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The action of GABA receptor agonists and antagonists on muscle membrane conductance in *Schistocerca gregaria*

V.F. Murphy & [1]K.T. Wann

Wellcome Research Laboratories, Langley Court, Beckenham, Kent, BR3 3BS

1 The properties of postsynaptic γ-aminobutyric acid (GABA) receptors in the extensor tibiae muscle of *Schistocerca gregaria* were studied by conventional electrophysiological recording techniques.

2 GABA and other active GABA receptor agonists produced rapid, dose-dependent, reversible increases in membrane conductance.

3 In two microelectrode experiments the ED_{50} for GABA was approximately 1 mM. In three microelectrode experiments (assuming short cable theory conditions) the ED_{50} for GABA was 2.3 mM. The Hill coefficient for GABA estimated from the latter experiments was 1.4

4 The relative potency of muscimol/GABA at the ED_{50} for GABA was 1.36. 3-Aminopropane sulphonic acid (3-APS) and isonipecotic acid were weakly active, baclofen and piperidine-4-sulphonic acid (P4S) were inactive. Isoguvacine produced depolarizations and increases in conductance in preparations which hyperpolarized in response to GABA. These depolarizations were enhanced by both picrotoxin and pitrazepin although the increases in input conductance were depressed.

5 Picrotoxin (20 μM), (+)-bicuculline (20–100 μM) and pitrazepin (1–10 μM) all reversibly antagonized GABA-induced responses. Such antagonism was not competitive in the case of picrotoxin and (+)-bicuculline but was competitive for pitrazepin. Schild plot analysis gave an average pA_2 value of 5.5 for pitrazepin.

6 The significance of these results is briefly discussed.

Introduction

It is now well established that γ-aminobutyric acid (GABA) is an important inhibitory transmitter in many invertebrate systems (for references see Nistri & Constanti, 1979). The properties of GABA-activated channels in several systems have been derived from studies of inhibitory junctional currents (Onodera & Takeuchi, 1976; Dudel *et al.*, 1977; Adams *et al.*, 1981; Cull-Candy, 1984) or from noise analysis (Dudel *et al.*, 1977; Cull-Candy & Miledi, 1981). Pharmacological analysis of the invertebrate GABA receptor has been studied principally with crayfish stretch receptor neurones (Hori *et al.*, 1978; Krause *et al.*, 1981), crustacean muscle (Takeuchi & Takeuchi, 1975; Constanti, 1977a,b) and molluscan neurones (Walker *et al.*, 1971). In the case of insect muscle GABA receptors, picrotoxin is known to be an antagonist (e.g. Usherwood & Grundfest, 1965) and the activity of aliphatic amino acids of different chain lengths has been studied (Scott & Duce, 1987a). Such studies reveal interesting differences between invertebrate GABA receptors and the mammalian $GABA_A$ type (Krogsgaard-Larsen *et al.*, 1980). In particular, potency ratios for receptor agonists which are almost equieffective in mammalian systems such as muscimol, 3-aminopropane sulphonic acid (3-APS), piperidine-4-carboxylic acid (isonipecotic acid) and 4,5,6,7-tetrahydroisoxazolo 5,4-c. pyridin-3-ol (THIP) vary over almost two orders of magnitude in an invertebrate system (Krause *et al.*, 1981). Also it is clear that (+)-bicuculline and picrotoxin are not true competitive and non-competitive antagonists (Werman & Brookes, 1969; Takeuchi & Onodera, 1972; Hori *et al.*, 1978; Constanti, 1978; Scott & Duce, 1987a).

We now describe the results of an investigation into the pharmacology of the GABA receptor in the

[1] Author for correspondence at present address: Division of Anaesthesia, Clinical Research Centre, Watford Road, Harrow, Middlesex HA1 3UJ.

extensor tibiae muscle of *Schistocerca gregaria*. Only about 10% of the fibres receive direct inhibitory innervation (Usherwood & Grundfest, 1965), fibres at both the proximal (Usherwood & Grundfest, 1965; May *et al.*, 1979) and the distal end (Duce & Scott, 1983) being sensitive to GABA. In this study the effects of bath-applied agonists and antagonists were tested on the membrane conductance of fibres in bundle 33 of the extensor tibiae muscle. The pharmacology of this GABA receptor system has received comparatively little attention, the sensitivity to a range of $GABA_A$-receptor agonists being unknown. Such information is potentially exploitable in the development of insecticidal agents. A preliminary note has appeared (Murphy & Wann, 1985).

Methods

Preparation

Experiments were carried out on individual fibres of the extensor tibiae muscle of male *Schistocerca gregaria*. The muscle was exposed in the isolated hind femur by removal of the flexor tibiae muscle and pinned out ventral side uppermost on the Sylgard base of a Perspex tissue chamber (2.5 ml volume). The muscle fibres were stretched to their maximum physiological length (about 2 mm). The preparation was maintained at 22°C and continuously superfused via a Watson-Marlow peristaltic pump (flow rate typically 4.5 ml min^{-1}). The composition of the locust saline was (mM): NaCl 170, KCl 10, $MgCl_2$ 10, $CaCl_2$ 2, HEPES buffer 10, pH 6.8.

Recording

For most experiments the central portion of superficial fibres in bundle 33 was impaled with two glass microelectrodes filled with 1 M potassium citrate, with resistances of 10–40 MΩ. Hyperpolarizing current pulses of 2–30 nA, 400–600 ms duration, 0.1 Hz were passed through one of the intracellular microelectrodes and the resulting electrotonic potential recorded by the other microelectrode (interelectrode distance $< 50\,\mu m$). Membrane potential and current recordings were displayed simultaneously on a Tektronix 5111 oscilloscope and a Gould 2200S pen recorder. For three microelectrode experiments a second voltage sensing electrode was inserted at the end of the same cell (i.e. a distance of half a muscle length from the first voltage-sensing electrode) where it joined the apodeme. Test compounds were bath applied for between 3 and 7 min, steady state being achieved within 2 to 4 min (e.g. Figure 1). No decline in the conductance responses to GABA or other agonists was observed during such long periods of drug application. Such desensitization has been observed previously with crab and cryfish muscle (Epstein & Grundfest, 1970; Dudel & Hatt, 1976). The full current/voltage (I/V) curve was determined before the addition of drug, once a steady-state was reached during drug application, and after washout.

Calculations

The membrane conductance of single muscle fibres and the conductance change induced by agonists was calculated essentially as in Takeuchi & Takeuchi (1967), Constanti (1977a) and Scott & Duce (1987b). The input conductance (G_0) of a semi infinite cable is given by $G_0 = \frac{1}{2}I/V_0$ where I and V_0 are the injected current and electrotonic potential respectively. However the space constant (λ) of locust muscle fibre is long with respect to the muscle length (e.g. mean $L/\lambda = 0.41 \pm 0.04$, $\bar{x} \pm 1 \times$ s.e., $n = 17$), thus it is not appropriate to treat the cell as a semi infinite cable. It is more relevant to adopt 'short' cable equations when the cable is assumed to terminate at an infinite resistance (Weidmann, 1952; Takeuchi & Takeuchi, 1967). The input conductance depends on both the membrane conductance and the muscle length/space constant ratio, and in order to compare the membrane conductance under different conditions (e.g. during agonist application) it was necessary to calculate the space constant in these conditions. This was achieved in some experiments by inserting a second voltage-sensing electrode at the end of the muscle fibre to determine the decrement in electrotonic potential and hence the space constant.

In the case of a short cable with an infinite resistance,

$$\frac{V_0}{V_L} = \cosh \frac{L}{\lambda} \tag{1}$$

$$\frac{2V_0}{I_0} = r_i \lambda \coth\left(\frac{L}{\lambda}\right) \tag{2}$$

$$\lambda = \sqrt{\frac{r_m}{r_i}} \tag{3}$$

Where V_0 and V_L are the electrotonic potentials at the centre and the end of the muscle fibre respectively; L is the half length of the muscle fibre (cm), λ is the space constant (cm), r_i is the longitudinal resistivity ($\Omega\,cm^{-1}$) and r_m is the transverse membrane resistance ($\Omega\,cm$). λ was calculated in three microelectrode experiments from (1).

From (3),

$$r_i = \frac{r_m}{\lambda^2} \quad \text{or} \quad \frac{1}{\lambda^2 g_m}$$

Substituting for r_i in (2)

$$\frac{2V_0}{I_0} = \frac{I}{\lambda^2 g_m} \lambda \coth\left(\frac{L}{\lambda}\right)$$

$$\therefore \quad g_m = \frac{I_0}{2V_0} \frac{1}{\lambda} \coth\left(\frac{L}{\lambda}\right) \qquad (4)$$

membrane conductance per cm (g_m) was calculated from (4) and the membrane conductance of single fibres was expressed as $g_m L$. The GABA-induced conductance change $\Delta G_m L$ was calculated from the difference in values of $g_m L$ in control and drug containing solutions.

At high doses of agonist where the conductance increased more than five fold, V_L was too small to measure accurately due to base line noise. Using a value of λ obtained from the same fibre under control conditions, an alternative method for calculating λ' in the presence of agonist was employed, as described by Takeuchi & Takeuchi (1967), using the ratio of the electrotonic potentials at the centre of the fibre in the presence (V_0') and absence (V_0) of agonist, where:

$$\frac{V_0'}{V_0} = \frac{L}{\lambda} \tanh \frac{L}{\lambda} \left(\frac{L}{\lambda'} \tanh \frac{L}{\lambda'}\right)^{-1} \qquad (5)$$

A mean of the control λ and V_0 values before drug application and after recovery was used for calculating λ', G_0 and $\Delta g_m L$. A BBC microcomputer was used for calculations involving equation (5). The degree of base line noise occurring in this preparation made the second method of calculating λ' (using equation (5)) likely to be the more accurate analysis. When comparing relative potencies of agonists input conductance (G_0) values were used. However, in quantitative studies of the action of GABA, conductance was calculated according to equations (4) and (5). For construction of the dose-response curves in Figures 2, 5 and 7 and for the Hill plot in Figure 3, the data were normalised by a method described by Constanti (1977a) since it was not always possible to obtain a true maximum response in all experiments. A reference dose of GABA was thus selected (0.33 mM) which produced a mean conductance change close to 1× the initial resting conductance (1.1 ± 0.1; $\bar{x} \pm$ s.e., $n = 66$). All other responses were expressed as a proportion of the response to the reference dose.

Drugs

GABA, muscimol, 3-aminopropane sulphonic acid (3APS), isonipecotic acid, picrotoxin and (+)-bicuculline were obtained from Sigma, piperidine-4 sulphonic acid (P4S) and isoguvacine from Cambridge Research Biochemicals, β-*p*-chlorophenyl GABA ((−)-baclofen) from Ciba-Geigy and pitrazepin from Sandoz. *Trans*-4-aminocrotonic acid was synthesized. All except picrotoxin and (+)-bicuculline were dissolved in Ringer solution. Picrotoxin was dissolved initially in ethanol to give a final concentration of <1% ethanol in the Ringer solution. Where picrotoxin was used, an equivalent concentration of ethanol was used throughout the experiment. (+)-Bicuculline was dissolved initially in 20 mM HCl and made up in Ringer solution to the required concentration immediately prior to application. This ensured that breakdown to the less active bicucine reported by Olsen *et al.* (1976) was unlikely to be significant. The antagonists (picrotoxin, (+)-bicuculline and pitrazepin) were applied to the preparation 5 min before and during the application of agonists.

Results

Properties of muscle fibres under control conditions

The resting potentials of the fibres selected for study were in the range −40 mV to −66 mV ($\bar{x} = -52$ mV, $n = 53$). Input conductance (G_0) values varied from 0.14 μS to 0.66 μS ($\bar{x} = 0.35$ μS, $n = 53$) and λ, when measured, had a mean value of 2.6 mm, ranging from 1.1 mm to 4.4 mm ($n = 15$). The membrane time constant (τ_m) for a short cable was calculated for five fibres according to Stefani & Steinbach (1969) and ranged from 36 ms to 80 ms ($\bar{x} = 61$ ms, $n = 5$). Muscle fibre length was between 1.6 mm and 2.3 mm ($\bar{x} = 1.9$ mm, $n = 21$) and the cells were 50–100 μm in diameter (measured by use of a microscope eye-piece graticule). Stable recording conditions could normally be maintained for several hours during multiple solution changes, although in some preparations a slow drift in membrane potential and/or input conductance was observed. Current-voltage relationships were normally linear through the origin within the normal range of values.

Effect of GABA on membrane conductance

The sensitivity to GABA increased slightly (an increase in the amplitude of the conductance change occurred) following the first application. 'Sensitization' to GABA (faster onset of response) has previously been reported by Brookes *et al.* (1973) in *Locusta migratoria*. A conditioning dose of GABA (1 mM) was therefore applied to the preparation at the start of each experiment, and washed out after 1 min. Subsequent drug application then produced reproducible dose-dependent conductance changes.

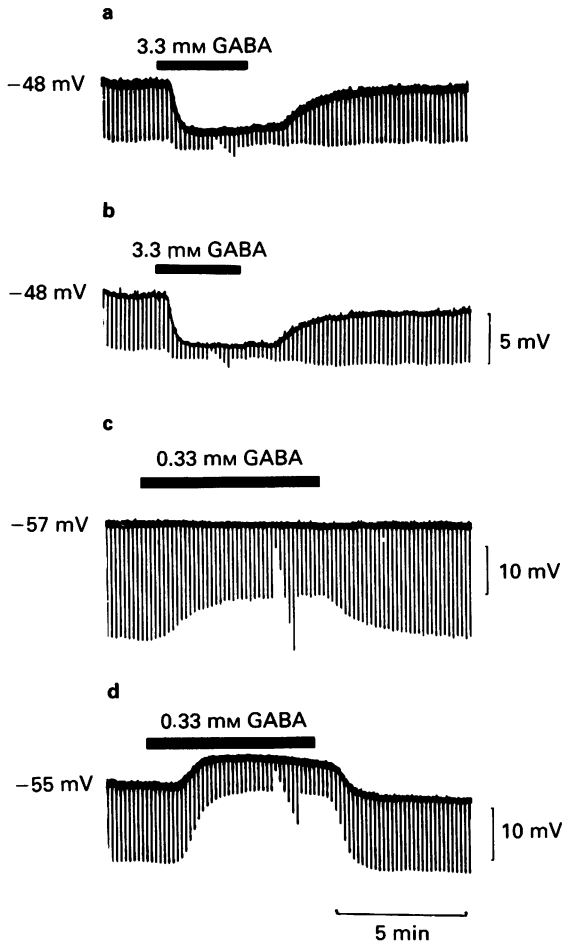

Figure 1 The effect of GABA on the hyperpolarizing electrotonic potentials (downward deflections) recorded at the centre, (a), (c) and (d) or end, (b) of locust muscle fibres in response to intracellular current pulses applied via a second central microelectrode (500 ms, 0.1 Hz): (a) and (b) were simultaneous recordings from a single muscle fibre, current pulse amplitude 6 nA (2–10 nA for measurement of the current-voltage relationship). Bath-applied GABA caused the cell to hyperpolarize, increased the membrane conductance from 0.6 μS to 2.6 μS and decreased the space constant (λ) from 3.9 mm to 1.6 mm (calculated according to short cable theory). (c) and (d) were two separate experiments where GABA (0.33 mM) caused no change in membrane potential and a depolarization respectively, and an increase in input conductance in both cases. Current pulses were: (c) 12 nA (4–20 nA for I/V plot), and (d) 15 nA (5–30 nA for the I/V plot).

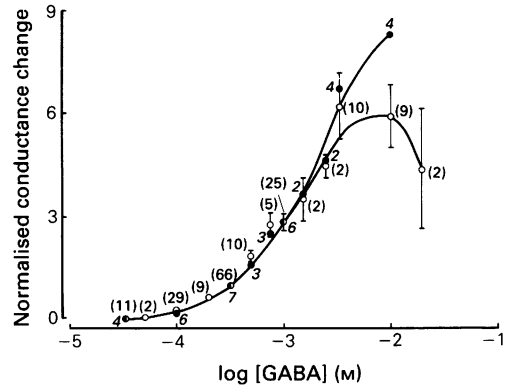

Figure 2 Normalized GABA dose-response curves obtained from 53 experiments (○), conductance changes calculated from $\frac{1}{2}I/\Delta V_0$ (number of measurements per point given in parentheses, bar heights represent 1 × s.e.), and 6 experiments (●), conductance changes calculated according to equation (4) (number of measurements per point given in italics). Curves were fitted by eye.

Membrane potential changes, however, were not maintained with repeated agonist application.

GABA produced a reversible dose-dependent change in input conductance which during early applications usually resulted in hyperpolarization of the membrane potential (Figures 1a and b). In a few preparations the membrane potential was unaffected or the cell depolarized (Figures 1c and d).

Pooled dose-response data for GABA calculated from $\frac{1}{2}I/V_0$ ratios or equation (4) are shown in Figure 2. In both cases the 'apparent' maximum conductance change is achieved at approximately 10 mM. From 53 experiments the apparent maximum represented a 6 fold change in input conductance calculated from the $\frac{1}{2}I/V_0$ ratio, and the ED_{50} was 1.05 mM. For the 6 experiments where conductance changes were calculated from equation (4) (λ' obtained using equation (5)), the apparent maximum represented a 9 fold change in input conductance and the ED_{50} was 2.3 mM. GABA 20 mM produced smaller responses than 10 mM (see Figure 2).

Data for the experiments shown in Figure 2 (up to 80% max conductance change) were used to construct a Hill plot (Figure 3) and a log dose/log response plot (not shown). The Hill coefficients obtained from these plots (as described by Constanti (1977a)) were 1.4

Effect of GABA analogues on membrane conductance

Muscimol produced responses similar to GABA (Figure 4a) and was slightly more potent (Figure 5). The relative potency (muscimol/GABA) at the ED_{50}

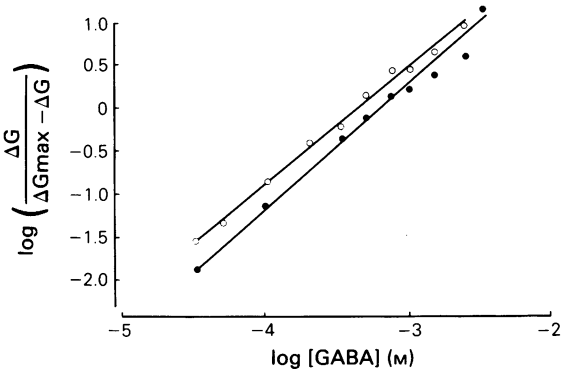

Figure 3 Hill plots derived from the GABA dose-response data shown in Figure 4 (○) conductance calculated from $\frac{1}{2}I/V_0$ and (●) conductance calculated using equation (4). The lines were fitted by eye, the Hill coefficients are 1.4 in both cases.

for GABA was 1.36. The maximum response for muscimol was not determined but 3.3 mM produced a 6.4 fold change in input conductance (3 experiments) which was similar to the apparent maximum response for GABA. Hill coefficients obtained from the dose-response curve or the limiting slope of the log dose-log response curve were 1.35 and 1.33 respectively. In one experiment *trans*-4-amino crotonic acid was equipotent with GABA. In two experiments P4S (>10 mM) was inactive in this preparation (Figures 4b and 5). (−)-Baclofen (<10 mM) was also inactive. 3-APS and isonipecotic acid had only very weak activity producing small conductance increases and hyperpolarizations at concentrations >1 mM (Figure 4c and d). 3-APS and isonipecotic acid at 10 mM produced only 0.14 and 0.27 fold changes respectively (3 experiments in each case) in input conductance (Figure 5). Potency ratios relative to GABA at the dose giving a 0.1 fold change in input conductance were 0.007 (3-APS) and 0.028 (isonipecotic acid). It was obviously not possible to compare the potency of these agonists at the GABA ED_{50} value.

In four experiments isoguvacine (2 mM) produced significant increases in input conductance accompanied by large depolarizations of the membrane, whereas GABA produced hyperpolarizations in the same preparations. Interestingly, the conductance change by 2 mM isoguvacine was greatest in cells which were least sensitive to GABA. Isoguvacine (1 mM) produced hyperpolarizations, depolarizations or no change in membrane potential in seven preparations. These variable reponses may also be related to the sensitivity of the preparation to GABA but further study is required to resolve this.

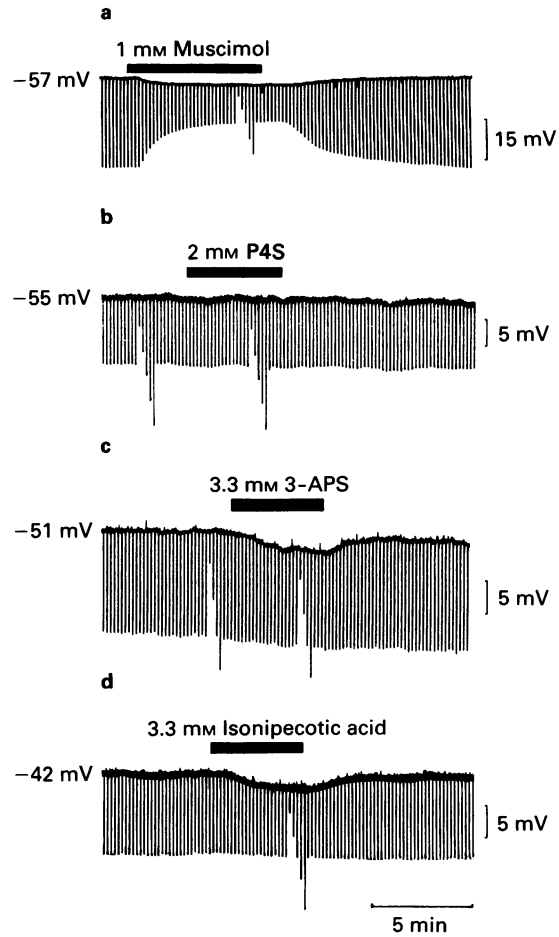

Figure 4 Examples of typical responses to GABA analogues from separate experiments: (a) muscimol (1 mM), test current pulse 6 nA, 2–10 nA for the I/V plot; (b) piperidine-4 sulphonic acid (P4S, 2 mM), 10 nA current pulse, I/V plot 4–20 nA, (c) 3-aminopropane sulphonic acid (3-APS, 3.3 mM), 15 nA current pulse, I/V plot 5–20 nA, and (d) isonipecotic acid (3.3 mM), 6 nA current pulse, I/V plot 2–10 nA.

In two experiments isoguvacine was able to evoke depolarizations in preparations that had been desensitized to their excitatory transmitter, glutamate, by preincubation in 1 mM glutamate (see Cull-Candy, 1984). In these experiments miniature excitatory junctional potentials were abolished and the preparation was unresponsive to high doses of glutamate (5 mM) (Figure 6b). Isoguvacine however, still produced dose-dependent reversible depolarizations and changes in input conductance (Figure 6c and e).

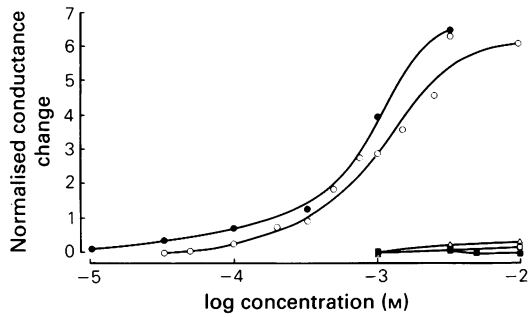

Figure 5 Normalized dose-response curves for GABA (○) (see also open symbols in Figure 2): muscimol (●), (6 experiments 2–6 measurements per point); 3-aminopropane sulphonic acid (□), (4 experiments, 2–4 measurements per point); piperidine-4 sulphonic acid (■), (2 experiments, 1–2 measurements per point) and isonipecotic acid (△), (4 experiments, 3–4 measurements per point). Conductance changes were calculated from G_0 values obtained before and during agonist application. Curves were fitted by eye.

Effects of GABA receptor antagonists

Picrotoxin (20 μM), (+)-bicuculline (20–100 μM) and pitrazepin (1–10 μM) all reversibly antagonized GABA responses. Picrotoxin and (+)-bicuculline effects were immediately reversed on washout, but full reversal of the effects of pitrazepin took between 30 and 60 min. Dose-response curves for GABA in the presence and absence of antagonist were constructed applying, where possible, alternate treatments of GABA and GABA plus antagonist to the preparation. This experimental protocol could not be used for pitrazepin because of the slow reversibility of the action of this drug. Full dose-response curves were obtained for GABA in the presence of 20 μM picrotoxin (not shown) and 20, 50 and 100 μM (+)-bicuculline (Figure 7), and a Schild plot analysis (see Simmonds, 1980) was carried out for (+)-bicuculline (not shown) and pitrazepin (Figures 8 and 9), in experiments where only the lower part of the dose-response curve was studied.

The antagonism by picrotoxin and (+)-bicuculline appeared not to be competitive. Picrotoxin (20 μM) reduced the apparent maximum by 20% and shifted the dose-response curve to the right (dose-ratio 1.3 at the dose giving a 1 fold change in input conductance). An interesting observation was the apparent small alteration in onset and recovery rates of the fibre in response to GABA in the presence of picrotoxin. Picrotoxin apparently slightly delayed the onset of the GABA response and in particular enhanced the rate of recovery on washout in this preparation. Experiments with better time resolution are necessary to examine this effect more reliably.

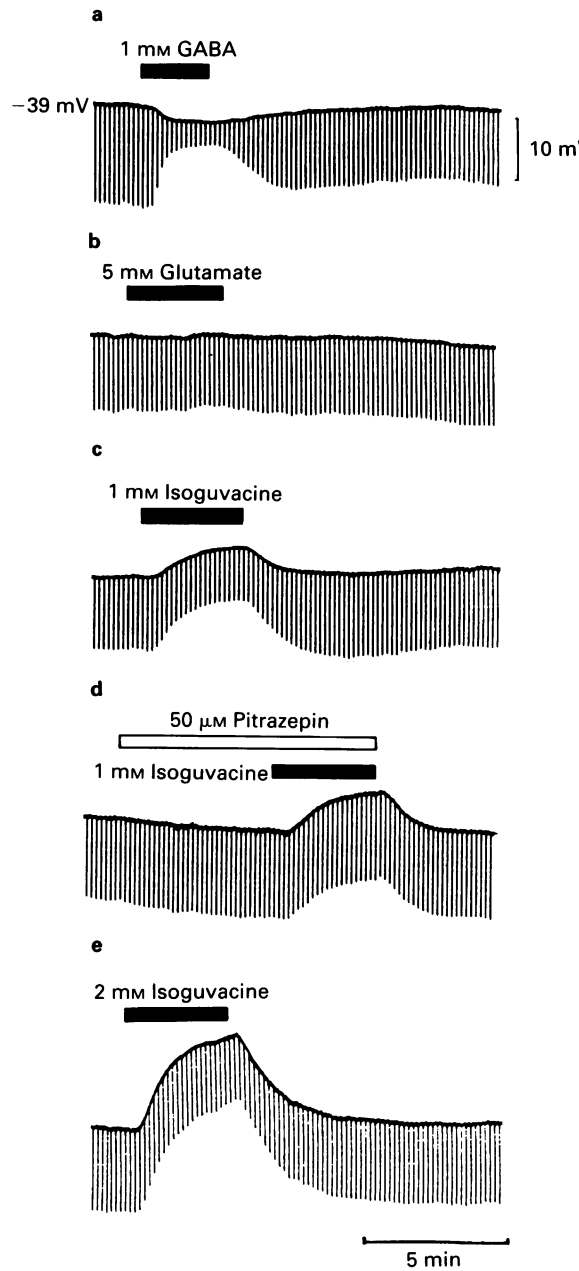

Figure 6 Data from a single muscle fibre desensitized to glutamate by preincubation in 1 mM glutamate. Note the absence of miniature excitatory junctional potentials (cf. Figures 1 and 4). Effects of (a) GABA (1 mM), (b) glutamate (5 mM), (c) isoguvacine (1 mM), (d) isoguvacine (1 mM) in the presence of 50 μM pitrazepin (note the increase in depolarization and the reduced conductance change) and (e) isoguvacine (2 mM). The standard test current pulse was 5 nA, 600 ms, 0.1 Hz.

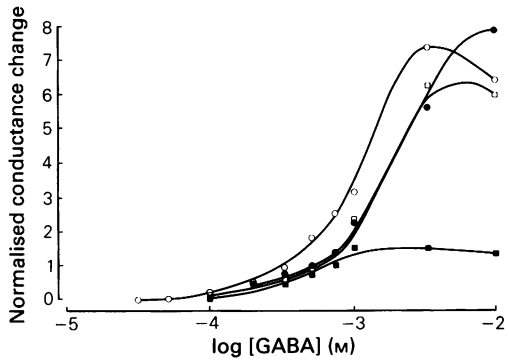

Figure 7 Normalized dose-response curves for GABA and GABA in the presence of (+)-bicuculline applied 5 min before and during the application of each dose of GABA: GABA control curve (○), (pooled data from 16 experiments, 1–21 measurements per point); GABA with 20 μM (+)-bicuculline (●), (8 experiments, 1–5 measurements per point); GABA with 50 μM (+)-bicuculline (□), (5 experiments, 1–3 measurements per point) and GABA with 100 μM (+)-bicuculline (■), (8 experiments, 1–5 measurements per point).

(+)-Bicuculline (20 μM) also caused a right shift in the dose-response curve (dose-ratio 1.5 at the dose giving a 1 fold change). The maximum response, however, was not supressed. (+)-Bicuculline at 50 and 100 μM also shifted the dose-response curve (dose-ratios 2 and 2.1 respectively) and suppressed

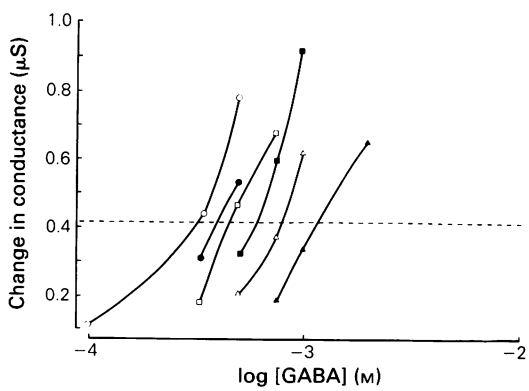

Figure 8 Dose-response curves for GABA and GABA in the presence of pitrazepin applied 5 min before and during the application of each dose of GABA. All points are taken from a single experiment: GABA control curve (○); GABA with 1 μM pitrazepin (●); GABA with 2 μM pitrazepin (□); GABA with 3.3 μM pitrazepin (■); GABA with 5 μM pitrazepin (△) and GABA with 10 μM pitrazepin (▲). Dashed line indicates reference conductance used for calculation of dose-ratios.

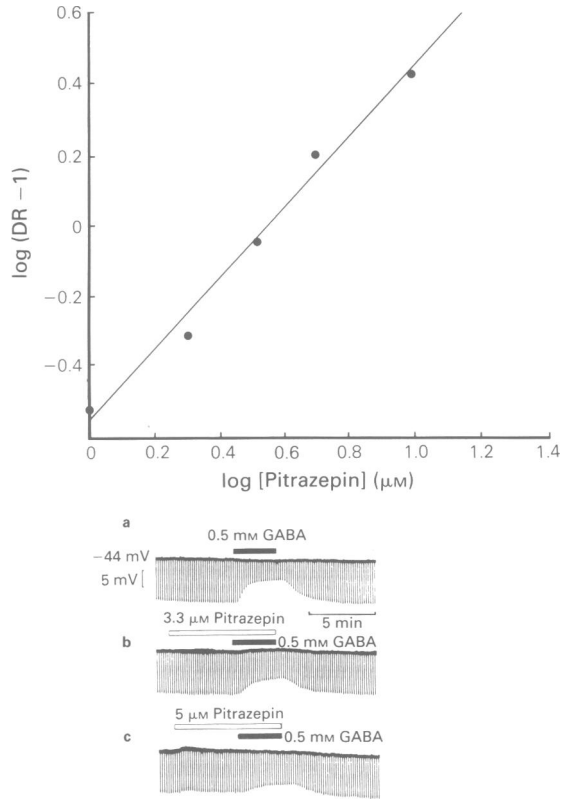

Figure 9 Schild plot of GABA-pitrazepin data from a single experiment $K_B = 3.6 \times 10^{-6}$ and $pA_2 = 5.5$. Insets: examples of the response to (a) GABA (0.5 mM) and (b) GABA (0.5 mM) in the presence of 3.3 μM pitrazepin and (c) GABA (0.5 mM) in the presence of 5 μM pitrazepin.

the maximum response by 15% and 79% respectively (Figure 7).

A Schild plot analysis was carried out on data from three experiments with (+)-bicuculline. A series of dose-response curves (the lower part only) were obtained with increasing concentrations of the antagonist. Dose-ratios (DR) were calculated at a response intersecting all curves (representing a 0.65 fold change in conductance). Schild plots of these data (log (DR − 1) against log antagonist concentration) gave non-linear plots in all three cases, and no significant part of any of the curves gave a slope of 1. pA_2 values varied from 3.9 to 4.4 ($\bar{x} = 4.11$). This indicates that the antagonism is not competitive. A Schild plot analysis of data from two experiments with pitrazepin gave linear plots and a regression analysis (allowing slope to vary) gave estimated slopes of 1 (i.e. 0.994) indicating competitive interaction. pA_2 values were 5.5 in both experiments.

Dose-response curves obtained for muscimol in the presence of (+)-bicuculline 20 μM (data not shown) all showed a non-parallel right shift indicating that the antagonism was not competitive. (+)-Bicuculline was equieffective against GABA and muscimol responses. The effects of pitrazepin on muscimol responses were not studied.

Preliminary data were obtained on the action of 100 μM picrotoxin and 50 μM pitrazepin on the depolarizing response of isoguvacine. The input conductance changes in response to 2 mM isoguvacine were reduced by 40% and 100% respectively, and in both cases the associated depolarizations were increased in the presence of the antagonists by 20% and 120% respectively (see e.g. Figure 6d).

Discussion

These experiments with the extensor tibiae muscle of *Schistocerca gregaria* provide the first electrophysiological analysis of a range of $GABA_A$ receptor agonists on an insect muscle GABA receptor. Previously only *trans*-4-aminocrotonic acid has been tested (Scott & Duce, 1987a). It is clear that there are both similarities and differences between the locust muscle GABA receptor system and the mammalian $GABA_A$ receptor. The sensitivity of this preparation to GABA is about 20 times less than that of lobster muscle (Constanti, 1977a). GABA sensitivity is also low compared with mammalian systems, but is consistent with previous published data (Brookes *et al.*, 1973; Duce & Scott, 1983). The variability between cells of GABA-induced membrane potential changes agrees with previous reports (Usherwood, 1973; Duce & Scott, 1985) and implies that intracellular chloride concentration is different from fibre to fibre. The observed reductions in membrane potential changes on repeated agonist application presumably indicates that bath-applied GABA leads to appreciable chloride loading in these cells.

A typical maximum conductance change to GABA in a single fibre was about 2 μS in this study. The mean conductance of a single chloride channel is reported as 22 pS at −80 mV (21°C) by Cull-Candy & Miledi (1981). Thus the observed maximum response represents about 90,000 channels in the open state. Cull-Candy (1984) has estimated the number of channels involved in an inhibitory junction current as about 30,000. Extrajunctional receptors, identified by Cull-Candy & Miledi (1981) may account totally for the extra conductance induced by bath-applied agonists or it may be indicative of multiple innervation or 'spare' receptors at the inhibitory synapse. Analysis of the dose-response data obtained here shows that there is some degree of cooperativity in the interaction of GABA with the receptor. The Hill coefficient of 1.4 indicates the likelihood of more than one binding site per receptor, and there was good correlation between the Hill coefficients obtained from the Hill plot and the log/log plot. The Hill coefficient from muscle bundle 33 obtained here is somewhat lower than the value of 1.7 obtained by Scott & Duce (1987b) for the same muscle bundle.

Muscimol was slightly more potent than GABA, P4S was inactive and 3-APS and isonipecotic acid were only weakly active, having 1/135 and 1/36 of the potency of GABA respectively. Conductance responses to isoguvacine were larger, but the depolarizations observed were not characteristic of GABA receptor responses. Picrotoxin and (+)-bicuculline were both fairly weak antagonists of the GABA response, and both were apparently not competitive in action. Pitrazepin was, however, a more potent competitive antagonist.

The agonists tested (with the exception of (−)-baclofen) were all recognised $GABA_A$ receptor agonists. In radiolabelled ligand binding experiments and in electrophysiological studies with mammalian systems, muscimol is invariably more potent and 3-APS and P4S at least as potent as GABA (see Nistri & Constanti 1979; Krogsgaard-Larsen *et al.*, 1980; Breckenridge *et al.*, 1981). Isoguvacine is reported to be more potent than GABA and isonipecotic acid equipotent with GABA in electrophysiological experiments (Krogsgaard-Larsen *et al.*, 1977), although they are slightly less potent in binding assays (Krogsgaard-Larsen & Johnston, 1978).

The low activity of the sulphonic acids (3-APS and P4S) observed in this preparation is consistent with data from the crustacean postsynaptic GABA receptor (Krause *et al.*, 1981) and with work on the somatic muscle of the nematodes *Ascaris* and *Ascaridia* (Green, Hodgson & Wann, unpublished observations). This thus represents a clear distinction between mammalian and invertebrate GABA receptors. The response to isoguvacine is evidently very different from that observed in mammalian studies. Our results suggest that isoguvacine has a dual effect on the cell membrane: a GABA receptor-mediated hyperpolarizing response and an opposing (non GABA receptor-mediated) depolarizing response which is more marked in the presence of a GABA antagonist. Desensitization of the preparation to glutamate (which produces a depolarization) did not block the depolarizing responses to isoguvacine. Further investigation of the action of isoguvacine is required.

In vertebrate preparations (+)-bicuculline is classically a competitive $GABA_A$ antagonist; however, in this study its action was clearly not competitive. This

agrees with biochemical studies in insect by Lummis & Sattelle (1985) showing that [^{3}H]-GABA binding in the cockroach central nervous system was not inhibited by bicuculline. Non-competititve antagonism by bicuculline has been demonstrated in crustacean muscle (Takeuchi & Onodera, 1972; Constanti, 1978). The apparent non-competitive antagonism observed here could be due solely to the blockade of chloride ion channels which mediate the response (Duce & Scott, 1983), although a 'mixed' (competitive and non-competitive) effect is also plausible. Pitrazepin is known to be a potent competitive antagonist of $GABA_A$ receptors (Gahwiler *et al.*, 1984) being 10 times more active than bicuculline. In locust muscle pitrazepin was also competitive, and was approximately 20 times more active than (+)-bicuculline.

The sensitivity of this preparation to muscimol and the finding that pitrazepin is a competitive antagonist indicate a degree of similarity between the locust GABA receptor and the vertebrate $GABA_A$ receptor; however, important differences have been highlighted by the effects of the sulphonic acids, isoguvacine and (+)-bicuculline. Such differences could be exploited in the design of insecticides.

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