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PHARMACOLOGY OF SKELETAL MUSCLE GABA-GATED CHLORIDE CHANNELS IN THE COCKROACH *PERIPLANETA AMERICANA*

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Summary

The pharmacology of γ-aminobutyric acid (GABA)-gated chloride channels of the coxal levator (182c,d) muscle of the cockroach *Periplaneta americana* has been investigated and the data compared with similar findings for the cell body of the cockroach fast coxal depressor motor neurone (D_f). Muscle GABA receptors resembled those of the motor neurone cell body in their sensitivity to picrotoxinin and insensitivity to bicuculline. However, muscle GABA receptors were insensitive to the neuronal GABA receptor agonists isoguvacine (10^{-4} mol l^{-1}) and 3-aminopropane sulphonic acid (10^{-3} mol l^{-1}). The benzodiazepine flunitrazepam, which at 10^{-6} mol l^{-1} greatly enhances the amplitude of the motor neurone GABA-induced responses, failed to affect muscle responses to GABA when tested at the same and at a higher (10^{-4} mol l^{-1}) concentration. The convulsant *t*-butylbicyclophosphorothionate was a weak antagonist of cockroach muscle GABA receptors, whereas several cyclodienes were much more effective antagonists. Thus, studies using a benzodiazepine and several convulsant antagonists reveal differences in the pharmacology of muscle and neuronal GABA receptors of the cockroach *Periplaneta americana*.

Key words: GABA receptor, muscle, cockroach, *Periplaneta americana*, flunitrazepam, *t*-butylbicyclophosphorothionate (TBPS), cyclodienes.

Introduction

GABA-gated chloride channels are membrane-bound proteins with an integral anion-selective pore and are members of the *cys*-loop family of ionotropic receptors (Karlin and Akabas, 1995). Such GABA receptors are present on many cell types and are widely distributed in the Animal Kingdom (Sattelle, 1990). The best characterized GABA-gated chloride channels are the $GABA_A$ and $GABA_C$ type receptors found in vertebrate central nervous systems (Stephenson, 1988; Olsen and Tobin, 1990). In insects, GABA-gated chloride channels are detected on both neurones and muscle cells (for reviews, see Sattelle, 1990; Rauh *et al.* 1990). To date, no detailed direct comparison of the pharmacology of muscle and neuronal GABA receptors exists for a single insect species. To address this problem, the coxal levator (182c,d) muscles in the cockroach *Periplaneta americana* have been used to study the physiology and pharmacology of muscle GABA receptors in an insect species for which data are also available for neuronal GABA receptors (Pitman and Kerkut, 1970; Walker *et al.* 1980), including identified neurones (David and Sattelle, 1984; Sattelle *et al.* 1988; Pinnock *et al.* 1988).

Responses of insect muscle to GABA that are chloride-mediated and blocked by picrotoxinin have already been described by Usherwood and Grundfest (1965). Usherwood (1973) detected both depolarizing and hyperpolarizing responses to GABA in the locust (*Schistocerca gregaria*) metathoracic coxal adductor muscle where the reversal potential for chloride (E_{Cl}) was found to be close to the resting potential. Brookes and Werman (1973) also described a GABA-gated chloride channel in locust (*Locusta migratoria*) flexor tibiae muscle. Detailed studies on the extensor tibiae muscles of the locust *Schistocerca gregaria* have described both junctional and extrajunctional GABA-gated chloride channels (Cull-Candy and Miledi, 1981; Cull-Candy, 1982). Scott and Duce (1987) have shown that GABA and the *trans*-isomer of 4-aminocrotonic acid are almost equally potent agonists at *Schistocerca gregaria* muscle GABA receptors. Picrotoxinin blocks this muscle GABA-gated chloride channel, whereas the barbiturate pentobarbitone enhances the GABA-induced increase in chloride permeability. The vertebrate $GABA_A$ antagonist bicuculline is ineffective.

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The present study investigates the actions on a cockroach skeletal muscle of a number of GABA receptor ligands and modulators and the findings are compared with data for an identified motor neurone in the same species. Evidence is provided for the existence of pharmacologically distinct populations of GABA-gated chloride channels in this insect.

Materials and methods

Animals

Adult male cockroaches (*Periplaneta americana*), reared at 27–31 °C with freely available food and water, were used in all experiments.

Skeletal muscle electrophysiology

Coxal muscle 182, branches c and d (see Pearson and Iles, 1971), were used in all experiments. Sections of muscle were removed from the third thoracic segment and secured in a recording chamber. Muscle fibres were impaled with one or two microelectrodes containing 3.0 mol l^{-1} KCl (resistance10–30 MΩ). Unless otherwise indicated, the preparation was continuously perfused with saline of the following composition (in mmol l^{-1}): NaCl, 214; $CaCl_2$, 10; $MgCl_2$, 20; KCl 3.2; D-glucose, 10; Hepes, 10; pH 7.2 adjusted with 1 mol l^{-1} NaOH. Methods of ligand delivery were as follows: (a) bath application; (b) pressure application from pipettes drawn on a Mecanex SA electrode puller (model BB-CH); or (c) delivery as a pulse (1–5 s duration) into the inlet tube of the experimental chamber *via* a motor-driven syringe pump (Sage Instruments). Putative antagonists and allosteric modulators were applied in the perfusing saline. In the case of pressure application, pipettes were filled with either GABA or muscimol (10^{-3} mol l^{-1}) in saline. Tetrodotoxin (10^{-6} mol l^{-1}) was used in conjunction with antagonist studies in order to block muscle contraction and action potentials in motor axons, thereby preventing neurally evoked release of transmitter.

Electrophysiology of an identified fast coxal depressor motor neurone

To provide a comparison with data from neuronal GABA receptors, experiments were performed on the fast coxal depressor motor neurone as described previously (David and Sattelle, 1984; Sattelle *et al.* 1988). Briefly, the metathoracic ganglion was removed from adult male cockroaches. The sheath was removed manually using sharpened forceps, and the preparation was mounted in an experimental chamber and perfused continuously with physiological saline of the same composition as used for the muscle recordings. The cell body of the fast coxal depressor motor neurone was impaled with one or two microelectrodes containing 3.0 mol l^{-1} KCl (resistance 10–30 MΩ). Ligands were applied as in experiments on muscle.

Chemicals

Isoguvacine, 4,5,6,7-tetrahydroisoxazolo-(5,4,c)pyridin-3-ol (THIP) and baclofen were obtained from Cambridge Research Biochemicals (Boston, MA, USA). Endrin, 12-ketoendrin, lindane and heptachlor epoxide were purchased from Chem Serv (Westchester, PA, USA), and the steroid 5α-pregnan-3α-ol-11,20-dione (alphaxolone) was obtained from Steraloids (Wilton, NH, USA). *t*-Butylbicyclo-orthobenzoate (TBOB) was synthesized by P. Y. Lam (DuPont). Picrotoxinin and all other drugs were obtained from Sigma Chemical Co. Ltd (St Louis, MO, USA).

Results

Actions of GABA and muscimol

Bath application, ionophoretic application or pressure application of either muscimol or GABA (Fig. 1A, trace a) to coxal levator 182c,d muscles reduced the input resistance of these cells. Replacing chloride ions in the bathing medium by equimolar isethionate reduced the conductance change and reversed the direction of the membrane potential response to pressure application of GABA (*N*=5, Fig. 1A, trace b). A dose-dependent increase in conductance was observed in response to bath-applied muscimol in the concentration range 10^{-5} to 10^{-3} mol l^{-1} (Fig. 1B). From such data (Fig. 1C), a Hill coefficient of 2.05±0.36 (mean ± S.E.M., *N*=5) was calculated. The response to GABA reversed at −52.6±4.1 mV (mean ± S.E.M., *N*=3; Fig. 1D). In Table 1, the reversal potential and Hill coefficient for cockroach muscle GABA-gated chloride channels determined in this study are compared with data for cockroach neuronal GABA-gated chloride channels.

Agonist profile

Four GABA agonists all reduced muscle input resistance, with an order of potency as follows: muscimol > GABA > THIP, all of which were full agonists, whereas 3-aminopropane sulphonic acid (3-APS) (10^{-3} mol l^{-1}) was largely ineffective (Fig. 1B). This agonist profile resembled that determined when the same compounds were tested on the cockroach *Periplaneta americana* fast coxal depressor motor neurone (D_f) (Sattelle *et al.* 1988). However, isoguvacine, a potent agonist of the motor neurone GABA-gated chloride channel, was ineffective on coxal levator muscles at concentrations up to 10^{-4} mol l^{-1} (data not shown). Estimated EC_{50} values (the concentration that elicits a half-maximal response) for agonists on muscle GABA receptors are summarized in Table 2 and compared with

Table 1. *A comparison of the reversal potentials and the Hill coefficients for cockroach (*Periplaneta americana*) muscle and neuronal GABA receptors*

	Coxal levator (182c,d) muscle	Fast coxal depressor motor neurone, D_f
Reversal potential (mV)	−52.6±4.11 (*N*=3)	−77.0±2.4 (*N*=22)[a]
Hill coefficient	2.05±0.36 (*N*=5)	2.3±0.1 (*N*=6)[b]

[a]Pinnock *et al*. (1988); [b]Sattelle *et al*. (1988).
All values are means ± S.E.M.

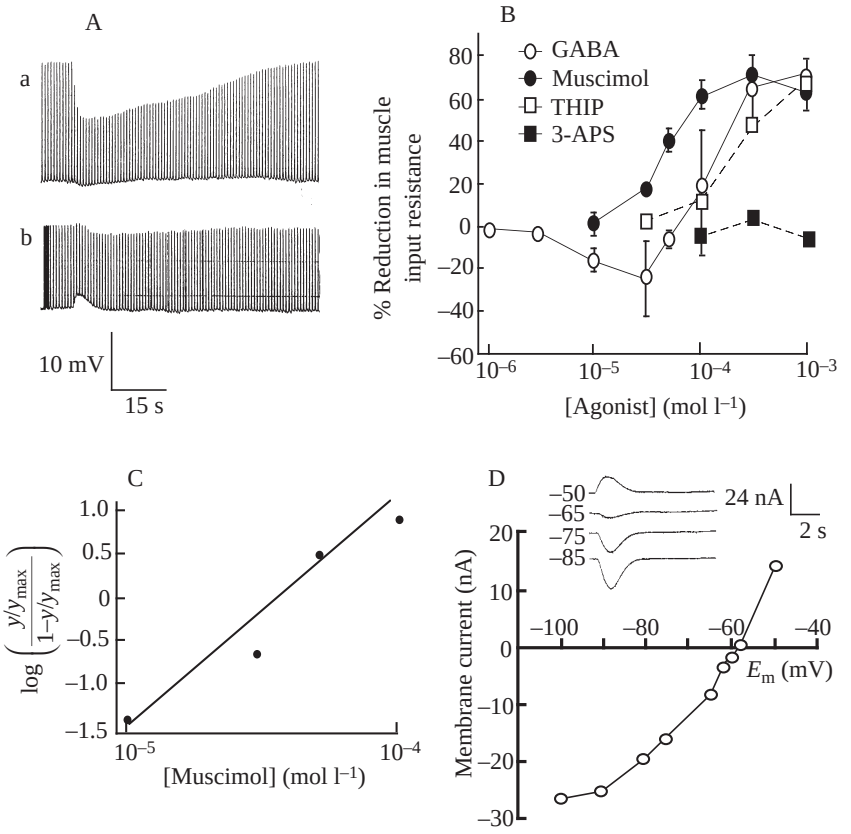


Fig. 1. Actions of muscimol and GABA on the cockroach coxal levator 182c muscle. (A) As shown in trace a, GABA applied to the coxal levator muscle by a brief pressure pulse results in a transient increase in membrane conductance. As shown in trace b, replacing chloride ions with isethionate results in a reversal in the direction of the current and suppresses the GABA-induced conductance increase. Muscle input resistance is measured by inserting two microelectrodes into a single muscle fibre. Through the first, a constant hyperpolarizing current (1–5 nA, 100 ms) is injected, and the second is used to detect changes in input resistance. Hyperpolarizing pulses appear as upward deflections because of the setting of the bridge balance on the amplifier. Data are taken from one cell, but the result is representative of five experiments. (B) The cockroach coxal levator muscle is responsive to a number of GABAergic agonists. Here, dose–response curves for bath-applied muscimol, THIP and 3-APS are presented with that for GABA as a comparison. A dose-dependent increase in conductance is observed in response to bath-applied muscimol in the concentration range 10^{-5} to 10^{-3} mol l^{-1} and to THIP in the range 3×10^{-5} mol l^{-1} to 10^{-3} mol l^{-1}. The muscle response to muscimol saturates at 3×10^{-4} mol l^{-1}. Saturation of the response to THIP could not be obtained in the concentration range tested, and higher concentrations could not be tested because of limitations in the solubility of THIP. Responses to 3-APS could not be obtained at concentrations below 10^{-4} mol l^{-1}. Values are means ± S.E.M., N=5. From the data for muscimol in B, a Hill plot was constructed (C) from which the Hill coefficient, determined from the slope of the line, was estimated to be 2.41 (for this experiment). A mean value of 2.05±0.36 (mean ±1 S.E.M.) for the Hill coefficient was estimated from five experiments. y is the amplitude of the response to each concentration of muscimol, y_{max} is the maximum amplitude response to muscimol. (D) The voltage-dependence of GABA-induced currents recorded from a coxal levator 182c muscle cell under voltage-clamp. Currents recorded at various membrane potentials E_m in the range −50 to −85 mV are shown (inset). The GABA-induced current in this cell reversed at approximately −58 mV. The current–voltage relationship is non-linear: less current than predicted is recorded at hyperpolarized potentials. Data shown are from one cell but are typical of the results of three similar experiments, from which a mean reversal potential of −52.6±4.1 mV (mean ±1 S.E.M.) was calculated.

previously published data (Sattelle *et al.* 1988) on motor neurone D_f GABA receptors.

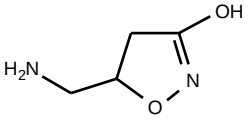
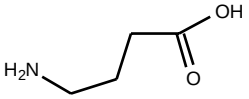
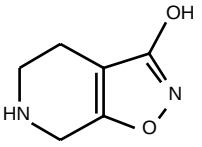
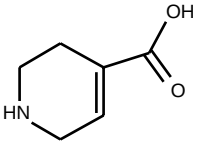
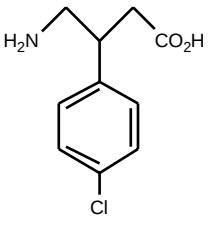
Actions of antagonists and modulators

Repeated brief applications of GABA at intervals of greater than 1 min yielded constant-amplitude, depolarizing responses in current-clamped cells (at membrane potentials in the range −90 mV to −80 mV). These responses were blocked by picrotoxinin (Fig. 2A). The absence of any significant desensitization under these conditions facilitated pharmacological studies with antagonists and modulators. Bicuculline methiodide, a vertebrate $GABA_A$ receptor antagonist, when tested at 10^{-4} mol l^{-1} (N=3), failed to block muscle responses to GABA (Fig. 2B). Responses to GABA were not modified by the barbiturate sodium pentobarbital (10^{-4} mol l^{-1}; N=3) (Fig. 2C). The benzodiazepines flunitrazepam (N=3) (Fig. 2D) or diazepam (N=5, data not shown) were also without effect at concentrations of 10^{-6} to 10^{-4} mol l^{-1}. The pregnane anaesthetic steroid alphaxolone and the ecdysteroid 20-hydroxyecdysone, when applied separately at 10^{-4} mol l^{-1}, also failed to modify responses of muscle GABA receptors to muscimol (N=3 in each case, data not shown). Table 3 compares the effectiveness of a range of vertebrate GABA receptor antagonists and modulators on cockroach muscle and neuronal GABA receptors.

Actions of bicyclophosphates and polychlorocycloalkanes

A number of polychlorocycloalkane insecticides were found to be highly effective blockers of muscle GABA-gated chloride channels (Figs 3, 4). Of these, 12-ketoendrin (Fig. 3B), endrin (Fig. 3C) and heptachlor epoxide (Fig. 3D) were found to be the most effective (Fig. 4). All polychlorocycloalkanes for which data are available for both muscle and neuronal GABA receptors exhibit a seven- to 180-fold greater potency on muscle GABA receptors than on neuronal receptors. TBPS has been widely used as a probe of a convulsant site on the vertebrate $GABA_A$ receptor. In contrast to findings for the cockroach motor neurone D_f, the

Table 2. *A comparison of EC_{50} values for GABA agonists tested on GABA-operated chloride channels in a cockroach muscle and neurone*

Ligand	Structure	EC_{50} (μmol l^{-1}) Coxal levator muscle	EC_{50} (μmol l^{-1}) Fast coxal depressor motor neurone, D_f
$GABA_A$ agonists			
Muscimol		40	62[c]
GABA		190	40[c]
THIP		200	>100
Isoguvacine		IA[a]	20[c]
3-APS		IA[b]	750[c]
$GABA_B$ agonist			
Baclofen		IA[a]	IA[a,c]

IA, inactive at concentrations up to [a]100 μmol l^{-1}, [b]1000 μmol l^{-1}; 3-APS, 3-aminopropane sulphonic acid; THIP, 4,5,6,7-tetrahydroisoxazolo-(5,4,c)pyridin-3-ol.

[c]Data from Sattelle *et al.* (1988).

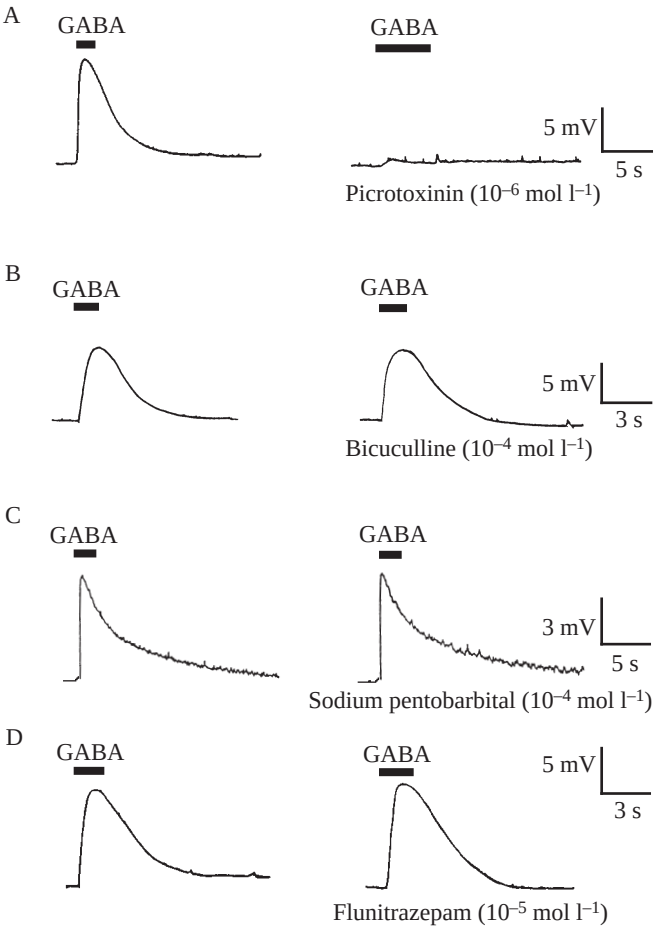


Fig. 2. Effects of putative GABA receptor ligands were tested on the responses of the coxal levator muscle to GABA. In all traces, except for top right. GABA (10^{-3} mol l^{-1}) was applied as a 2 s pulse (solid bar) into the inlet tube of the experimental chamber *via* a motor-driven syringe. Chemicals under investigation were then applied continuously for 10–20 min. The membrane potential was recorded using a single intracellular microelectrode. (A) The response to GABA was almost completely blocked by a 10 min exposure to saline containing 10^{-6} mol l^{-1} picrotoxinin. (B) Bicuculline (10^{-4} mol l^{-1}) had no effect upon the response to GABA. (C) Sodium pentobarbital (10^{-4} mol l^{-1}) was without effect on the amplitude or the time course of the GABA-induced muscle depolarization. (D) Flunitrazepam (10^{-5} mol l^{-1}) was also without effect on the muscle response to GABA. Experimental conditions were the same as in A.

amplitude of GABA responses recorded in coxal levator (182c,d) muscles was unaffected by TBPS concentrations up to 3×10^{-5} mol l^{-1} (Fig. 3A), a concentration that reduces the neuronal response to GABA by 50–80 % of control values (Table 4). TBOB was also ineffective at this concentration on the muscle GABA receptor (data not shown). Thus, although a convulsant site is clearly present on cockroach muscle GABA receptors, it differs from that observed for neuronal GABA receptors.

Discussion

A GABA-gated chloride channel is present on coxal levator (182c,d) muscle fibres of the cockroach *Periplaneta americana*, as indicated by the estimated reversal potential of GABA responses and their sensitivity to the concentration of external chloride ions. The chloride reversal potential for cockroach coxal levator muscle fibres is unknown, but a value of −57 mV has been measured for *Periplaneta americana*

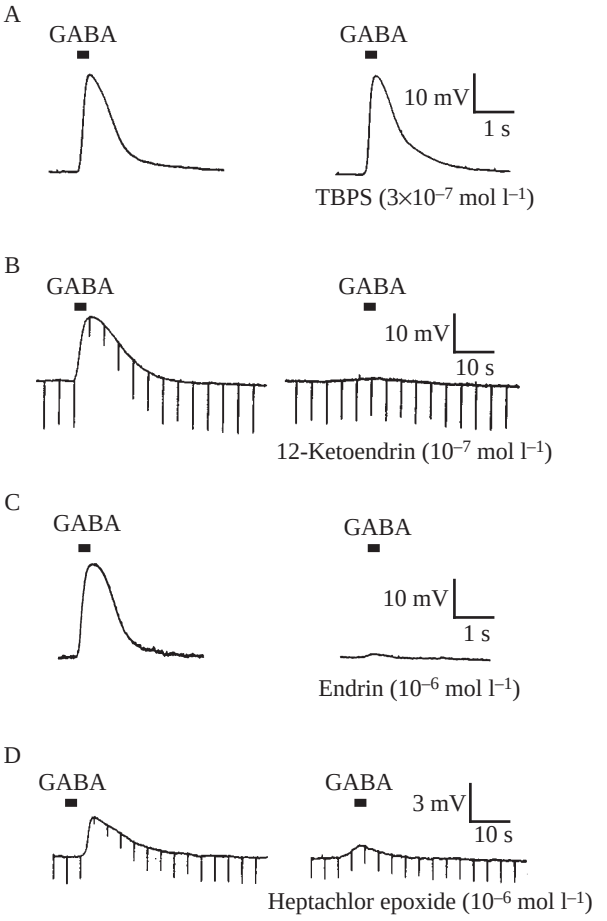


Fig. 3. Effect of putative antagonists on the cockroach coxal levator muscle response to GABA. In B and D, GABA (10^{-3} mol l^{-1}) was applied as a 2 s pulse by means of a motor-driven syringe pump, and in A and C, GABA was pressure-applied. All other ligands were applied for 10–20 min in the perfusing saline. (A) TBPS (3×10^{-7} mol l^{-1}) failed to alter depolarizations evoked by GABA. (B) GABA-evoked depolarizations were almost completely blocked by a 10 min exposure to 12-ketoendrin (10^{-7} mol l^{-1}). Muscle input resistance is monitored by passing regular 1.0 nA hyperpolarizing current pulses through a second microelectrode, as described for Fig. 1A. (C) A similar block of GABA responses was observed in the case of endrin (10^{-6} mol l^{-1}). (D) Heptachlor epoxide (10^{-6} mol l^{-1}) also strongly reduced the response to GABA.

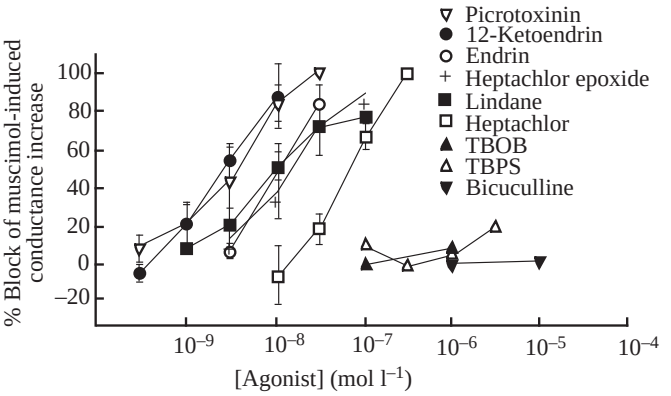


Fig. 4. Dose–inhibition curves for the response to GABA of the coxal levator muscle. The percentage block of the muscimol-induced conductance increase by putative antagonists is shown. Each point represents the mean of at least three separate experiments, vertical bars indicate ±1 S.E.M.

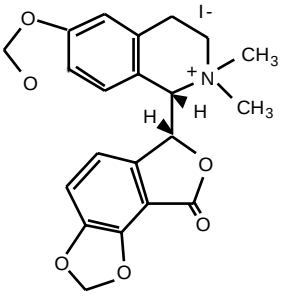
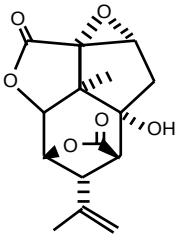
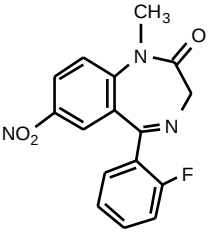
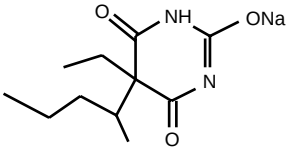
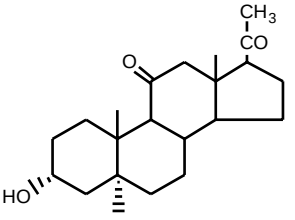
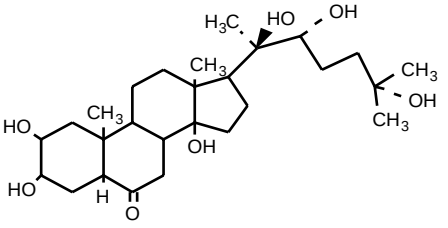
skeletal muscle (Wood, 1965). The Hill coefficient (2.05±0.36) estimated for cockroach muscle GABA receptors resembles previously reported values for insect GABA-gated chloride channels (see Sattelle, 1990 and Table 1). Our experiments have also shown that GABA receptors present on cockroach muscle are less sensitive to GABA (and, except in the case of muscimol, to GABA analogues) than are neuronal GABA receptors, as reflected by the EC_{50} values for each preparation.

In common with GABA-gated chloride channels of motor neurone D_f in the same insect, coxal levator muscle GABA receptors are insensitive to the vertebrate $GABA_A$ antagonist bicuculline methiodide (10^{-4} mol l^{-1}), but are blocked by 10^{-6} mol l^{-1} picrotoxinin (Fig. 2). This insensitivity to bicuculline contrasts with an earlier report that bicuculline blocks inhibitory postsynaptic potentials (IPSPs) (IC_{50} 2×10^{-5} mol l^{-1}) at cockroach coxal nerve–muscle preparations (Olsen *et al.* 1976). However, bicuculline is also reported to be inactive at the locust neuromuscular junction (Scott and Duce, 1987). As the responses reported here are largely from extrajunctional receptors, it cannot be ruled out that junctional and extrajunctional receptors of muscle differ in their sensitivity to bicuculline. In agreement with ^{36}Cl flux measurements on cockroach muscles (Matsumura and Ghiasuddin, 1983), electrophysiological results have shown that muscle GABA-gated chloride channels are sensitive to picrotoxinin (Table 3). Alphaxalone, a pregnane steroid anaesthetic which allosterically modulates vertebrate $GABA_A$ receptors (Harrison and Simmonds, 1984), is ineffective on cockroach muscle, as is the ecdysteroid 20-hydroxyecdysone. Similar findings have been observed for cockroach motor neurone GABA receptors (Rauh *et al.* 1990; see also Table 3). Thus, the GABA-gated chloride channels of cockroach muscle and neuronal membranes share some common pharmacological features.

However, differences emerge when the neurotransmitter recognition site is considered. Whereas 10^{-4} mol l^{-1} isoguvacine is ineffective on cockroach muscle GABA receptors, at the same concentration it is a potent neuronal GABA receptor agonist. THIP, a GABA agonist with an extremely rigid conformation (Roberts *et al.* 1981), is very active at vertebrate $GABA_A$ receptors. It is almost as effective as GABA on cockroach coxal levator muscle. In contrast, it is ineffective on the motor neurone D_f GABA receptor at . Thus, it appears that the receptor-active conformations of GABA may differ at the transmitter binding sites on cockroach muscle and neuronal receptors.

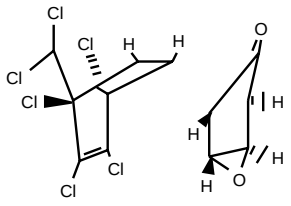
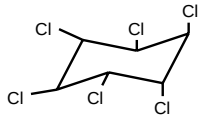
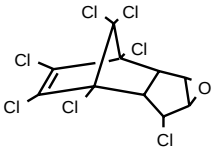
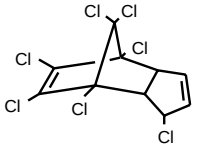
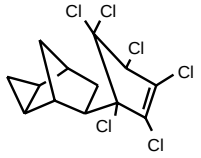
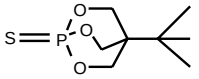
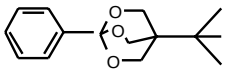
Muscle GABA receptors also differ from their neuronal counterparts in their sensitivity to certain benzodiazepines

Table 3. *Values of C_{50} for vertebrate $GABA_A$ receptor antagonists (IC_{50}) and modulators (EC_{50}) of GABA-operated chloride channels in a cockroach muscle and neurone*

Ligand	Structure	C_{50} (nmol l^{-1}) *Periplaneta americana* Coxal levator muscle	C_{50} (nmol l^{-1}) *Periplaneta americana* Fast coxal depressor motor neurone, D_f
Bicuculline methiodide		IA	IA
Picrotoxinin		41	100
Flunitrazepam		IA	1000
Sodium pentobarbital		IA	>100 000
Alphaxolone		IA	IA
20-Hydroxyecdysone		IA	IA

IA, inactive at 100 μmol l^{-1}.

Table 4. *IC_{50} values for block by polychlorocycloalkane insecticides and bicyclophosphates of muscle and neuronal GABA-operated chloride channels of insects*

Ligand	Structure	IC_{50} (nmol l^{-1}) *Periplaneta americana* Coxal muscle	Motor neurone, D_f
12-Ketoendrin		22	NT
Lindane		140	1000
Heptachlor epoxide		160	30 000
Heptachlor		750	90 000
Endrin		NT	500
TBPS		IA	>10 000
TBOB		IA	IA

TBPS, *t*-butylbicyclophosphorothionate; TBOB, *t*-butylbicyclo-orthobenzoate; IA, inactive; NT, not tested.

(Table 3). The benzodiazepine flunitrazepam, which enhances GABA responses in motor neurone D_f at a concentration of 10^{-6} mol l^{-1}, is ineffective on muscle GABA receptors at concentrations up to 10^{-4} mol l^{-1}. Diazepam is also ineffective on coxal levator muscle GABA receptors at concentrations in the range 10^{-6} to 10^{-4} mol l^{-1} (data not shown). Thus, benzodiazepines appear to be ineffective on insect muscle GABA receptors at concentrations that would have profound effects on insect neuronal GABA receptors. A [^{3}H]flunitrazepam binding site has been detected in the housefly thorax, but the binding of this radioligand is not affected by the presence of GABA (Abalis *et al.* 1983), and the thorax contains both neuronal and muscle tissues.

The barbiturates sodium pentobarbital and phenobarbitone are ineffective on the cockroach coxal levator (182c,d) muscle GABA receptors, although enhancement of locust *Schistocerca gregaria* muscle responses to GABA in the presence of phenobarbitone have been described, but only at very high (millimolar) concentrations (Scott and Duce, 1987). All benzodiazepines and barbiturates tested at high concentrations (10^{-4} mol l^{-1}) produce dose-dependent increases in the muscle input resistance (data not shown) which are not blocked by picrotoxinin. These results are similar to observations reported for pentobarbitone (Scott and Duce, 1987) that appear to be attributable to a separate site of barbiturate action, for which there is also evidence in vertebrate $GABA_A$ receptors (Turner *et al.* 1989).

By testing a number of channel-site ligands in addition to picrotoxinin, further differences between muscle and neuronal GABA receptors have been detected. TBPS is often used in radiolabelled ligand-binding studies to probe a site that is particularly sensitive to polychlorocycloalkane insecticides (Anthony *et al.* 1992; Deng *et al.* 1993). Although in vertebrates there is strong evidence that the TBPS binding site is part of the $GABA_A$ receptor, evidence that insect TBPS binding sites are coupled to GABA-gated chloride channels is less compelling. TBPS inhibits GABA-gated chloride channels in the cockroach motor neurone D_f at concentrations of 10^{-5} mol l^{-1} and above, whereas the binding constant (K_D) for [^{35}S]TBPS binding to cockroach nervous system membranes is 1.9×10^{-8} mol l^{-1} (Lummis and Sattelle, 1986). TBPS is ineffective on insect muscle GABA receptors at 3×10^{-7} mol l^{-1}. TBOB is ineffective on both preparations. Thus, it appears that TBPS displays low efficacy at a cockroach muscle GABA receptor convulsant site.

Several polychlorocycloalkane insecticides have proved to be very effective blockers of insect muscle GABA receptors (Figs 3, 4). Of these, 12-ketoendrin is the most effective. Those tested in the present study were more effective on muscle than on neuronal GABA-gated chloride channels. Thus, muscle GABA-gated chloride channels may be of significance in the toxic actions of these insecticides. However, immunocytochemical evidence shows that the cloned *Drosophila* GABA receptor subunit (RDL, *Resistant to Dieldrin*) that carries the target site for dieldrin is widely distributed in the *Drosophila* nervous system, but not in muscle (Aronstein and ffrench-Constant, 1995; Harrison *et al.* 1996).

Although coxal levator muscle membranes and fast coxal depressor motor neurone cell body membranes both possess bicuculline-insensitive, GABA-gated chloride channels, differences are noted in the sensitivity of these receptor/channel molecules to certain GABA receptor agonists, the benzodiazepine flunitrazepam, TBPS and several polychlorocycloalkane insecticides. These findings indicate that distinct types of muscle and neuronal bicuculline-insensitive GABA-gated chloride channels are present in

insects and that muscle receptors are highly sensitive to cyclodienes. Added to observations that a bicuculline-sensitive GABA receptor is also present in the insect nervous system (Waldrop *et al.* 1987; Walker *et al.* 1971), this indicates that, as in vertebrates (Olsen and Tobin, 1990), a family of GABA-gated chloride channels is present in insects, a finding consistent with the recent molecular cloning of multiple putative GABA receptor subunits (RDL, $LCCH_3$, GRD and several splice variants of RDL; ffrench-Constant *et al.* 1991; Henderson *et al.* 1994; Chen *et al.* 1994; Harvey *et al.* 1994). In view of the accumulating evidence for differences between insect GABA receptors and vertebrate $GABA_A$ receptors at sites for the action of barbiturates, benzodiazepines and steroids, molecular biological techniques applied to insect GABA receptors may assist in locating these modulatory sites to specific regions of GABA receptor molecules.

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