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# GAMMA-AMINOBUTYRIC ACID RECEPTORS ON CULTURED COCKROACH BRAIN NEURONES

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## SUMMARY

Gamma-aminobutyric acid (GABA) at $10^{-4}$ mol $l^{-1}$ inhibited spontaneous activity and produced conductance changes in 60% of cultured cockroach neurones tested. The reversal potential for the GABA-evoked response was between −65 mV and −75 mV. Under whole-cell voltage-clamp conditions, with 114 mmol $l^{-1}$ potassium chloride in the electrode, the reversal potential had a similar value to that predicted for a chloride current. The response was blocked by $10^{-5}$ mol $l^{-1}$ picrotoxin but was not affected by $10^{-5}$ mol $l^{-1}$ bicuculline. In the whole-cell voltage-clamp conditions, 50 $\mu$mol $l^{-1}$ GABA evoked an inward current that was accompanied by an increase in current noise. Fluctuation analysis of the noise gave a mean channel opening time of 11·8 ms for GABA and 6·5 ms for muscimol. The single-channel conductance was 18·6 pS for GABA and 15·2 pS for muscimol. When 50 $\mu$mol $l^{-1}$ GABA was applied in the presence of the benzodiazepine, flunitrazepam, there was an increase in both the evoked current and the accompanying current noise. Analysis of this noise gave values of 14·3 ms for the mean channel opening time and 18·3 pS for the single-channel conductance. The variance of the noise was increased by approximately 60% in the presence of flunitrazepam, suggesting that this drug potentiates the GABA responses of cockroach neurones by increasing the frequency of channel events.

## INTRODUCTION

Gamma-aminobutyric acid (GABA) is a putative inhibitory neurotransmitter in nervous systems across a broad range of animal phyla (Leake & Walker, 1980). In vertebrates there is now substantial evidence that the nervous system contains two distinct types of GABA receptor: bicuculline-sensitive $GABA_A$ receptors (Olsen *et al.* 1981) and baclofen-sensitive $GABA_B$ receptors (Bowery *et al.* 1984). Both types mediate the depression of excitatory transmission. The $GABA_A$ receptor is intimately associated with a channel for chloride ions and with modulatory sites for a

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variety of depressant and anticonvulsant drugs including the benzodiazepines (Turner & Whittle, 1983). In contrast, since it was first demonstrated that GABA had a potent inhibitory effect on cockroach neurones and that this was mediated by an increase in chloride permeability (Kerkut, Pitman & Walker, 1969; Pitman & Kerkut, 1970), little information has become available on the pharmacology of GABA receptors in the insect CNS (Sattelle, 1984). As there is now evidence that disruption of GABA-mediated synaptic function may play an important role in the toxicity of several classes of insecticides (Lawrence & Cassida, 1983; Tanaka, Scott & Matsumura, 1984) and that membrane fractions derived from insect central nervous tissue possess specific binding sites for the benzodiazepine, flunitrazepam (Robinson, MacAllan & Lunt, 1986; Lummis & Sattelle, 1985, 1986) it would appear that the physiological aspects of insect GABA receptors deserve a more detailed examination.

Recently, a number of *in vitro* insect neuronal preparations have been developed that facilitate pharmacological studies since they allow unrestricted access for the application of drugs onto cellular membranes. These preparations have been derived from locust ganglia (Usherwood, Giles & Suter, 1980; Giles & Usherwood, 1985) and embryonic cockroach brains (Beadle, Hicks & Middleton, 1982) and neurones in both of them respond to the application of GABA (Giles & Usherwood, 1985; Lees, Benson & Beadle, 1984). There is also evidence that the GABA responses of locust neurones are potentiated by flunitrazepam (Beadle, Benson, Lees & Neumann, 1986; Lees, Beadle, Neumann & Benson, 1987). Embryonic cockroach neurones can be maintained *in vitro* for at least 6 weeks, during which they undergo extensive physiological differentiation (Lees, Beadle, Botham & Kelly, 1985) and develop both nicotinic acetylcholine receptors (Lees, Beadle & Botham, 1983) and a high-affinity choline uptake system (Bermudez *et al.* 1985). In the present communication this culture preparation has been used to extend the previous preliminary investigations into GABA receptors by employing the whole-cell voltage-clamp technique to investigate the nature of the channels associated with the receptor. Preliminary accounts of part of this work have been published elsewhere (Beadle & Lees, 1986; Neumann, Lees, Beadle & Benson, 1987).

## MATERIALS AND METHODS

### *Cell culture technique*

Neuronal cultures were prepared from the brains of 21- to 23-day-old embryos of *Periplaneta americana* as described elsewhere (Dewhurst & Beadle, 1985). The cultures were initiated in a medium consisting of five parts Schneider's revised *Drosophila* medium and four parts of Eagles' basal medium containing streptomycin and penicillin. After 7 days growth they were transferred to a medium containing equal parts Leibovitz's L-15 and Yunker's modified Grace's medium containing streptomycin and penicillin. The cells were grown at 29°C in air in 50 mm Falcon Petri dishes using a modification of the hanging column method.

*Electrophysiology*

For electrophysiological experiments the growth medium was removed and cells were allowed to equilibrate in a saline solution that was modified from Pitman (1979) to meet the osmotic requirements of the cultured cells. The solution contained 210 mmol $l^{-1}$ NaCl, 10 mmol $l^{-1}$ $CaCl_2$, 3·1 mmol $l^{-1}$ KCl and 10 mmol $l^{-1}$ Hepes buffer at pH 7·2. Microelectrode recordings were made at room temperature using conventional electrophysiological techniques. Cells were impaled with capillary glass microelectrodes filled with 1 mol $l^{-1}$ potassium acetate solution, with tip impedances of 80–150 MΩ. Under phase optics the electrodes were positioned using Leitz micromanipulators and impalements were assisted by passing brief pulses of negative capacitance overcompensation through the electrode. Potentials from the electrode were recorded with respect to a Ag–AgCl reference electrode immersed in the recording saline through a precision electrometer (W. P. Instruments). Excitability and membrane resistance were monitored by passing depolarizing or hyperpolarizing rectangular pulses of current through the electrode using an active bridge circuit. Drugs were applied from coarse micropipettes, tip diameter 3–4 μm, by nitrogen pressure pulses (70 kPa) onto the impaled soma at a distance of 150–200 μm. Drug concentrations refer to those in the pipette. Antagonists were bath-applied.

For voltage-clamp experiments, the whole-cell clamp configuration of the 'patch-clamp' technique as described by Hamill *et al.* (1981) was used. In most experiments, the electrodes were filled with a solution containing 114 mmol $l^{-1}$ KCl, 5 mmol $l^{-1}$ EGTA, 1·6 mmol $l^{-1}$ $MgCl_2$, 0·2 mmol $l^{-1}$ $CaCl_2$ and buffered at pH 7·2 using 10 mmol $l^{-1}$ Hepes. The electrode resistance ranged from 3 to 5 MΩ. A List (L/M-EPCS) 'patch-clamp' amplifier was used and the results were stored on analogue tape (Ampex PR 500) and recorded on a Brush 280 ink chart-pen recorder.

Current fluctuation analysis was performed off-line using a spectrum analyser (HP 3582A) connected to a desk computer (HP 9825T) and a digital plotter (HP 9872A). The fluctuations were analysed for bandwidths between 0 and 250 or 500 Hz. A uniform filter was used throughout and spectra were averaged 8–64 times before being loaded into the computer memory and stored on digital cartridge tape. The spectral points were scaled and plotted on a double log scale. They were fitted with one or two Lorentzian functions, the steady-state values and the corner frequencies of which were varied until a reasonable fit was obtained. The variance was calculated by integrating the area under the curve. This value was used together with that of the mean current to evaluate the corresponding (hypothetical) single-channel current, the calculation being based on the assumption that the probability of opening (p) of any single channel is small (a reasonable assumption for small agonist concentrations such as those used in these experiments) and according to the following equations:

$$I = Nip, \tag{1}$$

$$\sigma^2 = Ni^2p(p-1), \tag{2}$$

$$i = \frac{\sigma}{I(1-p)}, \tag{3}$$

where i is the unitary current, I the total (macroscopic) current, and N the number of channels. Single-channel conductance was then calculated by dividing i by the driving force (i.e. the difference between the potential at which the membrane was held and the equilibrium potential of the response).

## RESULTS

GABA at $10^{-4}$ mol $l^{-1}$ inhibited spontaneous activity and produced conductance changes (Fig. 1A) in approximately 60% of cells tested when applied onto the somata of cultured cockroach neurones ($N > 100$). The responses were voltage-dependent, being hyperpolarizing in the majority of cells with a reversal potential in the range −65 mV to −75 mV (Fig. 1B) and depolarizing in cells with very large resting potentials (Fig. 1D). The response faded with prolonged application (>20 s) of relatively high concentrations of GABA ($>10^{-4}$ mol $l^{-1}$), but this was not accompanied by a change in conductance (Fig. 1C) and is therefore probably due to a redistribution of ions rather than to receptor desensitization. Since chloride ions are believed to mediate the GABA response in some cockroach neurones (Pitman & Kerkut, 1970) and the reversal potential of the response in the cultured neurones is in the range of the chloride equilibrium potential, it was decided to determine whether chloride ions were involved in the responses by changing the intracellular chloride ion concentration by means of the whole-cell patch-clamp method of intracellular perfusion (Hamill *et al.* 1981). With this method the solution inside a patch pipette of tip diameter around 1 μm used in the whole-cell voltage-clamp mode may be used to alter the internal ion concentration of small, spherical cells (<20 μm in diameter). With 114 mmol $l^{-1}$ potassium chloride in the patch electrode and 221 mmol $l^{-1}$ potassium chloride in the bathing saline the equilibrium of the GABA-evoked currents was −16 mV (Fig. 2) which is the value predicted by the Nernst equation if the current was caused by chloride ions.

In all cells tested ($N = 7$) the neuronal responses to GABA were insensitive to freshly prepared $10^{-5}$ mol $l^{-1}$ bicuculline (Fig. 3), a concentration previously reported to block GABA-induced currents in the cockroach $D_f$ neurone (Sattelle, 1984). Higher concentrations of bicuculline, $5\times10^{-5}$ and $10^{-4}$ mol $l^{-1}$, applied by bath perfusion for periods up to 30 min were also ineffective in blocking GABA-evoked responses whether the initial responses were hyperpolarizing or depolarizing. However, the neuronal responses to GABA were completely blocked by $10^{-5}$ mol $l^{-1}$ picrotoxin (Fig. 3) and $10^{-6}$ mol $l^{-1}$ picrotoxin reversibly reduced GABA-evoked responses by 90% ($N = 7$).

When 50 μmol $l^{-1}$ GABA was applied to cultured cockroach neurones for prolonged periods (>20 s) under whole-cell voltage-clamp conditions with either 114 or 221 mmol $l^{-1}$ potassium chloride in the intracellular electrode solution an inward current was seen (Fig. 4A) and with 114 mmol $l^{-1}$ potassium gluconate the current was outwards. Qualitatively similar responses were evoked by the GABA agonist muscimol (Fig. 4B). The sensitivity of the neurones varied considerably from one culture to another (compare Fig. 4A and 4C, for example) but, in general, was

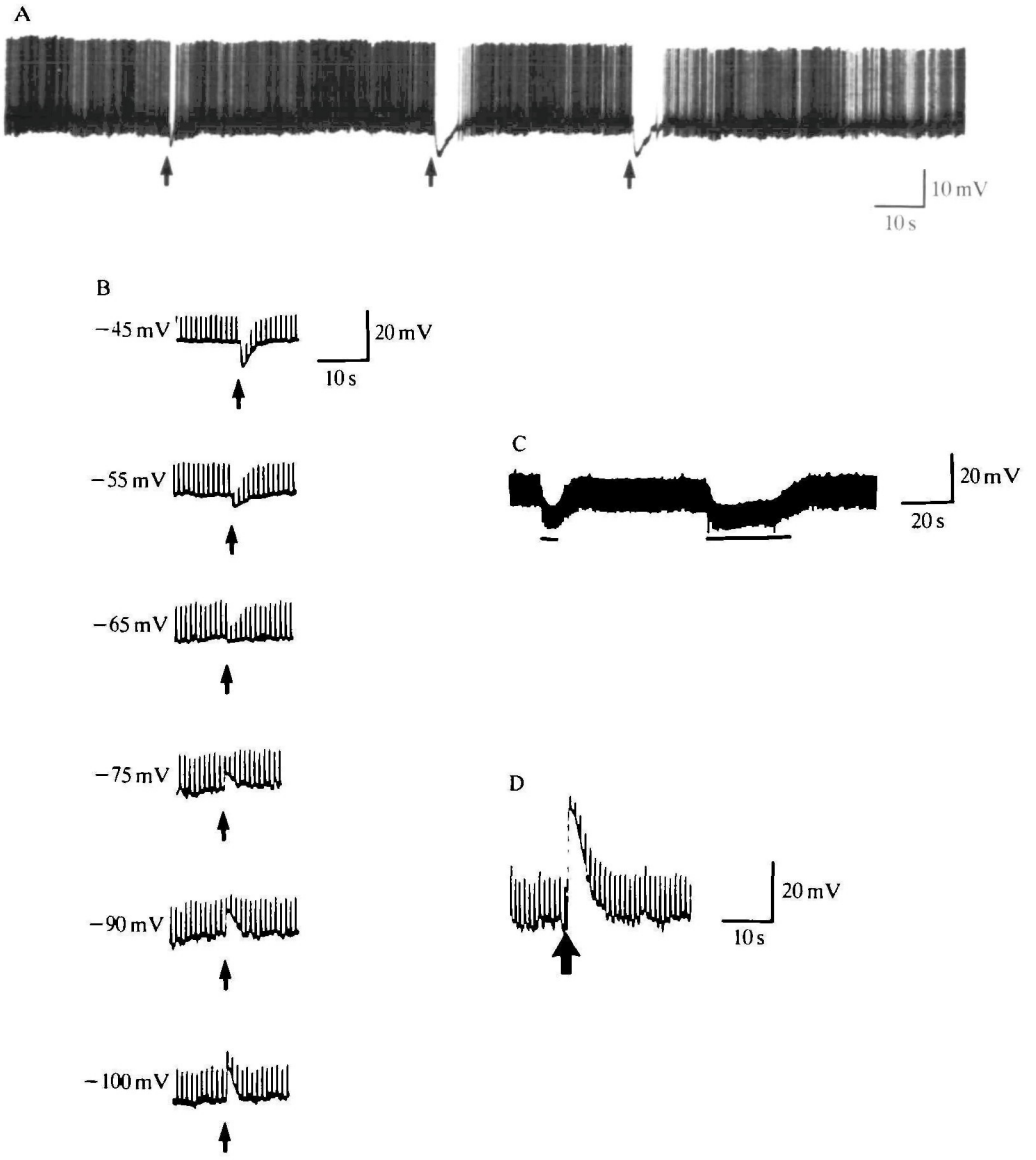


Fig. 1. Pressure application of GABA to cultured neurones. (A) Spontaneous action potentials recorded from a neurone maintained for 22 days *in vitro*. $10^{-3}$ mol $l^{-1}$ GABA applied as 100 ms or 600 ms pulses (arrows) inhibits spiking and hyperpolarizes the cell by up to 11 mV (resting potential, −45 mV). (B) Injection of hyperpolarizing direct current reveals the voltage-dependence of GABA-evoked polarity changes. In this case GABA was applied at $10^{-3}$ mol $l^{-1}$ as 1 s pulses (arrows) onto a neurone that had been maintained for 22 days *in vitro*. The polarity of the evoked response reverses at a resting membrane potential of about −65 mV. (C) Prolonged application of $10^{-4}$ mol $l^{-1}$ GABA (horizontal bars) results in response fading: following an initial peak hyperpolarization the response amplitude gradually decays throughout drug application but this is not accompanied by a change in conductance (resting potential, −44 mV). (D) A large depolarization and conductance increase evoked by $10^{-4}$ mol $l^{-1}$ GABA applied as a 100 ms pulse (arrow) onto the soma of a neurone with a resting potential of −87 mV. This cell had been maintained *in vitro* for 23 days.

similar within a single culture. The explanation for this is not clear at present, but a similar variation in sensitivity to acetylcholine between different cultures of ciliary ganglion neurones from the chick has recently been reported (Ogden, Gray, Colquhoun & Rang, 1984). With $50\,\mu mol\,l^{-1}$ GABA the inward current often reached a peak and then subsequently declined with a slow time course (Fig. 4A) even though the application of the agonist continued. This could represent desensitization but it might have been caused by a redistribution of chloride ions.

The inward currents evoked by $50\,\mu mol\,l^{-1}$ GABA were accompanied by an increase in current noise (Fig. 5) from a control variance of $1{\cdot}43\times10^{-24}\,A^2$ to a value of $2{\cdot}5\times10^{-23}\,A^2$ for $50\,\mu mol\,l^{-1}$ GABA (see, for example, Fig. 4A). When the values for variance were taken with the mean current to calculate the single-channel conductance, a value of approximately 18·6 pS was obtained for $50\,\mu mol\,l^{-1}$ GABA (Table 1) based on the assumption that chloride ions were the driving force of the evoked current. Further analysis of the increased membrane current noise evoked by $50\,\mu mol\,l^{-1}$ GABA at −50 mV holding membrane potential, by plotting the log spectral density against the log frequency, showed that the spectra obtained could be reasonably fitted by a single Lorentzian component, suggesting that a single class of ion channel was involved in the agonist-evoked response. The corner frequency ($f_c$) for GABA (Fig. 6A) was 13·5 Hz (Table 1) giving a mean channel-opening time of

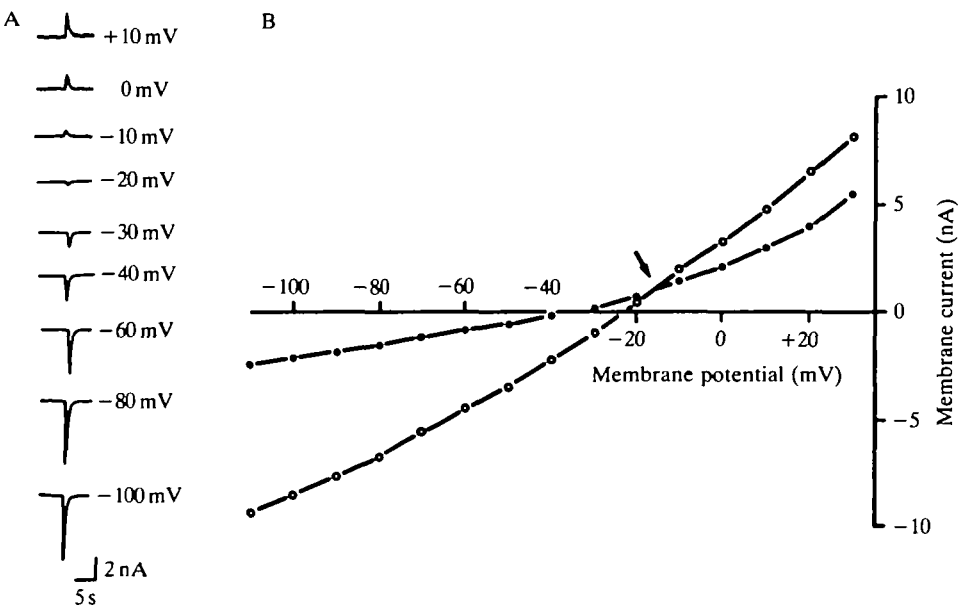


Fig. 2. (A,B) Whole-cell voltage-clamp currents evoked by 200 ms pressure pulses of $10^{-4}\,mol\,l^{-1}$ GABA at a range of holding potentials. This recording method effectively permits intracellular ionic manipulation. Observed reversal potentials are consistently close to those predicted by the Nernst equation for $Cl^-$. For the cell depicted here $[Cl^-]_o$ was 221 $mmol\,l^{-1}$ and $[Cl^-]_i$ was 114 $mmol\,l^{-1}$. The predicted $E_{Cl}$, assuming complete equilibration with the pipette contents, of −17·8 mV is close to the reversal potential observed for the GABA-evoked response (arrowed in B). (●) Currents in the absence of GABA; (○) GABA-evoked currents.

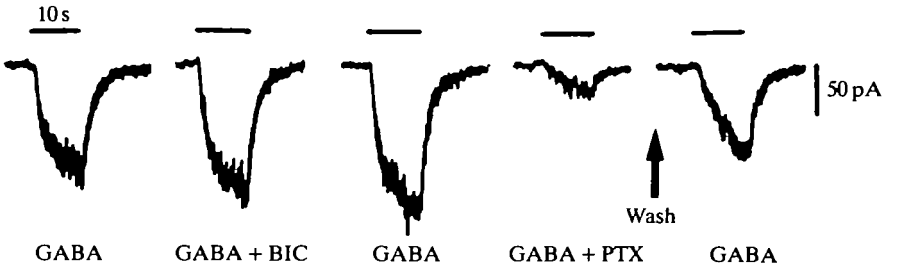


Fig. 3. Effect of antagonists on GABA-evoked responses. Currents recorded from a cultured cockroach neurone evoked by 10 s pressure pulses of 50 $\mu$mol $l^{-1}$ GABA. The evoked current is unaffected by exposure to 10 $\mu$mol $l^{-1}$ bicuculline (BIC) for 10 min but is almost completely abolished by exposure to 10 $\mu$mol $l^{-1}$ picrotoxin (PTX) for 5 min. The GABA response has partially recovered after 5 min of washing in normal saline.

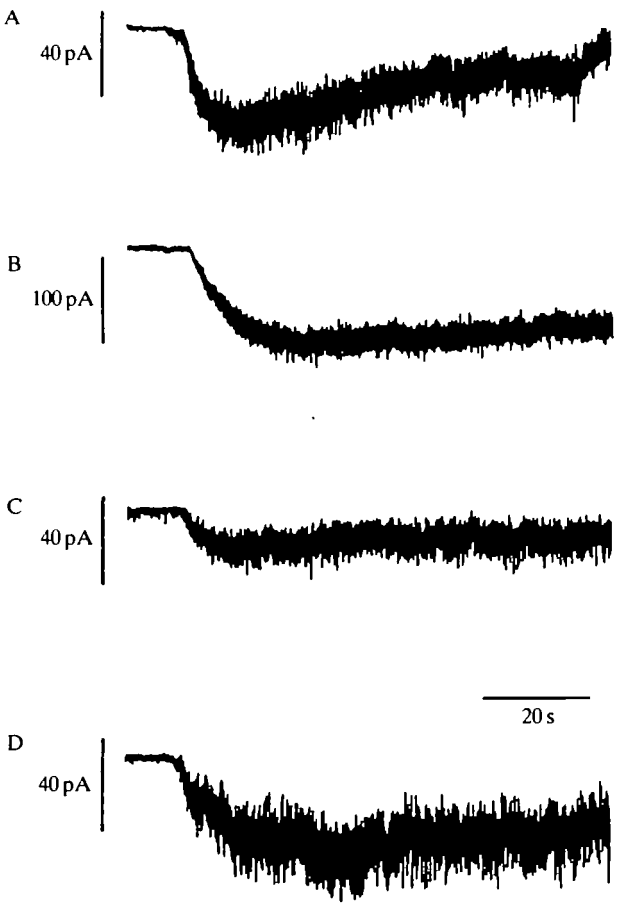


Fig. 4. Inward currents evoked by prolonged application of agonist onto neurones held under whole-cell voltage-clamp. (A) 50 $\mu$mol $l^{-1}$ GABA; (B) 5 $\mu$mol $l^{-1}$ muscimol; (C) 50 $\mu$mol $l^{-1}$ GABA; (D) 50 $\mu$mol $l^{-1}$ GABA in the presence of $2\times10^{-6}$ mol $l^{-1}$ flunitrazepam. The cells illustrated in C and D are from the same culture dish.

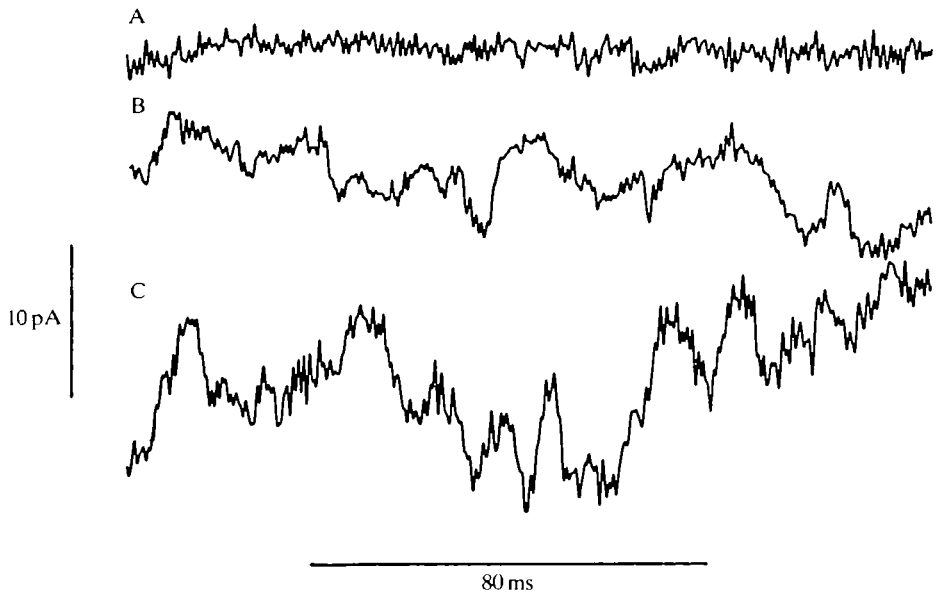


Fig. 5. Noise levels from currents evoked by 50 $\mu$mol $l^{-1}$ GABA (B) and 50 $\mu$mol $l^{-1}$ GABA in the presence of $2\times10^{-6}$ mol $l^{-1}$ flunitrazepam (C). (A) The control noise prior to application of agonist.

Table 1. *The effects of GABA, muscimol, and GABA in the presence of flunitrazepam on channel properties of insect neurones under voltage-clamp conditions*

| Agonist | Corner frequency (Hz) | Time constant (ms) | Single-channel conductance (pS) | Variance ($\times10^{-23}$ A$^2$) |
|---|---|---|---|---|
| 50 $\mu$mol $l^{-1}$ GABA | 13·5 ± 2·53 (11) | 11·8 | 18·6 ± 6·16 (6) | 2·37 ± 0·57 (6) |
| 50 $\mu$mol $l^{-1}$ GABA + $2\times10^{-6}$ mol $l^{-1}$ flunitrazepam | 11·1 ± 2·46 (4) | 14·3 | 18·3 (4) | 3·74 ± 0·25 (4) |
| 5 $\mu$mol $l^{-1}$ muscimol | 24·5 ± 3·08 (5) | 6·5 | 15·2 ± 5·3 (7) | — |

Results are mean ± S.D.
The numbers in brackets indicate the number of experiments.

11·8 ms. A similar analysis for muscimol-evoked currents gave a corner frequency of 24·5 Hz (Table 1) and a mean channel opening time of 6·5 ms.

Since it has been shown that the benzodiazepine, flunitrazepam, potentiates GABA responses in locust neurones (Lees *et al.* 1987), GABA was applied to cultured cockroach neurones in the presence of this compound. With 50 $\mu$mol $l^{-1}$ GABA applied to the neuronal membranes in the presence of $2\times10^{-6}$ mol $l^{-1}$ flunitrazepam both the inward current evoked and the corresponding noise increased when neurones within the same culture were compared (Figs 4C,D, 5B,C). When

the single-channel conductance and corner frequency were calculated for these responses (Fig. 6C), values of 18·3 pS and 11·1 Hz (Table 1) were obtained giving a mean channel opening time of 14·3 ms. A statistical analysis of the data in Table 1 (Student's two-tailed *t*-test) indicates that these values are not significantly different from those obtained with GABA alone. However, when the variance of the noise was compared, the values obtained were $2{\cdot}37\times10^{-23}\,A^2$ in $50\,\mu mol\,l^{-1}$ GABA and $3{\cdot}74\times10^{-23}\,A^2$ in $50\,\mu mol\,l^{-1}$ GABA in the presence of $2\times10^{-6}\,mol\,l^{-1}$ flunitrazepam (Table 1) and these values are significantly different at a probability level of 90%.

## DISCUSSION

The results presented in this communication show that at least 60% of the cultured neurones that were tested ($N > 100$) responded to pressure application of GABA and, since the cells were derived from the brains of embryonic cockroaches, this supports the long-held view that GABA is a major transmitter in the cockroach brain. The typical response evoked by GABA was a hyperpolarization accompanied by an increase in membrane conductance and a cessation of spiking activity. Similar results have been obtained with neurones derived from thoracic ganglia of nymphal locusts where all seven cells tested responded to GABA (Giles & Usherwood, 1985).

The responses described in the present study are reversed by the injection of chloride ions into the impaled cell and have a reversal potential that is close to the predicted value for chloride ions. They are also blocked by picrotoxin, an antagonist of chloride-mediated responses in the fast coxal depressor motoneurone of the cockroach (Wafford & Sattelle, 1986) and in many other invertebrates (Walker, James, Roberts & Kerkut, 1981). All of this evidence indicates that the GABA receptor is associated with a chloride ion channel and is therefore similar to the GABA receptor–ion channel complex of unidentified neurones in the thoracic ganglia of cockroaches (Kerkut *et al.* 1969; Pitman & Kerkut, 1970), the $D_f$ motoneurone of the cockroach (Sattelle, 1984) and the vertebrate $GABA_A$ receptor (Olsen *et al.* 1981). This complex differs from that of vertebrates, however, as it is not sensitive to bicuculline, the classic antagonist of the vertebrate receptor.

The sensitivity of insect GABA receptors to bicuculline is as yet unresolved. Mann & Enna (1980) reported that bicuculline-sensitive GABA binding was not detectable in the CNS of any invertebrate studied including *P. americana*, while Lummis & Sattelle (1985, 1986) reported that bicuculline was ineffective in inhibiting [$^3$H]GABA binding to extracts of cockroach CNS. Similarly, Lunt *et al.* (1984, 1985) found that it was very weak at displacing [$^3$H]muscimol bound to membrane fragments of housefly head and locust ganglia. However, at very high concentrations bicuculline did appear reversibly to block GABA-induced hyperpolarizations and IPSPs in unidentified dorsal midline cells of the cockroach $A_6$ ganglia (Walker, Crossman, Woodruff & Kerkut, 1971), although recently Walker *et al.* (1981) found that it was ineffective on cockroach GABA receptors. Beadle *et al.* (1986) and Lees *et al.* (1987) failed to find any effect of bicuculline on GABA-evoked currents over a

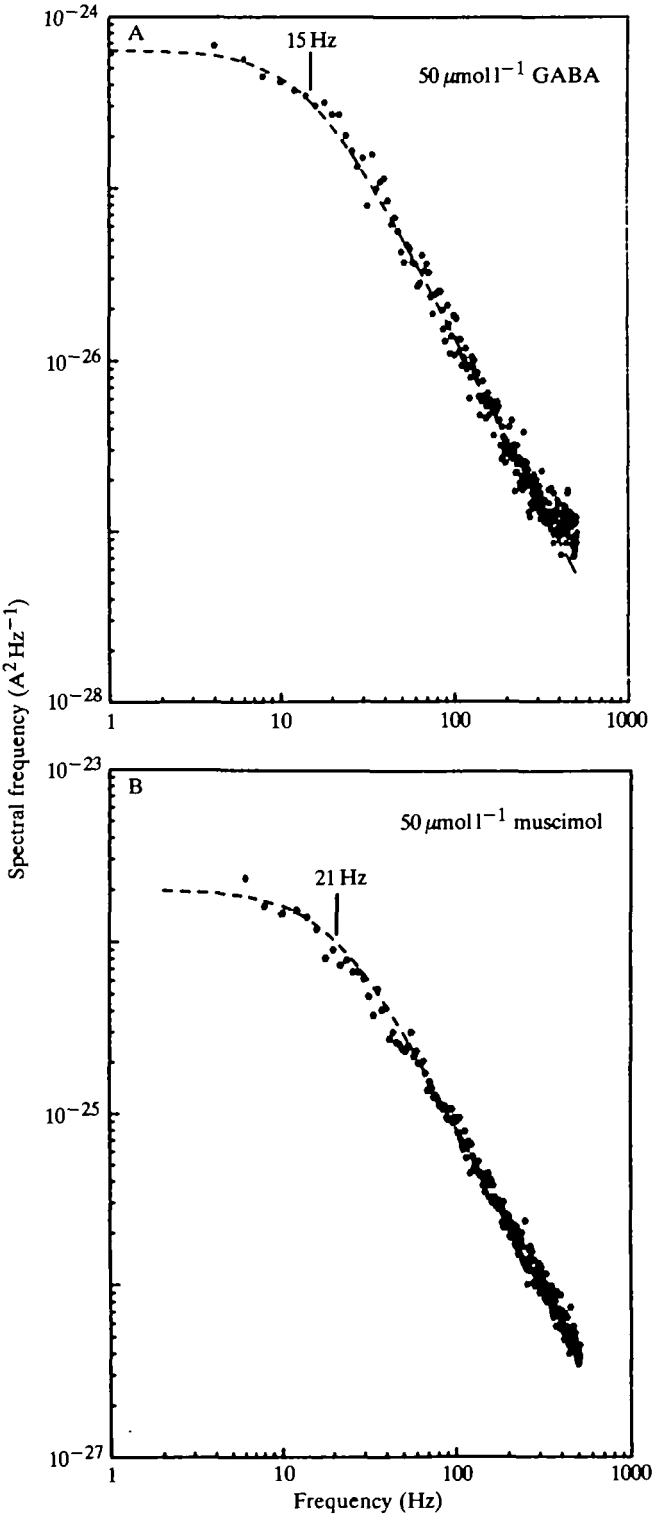
A
15 Hz
50 μmol l⁻¹ GABA
B
21 Hz
50 μmol l⁻¹ muscimol
Spectral frequency (A² Hz⁻¹)
Frequency (Hz)
10⁻²⁴
10⁻²⁶
10⁻²⁸
10⁻²³
10⁻²⁵
10⁻²⁷
1
10
100
1000

Fig. 6

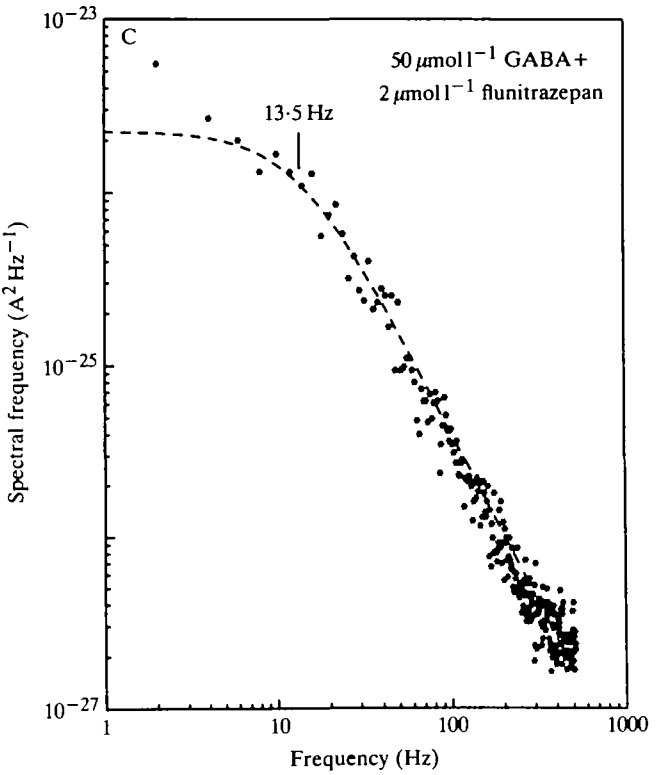


Fig. 6. Power spectra of the noise induced by pressure application of (A) 50 μmol $l^{-1}$ GABA, (B) 50 μmol $l^{-1}$ muscimol and (C) 50 μmol $l^{-1}$ GABA in the presence of $2\times10^{-6}$ mol $l^{-1}$ flunitrazepam onto cockroach neurones growing *in vitro*. In all three examples the spectra were fitted with a single Lorentzian component with corner frequencies of 15 Hz, 21 Hz and 13·5 Hz, respectively, at a holding potential of −50 mV.

wide range of membrane potentials in freshly dissociated locust neurones. Bicuculline at $10^{-5}$ mol $l^{-1}$ has been reported to block membrane currents in the $D_f$ motoneurone of the cockroach (Sattelle, 1984), although David, Pinnock, Sattelle & Wafford (1986) and Wafford *et al.* (1987) recently reported that it was without effect on this neurone. The results of the present study, that bicuculline is ineffective at antagonizing membrane responses to GABA in cultured cockroach neurones, tend to support the view that this compound is not an antagonist of insect neurone GABA receptors.

The application of GABA to neurones held under whole-cell voltage-clamp evoked inward currents that were accompanied by an increase in membrane current noise. Fluctuation analysis of the current noise revealed that a single class of channels was involved in the response with a mean channel opening time of approximately 11·8 ms for GABA and a single-channel conductance of 18–19 pS. Chloride channels associated with GABA receptors have previously been shown to have conductances of 15·4 pS in mouse spinal neurones (Barker & Mathers, 1981), 16 pS in rat cerebellar neurones (Cull-Candy & Ogden, 1985) and an 18 pS channel was one of three

reported in adrenal chromaffin cells (Borman & Clapham, 1985). The channel in cockroach neurones therefore appears to have similar properties to these mammalian channels. When GABA is applied to the neurones in the presence of the benzodiazepine, flunitrazepam, the channel properties in the presence of GABA remain essentially unchanged with a mean opening time of 14·3 ms and a conductance of 18·3 pS. However, there is a marked increase in the variance of the GABA-evoked current noise in the presence of this benzodiazepine. These effects are similar to those described for mammalian neurones where the potentiation of GABA responses by the benzodiazepines is thought to result from an increased frequency of channel events rather than from an increase in channel opening times or conductance (Mathers & Barker, 1982; Mathers, 1987). These results provide further evidence that GABA receptor complexes in insects possess modulatory sites similar to those found in vertebrates, a conclusion reached in a similar study of the effect of benzodiazepines on GABA responses in locust neurones (Beadle *et al.* 1986; Lees *et al.* 1987). They also provide physiological support for the discovery of flunitrazepam binding sites in membrane extracts from the locust and cockroach central nervous systems (Robinson *et al.* 1986; Lummis & Sattelle, 1985, 1986).

In conclusion, this study has shown that cockroach brain neurones possess a GABA receptor that is linked to a picrotoxin-sensitive chloride ion channel and possesses modulatory sites for the benzodiazepines. In this respect it resembles the vertebrate $GABA_A$ receptor (Simmonds, 1983). Furthermore, recent studies by Lees & Beadle (1986) have shown that insect neurones possess chloride channels that may be associated with GABA receptors and that can be activated by the insecticidal agents, the avermectins, and Eldefrawi, Abalis, Sherby & Eldefrawi (1986) have reported the inhibition of [$^3$H]muscimol binding to the honeybee brain GABA receptor by these compounds. These reports suggest that the insect neurone GABA receptor may also possess an avermectin binding site in common with its vertebrate counterpart (Iversen, 1984). However, the apparent insensitivity of this receptor to bicuculline supports the views expressed by Lummis & Sattelle (1985, 1986) and David *et al.* (1986) that its pharmacological properties are distinct from either of the mammalian GABA receptor subtypes. The variation in the responses of the cultured neurones to GABA and other drugs presumably reflects the mixed population of brain cells in each culture dish and the impossibility of ensuring that each culture contains exactly the same population of cells. Despite this, all of the GABA responses recorded and the effects of drugs on these were qualitatively similar in all neurones that were shown to possess GABA receptors.

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