Sodium pentobarbitone enhances responses of thalamic relay neurones to GABA in rat brain slices

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1 In rat isolated thalamic slices, intracellular recordings were made of responses to electrophoretically applied γ -aminobutyric acid (GABA) in the absence and presence of sodium pentobarbitone (NaPb).

2 Responses to electrophoretically applied GABA were biphasic in the majority of neurones studied. They consisted of an early, negative-going and later, positive-going phase, when recorded close to reversal potential.

3 An increase in the GABA ejection current caused an enhancement of the late positive-going phase, along with a shift in the reversal potential of the whole response to a more positive value. These changes were accompanied by an increase in GABA-induced conductance (decrease in resistance) and an increase in the duration of the response.

4 Application of NaPb to the GABA response produced similar effects, namely an enhancement of the late positive-going phase, a shift in the reversal potential to a more positive value, an increase in conductance and an increase in duration.

5 GABA-mediated inhibitory postsynaptic potentials (i.p.s.ps) were evoked by electrical stimulation of the nucleus reticularis thalami. Application of NaPb caused an increase in the duration of the i.p.s.p. and a shift in its reversal potential to a more positive value.

Introduction

A model, derived from experiments by Steriade and coworkers (Steriade *et al.*, 1971; 1977; 1986; Deschenes *et al.*, 1982; 1984; Roy *et al.*, 1984) implicates γ -amino-butyric acid (GABA)-mediated inhibition, in the mechanism underlying the transition from tonic to burst firing in thalamic relay neurones at the onset of slow wave sleep. Since the *in vivo* studies that give the most comprehensive model for slow wave sleep generation to date, were performed under barbiturate anaesthesia, it was of interest to determine whether barbiturates enhance GABA_A-receptor mediated responses in thalamus, and thus contribute to the ability of these drugs to promote slow wave sleep. *In vitro* studies in cultured mouse spinal neurones (Ransom & Barker, 1975; 1976), in hippocampal pyramidal neurones (Nicoll 1975; Scholfield, 1977; Gage & Robertson, 1985) and in sympathetic ganglion cells (Brown & Constanti, 1978) have

shown that barbiturates primarily increase GABA_A activated Cl⁻ channel open time.

Thalamic relay neurones disconnected from the nucleus reticularis thalami did not display the rhythmic, long-lasting inhibitory postsynaptic potentials (i.p.s.ps) typical of thalamic neurones during slow wave sleep (Steriade *et al.*, 1985). The reticularis thalami is therefore proposed to be the origin of these i.p.s.ps. Stimulation of the reticularis thalami *in vitro* (Thomson, 1988a,b), elicited i.p.s.ps in thalamic relay cells. These i.p.s.ps and responses to GABA were biphasic, the early component displaying a more negative reversal potential than the later component, although both components appeared to be mediated by GABA_A-receptors and Cl⁻ channels. The later component was, however, more sensitive to antagonists. Biphasic responses to GABA have also been recorded in the hippocampus (Alger & Nicoll, 1979; Anderson *et al.*, 1980). Here, the later phase was seen more clearly in the presence of barbiturates or benzodiazepines (Biscoe & Duchon, 1985a,b; Blaxter *et al.*, 1986). It was therefore of interest to

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Figure 1 Responses of a thalamic relay neurone to pulses of γ -aminobutyric acid (GABA) applied electrophoretically. All pulses were 36 nA. The top record illustrates the transition from a biphasic response in which the early, negative component predominated, to one in which the later positive-going component predominated during the application of Na pentobarbitone (9 nA, hatched bar). Twenty minutes later (middle record) the response was large and entirely positive-going, but the negative component reappeared on termination of the pentobarbitone. Approximately one hour later (bottom record) the control biphasic response to a 2 s GABA pulse became entirely negative-going when the pulse duration was reduced to 1 s. Membrane held at -70 mV by negative current injection throughout.

determine whether the later component of the i.p.s.p. and the GABA response was similarly more sensitive to barbiturate in thalamus. Accordingly, experiments were designed in which the i.p.s.ps evoked by stimulation of reticularis thalami and responses to GABA in thalamic relay neurones were challenged with sodium pentobarbitone (NaPb). A preliminary account of this work has been published (Sykes *et al.*, 1988).

Methods

Methods similar to those used here have been described previously (Thomson, 1988a). Female rats, body weight 120 to 180 g, were anaesthetised with halothane and decapitated. Coronal slices of brain, 400 μ m thick, containing the anterior portion of the

thalamus were cut and maintained at 35°C at the interface between warm humidified gas (O_2/CO_2 95%/5%) and medium containing (mm): NaCl 124, $NaHCO_3$ 25.5, KCl 3.3, KH_2PO_4 1.2, $CaCl_2$ 2.5, $MgSO_4$ 1.0 and D-glucose 15. Intracellular recordings were made using 3 M K-acetate-filled micro-pipettes with tip resistances of 60–100 M Ω . Conventional current clamp recording methods were used. Drugs were applied electrophoretically from a multibarrel pipette whose 3 barrels contained GABA (200 mm, pH 3.5), NaPb (20 mm, pH 9.5; Curtis & Lodge, 1977, or 100 mm, pH 9.5) and NaCl (150 mm, balance barrel). GABA was ejected with pulses of 1–4 s duration. NaPb when applied, was applied continuously. Ejection currents between 10 and 75 nA for GABA and between 10 and 45 nA for NaPb were found to be effective. I.p.s.ps were evoked by stimulating the reticularis thalami via a twisted bipolar wire stimulating electrode (50 μ m, Ni/Cr).

Figure 2 Current-voltage relations for a thalamic relay neurone obtained in the absence of γ -aminobutyric acid (GABA; $\circ$ — $\circ$) and during the application of GABA (36 nA, 2 s). (Δ) Current-voltage relation obtained during the early, more negative going phase of the GABA response and ($\square$) points obtained during the later, more positive going phase. These plots are repeated during the application of Na pentobarbitone (b, solid symbols), during recovery from pentobarbitone (c) and following an increase in the GABA ejection current to 74 nA (d). Arrows indicate the reversal potentials for the two phases.

Membrane potential was altered by continuous injection of current and accurate electrode balance monitored by observing voltage responses to small, relatively brief current pulses. Slope resistance was obtained from I-V plots, by measuring voltage responses to steady state current injection under control conditions and during GABA and/or NaPb applications.

Results

Electrical properties of thalamic neurones

Twenty neurones were recorded in anterior and intralaminar thalamic nuclei that displayed slow spike activity typical of thalamic relay neurones (Jahnsen & Llinas, 1984a,b; Crunelli *et al.*, 1987). Their mean resting potential (RP) was

$-60.8 \text{ mV} \pm 8.8 \text{ mV}$ (s.d.) and input resistance (R_i) was between 40 and 80 ($53 \pm 30 \text{ M}\Omega$), when measured close to RP. Fast spike amplitude measured from threshold was approximately 55 mV and spikes overshoot zero potential by $>5 \text{ mV}$. Spike width at half amplitude was $\leq 0.75 \text{ ms}$.

Responses evoked by GABA

Responses to brief electrophoretic pulses of GABA under control conditions and in the presence of NaPb, were recorded from 9 cells. Accurate electrode placement was found to be important. Small changes in the position of the electrophoretic pipette produced large changes in the amplitude of responses evoked by a given GABA pulse. Changes in position as small as $10 \mu\text{m}$ from the optimal could reduce the response by $>80\%$. Under control conditions,

typical responses were close to monophasic if the GABA pulse duration was short (1 s) (see Figure 1, lower records). These responses reversed close to RP (between 5 mV negative and 3 mV positive of RP) and produced a 20 to 50% drop in slope resistance measured close to reversal potential. Increasing the ejection current, and/or time, resulted typically in a biphasic response in which the early component reversed at a more negative membrane potential (mean -62.4 ± 7.1 mV) than the later component (-56.4 ± 5.39 mV) (see Figure 2). The mean RP for these cells was -61.7 ± 7.6 mV. Figure 1 shows the transition from a biphasic response to a monophasic response, when the GABA pulse was reduced from 2 s to 1 s. The decrease in slope resistance associated with the response to GABA was also enhanced when the GABA current was increased (see Figure 3). For example, in a cell whose control R_i was $50 \text{ M}\Omega$, 10 nA

GABA reduced R_i to $10 \text{ M}\Omega$, and 20 nA GABA reduced R_i to $3.3 \text{ M}\Omega$. Equivalent figures for another cell, were $54 \text{ M}\Omega$ (control), $37.4 \text{ M}\Omega$ (12 nA GABA) and $7.0 \text{ M}\Omega$ (20 nA GABA). Increasing the GABA ejection current beyond that required to evoke a biphasic response augmented the late phase, apparently at the expense of the early phase and shifted the reversal potentials of both phases in the positive direction. For example, in one cell whose RP was -60 mV, the early component reversed at -64 mV and the later at -61 mV with a GABA current of 12 nA (4 s). When the GABA current was increased to 40 nA, the two components reversed at -57 mV and -55 mV respectively. Another example is illustrated in Figure 2. Further increases in GABA application resulted in even more complex, e.g. triphasic responses, in some cells. Such complex responses were not studied in detail and are not described here.

Figure 3 Responses of a thalamic relay neurone to pulses of γ -aminobutyric acid (GABA, 20 nA, 4 s). The downward deflections are voltage responses to brief current pulses injected into the cell throughout the recording. Application of Na pentobarbitone (24 nA, hatched bar) decreased the amplitude of the voltage responses during the GABA response, i.e. it increased the conductance produced by GABA. Averages (each of 4) of these voltage responses are shown at a faster time base on the right. The lower record shows a similar increase in the conductance produced by GABA, when the GABA ejection current was increased from 20 to 40 nA. The transition from a negative going response to the 20 nA pulse, to a positive going response to the 40 nA pulse, was gradual.

Figure 4 Responses of a thalamic relay neurone to pulses of γ -aminobutyric acid (GABA, 36 nA, 2 s), recorded at four different membrane potentials in the absence (left) and presence of Na pentobarbitone (9 nA, hatched bar). Pentobarbitone increased the duration of the response and shifted its reversal potential in the positive direction.

Gradual transition from monophasic to biphasic responses when the GABA current is increased

The transition from monophasic to biphasic, following an increase (Figure 3), or from biphasic to monophasic response shape, following a decrease in GABA ejection (Figure 1), was gradual. In addition, in some cells there was a gradual change in response shape during the earliest 10 to 30 GABA applications. This typically involved the development of the later, more positive-going component. It is possible that this gradual change paralleled a gradual increase in the amount of GABA ejected with each pulse. However, GABA was always pulsed during the placement of the multibarrel pipette, i.e. several tens of GABA pulses had already been ejected from that barrel before the optimal position, from which responses to GABA could be obtained, was found. The first pulse applied in the final position was therefore not the first pulse from that pipette. This gradual change in response shape was not matched by a similarly gradual rise in the evoked conductance. The evoked conductance was enhanced with the first increased GABA pulse (see Figure 3), and plateaued before the change in response shape. A gradual redistribution of Cl^- could also account for the change in reversal potential. However, even responses that were apparently totally positive-going became more positive-going at rest and reversed at a more depolarized membrane potential when the GABA current was increased.

Enhancement of GABA responses by Na pentobarbitone

In brief, the effect of applying NaPb was similar to that produced by increasing the GABA pulse. Responses to small, brief pulses of GABA that were predominantly monophasic under control conditions, became biphasic, with the gradual development of a later, more positive going phase (see Figure 1). Responses that were biphasic under control conditions also changed, with a gradual increase in the later, positive-going phase and the apparent loss, or obscuring of the early phase (see Figures 1 and 4). Reversal potentials for both components shifted in the positive direction (mean ($\pm$ s.d.) for early phase to -58.1 ± 9.0 mV and for the later phase to -51.7 ± 6.6 mV) (see Figure 2). In addition, response duration and the conductance change evoked by GABA both increased. In 8 cells studied, the slope resistance decreased from a control value of 43 ± 19.0 M Ω to 16.97 ± 13.0 M Ω with GABA alone and to 7.85 ± 1.7 M Ω with GABA and NaPb. These effects gradually reversed on termination of the NaPb application (Figure 1).

Direct effects of Na pentobarbitone on recorded cells

Sodium pentobarbitone affected RP or R_i only when large currents were applied. Doses of NaPb that augmented responses to GABA produced no measurable effect on these parameters in the majority of

Figure 5 Inhibitory postsynaptic potentials (i.p.s.p.) recorded from a thalamic relay neurone in response to electrical stimulation of the nucleus reticularis thalami (see stimulus artifacts for stimulus timing). I.p.s.ps were recorded at several membrane potentials in the absence (a) and presence (b) of Na pentobarbitone. Each record is an average of 4 single sweeps. Pentobarbitone increased the duration of the i.p.s.p. and shifted its reversal potential in the positive direction.

neurones (e.g. Figures 1 and 3). There was no significant change in slow spike threshold, amplitude or duration in the presence of effective NaPb doses.

Stimulus-evoked i.p.s.ps: enhancement by NaPb

In two cells, electrical stimulation of the reticularis thalami evoked i.p.s.ps. These were similar to those described previously (Thomson, 1988a). One of these is illustrated in Figure 5. The effect of NaPb on these i.p.s.ps was similar to its effects on responses to GABA. The duration, amplitude and conductance increase associated with the i.p.s.p. increased and there was a positive shift in its reversal potential. In addition, trains of i.p.s.ps evoked by low frequency, repetitive stimulation (10 Hz) summed more readily in the presence of NaPb, with little or no repolarization between stimuli.

Discussion

The similarity of the responses to GABA described here, to those in the previous study (Thomson, 1988b) suggest that the majority of these were also predominantly mediated by GABA_A-receptor operated Cl⁻ channels. It is these channels that have been shown to be sensitive to barbiturates (Ransom & Barker, 1975; Nicoll, 1975; Scholfield, 1977; Brown & Constanti, 1978; Gage & Robertson, 1985; Olsen *et al.*, 1986). In the present study, the augmentation of GABA responses that was seen when NaPb was applied, was similar in several respects to the

augmentation produced by simply increasing the GABA application, a result that is in agreement with previous studies (Ransom & Barker, 1976). In both cases, the response increased in duration and in associated conductance. This is consistent with the proposal that barbiturates enhance the open time of the activated channel. Again, in both conditions, there was a shift in the reversal potential of the response in the positive direction, which appeared to be associated with a profound augmentation of the late component. Preferential augmentation of the later component of the GABA response is also apparent in hippocampus, where the early, more negative going phase predominates in control conditions, but the later, more positive phase is revealed in the presence of barbiturates, or benzodiazepines (Biscoe & Duchen, 1985b; Blaxter *et al.*, 1986).

The present results confirm our previous suggestion (Thomson, 1988b), that under the present conditions, much of the response of thalamic relay neurones to electrophoretically applied GABA is mediated by GABA_A-receptors. These results also demonstrate that a major action of NaPb on thalamic relay cells is augmentation of GABA mediated i.p.s.ps. The results are consistent with the view that enhancement of GABA-mediated inhibition may play a role in barbiturate promotion of slow wave sleep. They do not, however, dispute a role for GABA_B-receptors in thalamus, or in the initiation of burst firing in slow wave sleep. We have recorded i.p.s.ps and in one cell a response to GABA, that differed greatly from the responses described here, in that they had reversal potentials around -90 mV (unpublished results, see also Crunelli *et al.*, 1987).

The precise mechanisms underlying the biphasic nature of i.p.s.ps and responses to GABA remains a matter for debate. From the results obtained here it seems probable that at, or close to the soma, the Cl^- equilibrium potential is close to the RP and more negative than it is in the dendritic tree. The early, negative component was always the lowest threshold when the multibarrel pipette was positioned close to the soma, while monophasic depolarizing responses were more commonly observed when the pipette was some distance from the soma. It does, however, seem unlikely that the site of activation of the later component is very far from the soma, since this component was the most sensitive to GABA antagonists (Thomson, 1988b) and to barbiturates applied proximally. Whatever the geometrical arrangement, it appears that the later, more positive-going component is the one that is most readily enhanced by NaPb.

The results presented here indicate that the later component develops gradually with time. Although it is possible that the NaPb takes some time to reach full effect and similarly, some time to wash off, it seems less likely that an increase, or decrease in GABA ejection current would take several minutes and tens of applications to stabilize. Indeed, augmentations of the conductance produced by GABA were generally seen to precede changes in response shape, when the GABA ejection current was increased. Two relatively simple explanations could account for the gradual shift in reversal potential. Firstly, enhancement of the later component is gradual and as it develops, it slowly obscures the early component and leads to an apparent shift in the reversal potential for the whole response, without any significant redistribution of Cl^- .

This explanation would require some time-dependent modification in the responsiveness of the receptor-channel complex produced by a change in its level of activation.

The second explanation involves a gradual redistribution of Cl^- ions due to augmentation of the hyperpolarizing component. A negative-going response would involve an influx of Cl^- . Increasing the conductance would increase influx and successive GABA responses would have an additive effect if Cl^- pumping were saturated. However, responses that were apparently purely positive-going under control conditions also became more strongly positive-going and their reversal potential shifted. It remains possible, however, that a large positive-going component obscures the smaller negative-going component which causes Cl^- loading of the cell when augmented. This explanation would require more powerful Cl^- pumping in some parts of the cell. For example, relatively weak outward Cl^- pumping would occur at the soma, which would be saturated by augmented Cl^- influx, while powerful inward pumping in the dendrites would not be saturated by augmented Cl^- efflux. This does not seem entirely unlikely since the apparently different equilibrium potentials for Cl^- in the different cell compartments would require differences in Cl^- pumping anyway. Further experiments are, however, required to determine the precise mechanism underlying the time-dependence of this effect.

This work was supported by the Wellcome Trust and The Medical Research Council. T.C.F.S. was a Wellcome Trust Vacation Scholar. A.M.T. is a Wellcome Trust Lecturer.

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(Received October 24, 1988

Revised February 20, 1989

Accepted April 17, 1989)