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Modulation of $GABA_A$ receptor activity by alphaxalone

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1 The modulation of the γ-aminobutyric $acid_A$ ($GABA_A$) receptor by alphaxalone has been investigated by use of voltage-clamp recordings from enzymatically isolated bovine chromaffin cells maintained in cell culture.

2 Alphaxalone (> 30 nM) reversibly and dose-dependently potentiated the amplitude of membrane currents elicited by locally applied GABA (100 μM). The potentiation was not associated with a change in the reversal potential of GABA-evoked currents and was not influenced by the benzodiazepine receptor antagonist, Ro15-1788 (300 nM).

3 At relatively high concentrations (> 1 μM), alphaxalone directly elicited a membrane current. It is concluded that such currents result from $GABA_A$ receptor activation since they were reversibly suppressed by bicuculline (3 μM), dose-dependently enhanced by phenobarbitone (100–500 μM), and had a similar reversal potential (~0 mV) to currents elicited by GABA. Additionally, on outside-out membrane patches, alphaxalone activated single channel currents with amplitudes and a reversal potential similar to those evoked by GABA.

4 Alphaxalone (30 nM–1 μM) had no effect upon the amplitude of membrane currents elicited by locally applied acetylcholine (ACh) (100 μM). However, higher concentrations of alphaxalone (10–100 μM) reversibly suppressed ACh-evoked currents, the IC_{50} for blockade being 20 μM.

5 The β-hydroxy isomer of alphaxalone, betaxalone (100 nM–1 μM), did not potentiate GABA-induced currents, nor did higher concentrations of the steroid (10–100 μM) directly evoke a membrane current. However, over the latter concentration range, betaxalone suppressed the amplitude of currents elicited either by GABA or ACh.

6 The relevance of the present results to the anaesthetic action of alphaxalone is discussed together with the broader implications of steroidal modulation of the $GABA_A$ receptor.

Introduction

The demonstration by Selye in 1941 that some synthetic and hormonal steroids induce anaesthesia led to the development of alphaxalone (3α-hydroxy, 5α-pregnane-11,20 dione), a short acting steroidal general anaesthetic (Child *et al.*, 1971). It is now known that small changes in the structure of such steroids can produce large differences in their anaesthetic activity. The β-hydroxy isomer of alphaxalone, betaxalone, for example, is inactive as an anaesthetic (see Harrison & Simmonds, 1984), which suggests that the active form interacts with a specific membrane protein or 'receptor'.

The observation that alphaxalone, like pentobarbitone, caused a prolongation of inhibition of neurones in guinea-pig olfactory slices (Scholfield, 1980) suggested that alphaxalone may act to modulate transmission mediated by γ-aminobutyric acid (GABA). Support for this view was obtained by Harrison & Simmonds (1984). They observed that alphaxalone potentiated the GABA-evoked responses recorded extracellularly from slices of the rat cuneate nucleus and that it also increased the affinity of binding of muscimol, a GABA agonist, to a preparation of rat brain neuronal membranes.

In this study, the action of alphaxalone on GABA-induced currents was investigated on bovine adrenomedullary chromaffin cells maintained in dissociated cell culture. Bovine chromaffin cells were chosen because they have been shown to possess $GABA_A$ receptors, which are similar to those found on central neurones (Bormann & Clapham, 1985; Cottrell *et al.*,

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1985), and chemically-activated membrane currents may readily be recorded using the 'whole-cell' mode of the patch-clamp technique (Hamill *et al.*, 1981; Marty & Neher, 1983). A preliminary account of a part of this work has appeared in abstract form (Cottrell *et al.*, 1986a).

Methods

Dissociation and culture of chromaffin cells

Bovine adrenomedullary chromaffin cells were isolated and cultured by the method of Fenwick *et al.* (1982), with minor modifications. Fresh adrenal glands were retrogradely perfused with a calcium-deficient solution (composition, in mM: NaCl 119, KCl 4.7, $MgSO_4$ 1.2, $NaHCO_3$ 25, KH_2PO_4 1.2, glucose 11, bovine serum albumin (BSA) 0.5%, 5% CO_2) containing 1 mg ml^{-1} collagenase for 30 min. The partially digested medulla was then removed, minced with scissors, and the resulting tissue fragments incubated with the Ca-free medium containing collagenase for a further 30 min. Following trituration, the tissue fragment suspension was filtered through 'Nytex' gauze (pore size 80 μm) to remove undissociated cells, which were then reincubated in solution for a further 30 min. The dissociated cell fraction, resulting from each incubation, was sedimented by centrifugation (150 *g*, 10 min) and the supernatant discarded. Residual collagenase was removed by washing twice with 2 ml of Ca-free medium, each followed by centrifugation (100 *g*, 5 min) and aspiration of the supernatant. The cell fractions were pooled and layered on to a cushion of 25 mg ml^{-1} BSA in Ca-free Krebs solution. Following sedimentation under unit gravity for 1–1.5 h, the cells were resuspended in a growth medium consisting of 90% (vol/vol) Dulbecco's modified Eagle medium and 10% (vol/vol) heat inactivated foetal calf serum supplemented with gentamycin (40 μg ml^{-1}) and penicillin/streptomycin (50 iu ml^{-1}). Cells were plated at a density of 3×10^4 cells cm^{-2} into 35 mm diameter 'Nunclon' petri dishes and cultured at 37°C in 5% CO_2, 100% relative humidity for periods of 1 to 7 days before use.

Electrical recordings

Agonist-activated currents were recorded from 'whole-cell' and 'outside-out' membrane patches with a List Electronics L/M EPC-7 converter headstage and amplifier using standard techniques (Hamill *et al.*, 1981). To facilitate cell dialysis and voltage-clamping, cells were selected which were devoid of processes and 8 to 20 μm in diameter. Whole-cell and single channel currents were low pass filtered (Bessel characteristic) at the cut-off frequencies indicated in the Figure legends, and recorded on magnetic tape with an FM tape recorder (Racal Store 4 DS). Cells were continually superfused (3–5 ml min^{-1}) with a solution containing (in mM) NaCl 140, KCl 2.8, $MgCl_2$ 2, $CaCl_2$ 1.0 and HEPES-NaOH 10 (pH 7.2). The pipette solution employed to dialyse the cell interior comprised (in mM) CsCl 140, $MgCl_2$ 2.0, $CaCl_2$ 0.1, EGTA 1.1 and HEPES-NaOH 10 (pH 7.2). Cs was employed as the predominant internal cation to suppress the various K conductances of the cell membrane (Marty & Neher, 1985).

Drugs used

Drugs were applied either locally by pressure ejection (1.4×10^5 Pa) from modified patch pipettes, or directly into the medium. The following were used: GABA, (+)-bicuculline, collagenase type IV, nipecotic acid, phenobarbitone (Sigma); flunitrazepam, Ro 15-1788 (ethyl-8-fluoro-5,6-dihydro-5-methyl-6-oxo-4H-imidazo [1,5a] [1,4] benzodiazepine - 3 - carboxylase) (Roche), alphaxalone and betaxalone (Glaxo). With the exception of alphaxalone, betaxalone and flunitrazepam, which were prepared as concentrated stock solutions in ethanol, and bicuculline, prepared as a concentrate in HCl, all drugs were dissolved directly in the medium. Ethanol, at the maximum concentration employed in experiments (0.2% vol/vol), did not influence agonist-activated currents, or have any direct action on the cells. All experiments were performed at room temperature (17–21°C). Quantitative results are expressed as the arithmetic mean ± the standard error of the mean (s.e.mean).

Results

Potentiation of GABA-evoked currents by alphaxalone

Alphaxalone in the medium reversibly potentiated the amplitude of whole cell currents evoked by locally applied GABA (100 μM). The threshold concentration of alphaxalone for this effect was close to 30 nM; responses to GABA were increased by 17.2 ± 5.6% ($n = 8$) by 30 nM and 113.1 ± 27.4% ($n = 14$) by 100 nM alphaxalone. The dose-dependency of the alphaxalone-induced potentiation was examined in greater detail in the experiment shown in Figure 1 (a,b). Over the concentration range 30–300 nM, the increase in the amplitude of the GABA-evoked current was well maintained during exposure to alphaxalone. In contrast, with higher concentrations of alphaxalone (1–30 μM) the initial potentiation frequently declined to a lower and approximately constant level during continued exposure to the drug. This effect was particularly pronounced at the higher concentrations (10–100 μM) of alphaxalone studied

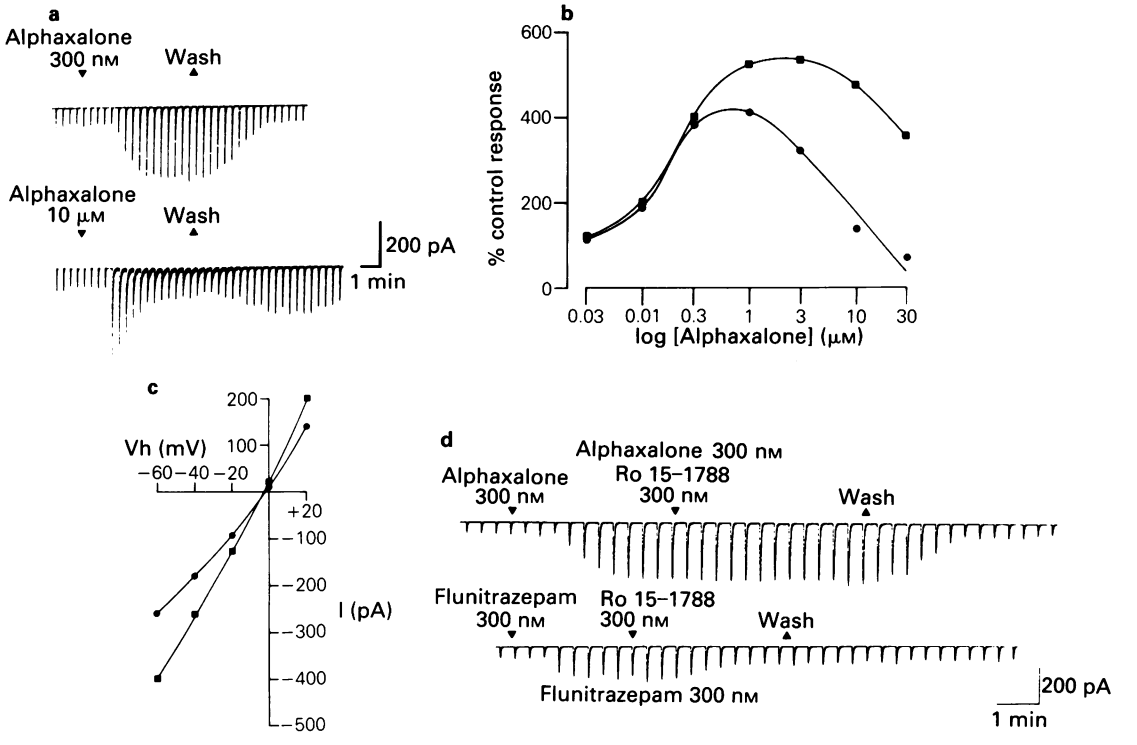

Figure 1 Potentiation by alphaxalone of whole cell currents evoked by locally applied γ-aminobutyric acid (GABA, 100 μM; 0.05 Hz, pressure applied at 1.4×10^5 Pa for 20 ms). (a) The upper and lower traces depict respectively the effect of 300 nM and 10 μM alphaxalone on GABA-induced currents recorded from a voltage-clamped chromaffin cell. Note that the potentiation of GABA-evoked currents in response to the higher dose of alphaxalone declines from an initial peak to an approximately steady state level during exposure to the drug and is accompanied by an increase in baseline current noise. (b) Dose-effect curve for the potentiating action of alphaxalone upon GABA-induced currents. The data points indicate the peak (■) and steady state (●) level of potentiation (plotted as a percentage of control response amplitude), as a function of the log of the concentration of alphaxalone in the bathing medium. Curves were fitted to the data points by eye. The graphs were constructed from data obtained from a single cell to which alphaxalone was non-cumulatively applied in increasing concentrations: representative traces appear in (a). (c) Relationships between response amplitude and holding potential for GABA (100 μM) applied locally in the absence (●) and presence (■) of alphaxalone (100 nM). The curves, fitted to the data points by eye, yield interpolated reversal potentials of − 2.0 and − 3.0 mV for GABA-induced responses in the absence and presence of alphaxalone (100 nM) respectively. All currents were low pass filtered at 500 Hz. (d) Influence of Ro 15-1788 (300 nM) upon the potentiation by alphaxalone (300 nM; upper trace) and flunitrazepam (300 nM; lower trace) of whole-cell currents evoked by locally applied GABA (100 μM). GABA-induced currents were recorded at a holding potential of − 60 mV and low pass filtered at 500 Hz. The data illustrated were obtained from the same chromaffin cell.

(Figure 1 a,b). In 6 cells, 100 μM alphaxalone first potentiated GABA-induced currents to 432.0 ± 124.4% of control, and then suppressed the response to GABA to 52.6 ± 27.4% of the control value within 5 min of exposure. Such observations may explain why alphaxalone has been observed either to potentiate (Harrison & Simmonds, 1984) or to suppress (Cullen & Martin, 1982), responses to GABA recorded from vertebrate central neurones.

The enhancement of GABA-evoked currents by alphaxalone could be due to one of several mechanisms, including a change in the reversal potential of the GABA response, or possibly an inhibition of the active uptake of GABA into the chromaffin cell (cf. Kataoka *et al.*, 1984). Both of these particular possibilities were excluded in the present experiments. As illustrated in Figure 1c, the potentiation of GABA-evoked currents by alphaxalone (100 nM) was not accompanied by any conspicuous change in the reversal potential of the response. In control experiments the GABA uptake blocker nipecotic acid (1 mM) had no effect upon the amplitude of GABA-evoked currents ($n = 4$).

Lack of effect of the benzodiazepine antagonist, Ro 15–1788

Bovine chromaffin cells possess high affinity binding sites for the benzodiazepine, [^{3}H]-flunitrazepam (Kataoka *et al.*, 1984), and diazepam potentiates GABA-evoked whole cell currents in these cells (Bormann & Clapham, 1985; Cottrell *et al.*, 1985). The diazepam-induced potentiation of GABA responses in bovine chromaffin cells is reversed by the benzodiazepine antagonist, Ro 15-1788 (Cottrell *et al.*, 1985). Ro 15-1788 (300 nM) did not, however, influence the alphaxalone-induced potentiation of whole cell GABA currents, although it did reverse the potentiation induced by flunitrazepam (300 nM) recorded from the same cell (Figure 1d). It is therefore unlikely that alphaxalone induces potentiation via an interaction with the benzodiazepine recognition site of the $GABA_A$ receptor (cf. Harrison & Simmonds, 1984).

The effect of betaxalone on GABA-evoked currents

The β-hydroxy isomer of alphaxalone, betaxalone, is inactive as an anaesthetic. In a sample of 7 cells, bath-applied betaxalone (100 nM–1 μM) had no detectable effect upon the amplitude of GABA-induced currents. In contrast, on all of the cells comprising this sample, alphaxalone (100 nM–1 μM) clearly enhanced the amplitude of the GABA-induced response, as in the experiment illustrated in Figure 2a. When higher concentrations of betaxalone were introduced into the medium, a reversible and dose-dependent suppression of the GABA-evoked current was observed. GABA-induced responses were reduced to 87.6 ± 6.5% ($n = 6$) and 35.9 ± 7.5% ($n = 5$) of their control values in the presence of 10 and 100 μM betaxalone respectively (Figure 2b).

Direct action of alphaxalone

In experiments where the potentiating action of relatively high (1–100 μM) concentrations of alphaxalone was examined, an increase in membrane noise was observed during the period of alphaxalone application (see, for example, the lower trace in Figure 1a). This effect could be detected by the appearance of discrete single channel currents on the whole cell when alphaxalone was examined, an increase in membrane noise was observed during the period of alphaxalone application to cells voltage clamped at negative potentials, the simultaneous activation of several channels gave rise to a small inward current. This direct action of the steroid was further investigated in experiments where alphaxalone (100 μM) was locally applied to cells by pressure ejection. Responses to locally applied GABA (100 μM) and alphaxalone (100 μM) recorded at a

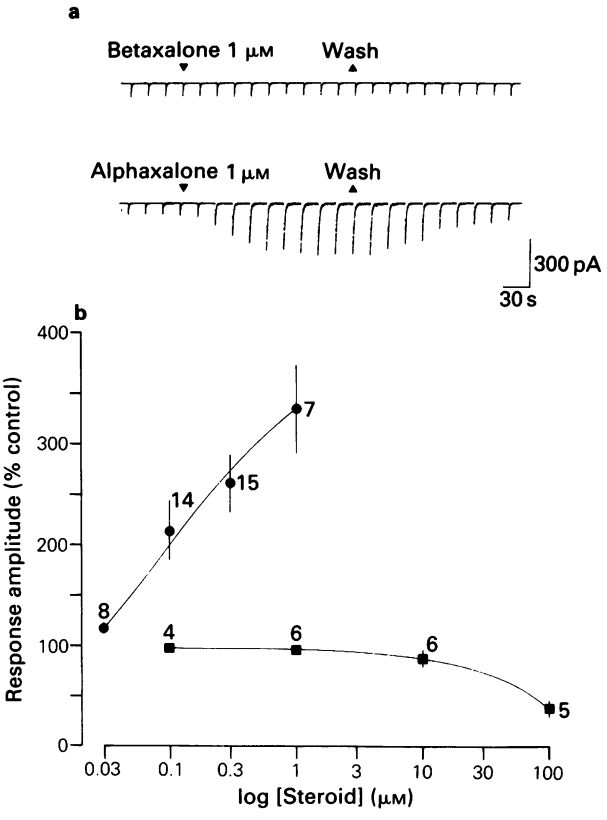

Figure 2 The effects of alphaxalone and betaxalone on whole cell currents evoked by locally applied γ-aminobutyric acid (GABA, 100 μM). (a) Comparison of the influence of equimolar concentrations (1 μM) of betaxalone (upper trace) and alphaxalone (lower trace) upon responses to GABA evoked from a cell voltage clamped at − 60 mV. Membrane currents were low-pass filtered at 500 Hz. (b) Graph comparing the actions of alphaxalone (●) and betaxalone (■) on GABA-induced currents. The amplitude of the GABA-evoked current, expressed as a percentage of its control value, is plotted against the log of the concentration of either alphaxalone or betaxalone in the bathing medium. Data points are the mean values of the number of observations indicated adjacent to each symbol. Vertical lines indicate the s.e.mean.

range of holding potentials from one chromaffin cell are shown in Figure 3. The responses to both agents displayed pronounced outward rectification, and reversed at a potential close to 0 mV. A previous investigation established that the GABA-evoked current in bovine chromaffin cells is carried predominantly by Cl ions (Cottrell *et al.*, 1985). With an approximately symmetrical distribution of Cl across the cell membrane ($[Cl^-]_o = 148$ mM;

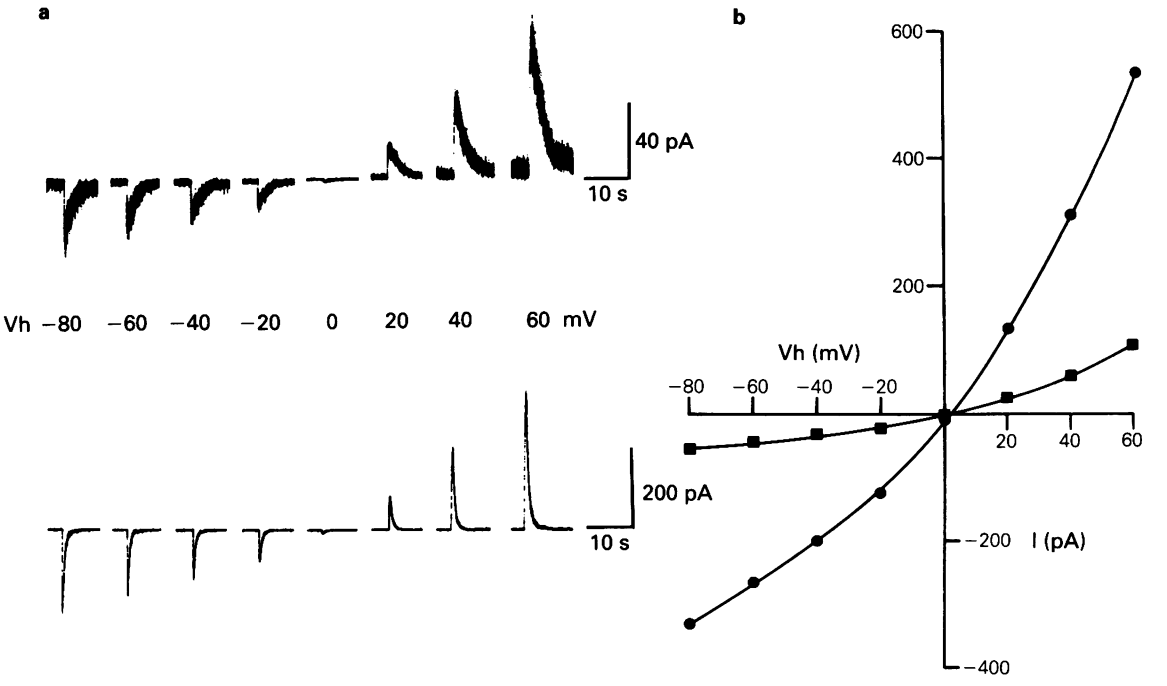

Figure 3 Comparison of the direct actions of alphaxalone and γ-aminobutyric acid (GABA) upon a voltage-clamped chromaffin cell. (a) The upper and lower rows of traces show respectively the currents evoked by locally applied alphaxalone (100 μM) and GABA (100 μM) at the holding potentials indicated adjacent to each response. All currents were low pass filtered at 500 Hz. Note the different calibration bars which apply to the GABA- and alphaxalone-induced currents. (b) Relationship between holding potential and response amplitude for GABA (●, 100 μM) and alphaxalone (■, 100 μM) derived from the data illustrated in (a). The GABA- and alphaxalone-induced currents have similar reversal potentials, determined by interpolation, of + 2.5 and + 1.5 mV respectively.

$[Cl^-]_i$ = 144 mM), the mean reversal potentials of the GABA- and alphaxalone-induced responses found in the present study were similar being 2.6 ± 0.5 (n = 6) and 0.4 ± 1.9 mV (n = 5) respectively.

The similarity of the reversal potentials of the alphaxalone- and GABA-induced responses is consistent with alphaxalone directly activating the GABA receptor-ion channel complex. In support of this view, inward currents evoked by locally applied alphaxalone (100 μM) were reversibly suppressed by bath application of the GABA$_A$ receptor antagonist, bicuculline (3 μM) (Figure 4a). Furthermore, phenobarbitone which is known to potentiate currents evoked by GABA in bovine chromaffin cells (Cottrell *et al.*, 1985), produced a striking enhancement of alphaxalone-induced currents. The results of such an experiment, illustrated in Figure 4b demonstrate that bath applied phenobarbitone (500 μM) reversibly enhanced the amplitude of inward currents evoked by locally applied alphaxalone (100 μM). In contrast, on cells in which a direct action of alphaxalone (100 μM) was evident, bath applied betaxalone (100 μM) did not induce an increase of membrane current noise (Figure 4c).

Further evidence for the view that alphaxalone exerts its agonist actions via an interaction with the GABA$_A$ receptor-ion channel complex was obtained on excised outside-out membrane patches. Bath-applied alphaxalone (1–10 μM) induced single channel current activity in patches that were previously quiescent (Figure 5). Such single channels exhibited similar conductance states to those of GABA and were antagonized by the GABA$_A$ receptor antagonist, bicuculline (not shown). Bath application of betaxalone (1–10 μM) did not directly activate ion channels.

Suppression of acetylcholine-evoked currents by alphaxalone

Bovine chromaffin cells possess nicotinic receptors that are pharmacologically similar to those of mammalian autonomic ganglia (Durant *et al.*, 1985; Lam-

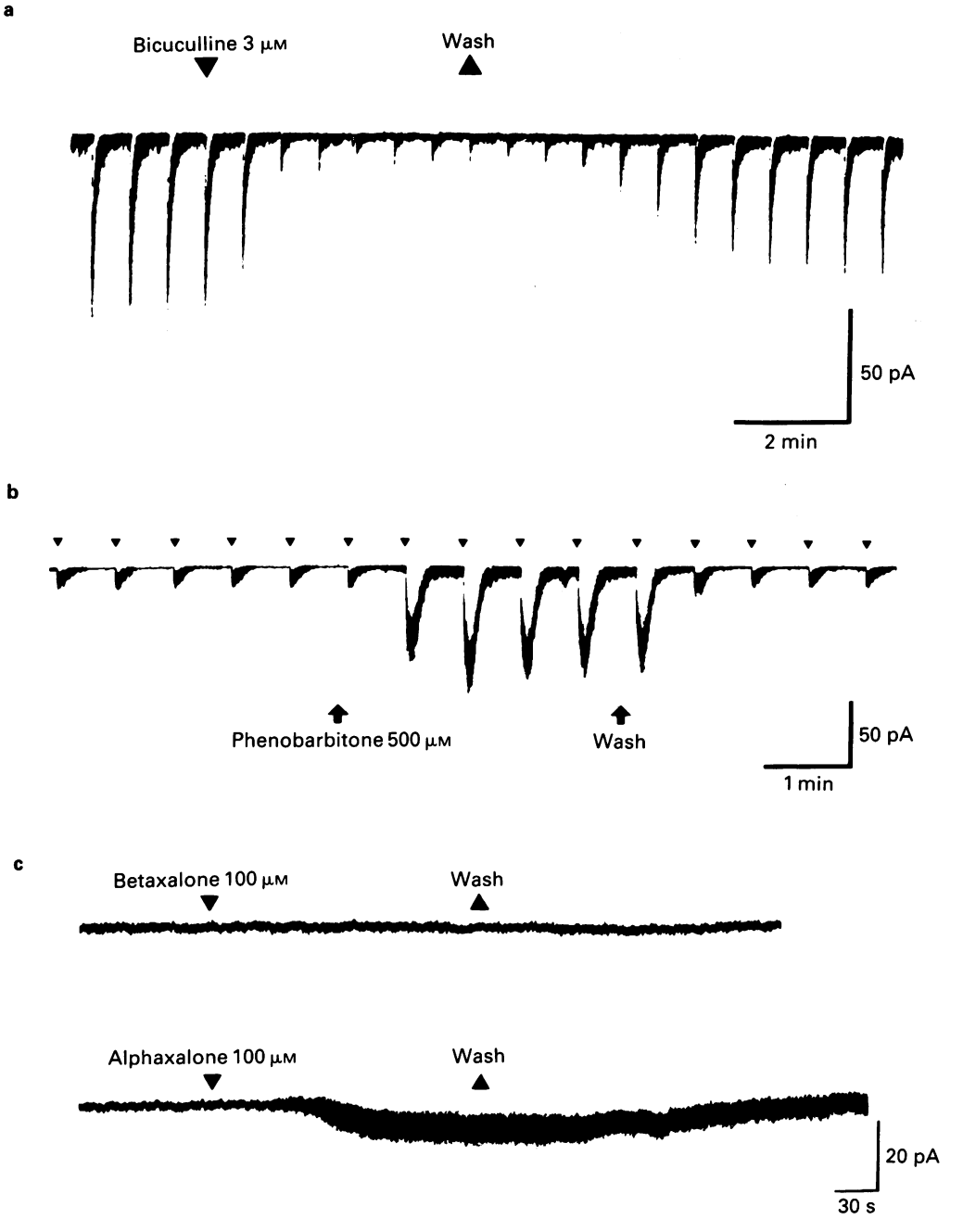

Figure 4 Modulation of alphaxalone-induced currents by compounds acting at γ-aminobutyric acid$_A$ (GABA$_A$) receptors and the lack of effect of betaxalone. (a) Reversible suppression of currents evoked by locally applied alphaxalone (100 μM) by bath-applied bicuculline (3 μM). (b) Reversible enhancement of alphaxalone (100 μM)-induced currents by bath-applied phenobarbitone (500 μM). (c) Bath-application of alphaxalone (100 μM), but not betaxalone (100 μM), directly elicited an inward current. Both agents were applied at a rate of 5 ml min^{-1}. All currents were recorded at a holding potential of − 60 mV and low pass filtered at 500 Hz.

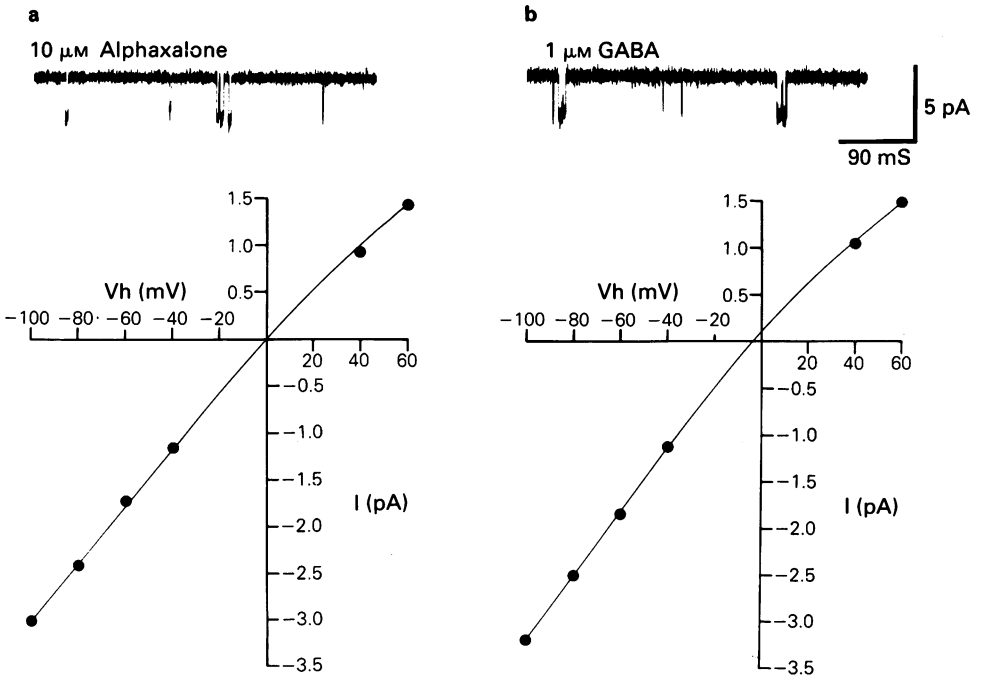

Figure 5 Single channel currents induced by alphaxalone and γ-aminobutyric acid (GABA) on outside-out membrane patches. Segments of data illustrating the predominant conductance state induced by (a) bath applied alphaxalone (10 μM) and (b) bath applied GABA (1 μM) are shown. The records in (a) and (b) were obtained from separate outside-out patches clamped at − 100 mV. Single channel currents were low-pass filtered at 2.0 kHz. Illustrated beneath each record are graphs showing the relationship between single channel current amplitude and transmembrane potential (range − 100 to + 40 mV). The slope of the line (fitted to the data points by eye) in the negative quandrant can be used to estimate single channel conductance. For alphaxalone- and GABA-activated channels the predominant conductance state was 29 pS and 32 pS respectively.

bert *et al.*, 1985; 1986). In order to gain information concerning the specificity of action of alphaxalone in the present system, experiments were conducted in which the influence of alphaxalone upon ACh-evoked whole cell currents was examined. To avoid complications in interpretation that might occur due to the direct agonist action of alphaxalone at the GABA receptor, such experiments were performed on cells that were insensitive to locally applied GABA (approximately 14% of the cell population, see Cottrell *et al.*, 1985).

Alphaxalone had no effect upon the amplitude of inward currents evoked by locally applied ACh (100 μM) when applied over a range of concentrations (300 nM–1 μM) sufficient normally to produce substantial enhancement of GABA-mediated responses. However, ACh-evoked currents were reversibly suppressed in a dose-dependent manner by the addition of higher concentrations (10–100 μM) of alphaxalone to the medium. An example of the blockade of ACh-induced responses produced by alphaxalone (30 μM) is illustrated in Figure 6. From pooled data obtained from 4 cells that were challenged with 10, 30 and 100 μM alphaxalone, the IC_{50} for blockade of ACh-evoked currents was estimated to be 20 μM (Figure 6). A somewhat lower IC_{50} for alphaxalone of 6 μM has been reported for the antagonism of ACh-evoked currents in cultured rat myoballs (Gillo & Lass, 1984).

Suppression of acetylcholine-evoked currents by betaxalone

In common with alphaxalone, betaxalone (10–100 μM) dose-dependently and reversibly, suppressed ACh-evoked currents. On one cell challenged with 3, 10, 30 and 100 μM betaxalone, the IC_{50} for blockade of the ACh-evoked currents was estimated to be 30 μM, a value similar to that found for alphaxalone.

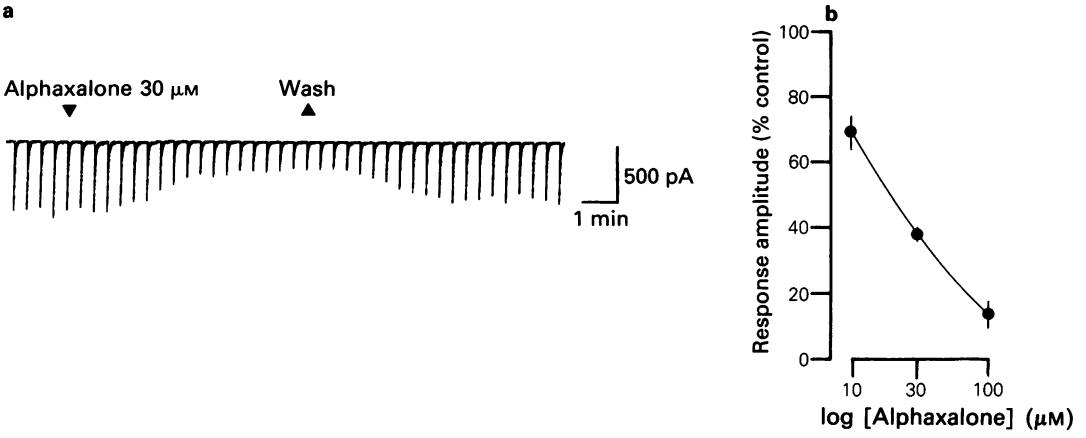

Figure 6 Effect of alphaxalone upon whole cell currents evoked by locally applied acetylcholine (ACh, 100 μM). (a) Reversible suppression of ACh-induced currents by bath-applied alphaxalone (30 μM). Currents were recorded at a holding potential of − 60 mV and low-pass filtered at 500 Hz. (b) Plot of the amplitude of whole cell currents evoked by locally applied ACh (100 μM) as a percentage of control against the log of the concentration of bath-applied alphaxalone. Data points are the mean of 4 observations. The vertical lines indicate s.e.mean. The curve, fitted to the data points by eye, yields an estimated IC_{50} for alphaxalone of 20 μM.

Discussion

The results described here confirm that under voltage-clamp conditions the steroidal anaesthetic alphaxalone can modulate the activity of $GABA_A$ receptors (cf. Harrison & Simmonds, 1984) and, in addition, show that higher concentrations of alphaxalone can directly activate the chloride channel linked to the $GABA_A$ receptor. Similar results have recently been reported for the actions of alphaxalone on $GABA_A$ receptors recorded from voltage-clamped rodent central neurones (Barker *et al.*, 1986; Cottrell *et al.*, 1986b). Alphaxalone does not influence glycine-evoked depolarizations in a slice preparation of the rat cuneate nucleus (Harrison & Simmonds, 1984), and far greater concentrations of alphaxalone are required to block ACh-induced currents and voltage-activated sodium currents than those required for modulation of the $GABA_A$ receptor (Cottrell *et al.*, 1986a). Collectively, these observations suggest that alphaxalone is a selective modulator of the $GABA_A$ receptor. Consistent with this view, alphaxalone does not modulate the binding to rat brain membrane preparations of ligands for muscarinic receptors or α_2-adrenoceptors, at concentrations that potenitate the binding of the $GABA_A$ receptor agonist, muscimol (Harrison & Simmonds, 1984).

Ethanol was used to dissolve alphaxalone in the present experiments. Ethanol (0.2%) had no effect on GABA-evoked whole cell currents nor did it activate ion channels (the highest concentration of ethanol used in the GABA potentiation experiments was 0.06%). The lack of effect of ethanol on GABA responses is consistent with previous observations made in a variety of neuronal preparations (Carlen *et al.*, 1982; Groul, 1982; Gage & Robertson, 1985) although others have suggested that ethanol may potentiate GABA-mediated neurotransmission (Nestros, 1980). Whilst the present experiments exclude an action of ethanol alone on GABA-evoked currents, we cannot completely exclude some synergistic effect between alphaxalone and ethanol.

The direct 'agonist' action of alphaxalone is unlikely to be due to a potentiation of background GABA as bovine chromaffin cells cultured in our laboratory do not contain GABA determined using high performance liquid chromatography techniques (D. Nicholls personal communication; cf. Kataoka *et al.*, 1984). Furthermore, alphaxalone-induced single channel currents on previously quiescent, isolated, outside-out membrane patches, deliberately positioned at least 5 mm away from the nearest cell.

Some barbiturate and benzodiazepine drugs potentiate GABA-induced responses in central neurones (e.g. Owen *et al.*, 1986) and bovine chromaffin cells (Bormann & Clapham, 1985; Cottrell *et al.*, 1985), apparently by different molecular mechanisms (Owen *et al.*, 1986). Studies employing fluctuation analysis suggest that pentobarbitone acts mainly to prolong greatly GABA channel open time, whereas some benzodiazepines act mainly to increase the frequency of GABA channel openings. In preliminary

experiments made on outside-out membrane patches, alphaxalone (300 nM), like pentobarbitone, greatly prolonged the single GABA channel open time confirming recent noise analysis studies (Barker *et al.*, 1986).

Specific binding sites for barbiturates and benzodiazepines (Squires & Braestrup, 1977; Johnston & Willow, 1982; Olsen *et al.*, 1986) closely associated with the $GABA_A$ receptor have been demonstrated. Of these two sites, an interaction of alphaxalone with the benzodiazepine site appears less likely, because the benzodiazepine antagonist Ro 15-1788 does not block the potentiating action of alphaxalone on GABA responses (present study, Harrison & Simmonds, 1984; Barker *et al.*, 1986). The prolongation of the GABA-activated channel by alphaxalone supports the suggestion that some barbiturates and alphaxalone may potentiate GABA via a common site or mechanism of action (Harrison & Simmonds, 1984). However, alphaxalone-induced currents were greatly potentiated by bath-applied phenobarbitone. Although not conclusive, such results do suggest that alphaxalone and barbiturates modulate GABA responses through different sites.

A number of general anaesthetics of different molecular structure from alphaxalone have been reported to potentiate GABA-mediated neurotransmission (Scholfield, 1980; Gage & Robertson, 1985) suggesting that such a mechanism may be involved in general anaesthesia. The concentration of alphaxalone in the brain during surgical anaesthesia in man is in the low micromolar range (Sear & Prys-Roberts, 1979). Although care must be taken in extrapolating the present results to those obtained *in vivo*, such concentrations of alphaxalone are consistent with the proposal that an enhancement of GABA-mediated synaptic transmission may in part be responsible for the anaesthetic effects of alphaxalone (Scholfield, 1980; Harrison & Simmonds, 1984). In support of this suggestion, the β-hydroxy isomer of alphaxalone, betaxalone, which is not an anaesthetic, neither potentiated GABA-evoked currents, nor activated the $GABA_A$ receptor. Indeed, high concentrations of betaxalone (10–100 μM) depressed, rather than enhanced, GABA-mediated responses. The observation that betaxalone is almost as effective as alphaxalone in blocking ACh-induced currents suggests that such a mechanism may not be important for alphaxalone in producing general anaesthesia.

Finally, the observations described here may be of physiological as well as pharmacological importance, as some endogenous progesterone metabolites have similar actions to those of alphaxalone, raising the fascinating possibility that steroids may act as endogenous modulators of the $GABA_A$ receptor (Barker *et al.*, 1986; Turner, 1986; Callachan *et al.*, 1986).

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