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Baclofen blocks postsynaptic inhibition but not the effect of muscimol in the olfactory cortex

C.N. Scholfield

Physiology Department, Medical Biology Centre, The Queen's University of Belfast, 97 Lisburn Road, Belfast, N. Ireland BT9 7BL

1 The olfactory cortex slice preparation from the guinea-pig brain was used to study the effects of baclofen on inhibition using intracellular recording. Stimulation of the lateral olfactory tract activates sequentially excitatory and inhibitory pathways. Inhibition is manifest as a period of increased membrane conductance (termed postsynaptic inhibitory conductance, IPSC).

2 Bath application of baclofen (0.2–500 μM) reversibly blocked the IPSC. Baclofen also produced a secondary increase in the amplitude and duration of the initial excitatory postsynaptic potential.

3 Baclofen (0.5–500 μM) slightly augmented the ability of bath-applied muscimol to increase the resting membrane conductance. Baclofen had no effect on cell excitability and membrane potential and no effect on the action of γ-aminobutyric acid (GABA), noradrenaline, glycine, taurine or 5-hydroxytrypamine.

4 These results confirm previous suggestions that baclofen at low concentrations acts outside the GABA receptor mediating the IPSC perhaps by reducing the release of the excitatory transmitter activating the inhibitory interneurones.

Introduction

Many inhibitory pathways in the mammalian central nervous system are known to release γ-aminobutyric acid (GABA) (Krnjević, 1973). GABA inhibits neurones by increasing the chloride conductance of the membrane so reducing the effect of depolarizing influences which might otherwise excite the cell (Scholfield, 1978). Baclofen (Lioresal or β-(4-chlorophenyl)-γ-aminobutyric acid) is used to reduce the excessive muscle tone in spastic patients. Its structural similarity to GABA has led people to think that it might somehow modify GABA-mediated inhibition. However, the complexity of experiments with intact animals has made it difficult to determine the site and mechanism of action (Benecke & Meyer-Lohman, 1974; Curtis, Lodge, Bornstein & Peet, 1974; Fox, Krnjević, Morris, Puil & Werman, 1978; Davies, 1981). Simpler *in vitro* preparations of spinal cord have been used (Nistri, 1975; Ault & Evans, 1981) but higher concentrations were applied than the 0.1 μM found in plasma during therapy (Knitsson, Lindblom & Martenssen, 1974). Many of the previous experiments have shown that baclofen depresses excitatory responses in the brain (Lanthorn & Cotman, 1981; Collins, Anson & Kelly, 1982; Cain & Simmonds, 1982). This reduced excitation probably results from a depression in the release of synaptic transmitter (Potashner, 1978; Bowery, Hill, Hudson, Doble, Middlemiss, Shaw & Turnbull, 1980; Collins *et al.*, 1982). Thus most previous work with baclofen suggests that it depresses excitation in at least some nerve terminals; less is known about the effects of baclofen at the neurone membrane and at the inhibitory synapse. However, in the olfactory cortex, Cain & Simmonds (1982) have shown that a manifestation of the inhibitory postsynaptic conductance (IPSC) recorded with pial surface electrodes (the I-wave) is also depressed.

It would therefore appear worthwhile to examine the effects of baclofen on the olfactory cortex where the effects of GABA on membrane conductance can be studied directly by means of intracellular recording electrodes and where the pharmacology of the GABA synapse has been extensively studied. GABA is difficult to use by bath application to an isolated preparation since its effects are reduced by its own rapid uptake into cells (Scholfield, 1981). Muscimol, however, acts at the same site but does not markedly suffer in this manner (see Scholfield, 1982); therefore muscimol was used instead of GABA. The olfactory cortex is a brain region containing a large homogeneous fibre tract, the lateral olfactory tract (LOT). These LOT fibres are a major excitatory

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input to the predominant neurone in the olfactory cortex, the superficial neurones. These neurones send out axon collaterals to similar nearby superficial neurones and also send axon collaterals to polymorph cells. These polymorph cells are thought to be inhibitory and they send back axons to the somata of the superficial neurones (Halliwell, 1976). This paper describes experiments showing that baclofen blocks GABA-mediated inhibition whereas the postsynaptic actions of muscimol are unaffected.

Methods

Slices of olfactory cortex (0.6 mm thick) were cut from the surface of guinea-pig brains and placed in a recording bath perfused with Krebs solution at 23–25°C. The transmembrane potential and resistance of cells were measured with single barrelled micropipettes drawn from 1 mm glass tubing, filled with a 4 M potassium acetate solution and connected to a preamplifier equipped with a current source to monitor membrane conductance (Scholfield, 1978). The cells were identified as neurones by their low input conductances and ability to generate action potentials. It was not possible to distinguish between the various types of neurone in the slice. Single stimuli were applied to the lateral olfactory tract (LOT) at more than 2 min intervals using constant current pulses of 0.1–0.4 nA and 200 ms duration. Agonists (usually muscimol) were applied to the bathing solution for 2 min periods at various concentrations alone and after adding baclofen for 30 min. Only one cell was used from each slice and more than one baclofen concentration was tested in some cells. In such cases the baclofen doses were added sequentially at 10 fold increasing concentrations. The extra membrane conductance (G_{agen}) produced by a drug was calculated from the relationship (Scholfield, 1982):

$$G_{agon} = [i/V_{test}] - [2i/(V_1 + V_2)]$$

where i is amount of constant current pulsed into the cell through the recording micro-electrode, V_{test} the resultant voltage excursion in drug solution and V_1 and V_2 the voltage excursions produced by the same current before adding and after recovery from the drug. This method of calculating agonist effect excludes any changes in resting membrane conductance. Graphs of the extra membrane conductance calculated by this procedure were plotted against log agonist concentration and a smooth curve drawn through the points. These curves showed an exponentially increasing trend with no identifiable maximum (see Figure 2). Thus an ED_{50} was unobtainable so instead, some other arbitrary point on each of the conductance-concentration curves was chosen. This point was the agonist concentration required to double resting membrane conductance, i.e., the value of the agonist conductance which was equal to the resting membrane conductance on initial impalement of the cell (see Scholfield, 1982).

Solutions and drugs

The Krebs solution had the following composition (mM): Na 144, K 5.9, Ca 2.5, Mg 1.3, Cl 127, HCO_3 25, SO_4 1.3, H_2PO_4 1.2, D-glucose, 11.0. (±)-Baclofen was obtained as a dry powder from CIBA-Geigy (batch number 000978) and muscimol from Fluka AG.

Results

Synaptic responses recorded on stimulating the lateral olfactory tract (LOT)

The neurones in the olfactory cortex slice had a transmembrane potential of − 60 to − 77 mV and an input resistance of 20 to 100 Mohm. Electrical stimulation of the LOT elicited a characteristic series of changes in membrane potential and conductance. Firstly, there was a brief reduction of the membrane potential presumably due to the release of substances (of unknown identity) from the LOT nerve terminals (Halliwell, 1976), lasting about 30 ms and of about 25 mV in amplitude (Figure 1, cell 1). This depolarization generated an all-or-nothing propagated action potential and it merged with an ensuing polysynaptic depolarization thought to result from feedback excitation (Halliwell, 1976; Scholfield, 1978). The polysynaptic depolarization coincided with and was outlasted by the inhibition generated through polysynaptic pathways. This inhibition is only revealed by measuring the membrane conductance on passing current pulses into the cell (Figure 1, cell 1) and is termed the postsynaptic inhibitory conductance (IPSC, Scholfield, 1980). It coincided with a slight depolarizing tail. Figure 1 (cell 2) also shows a response beginning with very little depolarization but still showing a period of increased membrane conductance.

Effect of baclofen on synaptic responses

Baclofen appeared to have its major effect on the IPSC. In cell 2, the IPSC was completely blocked by 0.2 μM baclofen. The record in Figure 1 shows that stimulation of the LOT failed to generate any conductance increase or potential changes in the presence of baclofen. The absence of the initial excitatory depolarization was atypical and the responses obtained from cell 1 were more usual (Figure 1). In cell

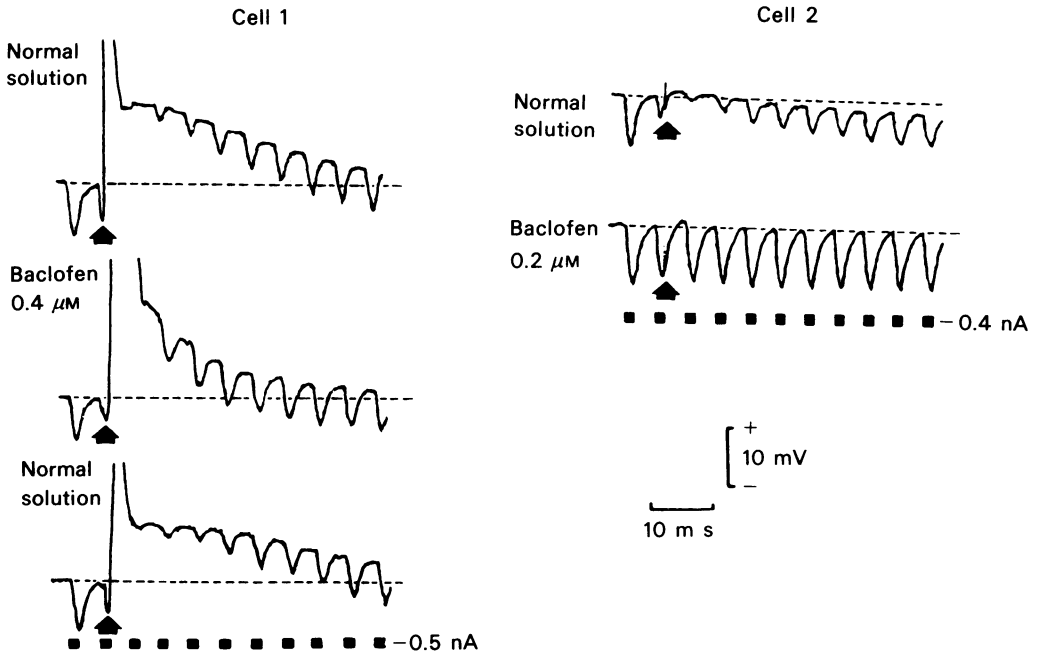

Figure 1 The effect of baclofen on synaptic responses evoked by electrical stimulation of the lateral olfactory tract (LOT). To monitor the changes in membrane conductance (or resistance) constant current pulses (0.5 or 0.4 nA) were passed into the cell during the periods marked by the filled bars below the records. These pulses produced downward voltage deflections with a time-constant of about 10 ms. In the upper record from cell 1, the LOT was stimulated at the arrow and this produced a depolarization lasting about 30 ms followed by a depolarizing tail accompanied by an increased membrane conductance as shown by a reduction in the amplitude of the downward voltage deflections. A similar period of increased membrane conductance was produced in cell 2 on stimulating the LOT but without the initial depolarization. Application of baclofen for 30 min reduced the increase in membrane conductance of both cells. This is particularly noticeable when comparing the amplitude of the current induced voltage changes soon after the stimulus. The initial depolarization in cell 1 was prolonged by about three fold. The potential and conductance changes were restored after washing out the baclofen for 60 min, (cell 1, lower trace). Voltage scale 10 mV, time scale 100 ms for all records.

1, baclofen blocked the period of high membrane conductance but also increased the duration and amplitude of the depolarization. The enlarged e.p.s.p. did not exceed 200 ms in duration and did not produce any of the multiple all-or-none discharges seen with bicuculline (Scholfield, 1980). This enlargement was observed at all baclofen concentrations tested (0.2–500 μM) (15 experiments on 12 cells). The IPSC was restored on prolonged washing out of the baclofen (0.5 to 2 h). However, a precise quantitative assessment of the baclofen effect was made difficult by the three synaptic responses merging into each other.

Effect on the action of muscimol

Since the reduced IPSC and enlarged depolarization are reminiscent of the effect of drugs which block the action of GABA (Scholfield, 1980), further experiments were performed to test the influence of baclofen on the effect of GABA. When GABA or muscimol is applied to the bathing solution, the chloride conductance of the cell membrane is greatly increased (Brown & Scholfield, 1979; Scholfield, 1982). In the present experiments muscimol was used since this drug is not removed by the GABA uptake system (see Introduction). Muscimol added alone increased the resting membrane conductance in a dose-related manner. The resultant curves had an exponentially increasing shape with no clear maximum (Figure 2).

The effect of muscimol on the conductance was not reduced by a range of baclofen concentrations (0.5–500 μM). On the contrary, there was a small but consistent rise in the effectiveness of muscimol at all the baclofen concentrations tested (Figure 2; Table 1). Baclofen also affected the cell membrane conductance in the absence of electrical stimulation and in the absence of muscimol: at 0.5 μM baclofen appeared to reduce membrane conductance slightly but

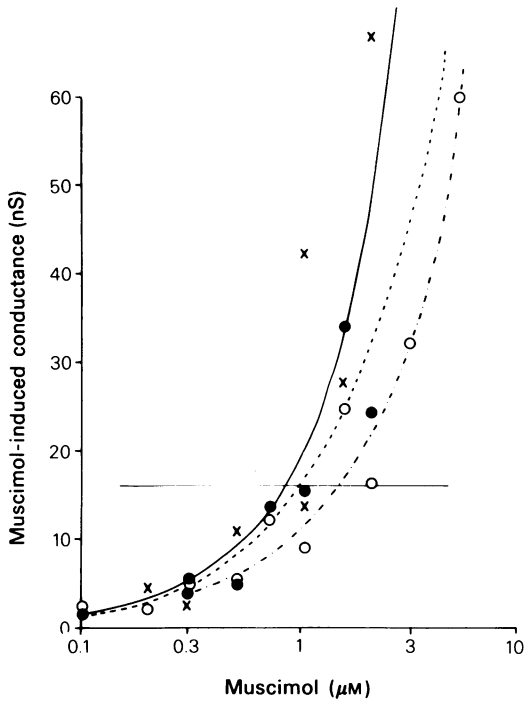

Figure 2 The effect of muscimol on the membrane conductance of a neurone in the guinea-pig olfactory cortex. Ordinate scale: increase in membrane conductance attributed to muscimol (nanoSiemen, nS); abscissa scale: muscimol concentration (μM). The preparation was bathed in normal solution and varying concentrations of muscimol added in random order (■). Baclofen (5 μM) was then added for 30 min and varying doses of muscimol again applied (×). The baclofen was washed out for 45 min and muscimol again tested (○). Points of 127 nS at 3 μM in baclofen solution and 109 nS at 5 μM muscimol for the initial control solution were omitted to reduce the length of the ordinate scale. The lines were drawn through the points 'by eye'. The horizontal line is the numerical value of the resting input conductance of the neurone before adding muscimol.

at the higher concentrations tested the membrane conductance was increased (Table 1). All these effects of baclofen were reversible.

Glycine (0.2–2 mM), 5-hydroxytryptamine (1 mM), taurine (2 mM) or GABA (0.01–0.5 mM) were applied in a similar manner to muscimol in two experiments. They also increased the cell input conductance but this effect was not antagonized by baclofen (0.5–5 μM).

None of the baclofen concentrations tested had any effect on the action potential, cell excitability or on membrane potential.

Discussion

Baclofen blocked the IPSC but failed to block the action of muscimol (and GABA) on the neurones in the olfactory cortex slice. This contrasts with the effects of bicuculline which blocks the IPSC and also muscimol at similar concentrations (Scholfield, 1982). There are two possible explanations for the discrepancy between the effects of baclofen and bicuculline: (1) GABA does not mediate the inhibitory conductance, or (2) baclofen acts in the chain of synaptic events at some point before the postsynaptic inhibitory site.

A large body of evidence favours GABA as the inhibitory substance in the olfactory cortex (Pickles & Simmonds, 1978; Brown & Galvan, 1979; Brown & Scholfield, 1979; Collins, 1979; Scholfield, 1980; Scholfield, 1982); therefore the former explanation is unlikely. Thus it would appear that baclofen acts either at the nerve terminal activating the inhibitory neurone, or it acts by depressing the excitability of the inhibitory neurone or by blocking the release of GABA. Baclofen appears not to interfere with the release of GABA from slices of olfactory cortex (Collins *et al.*, 1982) or cerebral cortex (Potashner, 1978) suggesting that baclofen acts before the GABA synapse.

Collins, Anson & Kelly (1982) have suggested that baclofen reduces the release of the excitatory substance activating the inhibitory cell since it reduces the release of excitatory amino acids (Potashner, 1978; Johnson, Hailstone & Freeman, 1980; Collins *et al.*, 1982). This idea is supported by the observation that baclofen depresses excitatory potentials in many parts of the central nervous system (see Introduction). Further support for this suggestion comes from the effect of baclofen on the polysynaptic depolarization generated by the synapses originating as collaterals from the superficial neurones (see Introduction). In electrical recordings of the compound field potentials from the slice, this polysynaptic depolarization is manifest as a surface positivity and baclofen reduces this field potential in the rat (Collins *et al.*, 1982) and the guinea-pig olfactory cortex either alone (C.N. Scholfield, unpublished) or after adding bicuculline (Cain & Simmonds, 1982). The polysynaptic depolarization was enlarged in the present intracellular recordings but this enlargement was notably smaller than that produced by the GABA antagonists (Scholfield, 1980). If both baclofen and bicuculline had the single effect of blocking inhibition, whether it be presynaptically or postsynaptically then the effect on other synaptic potentials should be the same. This was not the case: the polysynaptic depolarization revealed by baclofen was smaller than that produced by the GABA antagonists. So this observation suggests that baclofen has two effects: (i)

Table 1 The effects of baclofen on the ability of muscimol to increase membrane conductance and its effects on the cell membrane conductance in the absence of muscimol and electrical stimulation

Baclofen concentration (μM)	*Muscimol effect* (μM)			*Resting cell conductance without muscimol* (nS)		
	Normal solution	*With baclofen*	*Ratio*	*Normal solution*	*With baclofen*	*Ratio*
0.5	2.30	0.41	0.18	11.7	9.3	0.79
	0.90	0.90	1.00	21.0	13.6	0.65
	1.95	1.90	0.97	17.0	13.0	0.76
	Mean ± s.e.mean		0.71 ± 0.26			0.73 ± 0.0
5	1.17	0.84	0.72	13.6	17.8	1.31
	1.50	0.82	0.55	48.0	80.0	1.67
	1.22	0.69	0.56	23.0	23.0	1.00
	2.00	0.97	0.48	33.0	34.0	1.03
	1.15	1.25	1.09	10.8	22.0	2.04
	Mean ± s.e.mean		0.68 ± 0.11			1.40 ± 0.2
50	1.50	0.80	0.53	37.0	44.0	1.19
	2.00	0.97	0.48	18.5	18.5	1.00
	1.69	0.69	0.41	16.0	25.0	1.56
	Mean ± s.e.mean		0.47 ± 0.13			1.25 ± 0.13
500	0.90	0.70	0.78	12.7	27.0	2.13
	2.50	1.75	0.70	31.0	50.0	1.61
	Mean		0.74			1.87

The data were obtained from 10 cells and are expressed as mean ± s.e.mean. The changes were calculated for individual experiments and the results averaged. The 'muscimol effect' is expressed as the muscimol concentration required to double the resting membrane conductance.

an enlargement of the polysynaptic depolarization (due to reduced inhibition, as with the GABA antagonists), (ii) a depression of the polysynaptic depolarization. Since the nerve terminals which activate the polysynaptic depolarization probably originate from the same fibres that activate the inhibitory neurones (Halliwell, 1976), an action of baclofen on both sets of nerve terminals could explain its effect on the IPSC. Thus it appears that the two effects of baclofen on the polysynaptic depolarization (i and ii above) may result from the drug acting through some common mechanism. Whether or not this is via the bicuculline-insensitive $GABA_B$ receptor of Bowery *et al.* (1980) is uncertain from the present experiments. Direct verification of the effect of baclofen on the inhibitory neurone would require unequivocal electrical recordings and histological identification of these cells. This has not yet proved possible.

Baclofen appears to be quite potent since 0.2–1.0 μM could completely block the IPSC. Thus at least some blocking action would seem possible at the concentrations of 0.1 μM found in human cerebrospinal fluid during therapy (Knitsson *et al.*, 1974).

In conclusion, baclofen appears to block inhibition through an action outside the postsynaptic GABA-receptor, probably by reducing the ability of excitatory nerve terminals to activate the inhibitory cell.

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