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A GABA-activated chloride-conductance not blocked by picrotoxin on spiny lobster neuromuscular preparations

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1 Conductance increases to γ-aminobutyric acid (GABA) were recorded in the gm6b and opener muscle of the spiny lobsters, *Panulirus interruptus* and *P. argus*.

2 GABA-evoked responses were insensitive to picrotoxin at concentrations as high as 5×10^{-5} M. Some blockade by picrotoxin was observed at higher concentrations.

3 In normal physiological saline, the reversal potential of the *Panulirus* GABA-induced response was near the resting potential. The reversal potential was unaffected by reductions in sodium and calcium. Reduction of chloride by 50% resulted in a greater than 10 mV shift in the reversal potential of the GABA-induced response.

4 Muscimol was able to mimic the action of GABA while baclofen was without effect. Bicuculline was a weak blocker.

5 Avermectin B_1a irreversibly increased the chloride permeability of the gm6b membrane. This conductance increase was blocked by picrotoxin over a range of concentrations similar to those required for blockade of the GABA-induced response.

6 GABA-induced responses of the gm6b muscle of *Homarus americanus* were blocked almost completely by picrotoxin 10^{-6} M.

7 Sensitivity to picrotoxin is not invariably associated with GABA-activated chloride-mediated conductance increases. It is suggested that alteration in the binding-site for picrotoxin on the GABA-activated chloride-ion channel does not change other functional characteristics of the GABA-induced response.

Introduction

The alkaloid, picrotoxin, is known for its ability to block many inhibitory conductance increases activated by γ-aminobutyric acid (GABA) in both invertebrate and vertebrate systems (Nistri & Constanti, 1979; Simmonds, 1983; Tallman & Gallagher, 1985). Detailed electrophysiological analyses of the action of picrotoxin have been performed on crustacean preparations, including both neuromuscular systems (Grundfest *et al.*, 1959; Takeuchi & Takeuchi, 1969; Shank *et al.*, 1974; Earl & Large, 1974; Constanti, 1978) and the crayfish stretch receptor (Hagiwara *et al.*, 1960; Adams & Banks, 1980; Adams *et al.*, 1981; Aickin *et al.*, 1981). Despite this extensive investigation, molecular details of both the site(s) and mechanism of action of picrotoxin remain unresolved. Based primarily on non-competitive aspects of its block of GABA-activated conductance increases, the action of picrotoxin is widely held to involve some action on the chloride-permeable ion channel (Nistri & Constanti, 1979; Simmonds, 1983). However, such a view encompasses a broad range of possible mechanisms and little direct evidence in support of this idea has been advanced.

In addition to its action on GABA-activated chloride conductance increases, some other transmitter-activated conductances are somewhat picrotoxin-sensitive, including both cation and anion conductance increases (Marder & Paupardin-Tritsch, 1978; 1980; Lingle & Marder, 1981). Additionally, in the stomatogastric ganglion of the crab, *Cancer pagurus*, GABA-activated chloride-conductance increases are insensitive to picrotoxin concentrations as high as 10^{-4}M (Marder & Paupardin-Tritsch, 1978). We are interested in the possibility that the ability of

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picrotoxin to block GABA-activated chloride conductances may involve a site that is not functionally important to the anion permeation process. In this paper we extend earlier studies and show that GABA-activated chloride conductance increases on both foregut and opener muscle preparations are resistant to picrotoxin in the spiny lobsters, *Panulirus argus* and *Panulirus interruptus*. Since the GABA-induced response in *Panulirus* appears in all ways to be similar to the picrotoxin-sensitive GABA-induced responses of many other crustacean neuromuscular junctions, the results suggest that the integrity of the site of action of picrotoxin is not a requirement for the normal function of the GABA receptor/channel. Rather, picrotoxin-sensitivity cannot be considered to be invariably coupled to the GABA-activated chloride channel.

Methods

Preparations

The methods used were similar to those described previously (Lingle & Marder, 1981). *Panulirus interruptus* was obtained from Pacific Biomarine or Marinus; *P. argus* was obtained from the Florida Keys, courtesy of Dr. B. Ache, Whitney Marine Laboratory. The American lobster, *Homarus americanus*, was obtained from local markets in Boston. Foregut muscles are identified by the nomenclature of Maynard & Dando (1974). Muscles with intact ossicles were isolated from the animal and pinned in 1–3 ml Sylgard-coated superfusion dishes. For measurement of membrane resistance changes, muscle fibres were impaled with two microelectrodes, one for voltage recording and one for current injection. Electrodes contained either 3 M KCl or, for current injection, 4 M K-acetate/0.1 M KCl.

Drug application and saline substitutions were controlled by hand- or solenoid-activated switches in the perfusion line. Experiments on *P. interruptus* were done at 12–14°C and on *P. argus* at room temperature (22–24°C).

The physiological saline was the same for all animals (composition, mM): NaCl 479, KCl 12.7, $CaCl_2$ 13.7, $MgCl_2$ 10 and Tris-maleate 8.3 (pH 7.3–7.5); 50% sodium saline contained 240 mM choline chloride/239 mM NaCl as the primary salts. To produce changes in the chloride equilibrium potential, a 50% chloride saline containing 240 mM sodium propionate/240 mM sodium chloride was used. Previous work has indicated that propionate is impermeant through the GABA-activated conductance pathway in Crustacea (Takeuchi & Takeuchi, 1966). Although intracellular pH changes associated with the use of propionate (Sharp & Thomas, 1981) might be expected to influence the magnitude of the GABA-induced response (Takeuchi & Takeuchi, 1967), propionate was suitable for testing whether a transient shift in the chloride equilibrium potential would influence the reversal potential of the GABA-induced response. Junction potentials resulting from changes in the ionic milieu were measured and used to correct current voltage-curves to true membrane potential values. In many experiments, $MnCl_2$ 20 mM was added to the saline to stabilize electrode impalements for the long periods desired for these experiments. No change in sensitivity to GABA or in the action of picrotoxin on the GABA-induced response was noted between salines containing manganese and those without it. Manganese, (5 mM) has previously been reported to be without effect on GABA-activated conductance changes in the crayfish and lobster stretch receptor (Nichols & Nakajima, 1975).

The physiological and anatomical details of the decapod foregut neuromuscular system have been described elsewhere (Maynard & Dando, 1974; Govind *et al.*, 1975). Basically, the decapod foregut muscles are typical crustacean striated muscles receiving polyneuronal and multiterminal innervation. Unlike crustacean limb muscle, no inhibitory innervation to the foregut muscles has been described. The length of the gm6b muscle used in this study is typically at least 10 times its space constant. As a result, responses to GABA or other agents were calculated simply as changes in input resistance, i.e., the difference between the input resistance in control saline and the input resistance following perfusion with GABA. The degree of blockade produced by a drug was calculated as the percentage reduction in the GABA-induced change in input resistance in the presence of drug relative to the GABA-induced change in input resistance in the absence of the drug. For comparison among muscle fibres of different preparations, values were normalized to the responses evoked by GABA 10^{-4} M. Since the gm6b muscle behaves as a long cable, the use of input resistance as a measure of response is likely to result in an underestimate of the GABA-activated conductance changes and an overestimate of blockade produced by picrotoxin.

Drugs

3-Aminobutyric acid, picrotoxin, bicuculline methiodide, muscimol, β-guanidinopropionic acid, strychnine sulphate, imidazole acetic acid, 3-aminopropylphosphonic acid and β-alanine were obtained from Sigma Chemical Company. Baclofen was the generous gift of Ciba Pharmaceutical. Avermectin B_1a was the generous gift of Merck and Co. Avermectin was dissolved in saline with 2% dimethylsulphoxide (DMSO); when 2% DMSO was added alone to lobster

saline it produced no effect on resting input resistance properties of the gm6 muscle. Bicuculline was brought into solution with gentle heating at low pH, diluted into saline, and used within 1 h. The instability of bicuculline in lobster saline was indicated by a diminution in the blocking effects of bicuculline between GABA-induced responses elicited at intervals of 10 min. As a result, estimates of blocking potency of bicuculline are only approximate. When an agent was tested for antagonist activity, the preparation was first exposed to the drug alone for 2–5 min before a solution containing both GABA and the potential antagonist was introduced to the preparation. At the high concentrations of picrotoxin required for any effect on the GABA-induced response in *Panulirus*, washout from any picrotoxin blocking action was quite slow. In many cases, this precluded restoration of the GABA sensitivity that preceded picrotoxin application. It was therefore not possible to control adequately for gradual rundown of GABA sensitivity that results from desensitization. As a result, the observed blockade produced by high concentrations of picrotoxin may overestimate the true effect of picrotoxin in *Panulirus*.

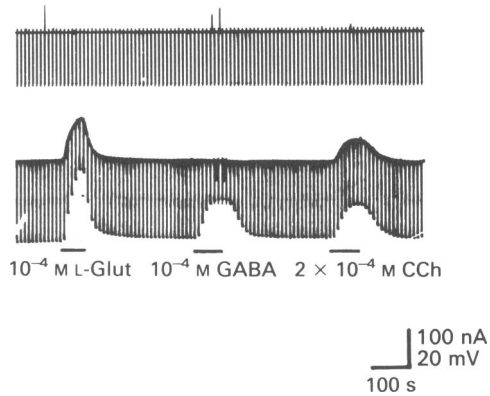

Figure 1 Sensitivity of the gm6b muscle of *Panulirus argus* to L-glutamate (L-Glut), γ-aminobutyric acid (GABA) and carbachol (CCh). Agonists were applied sequentially at 10^{-4} M L-glutamate, 10^{-4} M GABA, and 2×10^{-4} M carbachol for the periods indicated by the bars. The resting membrane potential was −78 mV. Top trace monitors injected current and bottom trace, membrane potential. Vertical calibration: 100 nA and 20 mV; horizontal calibration: 100 s.

Results

The gm6b muscle

The gm6b muscle of decapod crustaceans is innervated by a single excitatory motor neurone that appears to use glutamate as its neurotransmitter (Lingle, 1980; Lingle *et al.*, 1981). The gm6b muscle also has a number of responses to other neurotransmitters (Figure 1) including an excitatory conductance increase to acetylcholine ACh (Lingle, 1980), and an inhibitory conductance increase to GABA.

GABA-induced responses in Panulirus*: resistance to picrotoxin*

The gm6b muscle of both *P. interruptus* and *P. argus* responds to GABA at concentrations above 5×10^{-6} M. This response is a conductance increase with a reversal potential near the resting potential. The left-hand panel of Figure 2a shows the results of bath application of 2×10^{-4} M GABA to the gm6b muscle of *P. argus*. The dose-response curve for GABA in this muscle is shown in the control points of Figure 2b. In both species maximal decreases in input resistance approached about 80% at 2×10^{-4} M GABA in some fibres. The threshold concentration was between 5×10^{-6} M and 10^{-5} M in both species. Despite the marked sensitivity of these muscles to GABA, there is no known inhibitory innervation to these muscles.

The relative insensitivity of the GABA-activated conductance increase of the gm6 muscle to picrotoxin is illustrated in Figure 2. In Figure 2a, the effects of several concentrations of picrotoxin on the GABA-induced responses are illustrated. The lowest concentration shown (5×10^{-6} M) produces an almost complete blockade of GABA-induced responses on the crayfish (Takeuchi & Takeuchi, 1969) and *Homarus* opener muscle (Constanti, 1978) but has no blocking action on the *Panulirus* gm6b muscle. At picrotoxin concentrations above 5×10^{-5} M a small reduction in the GABA-induced response of the gm6b muscle was sometimes observed. However, even at 5×10^{-4} M picrotoxin, the highest dose tested, the reduction in the GABA-induced response did not exceed 50%. Thus, in comparison with the sensitivity of the *Homarus* opener muscle, the *Panulirus* gm6b muscle is about 1000 fold less sensitive to picrotoxin. The shape of the GABA dose-response curve in the absence and presence of 500 μM picrotoxin is illustrated in Figure 2b. This plot suggests that picrotoxin produced the same percentage block of the GABA-induced response at all GABA concentrations, consistent with a non-competitive mode of blockade. However, it is not our intent to present a detailed quantitative analysis of the blocking mechanism and our data are insufficient to exclude a mixed type of antagonism. The present results only serve to provide a general comparison with earlier more detailed studies of picrotoxin action (Constanti, 1978).

Since the opener muscles of the walking leg of the crayfish and of *Homarus* are the classical preparations

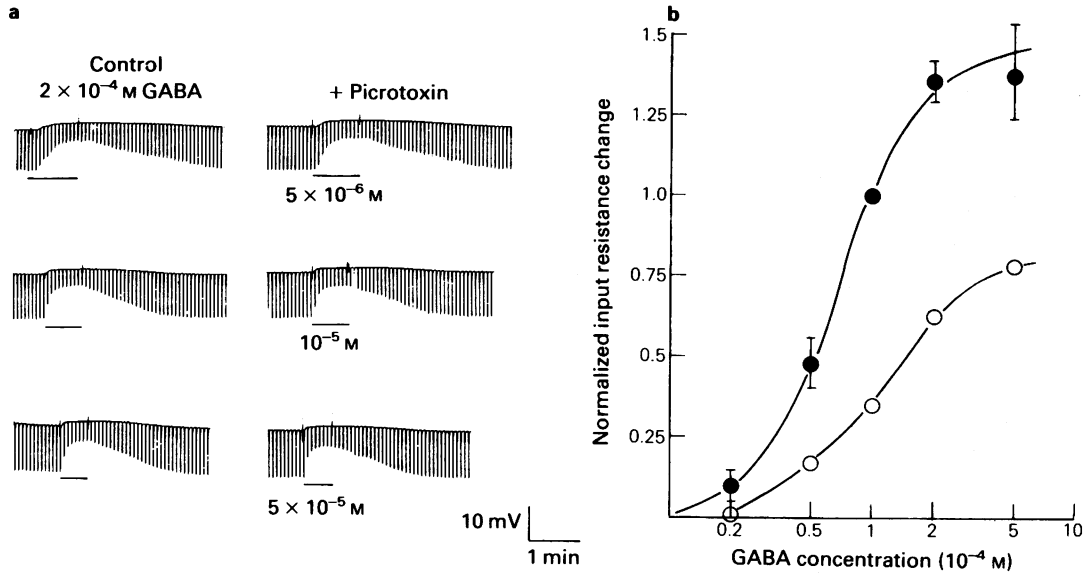

Figure 2 The γ-aminobutyric acid (GABA)-induced response and its insensitivity to picrotoxin in gm6b muscle. (a) Responses to 2×10^{-4} M GABA in the absence (left) and presence (right) of picrotoxin. Picrotoxin concentrations from top to bottom were 5×10^{-6} M, 10^{-5} M, and 5×10^{-5} M. Vertical deflections monitor responses to constant current injection. In each case picrotoxin alone was perfused over the preparation for 5 min before simultaneous GABA and picrotoxin administration. The resting membrane potential was −82 mV. Vertical calibration: 10 mV; horizontal calibration: 1 min. (b) Dose-response curve for the GABA-activated conductance increase is plotted along with a dose-response curve in the presence of 5×10^{-4} M picrotoxin. Conductance changes were normalized to the response at 10^{-4} M GABA.

for demonstration of picrotoxin-sensitivity of GABA-mediated inhibition (Grundfest *et al.*, 1959; Takeuchi & Takeuchi, 1969; Shank *et al.*, 1974; Earl & Large, 1974; Constanti, 1978), the relative insensitivity to picrotoxin of GABA-induced responses of gm6b muscle prompted us to examine the opener muscle of the walking leg of *P. argus*. One such experiment is illustrated in Figure 3 in which a series of GABA-induced responses are examined over a range of picrotoxin concentrations. Surprisingly, similar to the results obtained with the gm6b muscle, the opener muscle of *P. argus* exhibited slight or little sensitivity to picrotoxin.

Finally, the gm6b muscle of *Homarus americanus* was examined for sensitivity to picrotoxin. Figure 4 shows that the response of the gm6b muscle of *Homarus* to 5×10^{-4} M GABA was almost completely blocked by 10^{-6} M picrotoxin. These data support the interpretation that GABA-induced responses on *Panulirus* muscles differ from the homologous muscles in *Homarus* in their sensitivity to picrotoxin.

Picrotoxin sensitivity of avermectin B_1a-activated conductance increase

The antihelminthic agent, avermectin B_1a, has been reported to activate a chloride channel through an action on some site other than the GABA-receptor site (reviewed by Campbell *et al.*, 1983). At the lobster neuromuscular junction, activation of a chloride conductance by avermectin is irreversible, but the conductance retains its sensitivity to picrotoxin (Fritz *et al.*, 1979). In order to assess further the relative resistance of the *Panulirus* GABA-induced response to picrotoxin, the action of avermectin and its sensitivity to picrotoxin was examined.

Avermectin $10\,\mu g\,ml^{-1}$ was found to produce a dramatic and irreversible decrease in the gm6b muscle fibre input resistance (Figure 5a). This conductance increase is reduced by picrotoxin. A threshold effect of picrotoxin is detectable at 5×10^{-6} M. However, the dose-dependent reduction of the avermectin-induced change in input resistance produced by picrotoxin overlaps that of the sensitivity of the GABA-activated conductance to picrotoxin (Figure 5b). Thus, irrespective of whether the conductance is activated by GABA or avermectin, the effectiveness of picrotoxin remains about the same.

Other properties of GABA-induced responses in Panulirus

Several experiments were done to determine whether any other aspects of the GABA-activated conductance

change in *Panulirus* differs from that found in other crustacean preparations.

To assess the ionic basis of the GABA-induced response, the sodium content of the saline was reduced by half by substitution of sodium chloride with choline chloride (50% sodium saline). GABA-activated conductance increases were measured both in normal saline and during perfusion with 50% sodium saline. Both the magnitude and the reversal potential for GABA-induced responses were unchanged. Similarly, removal of calcium had no effect on the conductance change produced by GABA.

Similarly, the chloride content of the saline was reduced by substitution for 50% of the sodium chloride with sodium propionate (50% chloride saline). Only slight changes of the resting membrane potential were produced by perfusion with 50% chloride saline suggesting that the resting chloride conductance of this muscle is low. Figure 6a illustrates current-voltage curves for a muscle fibre in normal physiological saline and following application of 2×10^{-4} M GABA. In normal saline, the reversal potential for the GABA-induced response was approximately -78 mV. Subsequently, the fibre was perfused with 50% chloride saline which produced a negligible change in resting potential. Application of 2×10^{-4} M GABA in 50% chloride saline then resulted in a depolarizing conductance increase with a reversal potential of -57 mV. The magnitude of this shift is consistent with a chloride-dependent response. Similar results were obtained with two other fibres with the shift in reversal potential being greater than 10 mV in both cases. After about 15–30 min in low chloride saline, the reversal potential for the GABA-activated conductance change gradually approaches the resting potential. Although propionate is known to cause a decrease in intracellular pH in crab muscle (Sharp & Thomas, 1981), no significant effect of the propionate saline on the magnitude of the GABA-induced response was observed.

Because of the unique pharmacological sensitivity of the GABA-induced response in *Panulirus*, a variety of other agents known to mimic the action of GABA were examined. Muscimol was the most potent agonist producing changes in conductance with a threshold of about 5×10^{-6} M. The reversal potential for the muscimol response was near the resting potential. Imidazoleacetic acid produced conductance increases

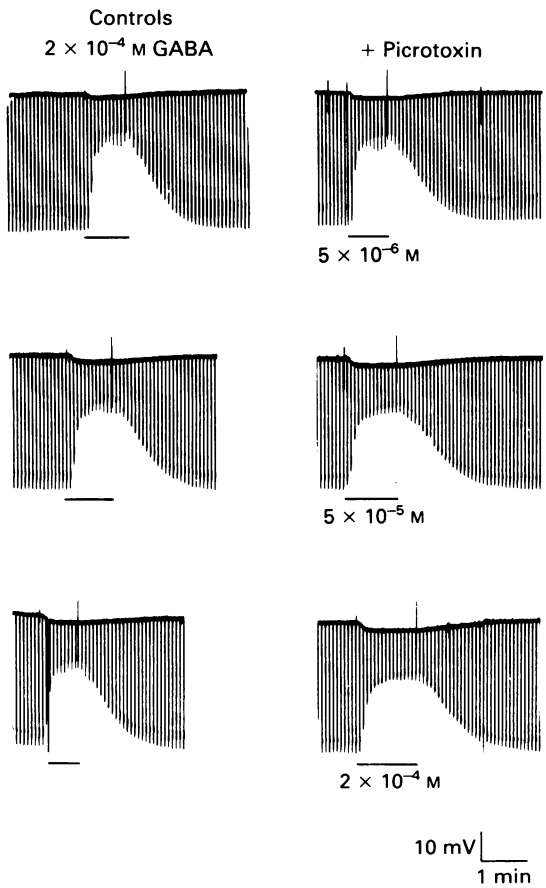

Figure 3 Lack of effect of picrotoxin on γ-aminobutyric acid (GABA)-induced response of *Panulirus argus* opener muscle. Similar to Figure 2, responses to 2×10^{-4} M GABA in the absence (left) and presence (right) of picrotoxin are displayed. Picrotoxin concentrations from top to bottom were 5×10^{-6} M, 5×10^{-5} M and 2×10^{-4} M. The resting membrane potential was between -70 mV and -72 mV for all traces. Vertical calibration: 10 mV; horizontal calibration: 1 min.

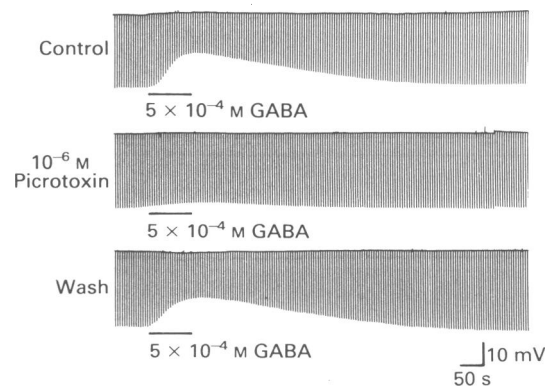

Figure 4 Effect of picrotoxin on the γ-aminobutyric acid (GABA)-induced response of gm6b muscle of *Homarus americanus*. The bars show the time of application of 5×10^{-4} M GABA. Vertical deflections monitored responses to constant current pulses. The middle panel was taken in the presence of 10^{-6} M picrotoxin. The resting membrane potential was -72 mV. Vertical calibration: 10 mV; horizontal calibration: 50 s.

at 5×10^{-4} M (Constanti & Quilliam, 1974), while 3-aminopropylphosphonic acid was without effect at up to 1 mM. β-Guanidinopropionic acid (BGP) produced slight decreases (> 10%) in input resistance of gm6b muscle at 5×10^{-4} and 1 mM. Simultaneous application of 1 mM BGP and GABA resulted in substantial reductions in the GABA-induced response. This is consistent with the idea that BGP occupies the receptor for GABA and activates the Cl^--channel.

Baclofen, an agonist of a lobster presynaptic GABA receptor (Barry, 1984), was entirely without effect on the gm6b muscle at concentrations as high as 1 mM. β-Alanine and glycine (1 mM) produced negligible effects.

Bicuculline, a rather weak antagonist of GABA-mediated inhibition in arthropods, produced a weak inhibition on the gm6b GABA-induced response with 50% blockade occurring between about 10^{-4}M and

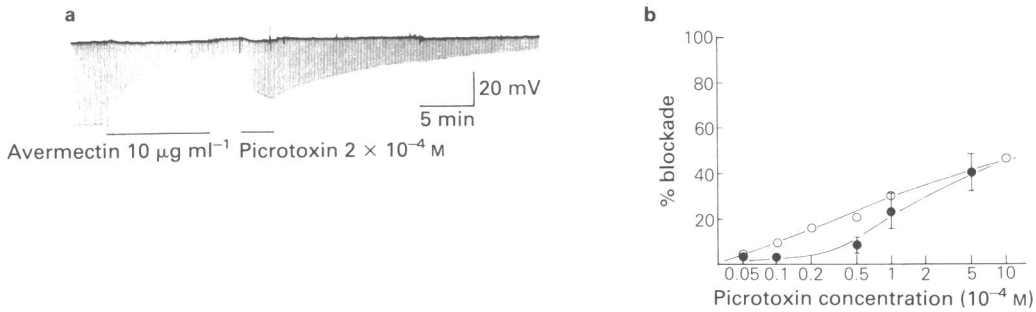

Figure 5 Sensitivity of the gm6b muscle to avermectin. In (a) avermectin B_1a 10 μg ml^{-1} in 2% DMSO elicited an irreversible conductance increase with a reversal potential at the resting potential. Picrotoxin 2×10^{-4} M reduced the conductance increase. Note the slow washout of the picrotoxin effect. The cell resting potential was −68 mV. Vertical calibration: 20 mV; horizontal calibration: 5 min. In (b) the percentage reduction of the avermectin- and GABA-induced input resistance changes produced by different picrotoxin concentrations is plotted: (○): picrotoxin blockade of avermectin-induced responses; (●): picrotoxin blockade of responses to GABA (concentrations between 10^{-4} and 5×10^{-4} M were used; note similar percentage blockade in Figure 1b). Error bars for GABA-induced responses indicate s.e.mean for 4 or more determinations.

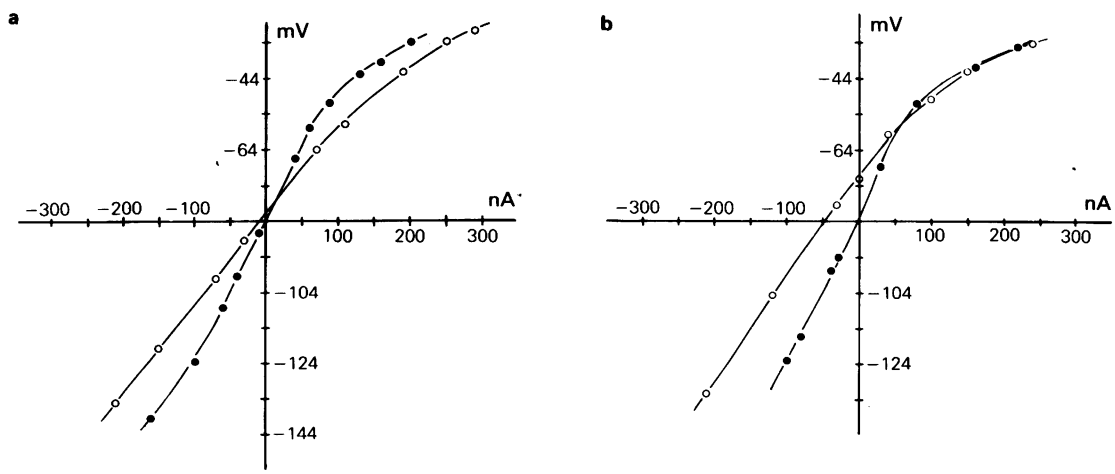

Figure 6 Shift of reversal potential of γ-aminobutyric acid (GABA)-activated conductance change in low chloride saline. In (a) current-voltage curves for a gm6b muscle fibre of *P. argus* in the presence (○) and absence (●) of 2×10^{-4} M GABA in normal lobster saline are shown. In (b) following perfusion of the muscle with 50% chloride saline (propionate substitution), current-voltage curves in the presence (○) and absence (●) of 2×10^{-4} M GABA were again generated. The plots in (b) has been corrected for an 8 mV junction potential offset resulting from perfusion with the low chloride saline.

5×10^{-4}M. In *Homarus* opener muscle, Zn^{2+} has been found to reduce GABA-activated conductance increases through action at a site distinct from the picrotoxin site (Smart & Constanti, 1982). In the gm6b muscle of *P. argus*, 10^{-4} M Zn^{2+} was found to produce a mean reduction of 74.7% (± 8.1 s.d.; $n = 4$) in the conductance change produced by 2×10^{-4} M GABA. This corresponds closely to the sensitivity of *Homarus* muscle to Zn^{2+}.

Discussion

Summary of properties of spiny lobster GABA response

The essential finding of this paper is that GABA-activated conductance increases on neuromuscular preparations in two different species of spiny lobster are relatively insensitive to picrotoxin. Specificially, picrotoxin insensitivity was observed on the opener muscle of the walking leg of the spiny lobster, *Panulirus argus*, and on the gm6 muscles of both *P. argus* and *P. interruptus*. For comparison, the gm6 muscle of *Homarus americanus* exhibited sensitivity to picrotoxin similar to that observed in *Homarus* opener muscle (Constanti, 1978).

Several tests were made that supported the idea that the GABA-induced response in *Panulirus* is similar to GABA-activated conductances found on other crustacean muscles. The response involves a chloride-mediated conductance increase. Muscimol, but not baclofen, was an effective agonist. β-Guanidinopropionic acid exhibited some weak agonist effects, while acting as an antagonist when applied together with GABA. As found in *Homarus* opener muscle (Smart & Constanti, 1982), Zn^{2+} produced a reduction in the *Panulirus* gm6b GABA-induced conductance change. Similar to its action on *Homarus* opener muscle (Fritz *et al.*, 1979), avermectin $B_{1}a$ produces an irreversible increase in chloride conductance presumably from an action on the GABA receptor/ion channel.

Comparison of picrotoxin sensitivity among crustacean species

The competitive and non-competitive aspects of picrotoxin action on other crustacean preparations preclude precise quantitative comparison to the present results. For a rough estimate of blocking potencies, the concentration of picrotoxin required to produce 50% reduction of GABA-induced responses at near saturating GABA concentrations will be used. In *Homarus* opener muscle, less than 4×10^{-7} M picrotoxin produced a 50% reduction in the conductance change to 1.6×10^{-4}M GABA (Constanti, 1978). In crayfish opener muscle, 2×10^{-6} M picrotoxin produced a 50% reduction of GABA-activated conductances (Takeuchi & Takeuchi, 1969), while the opener muscle of the hermit crab, *Eupagarus*, exhibited a sensitivity to picrotoxin intermediate to that of *Homarus* and the crayfish (Earl & Large, 1974). In contrast, in *Panulirus*, concentrations in excess of 5×10^{-4} M were needed to produce 50% blockade of the GABA-induced response. Thus, the GABA-activated Cl^--dependent conductance increase in *Panulirus* is at least 2.5 to 3.5 orders of magnitude less sensitive to picrotoxin than those observed in other crustacean species. Although other crustacean preparations have been used to study aspects of GABA-induced responses, the action of picrotoxin on these systems has not been reported (e.g. Sarne, 1976; Hochner *et al.*, 1976).

More information about the distribution of picrotoxin-insensitive GABA-activated Cl^- responses in other species would be of interest. In the stomatogastric ganglion of the crab, *Cancer pagurus*, 10^{-4} M picrotoxin had no effect on a GABA-activated chloride-dependent response, although it did produce a 50% reduction of GABA-activated potassium-dependent responses (Marder & Paupardin-Tritsch, 1978). Similar information is not available for neuronal responses to GABA in *Panulirus*. If there is similarity in picrotoxin sensitivity between neuronal and neuromuscular responses induced by GABA, neuromuscular GABA-activated responses of *Cancer pagurus* might be resistant to picrotoxin.

Minimally, the present results indicate that caution must be exercised in the use of picrotoxin as a diagnostic tool for GABA-mediated inhibition. This is further supported by evidence demonstrating that picrotoxin is somewhat effective in blocking a variety of non-GABA-activated and non-chloride dependent conductances (Marder & Paupardin-Tritsch, 1978; 1980; Lingle & Marder, 1981). Recent work indicating that resistance to picrotoxin may be a feature of at least some vertebrate $GABA_A$ receptors (Hankins & Ruddock, 1984) suggests that picrotoxin sensitivity may not always be associated with GABA-mediated inhibition in vertebrates either.

The sites and mechanism of picrotoxin action

Two primary features characterize the model proposed by Constanti (1978) to account for the mixed nature of picrotoxin action in Crustacea. First, GABA binding might influence the affinity of picrotoxin for its binding site, and, second, picrotoxin binding might initially produce an unblocked-bound state, leading subsequently to a blocked state. That the picrotoxin binding site may interact allosterically with the GABA binding site is also supported by work on vertebrate GABA receptors (Fujimoto & Okabayashi, 1981; Supavilai *et al.*, 1982).

The primarily non-competitive nature of picrotoxin action as determined from electrophysiological dose-response measurements and the lack of competitive effect of picrotoxin on GABA binding support the idea that the picrotoxin binding site may be the chloride-permeable ion channel. Similarly, the ability of chloride ion concentrations to influence the action of picrotoxin (Takeuchi & Takeuchi, 1969; Supavilai *et al.*, 1982) has been used to argue that picrotoxin acts at a site in close relation to the ion permeation pathway. However, these types of evidence are neither conclusive nor direct and, as yet, electrophysiological analyses of the effect of picrotoxin on GABA channel kinetic properties have failed to reveal a direct interaction of picrotoxin with the ion permeation pathway in either crustacean (Adams & Banks, 1980; Adams *et al.*, 1981) or vertebrate systems (Barker *et al.*, 1983). However, such studies do not exclude an open-channel block mechanism with slow dissociation rates or blockade of the channel while in a closed conformation.

To explain the relative insensitivity of the crustacean GABA-induced Cl^--dependent responses to picrotoxin described here, the simplest explanation is that a modification of the picrotoxin-binding site has resulted in a thousand fold reduction in affinity of picrotoxin for its binding site. This would be analogous to the tetrodotoxin-resistant sodium channels found in denervated mammalian muscle (Harris & Thesleff, 1973) in which other functional aspects of the channel molecule appear unaffected. Given the view that the picrotoxin site may reflect anion binding sites that are part of or close to the mouth of the ion channel (Tallman & Gallagher, 1985), alteration in that site might be expected to have other functional consequences. However, whatever accounts for the relative insensitivity reported here it is apparent that alteration of the picrotoxin binding site produced no detectable change in a variety of other properties of the GABA-activated conductance change.

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