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RESPONSES OF THE GUINEA-PIG ISOLATED OLFACTORY CORTEX SLICE TO γ-AMINOBUTYRIC ACID RECORDED WITH EXTRACELLULAR ELECTRODES

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1 Potential changes between the pial and cut surfaces of slices of guinea-pig olfactory cortex *in vitro* produced by γ-aminobutyric acid (GABA) were recorded with extracellular electrodes.

2 GABA, superfused over the pial surface (0.1 to 10 mM), produced a pial-negative potential deflection, accompanied by inhibition of the postsynaptic response to lateral olfactory tract (LOT) stimulation.

3 This effect was replicated by the following compounds (potency relative to GABA = 1, in brackets): 3-aminopropanesulphonic acid (5.3), δ-aminovaleric acid (0.07), β-alanine (0.07), β-amino-*n*-butyric acid (0.05), ε-aminocaproic acid, α-amino-*iso*butyric acid, L-leucine ($\ll$0.02).

4 L-Glutamate (1 to 10 mM) produced a very large surface negative shift, with relatively less synaptic inhibition. Glycine (1 to 10 mM) produced less surface negativity, accompanied by synaptic inhibition.

5 Responses to GABA were antagonized more effectively than those to glycine by bicuculline (3 to 30 μM) and picrotoxin (1 to 30 μM). Strychnine (1 to 10 μM) incompletely inhibited responses to glycine.

6 It is concluded that, while the locus within the slice of these effects is uncertain, the preparation may be useful for testing the interaction of drugs with cerebral GABA receptors.

Introduction

In the previous paper (Brown & Scholfield, 1979) a depolarizing action of γ-aminobutyric acid (GABA) on individual neurones in the guinea-pig olfactory cortex was described. The rather comparable depolarizing action of GABA on peripheral sympathetic neurones (Adams & Brown, 1975) can be easily recorded with extracellular electrodes (Bowery & Brown, 1974). This provides a more convenient way than intracellular recording of studying the pharmacological characteristics of the peripheral GABA-receptors. The aim of the present experiments was to see whether the depolarization of olfactory neurones *in vitro* can likewise be detected with extracellular electrodes, and to make a preliminary pharmacological assessment of the activity of some different agonists and antagonists in this tissue.

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Methods

Slices of olfactory cortex from guinea-pigs were prepared as described in the previous paper (Brown & Scholfield, 1979; see Harvey, Scholfield & Brown, 1974, for details). For extracellular recording, slices were mounted at 45° to the vertical, cut surface downwards, on the agar-base of a large diameter Ag/AgCl reference electrode (see Figure 1). The slice was superfused continuously with Krebs solution at about 25°C on both under (cut) and upper (pial) surfaces at 2 ml/min. A fine balsa-wick Ag/AgCl electrode was placed on the upper pial surface near the distal end of the lateral olfactory tract (LOT), to record drug- and synaptic-evoked potentials. A pair of platinum stimulating electrodes were placed either side of the proximal end of the LOT to stimulate the LOT fibres (0.1 ms duration square-wave pulses of supramaximal voltage at 10 s intervals). The stimulating electrodes also served to hold the slice in place. Evoked post-

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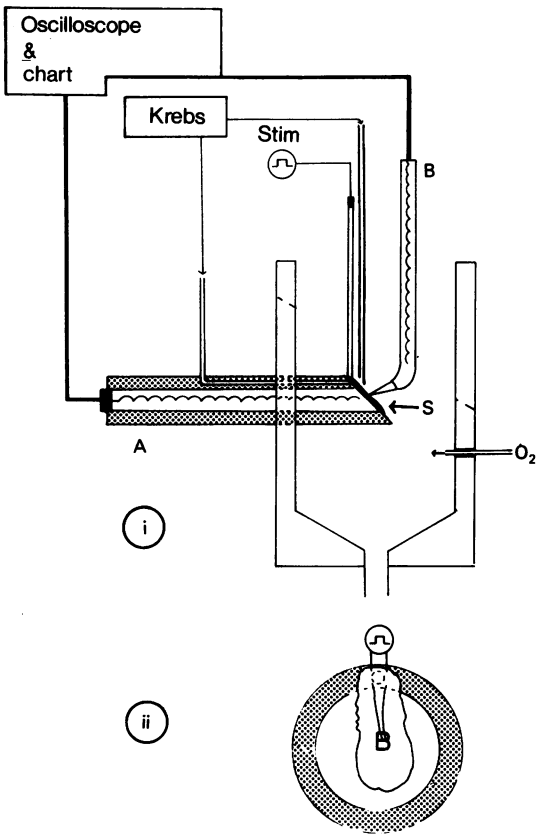

Figure 1 Diagram showing the method used for recording agonist-induced extracellular d.c. potential from the isolated olfactory cortex. (i) Lateral view of the reference electrode (A) supporting the cortex slice (S) at a 45° angle. The slice was held in place by the stimulating electrode (Stim) and superfused on its pial and cut surfaces. The active electrode (B) was placed at the end of the lateral olfactory tract (LOT), as shown in the vertical view in (ii). The slice was enclosed in an oxygen-enriched atmosphere.

synaptic field potentials were displayed on an oscilloscope with d.c. amplification and recorded on a hot-wire recorder (Devices).

Drugs were added to the superfusate in fixed concentrations and passed over the slice until a peak d.c. deflection was obtained. Agonists were applied for brief periods to the pial surface only; antagonists were perfused over both surfaces, to ensure full penetration of the slice. Drug-induced d.c. potential changes were displayed on a potentiometric recorder (Bryans 28000). The composition of the Krebs solution was (mM): NaCl 118, KCl 4.8, $CaCl_2$ 2.52, $NaHCO_3$ 25, KH_2PO_4 1.18, $MgSO_4.7H_2O$ 1.19, and D-glucose 11 (pH 7.4).

Results

Superfusion of GABA (0.1 to 10 mM) over the pial surface of the slice produced a transient negativity of the pial surface with respect to the reference electrode in contact with the cut surface ('surface negativity': Figure 2). The response was rapid in onset (<60 s) but faded appreciably during continued drug application, and sometimes reversed transiently on washing (see Figure 5).

The direction of the voltage change was the same as that evoked by glutamate or by K^+ ions (25 mM), and the same as the 'N-wave' evoked by LOT stimulation (see below).

Lateral olfactory tract stimulation

Stimulation of the LOT produces a pial-negative potential (the N-wave) which corresponds to the summed monosynaptic excitatory postsynaptic potentials e.p.s.p.) generated in the dendrites of the olfactory cells (Richards & Sercombe, 1968; Harvey *et al.*, 1974; Halliwell, 1976). GABA reduced the amplitude of this N-wave (Figure 3) in concentrations matching those producing the surface-negative shift (see Figure 2). At concentrations ≥1 mM GABA also depressed the action potential recorded from the LOT itself (Figure 3d). However, action potential depression alone probably did not account for the reduction in the postsynaptic response since the latter greatly exceeded that attained by a matching reduction in stimulus strength. Pickles (1978) has described a depolarization of LOT axons by GABA.

Comparison of γ-aminobutyric acid and other agonists

Both surface-negativity and synaptic depression were replicated by some other GABA-mimetic compounds (Figure 4). Approximate potencies from mean dose-response curves were: 3-aminopropanesulphonic acid, 5.3; GABA, 1; δ-aminovaleric acid, 0.07; β-alanine, 0.07; β-amino-*n*-butyric acid, 0.05; ε-amino-caproic acid, α-amino-*iso*-butyric acid and L-leucine, ≪0.02.

Glycine also produced a surface-negative response, but of lower amplitude than that produced by GABA (Figure 4a). L-Glutamate produced a very large surface negativity (Figure 4b), about 10 times greater than that produced by GABA.

All compounds with a potency >0.02 depressed the LOT-evoked N-wave. For a given surface-negativity, GABA inhibited transmission to a greater extent than glutamate but less than glycine. L-2,4-Diaminobutyric acid produced a low amplitude negativity at concentrations ≤3 mM; above this level large irreversible negative shifts occurred, accompanied by persistent depression of synaptic transmission. This may reflect

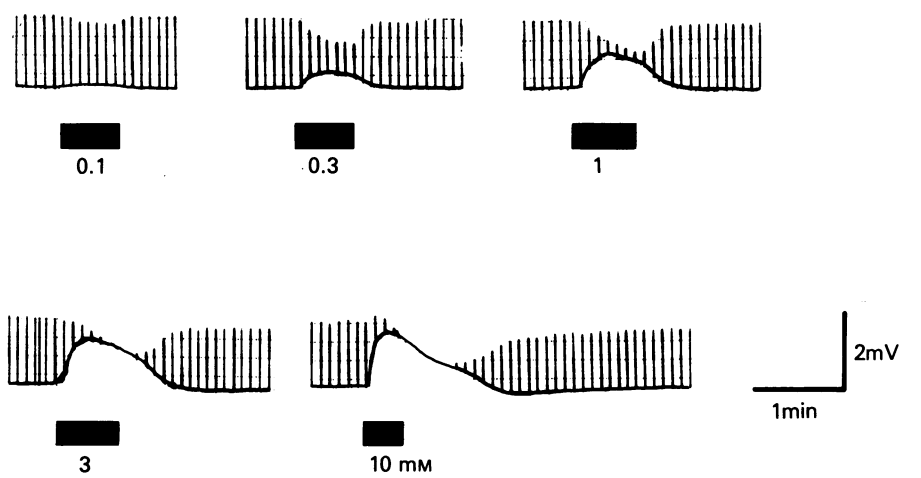

Figure 2 D.c. potential changes produced by superfusing increasing concentrations of γ-aminobutyric acid (GABA) over an olfactory slice at intervals of approximately 20 minutes. (Negativity at the pial surface, at electrode B in Figure 1, is represented by an upward deflection of the baseline.) The brief negative deflections are the synaptic responses to lateral olfactory tract stimulation (see Figure 3 for details).

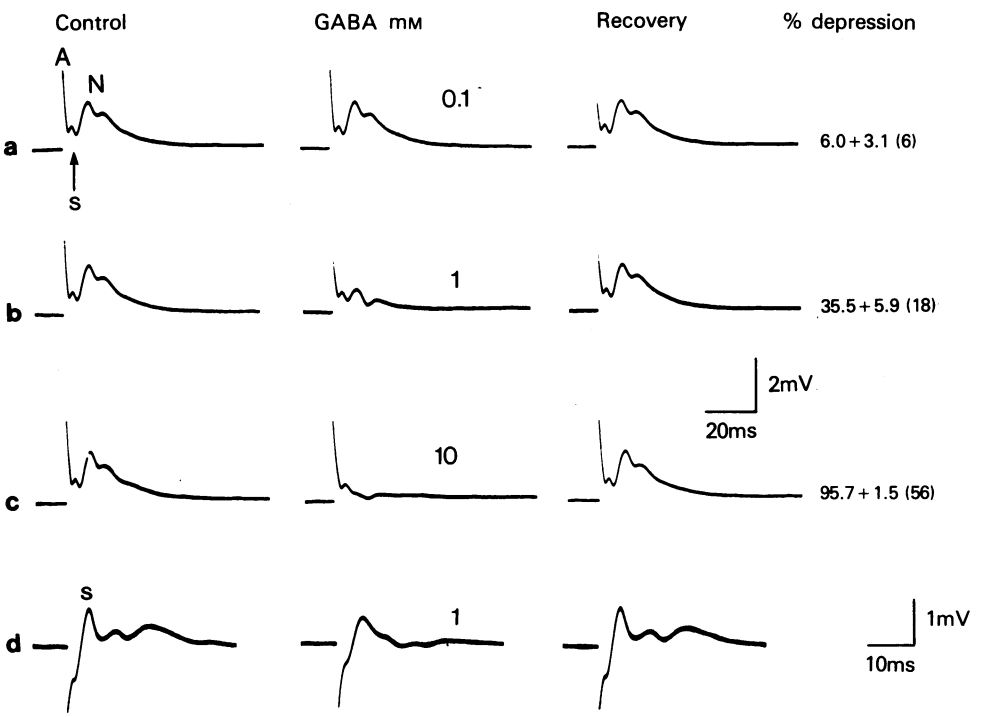

Figure 3 Effects of γ-aminobutyric acid (GABA) on the responses of the olfactory cortex to lateral olfactory tract (LOT) stimulation. Records (a-c) show the stimulus artefact (A), LOT spike (S) and postsynaptic surface-negative wave (N) (see Harvey *et al.*, 1974). Records in (d) show the LOT spike (S) recorded more directly from the LOT at a faster sweep speed (in a separate experiment). Increasing concentrations of GABA (centre column) progressively depressed the N-wave and partly reduced the LOT spike. Numbers on the right show the average % depression of the N-wave by each concentration of GABA (mean and s.e., *n* in brackets).

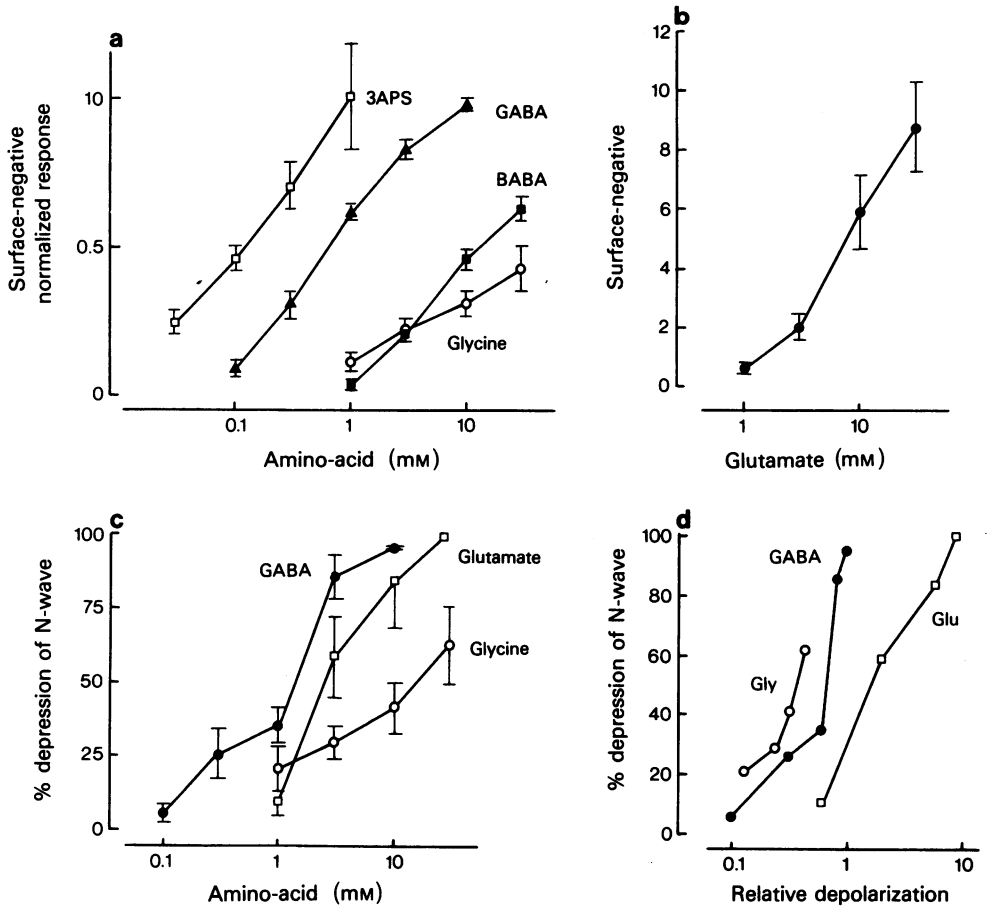

Figure 4 Surface-negative deflections (a and b) and depression of the N-wave (c) produced by different agonists. In each experiment responses were compared with those produced by γ-aminobutyric acid (GABA) and normalized with respect to responses to 10 mM GABA. Each point shows the mean of ⩾4 experiments; bars are s.e. Note that the scale for glutamate-induced surface negative responses (b) is 10 times that for the other agonists (a). The plots in (d) show the relationship between surface-negative deflections (abscissa scale, GABA 10 mM = 1) and % depression of the N-wave (ordinate scale) produced by increasing concentrations of three different agonists: thus, the amount of synaptic depression produced per unit of surface-negativity increased in the order glutamate (Glu) < GABA < glycine (Gly). 3APS = 3-aminopropanesulphonic acid; BABA = β-amino-*n*-butyric acid.

its neurotoxic action (O'Neal, Chen, Reynolds, Meghal & Koeppe, 1968).

Effects of antagonists

Surface negativity produced by GABA was reduced (though not abolished) by picrotoxin or bicuculline at concentrations >1 μM (Figures 5 and 6). Both antagonists were more effective against GABA than against glycine (though far from completely selective). In contrast, strychnine was appreciably more effective against glycine than against GABA but did not completely prevent the action of glycine (Figure 6c).

Discussion

The surface-negative response to GABA probably results from depolarization. Thus, the potential change was in the same direction as that produced by glutamate or K^+ ions, and that of the depolarizing e.p.s.p.

An appropriate intracellularly-recorded depolarization was described in the preceding paper (Brown & Scholfield, 1979). However, as pointed out in that paper, the action of GABA on the olfactory cortex is unlikely to be restricted to the soma of the olfactory neurones. For example, Pickles (1978) has described

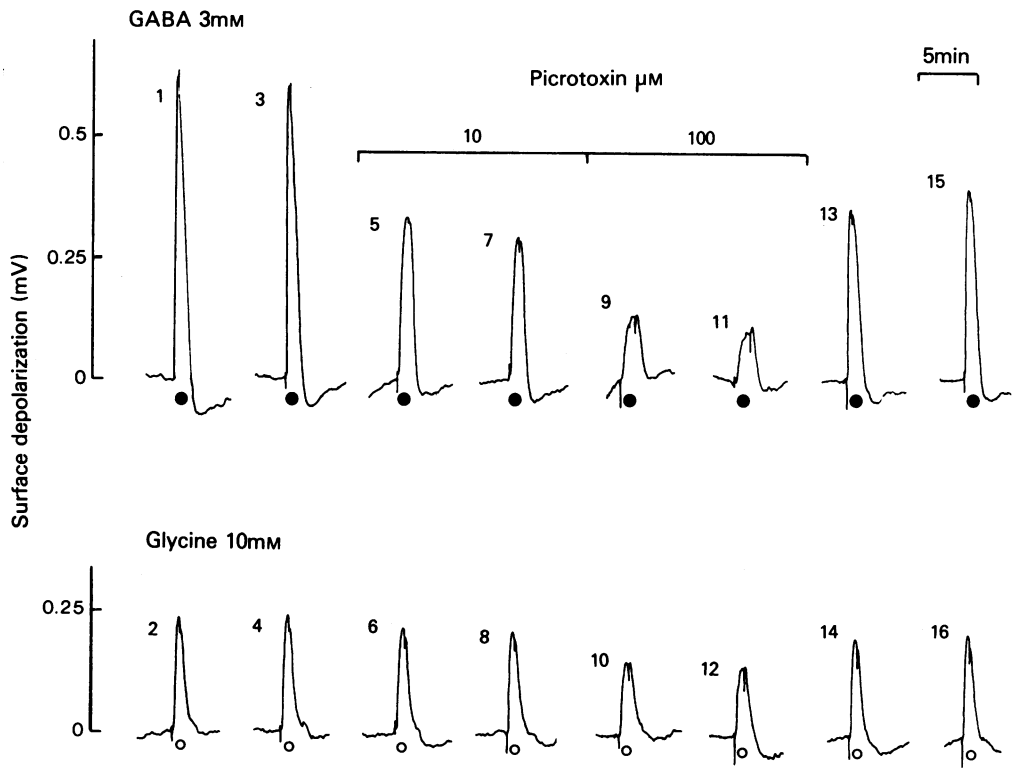

Figure 5 Effects of 10 and 100 μM picrotoxin on surface-negative responses to 3 mM γ-aminobutyric acid (GABA, ●) and 10 mM glycine (○). Agonists were applied to peak response (<1 min) at approximately 20 min intervals in the order indicated, except for responses 15 and 16 (determined 90 min after washing out the picrotoxin).

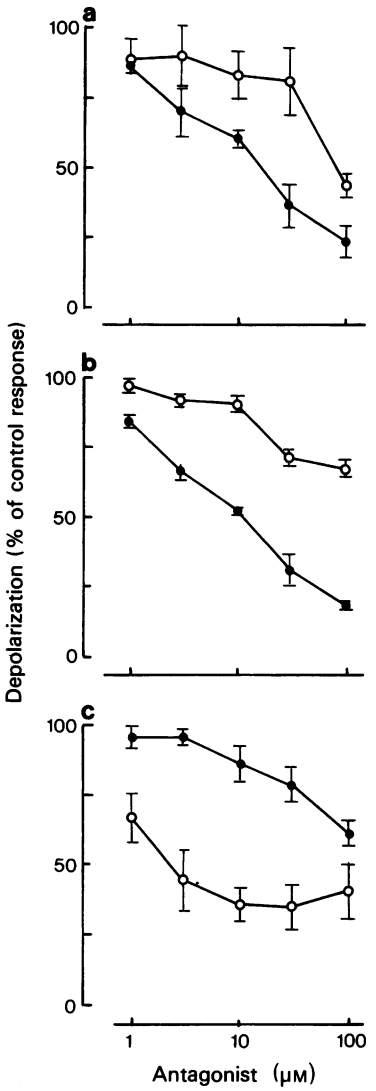

Figure 6 Inhibition of surface negative responses to 3 mM γ-aminobutyric acid (GABA, ●) and 10 mM glycine (○) produced by increasing concentrations of (a) bicuculline, (b) picrotoxin and (c) strychnine. Each point represents the mean of at least 4 experiments (bars show s.e.). Responses are expressed as % of those obtained before adding antagonist; depression refers to steady-state responses in the presence of incremental antagonist concentrations.

a depolarization of LOT axons by GABA, and the reduction of the LOT action potential described in the present paper accords with this. Hence, it would be unwise to equate the surface-response directly with the previously-recorded neuronal depolarization.

The high concentration of GABA required to evoke a surface-depolarization when compared with (say) the sympathetic ganglion (Bowery & Brown, 1974) or cuneate fibres (Simmonds, 1978) may be attributed to clearance by neuronal and glial uptake mechanisms en route to the receptive sites (see Galvan & Scholfield, 1978). The much greater potency of 3-aminopropanesulphonic acid presumably stems from its relatively low affinity for GABA-carriers (Bowery & Brown, 1972; Beart & Johnston, 1973; Olsen, Bayless & Ban, 1975). Likewise, antagonists were effective in low concentrations because they were applied for prolonged periods and hence, being less susceptible to clearance mechanisms, could diffuse readily to the receptive sites.

The lesser depolarization produced by glycine also accords with intracellular observations (Brown & Scholfield, 1978). In this case, there is perhaps more justification for assuming a closer identity between the sites of action recorded with intracellular and extracellular electrodes, since glycine does not duplicate the ubiquitous effects of GABA on axons in the central (Simmonds, 1978; Pickles, 1978) and peripheral (Brown & Marsh, 1978) nervous systems. The intracellularly recorded depolarization was accompanied by a large conductance increase (Brown & Scholfield, 1978), as expected for replication of a transmitter function. However, the extracellularly recorded response was incompletely antagonized by strychnine, suggesting an aberrant component to the depolarization, perhaps Na-coupled uptake by a high velocity neutral amino-acid carrier (see Kehoe, 1975).

Overall, our conclusions from these experiments are that, even though interpretation of agonist responses recorded in this way is difficult, the method may be useful for testing the effects of antagonists on amino acid-induced depolarization, provided due allowance is made for (a) influence of cellular transport and (b) possible differences between synaptic and extrasynaptic receptors.

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