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INTERACTION OF PENTOBARBITONE AND γ-AMINOBUTYRIC ACID ON MAMMALIAN SYMPATHETIC GANGLION CELLS

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1 Interactions of bath-applied pentobarbitone and γ-aminobutyric acid (GABA) on neurones in isolated superior cervical ganglia of the rat have been examined with intracellular microelectrodes.

2 Pentobarbitone itself (30 μM–1 mM) showed no clear or consistent GABA-like effects: changes in resting input conductance and membrane potential were small and variable.

3 Pentobarbitone (100 μM) strikingly enhanced the conductance increases produced by GABA and 3-aminopropanesulphonic acid, and reversed the depression of GABA-evoked responses by bicuculline.

4 It is concluded that reversal of bicuculline action at the membrane conductance level might be explained by augmentation of GABA-action. This augmentation cannot be attributed to 'partial agonist' properties of pentobarbitone or to interference with glial transport processes.

Introduction

Bowery (1976) and Bowery & Dray (1976; 1978) have described an interesting reversal of convulsant-GABA antagonism in sympathetic ganglia (and on medullary neurones) by pentobarbitone. In ganglion cells, GABA increases chloride conductance and produces a membrane depolarization (Adams & Brown, 1975). Bowery (1976) measured this depolarization extracellularly, and found that, when the depolarization was depressed by bicuculline or other GABA antagonists, addition of low concentrations (10 to 100 μM) of pentobarbitone restored the response to the normal level. There was no comparable reversal of acetylcholine antagonism by pentobarbitone. Since pentobarbitone did not consistently increase the depolarization produced by GABA in the *absence* of a GABA-antagonist, a direct interaction between pentobarbitone and the GABA-antagonist itself was suggested. However, other authors have reported both a potentiation of the response to GABA in the absence of an antagonist and/or a direct GABA-like action of pentobarbitone alone, in both ganglia (Evans, 1977) and in other preparations (Nicoll, 1975; Nicoll, Eccles, Oshima & Rubia, 1975; Ransom & Barker, 1975; 1976; Curtis & Lodge, 1977; Scholfield, 1978); how far such effects contribute to the 'GABA-antagonist reversal' phenomenon in ganglia is unclear.

The aim of the present experiments was to see how the effect described by Bowery (1976) and Bowery & Dray (1976; 1978) appeared when ganglion cell *conductance* changes rather than potential changes were recorded. We wished to answer three specific questions: (i) is the bicuculline-antagonized GABA conductance change reversed by pentobarbitone?; (ii) does pentobarbitone increase GABA-induced conductance changes in the *absence* of an antagonist and, if so, would this be sufficient quantitatively to explain the antagonist-reversal?; and (iii) does pentobarbitone itself act like GABA, i.e. could reversal stem from a partial agonist effect?

Methods

Superior cervical ganglia were isolated from Wistar rats (about 200 g weight) anaesthetized with urethane (1.5 g/kg). The connective tissue sheath was cut open and the preparation pinned down in a bath of flowing Krebs solution (20 to 25°C, bubbled with 95% O_2 and 5% CO_2 gas mixture; pH 7.4) perfusing at a sufficient rate to give a filling time of 3 to 5 seconds. Methods for intracellular recording, stimulation and drug application (via the bathing fluid) were as described by Adams & Brown (1975) with some small modifications as follows: (i) Microelectrodes were drawn from 1 mm o.d. borosilicate glass with an indwelling glass fibre (Clark Electromedical GC 100

F-6). They were filled with 4 M potassium acetate solution buffered to pH 7 with acetic acid and had a tip resistance >100 MΩ. (ii) The input probe and current injection circuit used was that of Colburn & Schwartz (1972), with facility for neutralizing capacitance and electrode resistance up to 500 MΩ. The preganglionic nerve trunk was stimulated with a suction electrode ('orthodromic stimulation'). Responses were displayed on a storage oscilloscope and potentiometric recorder and stored for analysis on magnetic tape (3 M 220) using a Racal Thermionic Store-4 tape recorder. Data analysis has been restricted to only a few (7) of many cells impaled and partially tested, since the experiments required stable membrane potentials and input resistances (with consistent responses to GABA) for several hours in order to complete an appropriate sequence of drug administrations. Sodium pentobarbitone was used throughout: it did not change the pH of the bathing solution by more than 0.1 unit at the highest concentration used.

Results

Action of pentobarbitone alone

Effects of pentobarbitone (30 μM to 1 mM) on membrane potential and neurone input conductance (G_i) are summarized in Table 1. No consistent 'GABA-mimetic' action, i.e. increased input conductance and depolarization, was detected. On the contrary, in 4 out of 6 cells tested, pentobarbitone *increased* membrane potential by up to 7 mV. This effect did not appear to be concentration-dependent. Input conductance changes, measured from the slope of the current-voltage curves at the *resting* membrane potential (see Figure 1c) were variable and small, ranging from 17% decrease to 23% increase. In some cells the decrease in input conductance seemed to be secondary to the hyperpolarization since it could be replicated by passing sustained hyperolarizing current through the microelectrode or annulled by restoring the membrane potential to its initial level with positive current.

At concentrations up to 100 μM there was no clear change in the amplitude or voltage-threshold of ganglion cell action potentials evoked by injecting positive current through the microelectrode or by orthodromic stimulation. At 500 μM the orthodromic spike was delayed and the synaptic potential reduced in amplitude (Figure 1a). This was not the result of a reduced input resistance (Figure 1b and c). It might reflect an action on nicotinic receptor mechanisms (Brown & Quilliam, 1964; cf. Adams, 1976).

Effect of pentobarbitone on responses to γ-aminobutyric acid

Pentobarbitone strikingly enhanced the conductance increases produced by GABA, particularly when the initial conductance increase was relatively small. Figure 2 illustrates this effect. The potentiating action of pentobarbitone increased over the first 15 to 30 min exposure, then reversed over a comparable time scale on washout. It was quite repeatable within the same experiment.

Table 2 shows some measurements of this potentiation in 4 cells in which both potentiation and sub-

Table 1 Effect of pentobarbitone itself on membrane potential (E_m) and input conductance (G_i). Input conductance was calculated from the slope of current-voltage curves at the *resting* value of E_m, unless otherwise indicated.

Concentration	*Cell*	E_m (mV)	ΔE_m (mV)	G_i (nS)	ΔG_i (nS)	g ($\Delta G_i/G_i$)
30 μM	3	−49	−2	21	±0	±0
30 μM	4	−65	−7	5.1	+0.4	+0.08
30 μM	5	−54	−4	11.1	−0.5	−0.05
80 μM	1	−30	+1	9.7	+1.2	+0.12*
100 μM	3	−49	−1.5	43	−10.1	−0.23
100 μM	5	?	−3	10.5	±0	±0
100 μM	6	−40	−6	5.6	+0.3	+0.06
100 μM	7	−34	+2	21	+3.7	+0.17
500 μM	3	−52	−1	42	−8	−0.20
	5	?	−4	13	−1.2	−0.09*†
1 mM	1	−32	+1	8.5	+0.6	+0.07

* Measured from potential deflections (⩽15 mV) produced by constant-current responses.
† No change in G_i on restoring E_m with applied current.
? Uncertain, because of d.c. drifts.

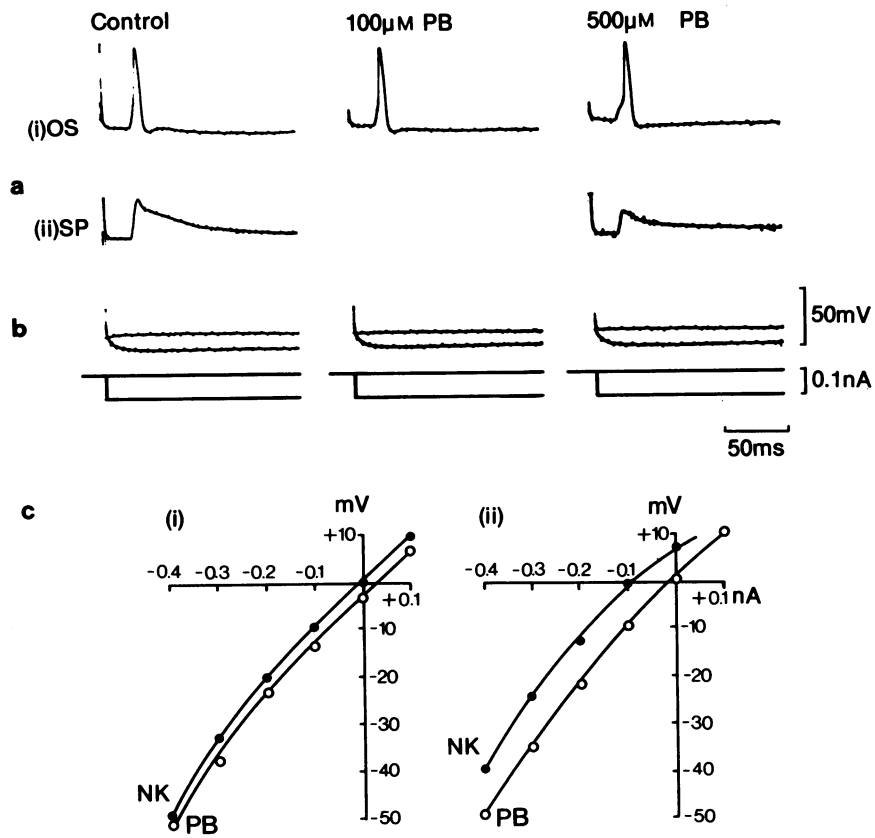

Figure 1 Effects of pentobarbitone (PB) on orthodromic transmission (a) and input conductance (b and c) in a sympathetic ganglion cell. In the part of the experiment from which records (a) and (b) were obtained, orthodromic stimuli (OS: 2ms, 70 V) and negative intracellular current pulses (b: 300 ms, 0.1 nA) were applied alternately, each at 0.2 Hz. Records were obtained 2 min before (control) and then 1.5 min after adding 100 μM PB, and finally 2 min after raising the concentration of PB to 500 μM. Records in (a ii) show the synaptic potentials (SP) revealed by injecting 0.4 nA negative current, which hyperpolarized the cell by about 40 mV and blocked the orthodromic spike. In (b) the responses to the negative current pulses are superimposed on the resting traces. The plots in (c) are current-voltage curves obtained in an earlier part of the same experiment (i) before (●) and 1.5 min after (○) adding 100 μM PB and then (ii) after 19 min in the PB solution (○) and 8 min after washing out the PB (●, NK: normal Krebs solution). Points refer to plateau potential deflections produced by 300 ms current pulses. The cell hyperpolarized by 3 mV on adding the PB and then depolarized by 6.5 mV on washing out the PB. The average resting potential of the cell throughout the experiment was about −50 mV and average input resistance 110 MΩ (input conductance 9.1 nS).

sequent recovery could be reliably determined. Potentiation is expressed as the ratio of the GABA-induced conductance increase in pentobarbitone (PB) solution to that observed in normal Krebs solution (NK) just before the addition of pentobarbitone ($\Delta G_i^{PB}/\Delta G_i^{NK}$). Low-level GABA responses ($\Delta G_i/G_i \leqslant 0.2$) were increased 6–20 times; larger responses ($\Delta G_i/G_i \geqslant 0.2$) were potentiated 2–3 times. Clearly, it would be desirable to observe changes in the complete GABA dose-conductance curve, but this was rather impracticable given the necessary time-scale, particularly in view of the progressive effect of pentobarbitone. As a guide, it appeared that the effect of 100 μM GABA in normal Krebs solution was approximately replicated by 30 μM GABA in 100 μM pentobarbitone solution.

Estimates of GABA reversal potentials (E_g) from the amplitudes of applied constant-current pulses (text-eqn. (3) in Adams & Brown, 1975) gave the im-

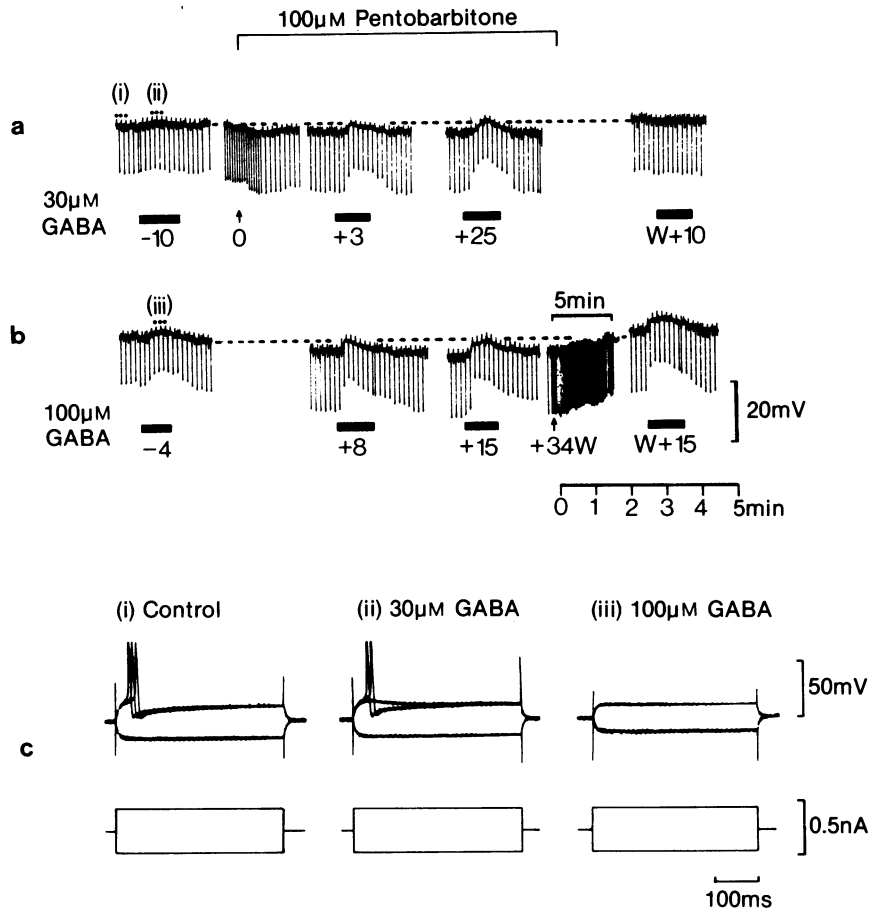

Figure 2 Effect of pentobarbitone 100 μM on the changes of membrane potential and input resistance of a ganglion cell produced by (a) 30 μM and (b) 100 μM γ-aminobutyric acid (GABA). Membrane potential was recorded continuously with a potentiometric recorder; downward deflections of the voltage record are the plateau responses to negative intracellular current pulses (300 ms, 0.2 nA, 0.2 Hz). GABA was applied for the duration indicated by the horizontal bars (either 1 min or to peak response). The numbers below each bar show the time before (−) or after (+) adding pentobarbitone (at the arrow marked 0), or after washing out the pentobarbitone (W). The oscilloscope records in (c) are photographs of 3 superimposed responses to injected positive and negative current pulses delivered alternately (i) before GABA, (ii) during 30 μM GABA and (iii) during 100 μM GABA at the times indicated by dots in the potentiometric records in (a) and (b). (Responses to the positive current pulses are omitted from the potentiometric record because of distortion introduced by the attenuated spike responses). The initial resting potential of this cell was about −50 mV (indicated by the dashed line) and initial input resistance 83 MΩ (input conductance 12 nS). Pentobarbitone hyperpolarized the cell by about 5 mV and appeared to reduce input conductance by 2.3 nS as judged from the amplitudes of the responses to constant-current pulses. However, restoration of the membrane potential by depolarizing current (not shown) reversed this conductance decrease.

pression that E_g was somewhat more hyperpolarized (by up to 8 mV) in pentobarbitone solution than in normal Krebs solution (Figure 3). Although replicated in two cells where sufficiently consistent estimates of E_g could be obtained, this conclusion should be treated with caution since the estimates assume linearity of the current-voltage curves within ±15 mV of resting potential (cf. Figure 1c) and the reliability of membrane potential records over sustained periods is questionable. A further point of note is that low

concentrations of GABA, producing $\leqslant 10\%$ increase in input conductance, frequently *hyper*polarized the neurone rather than depolarizing it (see, for example, the last response in Figure 2a and the third responses in Figure 4a), suggesting that E_g might change with the concentration of GABA used (cf. Takeuchi & Takeuchi, 1971).

Responses to 3-aminopropanesulphonic acid (APS)

This substance is a powerful GABA-mimetic agonist in ganglia (Bowery & Brown, 1974) but a poor substrate for the glial transport process for GABA (Bowery & Brown, 1972; Bowery, Brown, Collins, Galvan, Marsh & Yamini, 1976). Hence, if pentobarbitone acts by inhibiting glial uptake, the action of APS should not be affected. In practice, APS was potentiated with equal or greater facility than GABA (Figure 4).

Pentobarbitone-bicuculline interaction

In confirmation of Bowery (1976) and Bowery & Dray (1976; 1978) pentobarbitone reversed the depression of GABA-induced conductance changes produced by 25 μM bicuculline (Figure 5). This effect was temporary, so that blockage was restored on removal of the pentobarbitone. Comparison with the effect of pentobarbitone in the same preparation in the *absence* of bicuculline (Figure 5a) indicated that the increased response in bicuculline solution was no greater than that in normal Krebs solution for matched pre-pentobarbitone conductance changes.

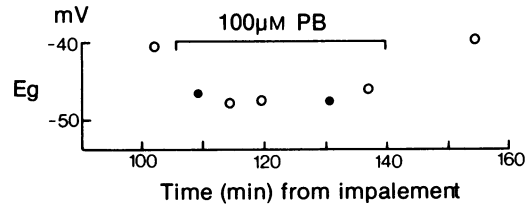

Figure 3 Estimates of reversal potential at the peak of responses to 30 μM (●) and 100 μM (○) γ-aminobutyric acid (GABA) made from the experiment illustrated in Figure 2. The reversal potential (E_g) was calculated from extrapolated responses to constant negative current pulses delivered before and during the application of GABA according to text-equation 3 of Adams & Brown (1975):

$$E_g = E_m + \Delta E[V_1/(V_1 - V_2)]$$

where E_m = resting potential before GABA, ΔE = membrane potential change produced by GABA, and V_1 and V_2 are the voltage deflections produced by constant current pulses before and during GABA application. See text for further interpretation.

Discussion

Bowery & Dray (1976; 1978) have concluded that the ability of pentobarbitone to reverse convulsant antagonism of GABA stems from an interaction with the antagonist site, rather than with that of the agonist,

Table 2 Effect of pentobarbitone (PB) 100 μM on conductance increases (ΔG_i) produced by GABA ($g_{NK} = \Delta G_i^{NK}/G_i^{NK}$; $r = \Delta G_i^{PB}/\Delta G_i^{NK}$; NK = normal Krebs solution; t = time in pentobarbitone solution, min. s).

		30 μM GABA			*100 μM GABA*	
Cell	g_{NK}	r	t	g_{NK}	r	t
*1	0.70	2.28 2.16	1.20 4.40	—	—	—
3	0.04	6.7 6.7	5.30 18.10	0.33	2.12 2.40	9.40 15.25
5	0.04	7.1 16.0	3.0 25	0.21	2.39 2.30 2.31	8.00 13.40 30.40
5 (repeat)	0.08	6.17	7	—	—	
7	0.16	1.47 19.6	16.15 30.40	—	—	

* 80 μM pentobarbitone.

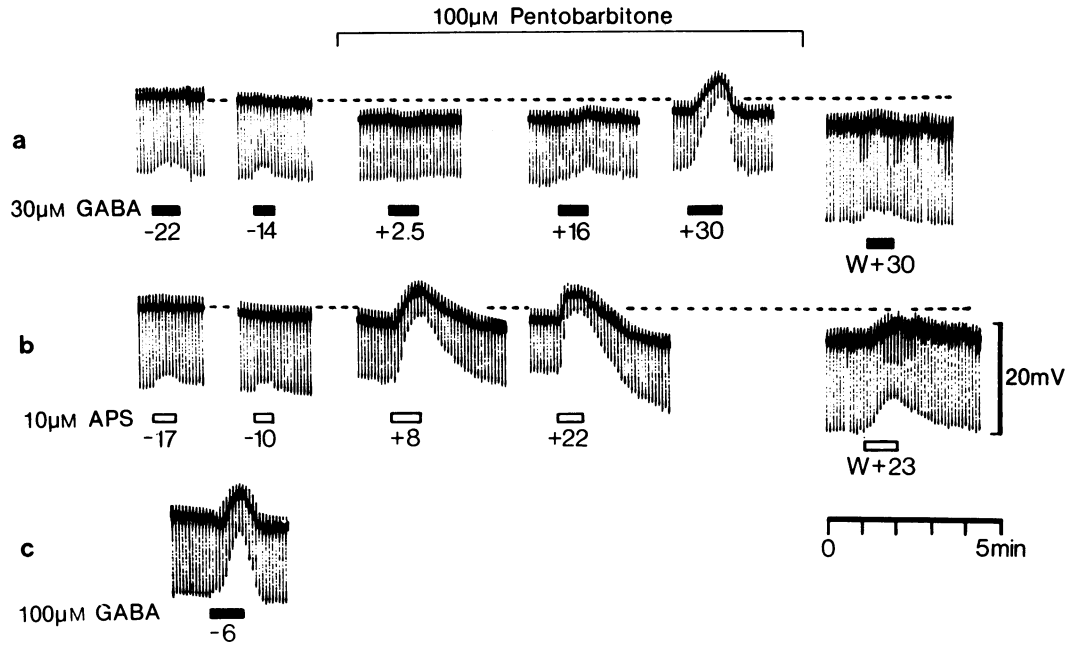

Figure 4 Effect of pentobarbitone 100 μM on responses of a ganglion cell to (a) 30 μM γ-aminobutyric acid (GABA) and (b) 10 μM 3-aminopropanesulphonic acid (APS), applied alternately; (c) shows the response to a single 100 μM concentration of GABA. See Figure 2 for details of recording and labelling. The membrane potential of this cell was rather low (about −40 mV, dashed line) but uncertain due to drifting; pentobarbitone appeared to increase the membrane potential but this did not reverse on washing. Input conductance estimated from the constant current pulses was increased from 20 nS to 22 nS on addition of pentobarbitone and fell to 17–18 nS when the pentobarbitone was washed out.

on the grounds that pentobarbitone did not consistently increase the depolarizing action of GABA in the absence of a convulsant. We have confirmed this reversal using intracellular conductance measurements. However, we also found that pentobarbitone did increase the conductance change produced by GABA in the *absence* of an antagonist. Further, the extent of this potentiation in unblocked cells appeared sufficient to explain the reversal in blocked cells, though this does not, of course, exclude a direct interaction with the antagonist site as the cause of the reversal.

The potentiated conductance response was consistent and substantial, and was accompanied by an increased voltage deflection. Hence, it becomes difficult to understand why changes in the extracellular voltage signal recorded by Bowery & Dray (1978) were so small. Since our experiments have been conducted on a relatively small sample of cells, there could be a sampling bias. Alternatively, pentobarbitone could alter the ionic selectivity of the GABA-ionophore in such a manner as to shift the reversal potential in a hyperpolarizing direction, so that the relationship between conductance and voltage deflection changes. Some of our observations on extrapolated reversal potentials were compatible with this, but need confirmation by more rigorous methods. Ransom & Barker (1976) have also obtained evidence for a pentobarbitone-induced shift in E_g in tissue-cultured spinal neurones, but in the opposing depolarizing direction.

The ability of pentobarbitone to potentiate GABA or inhibitory synaptic transmission, plausibly mediated by GABA, has been observed in a number of preparations (e.g. Nicoll, 1972; 1975; Nicoll *et al.*, 1975; Ransom & Barker, 1975; 1976; Curtis & Lodge, 1977; Scholfield, 1978). As suggested by Ransom & Barker (1976) and Barker, MacDonald & Ransom (1977) a direct interaction at the receptor-ionophore level seems most likely. Alternatives such as interference with clearance mechanisms or a partial agonist action may be excluded in ganglia for the following reasons:

(a) *Clearance mechanisms.* In ganglia, GABA is cleared by a neuroglial transport system (Bowery & Brown, 1972; Brown & Galvan, 1977). When the car-

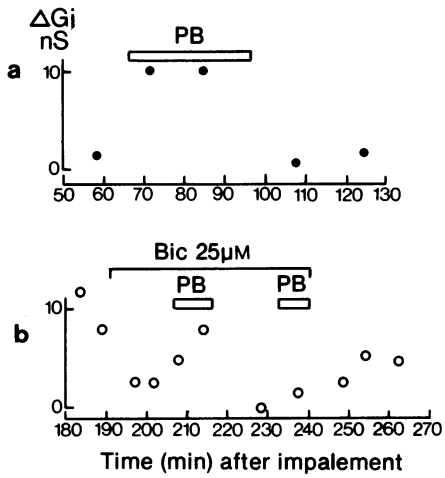

Figure 5 Comparison within a single cell of (a) the extent to which the conductance increase produced by γ-aminobutyric acid (GABA) is potentiated by pentobarbitone (100 μM, PB) and (b) the extent to which the blocking action of bicuculline (25 μM, Bic) is 'reversed' by pentobarbitone. Ordinates give the peak increase in input conductance (ΔG_i, nS). In (a) a concentration of 30 μM GABA was applied, producing a conductance increase of around 1 nS, which was increased to around 10 nS in pentobarbitone solution. Later, in the same experiment (b) a concentration of 100 μM GABA was applied, producing initially about 8–12 nS ΔG_i. In the presence of bicuculline this effect was reduced to less than 2 nS; subsequent admixture of pentobarbitone restored the response to 8 nS. (There is a gradual decline in the conductance response to GABA during this latter stage of the experiment.) Input conductance throughout was estimated from hyperpolarizing responses to constant intracellular negative current pulses. Bicuculline did not alter resting membrane potential or input conductance. Pentobarbitone hyperpolarized the cell by about 3 mV and reduced apparent input conductance from 40 to 35 nS on the first application. In the presence of bicuculline, pentobarbitone hyperpolarized the cell by 1–2 mV but did not change input conductance.

rier is inhibited by (e.g.) nipecotic acid, the effect of GABA is augmented but not that of 3-aminopropanesulphonic acid (Brown & Galvan, 1977) since the latter is not a substrate for the carrier (Bowery *et al.*, 1976). In contrast, pentobarbitone potentiated both agonists. Release of subthreshold but additive amounts of GABA from the glial cells by pentobarbitone can also be excluded since pentobarbitone *reduces* the rate of GABA efflux from the glial cells (N.G. Bowery, personal communication).

(b) *Partial agonism* We observed no clear agonist action of pentobarbitone in concentrations up to 1 mM. P. Feltz (personal communication) reports that pentobarbitone also shows no clear GABA-mimetic action on rat dorsal root ganglion cells *in vivo*. Although pentobarbitone increased membrane conductance in some cells, this effect was small ($\leqslant 15\%$) and was usually accompanied by membrane hyperpolarization. Further, in other cells, membrane conductance appeared to decrease rather than increase. The form of interaction between pentobarbitone and GABA was also contrary to that expected for a partial agonist effect: potentiation at low GABA doses was too great and there was no inhibition at high doses.

We cannot exclude a very weak GABA-like action on membrane conductance which, if translated into voltage change in an undamaged cell where $E_m > E_{Cl}$, might produce appreciable depolarization because of the hyperbolic relationship between ΔG and ΔE. This may account for the low-amplitude depolarization of ganglia reported by Evans (1977) and Bowery & Dray 1978) using extracellular recording, though the evidence that this effect is 'GABA-like' in terms of ionic mechanism and receptor specificity is not yet convincing.

Bowery & Dray (1978) also report that several other types of drug with a 'sedative-hypnotic' action can also reverse the action of GABA antagonists. We have not examined all of these other compounds but have observed a qualitatively-similar potentiation of GABA-induced conductance changes by one of them, chlordiazepoxide (30 μM). This effect of chlordiazepoxide on ganglion cells was very similar to that recently described for tissue-cultured spinal neurones by Choi, Farb & Fischbach (1977). Hence, the action of pentobarbitone described in the present paper may be common to a number of compounds. Future mechanistic interpretations will need to take this into account.

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