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Actions of anaesthetics and avermectin on $GABA_A$ chloride channels in mammalian dorsal root ganglion neurones

[1]Brian Robertson

Department of Physiology, John Curtin School of Medical Research, Australian National University, Canberra, A.C.T. 2601, Australia

1 The γ-aminobutyric acid (GABA)-mimetic actions of some anaesthetics and the antehelminthic avermectin B_{1a} were examined on freshly isolated mammalian dorsal root ganglion (DRG) neurones by use of suction electrodes and a single electrode voltage clamp.

2 Pentobarbitone (60 μM–3 mM), chloralose (600 μM–1 mM), etomidate (10–100 μM), alphaxalone (10–60 μM) and avermectin (10–60 μM) directly activated chloride channels in GABA-sensitive DRG neurones. The agonist action was sensitive to block by bicuculline and picrotoxinin.

3 Steady-state current-voltage (I-V) curves for the anaesthetics were either linear, or rectified in the opposite direction to steady-state I-V curves obtained with GABA. Current relaxations in response to voltage jumps were also of the opposite direction. An extra surge of current ('bounce') was commonly observed on washout of some of these agonists.

4 Pentobarbitone was ineffective as an agonist at alkali pH (10.4 and 9.4), but was approximately twice as effective at acid (5.4) than at normal (7.4) pH values.

5 These results suggest that some anaesthetics and avermectin are capable of 'blocking' GABA channels in addition to activating them.

Introduction

Several general anaesthetics enhance the actions of γ-aminobutyric acid (GABA) both in the central and peripheral nervous systems. In the case of pentobarbitone and some other anaesthetics, at least part of this action may be explained by an increase in duration of the open-time of GABA-activated channels (e.g. Jackson *et al.*, 1982). However, it has also been shown that a few anaesthetic drugs act on vertebrate neurones in a manner similar to GABA (Nicoll, 1975; Harrison & Simmonds, 1984), and this action is antagonized by bicuculline and picrotoxin. It has been suggested that these substances, which bear no obvious structural relation to the classical GABA-mimetic agonists, can directly activate GABA receptor-channel complexes. It is not known whether the agonist action occurs at the receptor site or at the ion channel. The present study examines the actions of various anaesthetic substances and the antehelminthic avermectin on $GABA_A$ channels in freshly isolated mammalian dorsal root ganglion (DRG) neurones, in an attempt to examine their kinetic behaviour in response to rapid jumps in membrane voltage and compare these responses to those obtained with GABA and structurally related agonists (Robertson, 1989).

Some of these results have been communicated previously (Robertson, 1985; 1986).

[1] Present address and address for correspondence: Electrophysiology Laboratory, Wyeth Research, Huntercombe Lane South, Taplow, Maidenhead SL6 0PH.

Methods

Experiments were conducted on freshly isolated dorsal root ganglion neurones using voltage clamp methods. Full details of our experimental methods are given in Robertson & Taylor (1986) and Robertson (1989). Briefly, ganglia (thoracic, lumbar and sacral) were obtained by dorsal laminectomy of adult rats and cats. Cells were cleaned enzymatically (with 0.5% trypsin and 0.5% collagenase), and recordings performed with a suction microelectrode in conjunction with a single electrode voltage clamp system (ASF2, Axon Instruments). Clamp 'switching'

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rates of 60–65 kHz were achieved as a consequence of low electrode resistance (~0.3 MΩ) and by reducing input capacitance. Voltage clamp traces were captured and subsequently analyzed on computer.

The external solution used in recording agonist-induce chloride responses was as follows (in mM): choline chloride, 135, CsCl 5, $MgSO_4$ 10, HEPES 5 and glucose 10; adjusted to pH 7.4 with CsOH. The suction electrode (intracellular) solution contained (mM): CsCl 140, HEPES 5, EGTA 2; adjusted to pH 7.4 with CsOH. Experiments were conducted at 22–24°C.

GABA was obtained from Sigma Chemical Company. Sodium pentobarbitone (Prosana or Abbott Pharmaceuticals) and etomidate (Janssen Pharmaceuticals) were kindly provided by Prof. David Curtis. α-Chloralose (Fluka) was a gift from Prof. W. Levick. Avermectin B1a (Merck Sharp and Dohme Laboratories) was dissolved in 2% dimethyl sulphoxide (DMSO) to give a final concentration of 10 μM drug and 0.1% DMSO for experiments. Alphaxalone (Glaxo Group Research) was dissolved and stored in 0.2 mM ethanol. Drugs were bath applied and solutions switched rapidly (less than 1 s), revealing transient components of responses.

Results

γ-Aminobutyric acid

Application of GABA (usually 60 μM) produced an inward current that reversed at the equilibrium potential for chloride ions predicted by the activity of chloride in the intracellular and extracellular solutions. Current relaxations were measured in response to voltage jumps in control and drug solutions and subtracted on-line to give the net agonist-induced current. The net agonist-induced current is shown in all figures. The instantaneous current, measured at the point where the command voltage is achieved, was linear in symmetrical chloride solutions (Figure 1a), while the steady-state current (measured at the end of the voltage step) showed rectification (Figure 1b). The direction of the rectification was consistent with a reduction in the number of open channels at hyperpolarized potentials, due to the reduction in GABA channel open time as the membrane voltage was made more negative (e.g. Robertson, 1989).

Pentobarbitone

Racemic pentobarbitone was bath applied to DRG neurones at concentrations between 60 μM and 3 mM. In every cell where pentobarbitone was effective ($n = 53$), GABA responses could also be elicited.

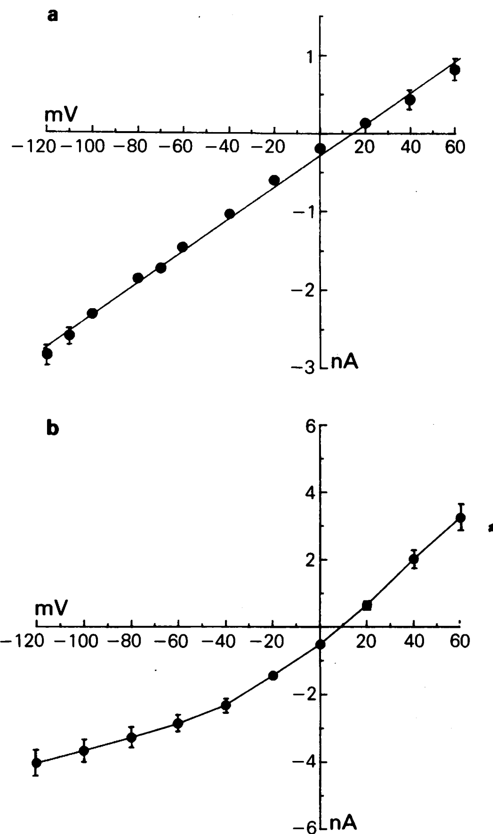

Figure 1 (a) Instantaneous current-voltage relation for 60 μM GABA with 140 mM chloride on both sides of the cell membrane. Data points are mean for 8 cells (with s.e.mean shown by vertical bars) normalized to −1 nA at −40 mV. (b) Steady-state current voltage relation for the same cells. The rectification in this curve is due to the voltage sensitivity of GABA channel lifetime. Data points also mean with s.e. shown by vertical bars.

However, if cells were unresponsive to GABA then no response to pentobarbitone was obtained ($n = 10$).

Where pentobarbitone was applied at the same concentration as GABA, the anaesthetic was less effective than GABA (60 μM pentobarbitone was only 10% as effective as 60 μM GABA, $n = 3$; 1 mM pentobarbitone was 40% as effective as 1 mM GABA, $n = 3$). The agonist action of pentobarbitone was rapidly and reversibly blocked by 50 μM of either bicuculline methiodide or picrotoxinin.

In four experiments the effects of 60 μM GABA and 1 mM pentobarbitone applied separately and together were assessed. GABA (60 μM) alone evoked on average −1.2 nA of inward current, 1 mM pento-

barbitone produced −2.1 nA, but together these agonists produced a mean of −11.1 nA of current.

When pentobarbitone concentration was increased to 600 μM and above, the current response increased rapidly to a peak and then quickly declined to a plateau level. However, an unusual response was observed when the drug was washed out of the bath. Immediately after the solution switch, a marked transient increase in current was observed. This 'bounce' of current then decayed to pre-drug baseline level. In contrast, current responses to high concentrations of GABA showed desensitization only, and never 'bounce' on washout.

Current responses to 1 mM pentobarbitone at −40 and +40 mV are illustrated in Figure 2a. At least part of the decline in peak current in Figure 2a was due to desensitization, but there may also have been a contribution from channel 'block' by pentobarbitone (see below).

Voltage jumps were performed before and during pentobarbitone exposure to measure the voltage-dependence of the agonist action of pentobarbitone on GABA channels. Figure 2b compares the relaxations produced by 60 μM GABA and 1 mM pentobarbitone upon a potential step from −40 to −100 mV. The current relaxations for the two agonists have very different characteristics. The GABA response showed the usual exponential decrease in current to a new, lower steady state value, while the pentobarbitone response showed a more rapid relaxation in the opposite direction, such that current increased to a new steady state value after the instantaneous jump. At the end of the hyperpolarizing voltage step, the relaxation for the two agonists were again opposite in direction, consistent with a reduction in the average number of open channels during the hyperpolarizing voltage step with GABA, and an increase in the number of open channels on the same voltage step with pentobarbitone. These unusual relaxations were a typical feature with pentobarbitone at concentrations greater than 100 μM.

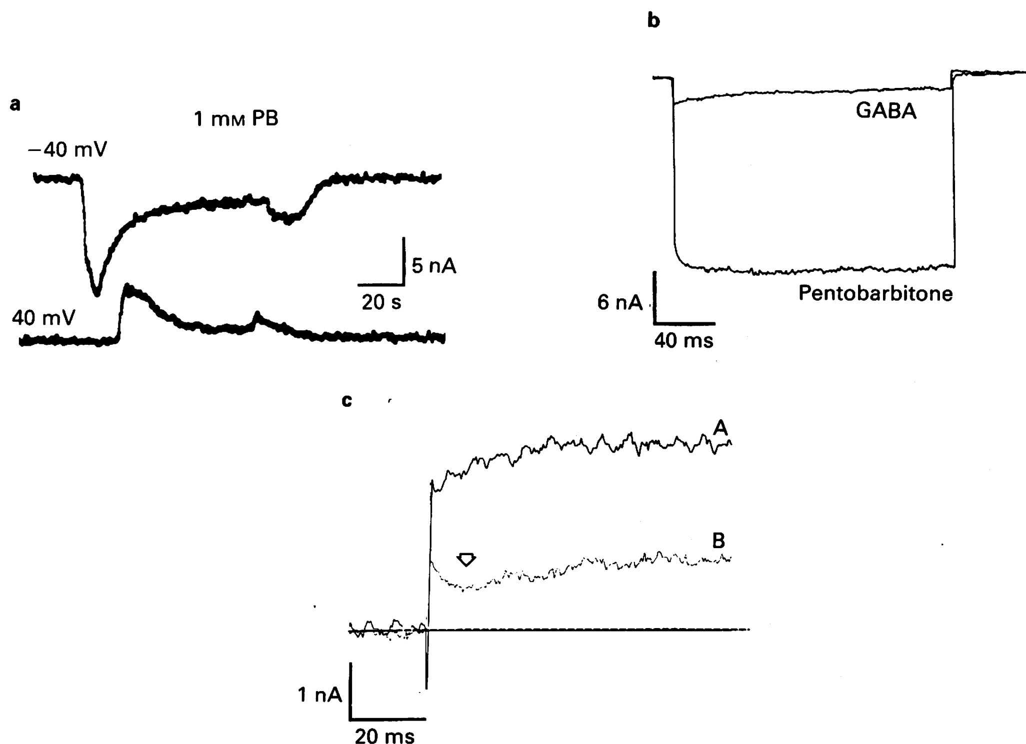

Figure 2 (a). Agonist action of 1 mM pentobarbitone at two potentials. Pentobarbitone produces a large increase in current, which declines to a plateau, and is followed by a secondary increase in current ('bounce') on washout of the drug. (b) Voltage jump relaxations with 60 μM GABA and 1 mM pentobarbitone in the same cell on a 60 mV hyperpolarizing step from −40 mV. The relaxations are opposite in direction for the two agonists. (c) Relaxations in response to depolarizing steps to +60 mV from −40 mV with 60 μM GABA (A) and 600 μM pentobarbitone (B). Note that the GABA-induced current increases steadily after the instantaneous current, while the outward current in pentobarbitone initially decreases (open arrow), before increasing to a new steady state value.

Figure 2c illustrates expanded relaxations in response to depolarizing voltage jumps with 600 μM pentobarbitone and 60 μM GABA on the same cell. The GABA current increased steadily after the instantaneous current, but the current induced by pentobarbitone showed an initial sharp decrease after the ohmic current, and then increased steadily. (The open arrow highlights this phenomenon). Similar behaviour was observed with 10 other cells exposed to pentobarbitone.

As a consequence of the different modes of current relaxation, the steady state current-voltage relationship for cells exposed to 600 μM pentobarbitone showed rectification in the opposite direction from channels gated by GABA or GABA-like agonists, with less current at positive membrane potentials.

Since pentobarbitone is 75% in the uncharged form at physiological pH and 25% in an anionic form, experiments were conducted to test which form of the anaesthetic was responsible for the reduced current at positive potentials, that is to determine whether the anionic or neutral forms were responsible for the agonist action of pentobarbitone.

Experiments were performed measuring the response to 1 mM pentobarbitone at pH 7.4, then changing pH to 10.4 or 9.4, and comparing the response at the new alkaline pH. In two separate experiments pentobarbitone produced −4.4 and −7.0 nA at pH 7.4, but there was no response to this agonist at pH 10.4. Similarly, in four experiments at pH 7.4 and 9.4, pentobarbitone produced a mean response of −5.5 nA at pH 7.4, but a mean current of only −0.2 nA at pH 9.4. (In three applications pentobarbitone caused no current change at pH 9.4, the other cell showed a maximum current that was only 10% of the full response at pH 7.4). One of these experiments is illustrated in Figure 3a.

Changes in holding current in control solutions at alkaline pH were negligible. In order to determine if the GABA receptor/channel complex itself was disturbed by alkaline solutions, experiments were conducted to measure the effectiveness of 60 μM GABA at pH 9.4. In all three experiments, GABA responses were 84 ± 2.6%, (mean ± s.e.) of their value at pH 7.4, suggesting that the receptor/channel was not substantially altered at pH 9.4.

Further experiments were carried out with pentobarbitone at pH 5.4. In 6 cells with 8 applications of pentobarbitone at pH 7.4 and pH 5.4, 1 mM pentobarbitone produced 1.2 to 2.9 fold more current at acid pH values. The mean increase in current was 1.9 ± 0.3 fold at −40 mV. The steady state I-V relationship from 4 cells with paired applications of 1 mM pentobarbitone at both pH values is illustrated in Figure 3b. Pentobarbitone was more effective at pH 5.4 at all potentials examined; however current responses still rectified at positive potentials. Additionally, the bounce of current observed upon washout of pentobarbitone was still present at pH 5.4.

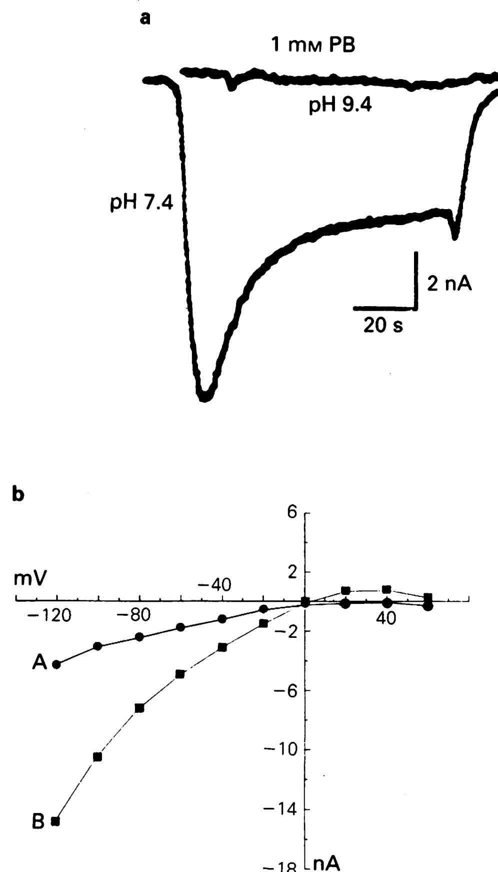

Figure 3 (a) The agonist action of pentobarbitone at normal and alkali pH. Pentobarbitone (1 mM) elicited a large inward current at pH 7.4, but was ineffective at pH 9.4. On reapplication of the drug at pH 7.4, the usual inward current was observed (not shown). Cell holding potential −40 mV. (b) Steady state I-V for 4 cells with 1 mM pentobarbitone applied at pH 7.4 (A) and pH 5.4 (B).

Chloralose

α-Chloralose activated GABA receptor/channels at concentrations of 100 μM and above ($n = 34$). Current responses to 600 μM chloralose were blocked by both 50 μM picrotoxinin and 50 μM bicuculline methiodide. Chloralose responses were not observed on GABA-insensitive cells ($n = 5$). When the potency of 60 μM GABA and 600 μM chloralose were compared on the same cells ($n = 7$), GABA produced a

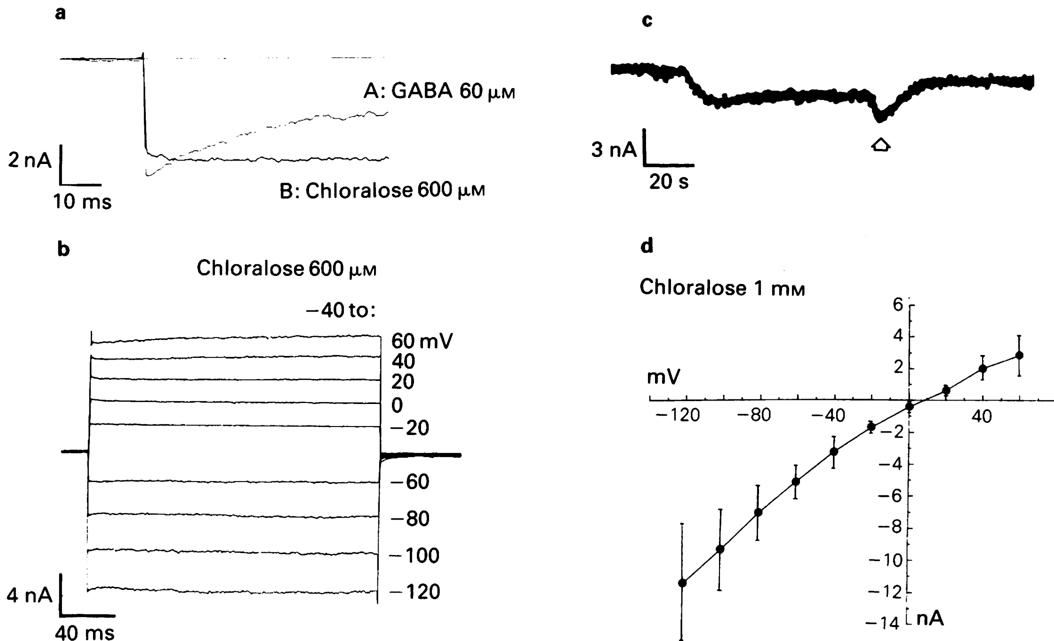

Figure 4 (a) Voltage jump relaxations on a −80 mV step from −40 mV with 60 μM GABA (A) and 600 μM chloralase (B). (b) A family of current relaxations from another cell with 600 μM chloralose, stepping from −40 mV to the potentials indicated at the right hand side of the current traces. (c) The response of a DRG neurone to 1 mM chloralose. Note the decline after the initial peak and the bounce (arrow) on washout of this anaesthetic agonist. Clamp potential −40 mV. (d) Steady-state current voltage relation for 1 mM chloralose. Points are the mean values from 3 cells.

mean inward current of -2.25 ± 0.47 nA, and 600 μM chloralose gave -2.51 ± 0.4 nA.

There was no evidence for 'bounce' or desensitization with 600 μM chloralose. Voltage jump relaxations (Figures 4a and b) in 600 μM chloralose did not show the form of relaxations observed when GABA was used to activate the channels. As with pentobarbitone, if relaxations were seen, they were of the opposite direction to those obtained with GABA (i.e. increasing current on hyperpolarizing steps).

With higher concentrations of chloralose (1 mM), current responses showed a decline after an initial peak, and a slight bounce after removal of the agonist (see Figure 4c). The steady state I-V for 1 mM chloralose on 3 cells is shown in Figure 4d.

Etomidate

The non-barbiturate anaesthetic etomidate was effective in eliciting conductance changes on DRG neurones at concentrations of 30, 60 and 100 μM ($n = 28$). These responses reversed at the chloride equilibrium potential and were blocked by the GABA antagonists bicuculline methiodide and picrotoxinin (50 μM), and cells unresponsive to GABA did not respond to etomidate ($n = 6$). In 11 cells where 60 μM etomidate was compared to 60 μM GABA, the average response in etomidate was -1.27 ± 0.16 nA and -2.24 ± 0.39 nA in GABA. However, these figures represent only an approximate index of potency, since as Figure 5 shows, there was a large and rapid bounce of current as eto-

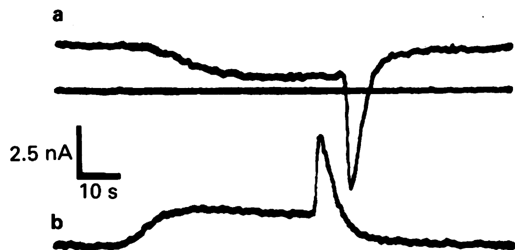

Figure 5 Response to 60 μM etomidate at −40 mV (a) and +40 mV (b). The current rises slowly to a plateau, and there is a large transient increase in current when etomidate is washed out of the bath. The middle trace is the clamp potential during exposure to the anaesthetic.

midate was washed out of the recording bath. The bounce could be as large as four times the 'steady state' (plateau) response. In the cell illustrated in Figure 5, 60 μM GABA produced −4.6 nA of inward current at −40 mV, but 60 μM etomidate gave a plateau current of −1.5 nA followed by a bounce reaching −6 nA. If etomidate was applied for long periods (greater than 2 min) the plateau response declined slightly, but a large bounce was still observed on washout.

In 3 experiments where GABA and etomidate were applied separately, then additively, the response to the two agonists was greater than the algebraic sum of the individual responses.

Current responses to voltage jumps in 60 μM GABA and 100 μM etomidate are illustrated in Figure 6. As was observed for pentobarbitone and chloralose, these currents did not 'relax' in the manner of GABA-induced responses. The voltage jump responses in etomidate were essentially flat, with a small increase in current being observed on hyperpolarizing voltage jumps. The steady state current voltage relation in 60 μM etomidate is shown in Figure 6b. There appeared to be little overall voltage-dependence of the etomidate response, and any voltage sensitivity was opposite to that seen with GABA-induced responses.

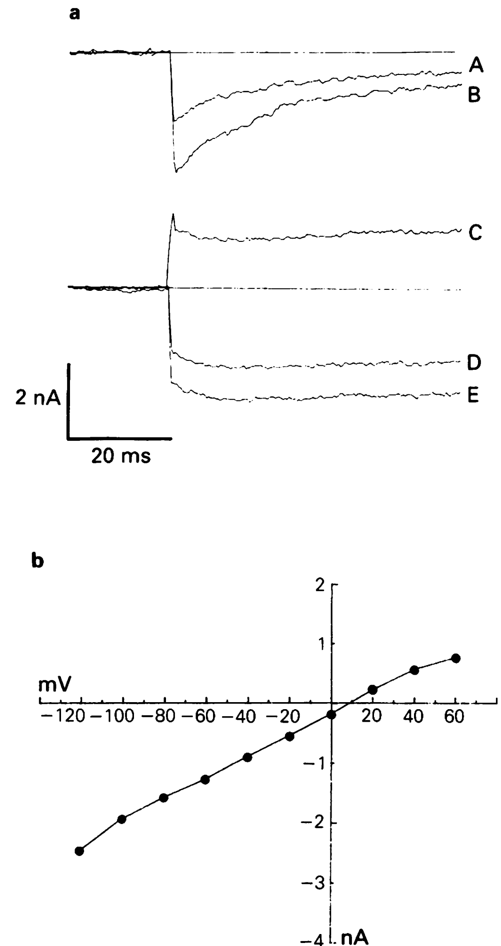

Figure 6 (a) Voltage jump relaxations in response to hyperpolarizing steps from −40 mV in 60 μM GABA (A: step to −120 mV; B: step to −100 mV) and 100 μM etomidate in the same cell. Jumps in etomidate are to +60 mV (C), −100 mV (D) and −120 mV (E). The outward relaxations on hyperpolarizing steps in etomidate are quite dissimilar to those responses in GABA, as is the reduction in current after the instantaneous current in trace (C). (b) Steady state current voltage relation in 60 μM etomidate (mean of 7 applications). Note the linearity of the I-V.

Alphaxalone

The steroid anaesthetic alphaxalone (10 to 60 μM) activated a chloride conductance in 20 DRG neurones, and these responses were blocked by 10 and 50 μM picrotoxinin. Cells that were not responsive to GABA did not produce currents in response to alphaxalone ($n = 4$). Current responses to 10 μM alphaxalone showed considerable desensitization with only occasional bounce on removal of the agonist.

In 4 cells the currents in response to 10 μM alphaxalone and 60 μM GABA were compared: the anaesthetic produced a mean current of -2.3 ± 0.7 nA while GABA gave a mean current of -5.6 ± 2.1 nA.

Voltage jump relaxations with alphaxalone were again mainly flat, with small fast relaxations in the opposite direction to GABA currents.

Avermectin B_{1a}

The insecticide avermectin B_{1a}(AVM) is structurally quite dissimilar to any of the other anaesthetic agonist-like compounds examined, and has been reported to have GABA-like agonist properties in some invertebrate preparations. At 10 μM, AVM activated picrotoxinin-sensitive chloride channels in all 5 cells tested; these cells were also GABA-sensitive. Responses to AVM were typically much slower in onset and decay than current responses to the other agonists examined, often taking minutes to reach a peak response. The chloride currents induced by AVM were however fully reversible; no irreversible increases in Cl^- permeability were seen (see Duce & Scott 1985). AVM 10 μM elicited currents of amplitudes between −1 and −5 nA, with the mean response being -3.1 ± 0.9 nA, compared to -8.3 ± 2.3 nA with 60 μM GABA on these same

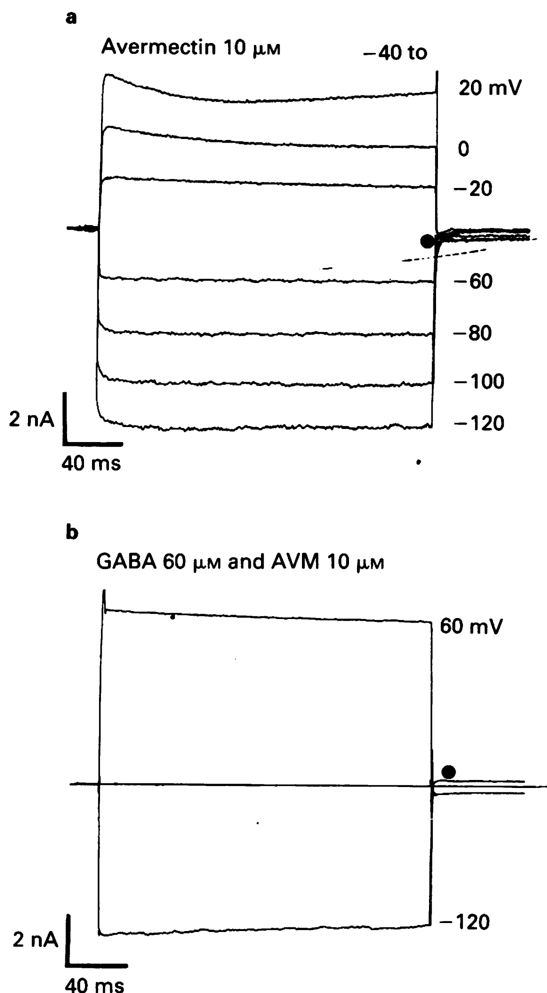

Figure 7 (a) Relaxations in response to voltage jumps in 10 μM avermectin are very different from those seen with GABA. Currents were obtained in response to steps from −40 mV to the potentials indicated on the right hand side of the figure. Note that the current increases at the beginning of the hyperpolarizing step, and then relaxes back to the baseline again at the end of the step (indicated by ●). (b) Simultaneous application of 10 μM avermectin (AVM) and 60 μM GABA results in altered current relaxations in response to potential steps. In the relaxation obtained on a step from −40 to −120 mV, AVM markedly slows the inward relaxation due to GABA channel activation, and slows the 'off' relaxation at the end of the voltage step (●).

cells. Despite the slow rise and decline of the AVM-induced current, this could be very rapidly and completely blocked by including the GABA antagonist picrotoxinin in the perfusate. Block by the antagonist was fully reversible. No tests of bicuculline sensitivity were made.

Current responses to voltage jumps in AVM are illustrated in Figure 7a and the steady state current voltage relationships was approximately linear. AVM currents displayed small outward relaxations on hyperpolarizing potential steps, and inward relaxations on depolarizing steps. These results are consistent with there being an increase in the number of conducting channels at more negative potentials.

No obvious bounce was detected on washing AVM out of the bath, instead the current declined slowly back to control values. In one experiment, 10 μM AVM was applied with 60 μM GABA (Figure 7b). AVM appeared to slow down the normal GABA relaxations at hyperpolarized potentials considerably. This can be seen with the slow change in current amplitude during the hyperpolarizing voltage step.

Due to the poor solubility of AVM, this substance was dissolved in 0.1% DMSO. In 3 experiments, application of DMSO alone had no effect on the resting conductance of DRG neurones, or on GABA responses.

Halothane, ketamine and diazepam

No conductance change was observed on applications of halothane (1 mM, $n = 3$), 1 mM ketamine ($n = 3$), or 100 nM and 20 μM diazepam ($n = 6$), although all of these neurones responded vigorously to 60 μM GABA.

Discussion and conclusions

Pentobarbitone activated GABA channels in DRG neurones at concentrations as low as 60 μM, and up to 3 mM. The anaesthetic range for pentobarbitone is approximately 100–200 μM (Richards 1972), and it is possible that the GABA-mimetic action of this drug plays a role during surgical anaesthesia. Addition of pentobarbitone and GABA together produced currents that were 3–4 times greater than the algebraic sum of individual GABA and pentobarbitone responses, due both to the potentiation of GABA responses as well as the agonist action of pentobarbitone. Similar observations of the agonist-like effects of pentobarbitone have previously been made on a variety of neurones (Nicoll, 1975; Nicoll & Wojtowicz, 1978; Barker & Ransom, 1978; Connors, 1981; Akaike *et al.*, 1985).

As well as exerting agonist actions, the presence of 'bounce' on washout of pentobarbitone suggested that this compound may also be blocking GABA receptor/channels. Bounce has also been observed with pentobarbitone by Akaike *et al.* (1985, 1987),

and these authors have proposed a channel blocking mechanism to account for this phenomenon. Adams (1975) observed a bounce on washout of high concentrations of certain nicotinic agonists from frog muscle and suggested that these current surges were due to the agonist binding to 'hypothetical non-receptor sites' and that the agonists were released from these reservoirs during washout. This possibility was excluded during the present experiments by changing the solution very rapidly around a single DRG neurone. The results obtained here were more consistent with a rapid blocking of the GABA receptor/channel by pentobarbitone, with these channels becoming unblocked and available to carry current during washout of the agonist. Peters *et al.* (1988) have also observed a marked bounce on washout of high concentrations of pentobarbitone, and suggest these high concentrations had blocking activity on $GABA_A$ chloride channels. A depressant or blocking action of pentobarbitone has been well described at acetylcholine (ACh)-gated chloride channels in invertebrates (Adams *et al.*, 1982; Judge & Norman, 1982; Wachtel & Wilson, 1983). In the present experiments the characteristics of the pentobarbitone induced currents during voltage jumps (where the current increased on a hyperpolarizing step) and the consequent curvature of the anaesthetics' steady-state I-V relationship suggests that there may be a greater degree of block by this agonist at positive membrane potentials. Since pentobarbitone exists in both anionic and neutral forms at pH 7.4, it was possible that the anionic form might be entering the channel and transiently reducing ionic conductance at positive potentials. In experiments with alkaline solutions, pentobarbitone produced little or no conductance change. At this pH most of the anaesthetic is in the anionic form, and may have been completely blocking the channels. However, at pH 5.4, where over 99% of the drug would be uncharged, the steady state I-V still rectified significantly at positive potentials and bounce was still observed on washout of the agonist. The simplest interpretation of these results is that agonism and blocking are associated with the neutral and not the anionic form of pentobarbitone.

Etomidate was the most potent anaesthetic in activating GABA receptor/channels, indeed this compound had a potency comparable to GABA when the blocking action of etomidate was taken into consideration. The GABA-agonist action of etomidate has previously been observed by Evans & Hill (1978), who found that the agonist effects were apparent at concentrations similar to those measured in the rat brain during hypnotic and behavioural effects. The marked bounce on washout and the shape of the voltage jump relaxations in etomidate can also be interpreted in terms of a sequential channel block by this anaesthetic after activation. Indeed computer simulations of the current induced by etomidate (unpublished observations) would suggest that at 60 μM etomidate blocks about 80% of the activated receptor/channels at steady state. It is suggested that the channel blocking kinetics have opposite sensitivity to membrane potential than the normal kinetics seen when GABA or related agonists activate the channel (Robertson, 1989). Judge & Norman (1982) found that 100–500 μM etomidate depressed ACh-evoked chloride responses in *Helix* neurones, while Cullen & Martin (1982) have reported that metomidate (a close analogue of etomidate) depressed GABA-induced chloride responses in lamprey medullary neurones.

The general anaesthetic α-chloralose was also effective in activating GABA channels directly. Similar results have been obtained previously by Nicoll & Wojtowicz (1980) and Nicoll & Madison (1982). This anaesthetic also produced steady state currents and relaxations opposite in direction to those seen with GABA, which are consistent with some form of blocking behaviour. Chloralose is well known for its excitatory actions during anaesthesia, and the basis for this action should be examined at the electrophysiological level. It is possible that the GABA blocking action of chloralose becomes significant at higher doses, leading to a temporary reduction in synaptic inhibition causing an increase in excitability (e.g. see Nicoll, 1972).

Harrison & Simmonds (1984) have observed that low doses of the steroid anaesthetic, alphaxalone, potentiated the depolarizing responses to GABA and muscimol in rat cuneate neurones. These authors also found that doses of 10 μM and above alphaxalone could directly activate GABA receptor/channels, and that this action was only seen with the α- and not the β-isomer. The experiments reported here show that alphaxalone also activates GABA channels directly on DRG neurones. This supports observations made by Barker *et al.* (1987) and by Cottrell *et al.* (1987) on central neurones and chromaffin cells respectively. (Barker *et al.*, 1987 have also observed 'bounce' on washout of alphaxalone; see Figure 9 of their paper).

Avermectin B_{1a} activated GABA channels in DRG neurones, although with a much slower time course than the other agonists tested. The activation was rapidly and completely antagonized by picrotoxinin, suggesting that this large macrocyclic compound is capable of activating and opening GABA channels directly. However, Duce & Scott (1985) have reported that AVM can induce increases in chloride permeability in locust muscle fibres that are insensitive to GABA, and suggest that AVM has other, less specific membrane actions. In this connection, it is interesting to note that Martin & Pen-

nington (1989) have recently reported that AVM can induce cation channels in patches of *Ascaris* muscle. It would seem then that AVM probably has other 'nonselective' actions on biological membranes, and further experiments on mammalian neurones would be necessary in order to determine if these occur in higher animals.

AVM is a potent antehelmintic and insecticide, and its mode of action has been explained by the GABA agonist like actions of this compound (see e.g. Wang & Pong, 1982). Avermectin binds specifically to brain membranes, and enhances GABA binding (Wang & Pong, 1982). Supavilai & Karobath (1981) have also suggested that avermectin enhances benzodiazepine binding to a novel modulatory site on the GABA-benzodiazepine receptor/complex. Whether these effects are correlated with the slow agonist actions of this compound is not yet known. Because of the slow rise times of the avermectin responses observed in the present experiments, it is possible that this compound is acting by stabilizing spontaneous openings of GABA channels (see e.g. Jackson, 1986, for details of this phenomenon in ACh receptors). However, preliminary experiments with application of GABA antagonists and large voltage gradients (designed to reveal any current generated by spontaneous GABA channel openings) suggest that any contribution from such spontaneous openings to the background conductance of DRG neurones would be vanishingly small. Additionally, other agents which stabilize GABA channels in an open conformation should also act as agonists on this basis, however neither ketamine nor halothane, which potentiate GABA responses in central neurones (Scholfield, 1980; Gage & Robertson, 1984) showed any GABA receptor agonist action.

The benzodiazepine diazepam was also tested for potential agonist activity since Barker and colleagues (Barker, 1983; Barker *et al.*, 1984) have reported that diazepam is an extremely potent agonist at GABA receptor channels. In contrast to results obtained on central neurones in the present study, 100 nM and 20 μM diazepam were completely ineffective in eliciting a conductance change, even though GABA responses were quite marked in these neurones. It may be that in cultured central neurones benzodiazepines may be enhancing the action of endogeneously released GABA.

The activation of GABA channels by anaesthetic agents whose chemical structures vary widely and are not easily reconciled with the classical requirements for GABA agonists (Curtis & Watkins, 1960) is unusual for the agonist-gated channels studied to date. It will be interesting to determine precisely how these anaesthetics activate GABA channels, and whether this occurs by interactions at the receptor site, or if the ionophore is opened directly. Given the complex nature of the interaction of anaesthetics with $GABA_A$ channels (modulation, agonism and blocking), it would be necessary to use single channel techniques to resolve their kinetics properly. Perhaps such studies will tell us more about the molecular processes involved in receptor/channel activation in general.

I would like to thank Dr Caroline Herron for her critical comments on the manuscript.

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