)

$$\Delta E_m = (E_g - E_m)\left(\frac{\Delta G}{G_m + \Delta G}\right). \qquad (2)$$

If the reversal potential were constant throughout the agonist dose-range, and the current voltage curve assumed linear, the data points in Figure 6 should lie on a straight line extrapolating to $\Delta E_m = (E_g - E_m)$ at $\Delta G/(G_m + \Delta G) = 1$. In practice (see above) the reversal potential appeared to shift in a depolarizing direction with increasing agonist concentration, hence the curves tend to convexity. Nevertheless, the results suggest that the reversal potential for β-alanine and glycine is less positive than that for GABA and 3-aminopropanesulphonic acid.

Antagonists

Bicuculline (0.1 to 10 μM) or picrotoxin (10 μM) induced repetitive 'seizure like' discharges of olfactory neurones following stimulation of the LOT. At these concentrations responses to GABA were not obviously reduced.

Discussion

Superficially, the depolarization of olfactory neurones by GABA and also by glycine seems to be at variance with the expected effect of a possible inhibitory neurotransmitter (see Krnjević, 1974). There have been no previous reports of the effect of GABA on the membrane potential of olfactory neurones, either *in vivo* or *in vitro*, but Dreifuss, Kelly & Krnjević (1969) observed hyperpolarization in other regions of the cerebral cortex *in vivo*. However, this difference may,

to a substantial extent, result from the far higher resting potential of olfactory neurones *in vitro* (possible reasons for which have been discussed previously: Scholfield, 1978a). More importantly, the reversal potential for the depolarization is no less negative than that deduced for cerebral cortex neurones *in vivo* (Dreifuss, *et al.*, 1969). In fact, reported reversal potentials for the action of GABA on different mammalian neurones under different experimental conditions show a remarkable variation. Some examples are: mammalian motoneurones *in vivo*, −75 to 80 mV (Curtis, Hosli, Johnston & Johnston, 1968); spinal cord neurones in tissue culture, −35 to 70 mV (Ransom, Bullock & Nelson, 1977); cerebral cortex neurones *in vivo*, −45 to −55 mV (Dreifuss *et al.*, 1969); mammalian sympathetic ganglion cells *in vitro*, −40 to −45 mV (Adams & Brown, 1975) and in tissue culture, −20 mV (Obata, 1974); mammalian sensory ganglion cells *in vivo*, −34 to −37 mV (Deschenes, Feltz & Lamour, 1976) and *in vitro*, −24 mV (Gallagher, Higashi & Nishi, 1978). Nevertheless, in each case a primary conductance increase to chloride ions has been deduced. The present experiments are compatible with a primary increase in Cl^- current as the generator for the depolarization of olfactory neurones, but do not conclusively eliminate contributory cation currents.

Particular difficulties arise from the positive shift in the reversal potential with increasing GABA concentrations, and the apparent difference between the reversal potentials of GABA and glycine. The former has some resemblance to the time-dependent positive shift in E_g in spinal neurones recently reported by Krnjević, Puil & Werman (1977): this was attributed to Na^+-coupled uptake, but this seems unlikely for olfactory neurones since most neurones fail to show autoradiographically-visible uptake (M. Galvan, unpublished observations). Other possibilities include an additional component of increased Na^+-conductance at high concentrations or secondary K^+ shifts (see Deschenes & Feltz, 1976). A major interpretational problem arises from the undoubtedly widespread action of GABA in the olfactory cortex. Thus, lateral olfactory tract axons are clearly affected (Pickles, 1978; Brown & Galvan, 1979) and efferent axons from impaled neurones are also responsive, to judge from the higher current threshold for antidromic stimulation (see Figure 5). Experiments on hippocampal neurones (Andersen, Bie, Ganes & Laursen, 1978) and tissue-cultured spinal neurones (Ransom *et al.*, 1977) suggest that dendrites may also be sensitive to GABA. Such extra-somatic effects might well vary with concentration, given the remote form of GABA-application and consequential concentration gradients cross the tissue. This introduces appreciable error in estimating reversal potentials by single point (somatic) current injection (see Ginsborg, 1967). Until such extrasomatic effects can be eliminated by (for example) finely-localized iontophoresis, further discussion of ionic mechanisms seems unrewarding.

With respect to the possible transmitter function of GABA in this preparation, two points may be made. Firstly, notwithstanding the depolarization, GABA is clearly inhibitory in its effect on both excitatory transmission and on neuronal excitability This affords an illustration of the point clearly made by Ginsborg (1967), that a transmitter need not hyperpolarize to inhibit, the only requirement being that the equilibrium potential for the conductance increase is sufficiently negative to shunt the e.p.s.p. to a value below the theshold for spike generation. Secondly, the recurrent inhibitory postsynaptic potential is also depolarizing in direction under these experimental conditions, with a reversal potential (−63 mV) comparable to that for low GABA concentrations, and with a similar dependence upon external chloride concentration (Scholfield, 1978b). To this extent, GABA remains a plausible candidate for the recurrent inhibitory transmitter. The previously reported ability of low concentrations (≤ 10 μM) of bicuculline or picrotoxin to reduce the recurrent i.p.s.p. (Scholfield, 1978c and d) might be regarded as further evidence in support of this view. However, we feel that this should be interpreted with some caution since the activity of these compounds against exogenously-applied GABA was not striking. Some further experiments concerning the pharmacological nature of the GABA-receptors in this tissue is presented in the companion paper (Brown & Galvan, 1979).

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