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ELECTROPHYSIOLOGICAL INVESTIGATION OF GABA-MEDIATED INHIBITION AT THE HERMIT CRAB NEUROMUSCULAR JUNCTION

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SUMMARY

1. The inhibitory neuromuscular junction of the abductor muscle of the large claw of the hermit crab (*Eupagurus bernhardus*) was investigated using electrophysiological intracellular techniques in order to elucidate further the relative contributions of the pre- and post-synaptic mechanisms of action of GABA and of neural inhibition.

2. The electrical constants of the post-synaptic membrane, calculated using the equations for a 'short cable' model, were characteristic of a poorly developed electrical excitability; the specific membrane resistance was usually $< 1000\ \Omega\ cm^2$ and the specific membrane capacitance was $> 40\ \mu F/cm^2$.

3. Stimulation of the excitatory axon to the abductor muscle of the large claw at a frequency of 20 Hz evoked highly facilitating excitatory junction potentials (e.j.p.s); stimulation of the inhibitory axon (60–220 Hz) during the excitatory train elicited inhibition which was manifest as an attenuation of the e.j.p.s.

4. The addition of γ-aminobutyric acid (GABA) to the bathing solution produced a dose-dependent reduction of e.j.p. amplitude and membrane resistance. The inhibitory effect of concentrations (5×10^{-5} and 1×10^{-4} M) which caused a 40–75 % e.j.p. attenuation could largely be accounted for by a post-synaptic action on membrane conductance.

5. Experiments with picrotoxin suggest that presynaptic inhibitory mechanisms have an important role in neurally evoked inhibition.

6. Picrotoxin ($1–5 \times 10^{-5}$ M) effectively blocked neural inhibition and the actions of GABA in this preparation, whereas bicuculline proved to be considerably less potent and therefore less useful as a physiological tool for studying GABA-mediated inhibition in crustacea.

INTRODUCTION

One of the striking features of crustacean skeletal muscle is that, unlike that of vertebrates, it possesses an inhibitory innervation in addition to its excitatory nerve supply. Inhibition at the neuromuscular synapse is achieved by both pre- and post-synaptic mechanisms. There is strong evidence (see Gerschenfeld, 1973) that the inhibitory neurotransmitter at the crustacean neuromuscular junction is γ-aminobutyric acid (GABA) which has been shown to mediate both types of inhibition by a selective increase in membrane conductance for chloride ions (Dudel & Kuffler, 1961; Boistel & Fatt, 1958; Takeuchi & Takeuchi, 1966). Moreover in the crayfish it has been reported that the presynaptic component of the GABA inhibition is more potent than the post-synaptic one (Dudel, 1965), although subsequently little work has been published concerning this problem.

In these experiments we have studied the inhibitory synapse of the abductor muscle of the large claw of the hermit crab (*Eupagurus bernhardus*). The advantages of this species are, firstly, that the abductor muscle is innervated by a single excitatory axon and a single inhibitory axon which are present in separate nerve bundles, thus obviating the need to split the bundles (Wiersma & van Harreveld, 1934), and secondly, that hermit crabs are readily obtainable in Britain throughout the year.

This study was undertaken to elucidate further the relative contributions of the pre- and post-synaptic mechanisms of action of GABA and of neural inhibition. It was also of interest to compare the usefulness of picrotoxin and bicuculline as physiological tools in the study of GABA-mediated inhibition in crustacea. A preliminary account of some of the work has been published (Earl & Large, 1972).

METHODS

Preparation. Specimens of *Eupagurus bernhardus* were obtained weekly from the Marine Biological Association, Plymouth and were kept in an aquarium in our laboratory. The sea water was constantly circulated to allow adequate aeration and was filtered through a charcoal bed. The temperature was maintained at 12–14° C and under these conditions the crabs could survive for several weeks.

The large claw was removed at the natural point of autotomy between the ischiopodite and meropodite and the nerve bundles were exposed in the latter by removal of the overlying flexor muscle. The bundle containing the inhibitory axon to the abductor is considerably thicker than that containing the excitatory axon thus permitting ready identification of each. In order to prevent physical movement due to stimulation of whole bundles, all tendons except that of the abductor were cut and the claw was tied in the closed position. After exposure of the abductor muscle, the claw was anchored with Plasticine in a Perspex bath. The Ringer composition, modified from that of Davenport (1941), was NaCl 445 mM; KCl 12·2 mM; $CaCl_2$ 29·6 mM, $MgCl_2$ 5·75 mM, $NaHCO_3$ 1·79 mM. The pH was 6·8–7·0 and

the temperature was maintained at 16–18° C. Surface fibres were illuminated by reflected light and viewed through a binocular microscope.

Stimulating and recording conditions. Conventional electrophysiological techniques for stimulation and intracellular recording were used. A Devices 'Digitimer' was used to deliver trains of pulses to suction electrodes containing the nerve bundles. When measuring membrane conductance, rectangular hyperpolarizing pulses were injected through an intracellular micro-electrode; in these experiments a 200 MΩ resistor was included in the circuit to minimize fluctuations in current arising from changes in micro-electrode resistance. The current was monitored by measuring the voltage drop across a 10 kΩ resistor. Both intracellular stimulating and recording micro-electrodes were filled with 3 M-KCl and had tip resistances of 5–30 MΩ. Changes in potential were displayed on an oscilloscope and photographed with a Polaroid camera or recorded with a Mingograf 34.

It was often possible to study synaptic events for 1–2 hr, but sudden, unexplained failure of initially satisfactory preparations was a constant problem.

The measurement of electrical constants of excitable membranes, assuming an infinite cable theory, has been described by Hodgkin & Rushton (1946). Applying this model to the hermit crab fibres, the calculated length constant (λ) was always more than half the fibre length and therefore, as for other crustacea, we used the modified 'short cable' equations (Weidmann, 1952), which assume that the fibre is terminated at each end by an infinite resistance. For measurement of membrane resistance and capacitance, two intracellular micro-electrodes were used. The current-passing electrode was impaled in the middle of the fibre. The recording electrode was placed firstly at the end of the fibre and then within 50 μm of the current electrode. The value of L/λ, and hence λ (where L = half-length of fibre; λ = length constant) was calculated from the ratio of electrotonic potentials at the middle (V_0) and end (V_L) of the fibre, according to the equation (Takeuchi & Takeuchi, 1967)

$$V_0/V_L = \cosh(L/\lambda). \tag{1}$$

For the short cable model, the following equation holds when both stimulating and recording electrodes are in the middle of the fibre

$$V_0/I_0 = (r_i/2)\,\lambda \coth(L/\lambda), \tag{2}$$

where I_0 = electrode current; r_i = internal resistance of fibre per unit length (Ω cm^{-1}).

Since $\lambda = (r_m/r_i)^{\frac{1}{2}}$, where r_m is the membrane resistance × unit length (Ω cm), eqn. (2) can be rearranged:

$$r_m = (2V_0/I_0)\,\lambda\,(1/\coth L/\lambda) \tag{3}$$

and hence the specific membrane resistance, R_m, can be calculated as $R_m = 2\pi a r_m$, where a is the fibre radius. The fibre diameters and lengths were measured using a calibrated graticule in the eyepiece of the microscope and the values obtained were used in the calculations. These measurements possibly introduced two main errors in the calculations; firstly, it was not possible to observe the 'shell end' of the fibre as it was necessary to leave some exoskeleton to prevent detachment of the muscle and therefore an estimate of length was made assuming the muscle continued to the vertical edge of the shell. Secondly, the diameter was estimated assuming the fibres to be cylindrical and there to be no overlap of adjacent fibres.

Membrane capacitance (C_m) was calculated from $C_m = \tau_m/R_m$, where τ_m is the membrane time constant. For an infinite cable, τ_m is taken as the time for $V_{0,t}$ ($V_{0,t}$ = value of V_0 at time t) to attain 85% of its maximal value V_0. However, as pointed out by Stefani & Steinbech (1969), a correction is necessary for a short cable, where the value of $V_{0,t}/V_0$ depends on the ratio of $2L/\lambda$.

Membrane conductance was measured by the method of Takeuchi & Takeuchi (1967) using the relation

$$g_m L = (I/2V_0)\,(L/\lambda)\ \coth\ (L/\lambda), \tag{4}$$

where g_m is membrane conductance/unit length (Ω^{-1} cm^{-1}). The value of L/λ in normal Ringer was calculated according to eqn. (1). Since GABA not only changes the input conductance but also L/λ, it was necessary to calculate this ratio for each GABA concentration from the following equation:

$$\frac{V_0}{V_0'} = \frac{L/\lambda' \tanh\,(L/\lambda')}{L/\lambda \tanh\,(L/\lambda)}. \tag{5}$$

V_0 and L/λ are the values obtained in control conditions and V_0' and L/λ' are those calculated for each GABA concentration. The expression $L/\lambda' \tanh\,(L/\lambda')$ in (5) was solved by iteration using the Newton–Rapson method and calculated on a Digital PDP 12 computer. Input resistance was read as the slope of the voltage–current plot, obtained when both electrodes were impaled in the middle of the fibre 50–100 μm apart. After successive applications of Ringer containing increasing concentrations of GABA, dose–response curves of $g_m L$ against log concentration of GABA were plotted. Readings were taken 1 min after the addition of each concentration of GABA. In preliminary experiments, it was found that reproducible curves were obtained if the preparation was pretreated with a low dose of GABA (Feltz, 1971).

RESULTS

Physiological observations in normal Ringer

Electrical constants of the resting muscle fibre. Table 1 gives the membrane constants for eight typical fibres, from six preparations. All values were calculated for a short cable, except for the figures in the last two columns, which were calculated assuming the infinite model and are included for comparison. The most striking feature is the large spectrum of values for C_m (range 37–144 μF cm^{-2}) and R_m (172–1018 Ω cm^2). The low values for input resistance were characteristic of the *Eupagurus* muscle membrane and were not attributable to the recording conditions. The same electrodes and current were used to measure the input resistance of frog sartorius muscle and the expected higher values were obtained.

Junction potentials. Resting membrane potentials of 55–75 mV were observed and throughout an experiment did not change by more than 5 mV. Stimulation of the bundle containing the excitatory axon at low frequencies ($\leqslant$ 5 Hz) evoked excitatory junction potentials (e.j.p.s.) of relatively small amplitude. High-frequency stimulation ($\geqslant$ 25 Hz) usually produced movement, which was not necessarily accompanied by action potentials. However, at a frequency of 20 Hz, facilitation of e.j.p.s. was typically observed, with amplitudes attaining 1·5–13 mV. Delivery of a 500–700 msec train of pulses at 30 sec intervals minimized failure due to transmitter depletion. A typical train of e.j.p.s is shown in Fig. 1(*a*), in which maximal facilitation has occurred by the 6th e.j.p.

TABLE 1. Electrical constants of hermit crab muscle fibres

Fibre diameter (μm)	Resting potential (mV)	Input resistance $V_0/I_0 \times 10^4$ (Ω)	Length constant λ (cm)	Time constant τ_m (msec)	Specific membrane resistance R_m (Ω cm^2)	Specific membrane capacitance C_m (μF/cm^2)	Specific membrane resistance calculated as for infinite cable R_m (Ω cm^2)	Specific membrane capacitance calculated as for infinite cable C_m (μF/cm^2)
350	60	3·35	0·20	40	987	40·5	1988	29·2
325	70	1·33	0·12	27	304	89	571	65
200	72	1·20	0·11	26	201	129	445	74
300	55	5·0	0·20	38	1018	37	2638	19
240	71	1·50	0·10	29	201	144	477	84
250	55	4·2	0·25	40	524	76	2308	23·8
300	67	0·94	0·11	23	172	134	317	85
240	70	2·10	0·20	46	383	120	791	85

When selectively stimulating the bundle containing the inhibitory axon, frequencies of $\geqslant$ 60 Hz were needed for a change to be observed. As shown in Fig. 1 (*b*), the effect is one of hyperpolarization representing summation of individual inhibitory junction potentials (i.j.p.s.). However, in many experiments it was not possible to produce a measurable response, presumably because the membrane potential was very close to the reversal potential of the i.j.p.s. Therefore to study inhibition we superimposed stimulation of the inhibitory axon for a short period during the excitatory train. This resulted in attenuation of the e.j.p. amplitude. Fig. 2 illustrates

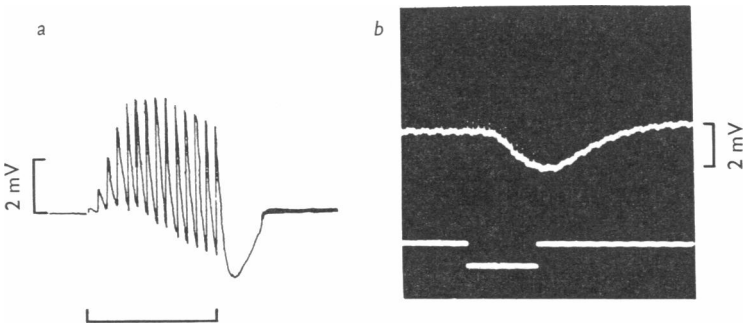

Fig. 1. (*a*) Train of excitatory junction potentials recorded intracellularly from the abductor muscle of the large claw of the hermit crab. The excitatory axon was stimulated at a frequency of 20 Hz for 700 msec, indicated by the horizontal bar (retouched). (*b*) Hyperpolarization produced by stimulation of the inhibitory axon at a frequency of 130 Hz for the 100 msec period indicated by the horizontal bar. This hyperpolarization represents summation of individual inhibitory junction potentials.

progressive decline of e.j.p. amplitude with increasing frequency of inhibitory axon stimulation. In this example the inhibitory axon was stimulated for 100 msec during a 500 msec excitatory train and an effect was observed with frequencies $\geqslant$ 60 Hz. Complete inhibition of the second e.j.p. during the inhibitory stimulation was achieved with frequencies of 200 Hz. In other experiments complete inhibition was usually observed with frequencies of 100–150 Hz. E.j.p. attenuation produced in this way presumably is caused by both pre- and post-synaptic inhibition and this experimental procedure does not permit distinction between the two components.

Effect of GABA on e.j.p. amplitude

The effect of GABA on e.j.p. amplitude was studied by replacing the bath solution with increasing concentrations of GABA, without intermediate washing, with continued recording from the same fibre. In effective concentrations, GABA produced a rapid reduction in e.j.p. amplitude, its

maximal effect being achieved within 1 min (Fig. 3). This attenuation was dose-dependent, the threshold concentration being $1–2 \times 10^{-5}$ M. 5×10^{-5} M produced 40–60 % reduction; 90 % reduction was generally achieved with $4–8 \times 10^{-4}$ M. With the latter concentrations, total abolition of e.j.p.s was sometimes observed, although in other experiments concentrations as high as $3{\cdot}2 \times 10^{-3}$ M achieved a maximal effect without abolition. Using this cumulative dosage regimen, recovery took 20–30 min, with several Ringer changes, and even then the control amplitude was rarely attained. Some fibres were insensitive to the effects of GABA and even concentrations of $> 5 \times 10^{-4}$ M produced little attenuation of e.j.p. amplitude. Assuming the exogenous GABA has access to both pre- and post-synaptic

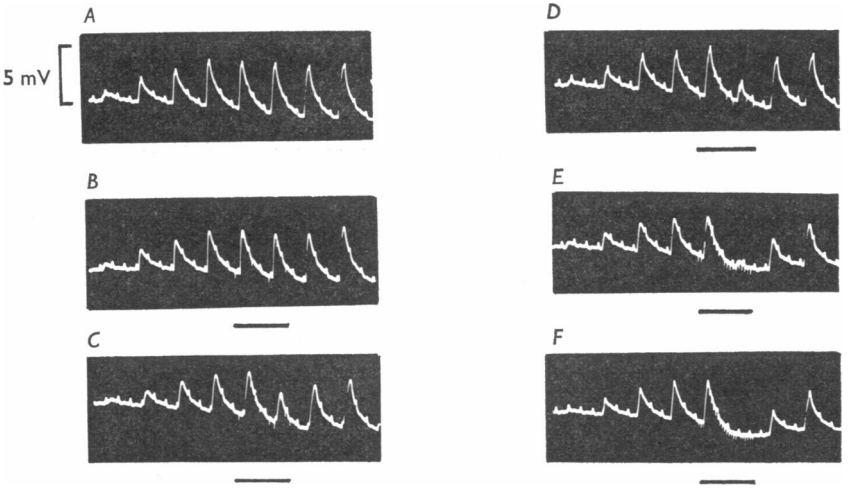

Fig. 2. Progressive attenuation of e.j.p. amplitude with increasing frequency of inhibitory axon stimulation. Each trace shows a 500 msec train of e.j.p.s, evoked by stimulation of the excitatory axon at 15 Hz. *A* shows the control excitatory response. The inhibitory axon was stimulated, in *B–F*, for the 100 msec period indicated by the bar at the following frequencies: *B*, 60 Hz; *C*, 80 Hz; *D*, 100 Hz; *E*, 160 Hz; *F*, 200 Hz.

receptors then its effect on e.j.p. amplitude will be achieved through both inhibitory mechanisms. However, it should be noted that whereas total abolition of e.j.p.s was produced by neural stimulation, this was not always the case after the addition of GABA, even in high concentrations.

Effect of GABA on membrane conductance

In contrast to the above procedures, measurement of changes in muscle membrane conductance after addition of GABA enables selective evaluation of the post-synaptic component of its action.

The shape of the dose–response curve was sigmoid, with a threshold change in input conductance becoming apparent with 1×10^{-5} M-GABA (see Fig. 6 control curve). The maximal increase in membrane conductance was in the order of two- to threefold and was usually observed with $1–4 \times 10^{-4}$ M GABA. Frequent washing for 10–30 min was necessary to restore the membrane conductance to its control value. However, it should be noted that the membrane conductance of a small proportion of fibres was not altered by addition of GABA, even using high concentrations.

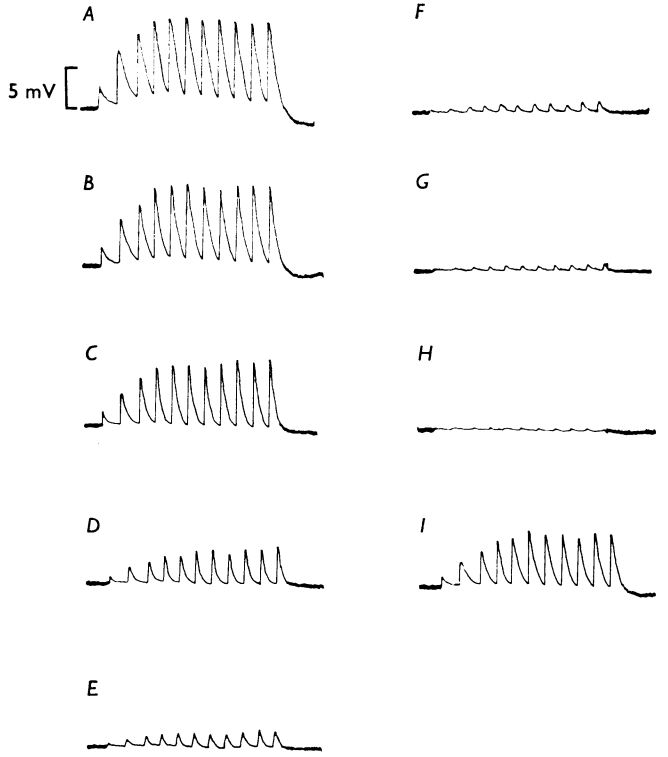

Fig. 3. The effect of GABA on e.j.p. amplitude. Each trace is an 1100 msec train of e.j.p.s evoked by stimulation (10 Hz) of the excitatory axon 1 min after addition of either normal Ringer or GABA. *A*, control; *B*, 1×10^{-5} M; *C*, 2×10^{-5} M; *D*, 5×10^{-5} M; *E*, 1×10^{4} M; *F*, 2×10^{-4} M; *G*, 4×10^{-4} M; *H*, 8×10^{-4} M-GABA; *I*, 25 min after washing (retouched).

In order to assess the importance of the post-synaptic effect in reducing e.j.p. amplitude, we compared the decrease in membrane resistance with the % decrease in e.j.p. amplitude. In this preparation, since the synapses are widely distributed over the muscle surface, a synaptic potential is expected to be proportional to the membrane resistance, i.e. if the action of GABA is purely post-synaptic, a 50% reduction in membrane resist-

ance would produce an equal decrease in e.j.p. amplitude. In twenty fibres, 5×10^{-5} and 1×10^{-4} M-GABA reduced the membrane resistance by 38 and 61 % respectively (mean values); these same concentrations attenuated e.j.p. amplitude by 45 and 72 % respectively. Thus from these results, the post-synaptic effect accounts for approximately 80 % of the observed reduction in e.j.p. amplitude produced by the addition of GABA. However, the conductance changes produced by higher concentrations of GABA ($2–8 \times 10^{-4}$ M) were hardly greater than that produced by 1×10^{-4} M-GABA, although 90–100 % e.j.p. attenuation was achieved with these high doses.

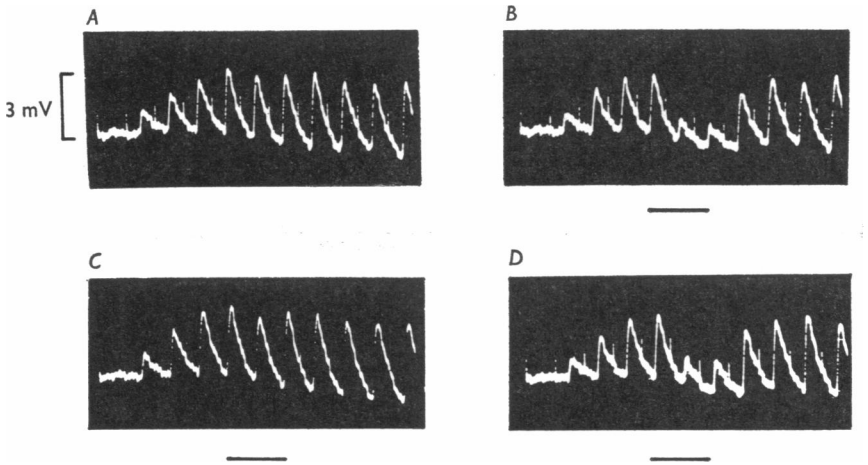

Fig. 4. The effect of picrotoxin on neurally evoked inhibition. Each trace is a 500 msec train of e.j.p.s; the horizontal bar in *B–D* indicates simultaneous stimulation (100 msec) of the inhibitory axon (220 Hz). *A* and *B*, controls; *C*, 2 min after 4×10^{-5} M picrotoxin; *D*, 35 min after wash.

Effects of picrotoxin and bicuculline on GABA-mediated inhibition

In other species of crustacea, picrotoxin has been the only compound to block inhibitory neuromuscular transmission in low concentrations (Grundfest, Reuben & Rickles, 1959; Takeuchi & Takeuchi, 1969) and therefore we investigated its action in the hermit crab. The addition of picrotoxin did not alter grossly the resting potential or the amplitude of control e.j.p.s. However, picrotoxin (1×10^{-5} to 5×10^{-5} M, twelve expts.) effectively reduced the neurally evoked inhibition within 2–20 min depending on the concentration. Fig. 4 shows a typical experiment. Two min after replacement of the bath solution with Ringer containing 4×10^{-5} M picrotoxin, the inhibition was totally abolished; 35 min after repeated washing the inhibitory response was restored. Characteristically, difficulty in washing out the picrotoxin was experienced; 20–60 min washing was

often necessary to achieve partial or total recovery. Moreover, in certain experiments, it was impossible to reverse the block, but it cannot be discounted that this might have been due to failure of the inhibitory synapse. We also investigated the effect of picrotoxin on the e.j.p. attenuation produced by addition of 5×10^{-5} M GABA. This action of GABA was blocked when the muscle was pretreated with 5×10^{-5} M picrotoxin for 5 min (Fig. 5). This exposure time was selected since in our experiments on neurally evoked inhibition it was sufficient for complete block to occur with 5×10^{-5} M picrotoxin.

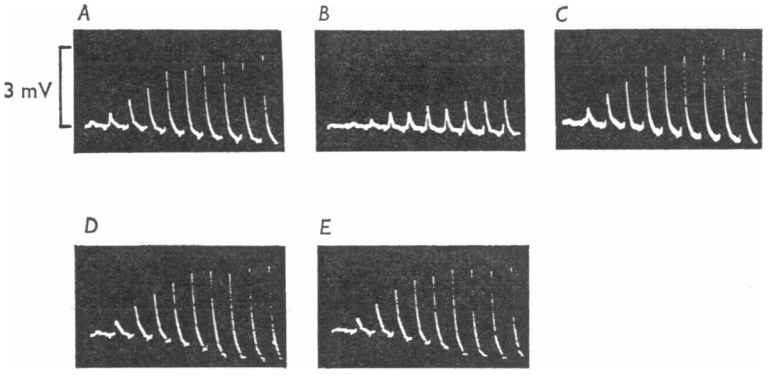

Fig. 5. The effect of picrotoxin on GABA-induced depression of e.j.p.s. Each trace is a 500 msec train of e.j.p.s. *A*, control; *B*, 2 min after 5×10^{-5} M-GABA; *C*, 5 min after wash; *D*, 5 min after 5×10^{-5} M picrotoxin; *E*, 2 min after mixture of 5×10^{-5} M picrotoxin and 5×10^{-5} M-GABA.

Picrotoxin produced a dose-dependent block of the GABA-induced increase in membrane conductance (Fig. 6). As in all experiments of this type, the conductance curves were plotted after the preparation had been exposed to picrotoxin for 5 min, during which time there was usually no change in the input resistance of the membrane. Sometimes, however, a small increase was observed. This could be due to three factors: firstly, picrotoxin may have a direct effect on membrane resistance, which may only occur when the resting membrane resistance is low; secondly, if in some preparations there was a comparatively large spontaneous output of GABA, the addition of picrotoxin would increase the membrane resistance by preventing the effect of this transmitter; and finally, some GABA may still have been present in the tissue from the previous applications. It can be seen that with increasing concentrations of picrotoxin, the maximal conductance change produced by GABA was progressively reduced and furthermore the slopes were non-parallel. These findings are qualitatively similar to those reported by Takeuchi & Takeuchi (1969) for the crayfish, although these workers used slightly lower concentrations of $1–5 \times 10^{-6}$ M.

McLennan (1970), when studying the inhibitory synapse of the crayfish stretch receptor neurone, reported that the alkaloid bicuculline not only antagonized the effects of GABA but was also ten times more potent than picrotoxin on this preparation. It was therefore of interest to investigate the effects of bicuculline at an inhibitory neuromuscular synapse.

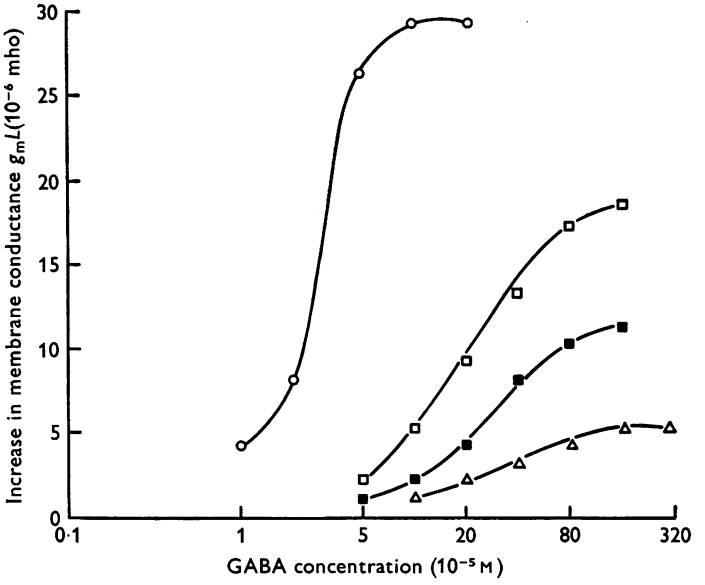

Fig. 6. The effect of picrotoxin on the relation between the GABA-induced increase in membrane conductance, g_mL (ordinate) and the concentration of GABA (abscissa). ○, in normal solution; □, in 5×10^{-6} M; ■, in 1×10^{-5} M; △ in 2×10^{-5} M picrotoxin.

In ten experiments, bicuculline (2×10^{-5} to 2×10^{-4} M) failed to antagonize neurally evoked inhibition during 30–60 min exposure. In the experiment illustrated in Fig. 7, 5×10^{-5} M bicuculline was without effect after 30 min, though with continued recording from the same fibre, subsequent addition of 5×10^{-5} M picrotoxin produced a reversible block of inhibition within 2·5 min. When 1×10^{-3} M bicuculline was added, rapid diminution of both inhibition and excitation was observed which suggested a non-specific interference with the membrane properties. Bicuculline also failed to block the reduction of e.j.p. amplitude (five expts.) due to added GABA; on two occasions subsequent addition of picrotoxin was again effective.

In contrast to the above findings, bicuculline (seven expts., $1–4 \times 10^{-4}$ M) was found to antagonize the increase in membrane conductance produced by GABA. In these experiments, the addition of bicuculline produced a small increase in membrane resistance; this increase was similar to that

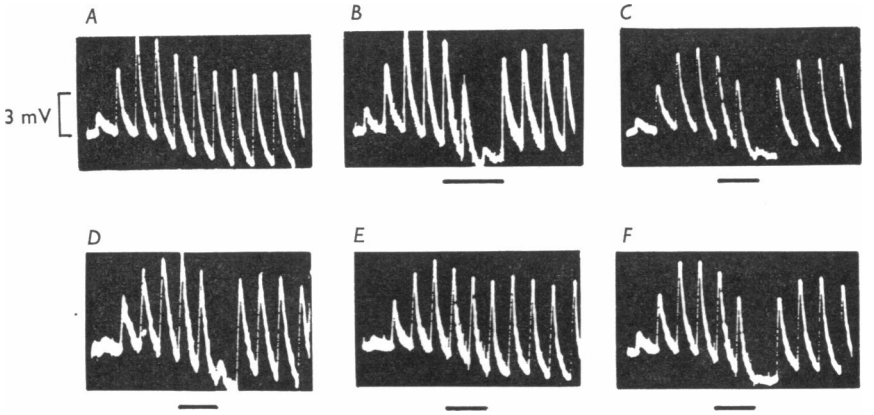

Fig. 7. Comparison of the effects of bicuculline and picrotoxin on neurally evoked inhibition. Each trace is a 500 msec train of e.j.p.s; the horizontal bar in *B–F* indicates simultaneous stimulation (100 msec) of the inhibitory axon (130 Hz). *A* and *B*, controls; *C*, 30 min after 5×10^{-5} M bicuculline; *D*, after wash; *E*, 2·5 min after 5×10^{-5} M picrotoxin; *F*, 30 min after wash.

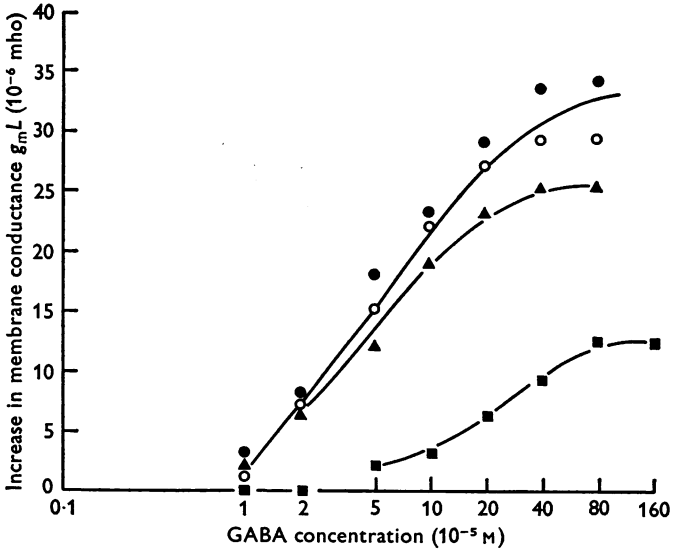

Fig. 8. Comparison of the effects of bicuculline and picrotoxin on the GABA-induced increase in membrane conductance. Ordinate: increase in membrane conductance $g_m L$; abscissa: GABA concentration. ○, in normal solution; ▲, in 2×10^{-4} M bicuculline; ●, in normal solution after bicuculline; ■, in 1×10^{-5} M picrotoxin.

observed with picrotoxin and possible causes have already been suggested. In four experiments, picrotoxin was subsequently added to compare its potency with that of bicuculline. Fig. 8 shows one experiment of this type. 2×10^{-4} M bicuculline depressed the maximal conductance increase caused by GABA and there appeared to be a non-parallel shift of the dose–response curve. This action of bicuculline was reversed by washing. The lowest curve shows the effect of 1×10^{-5} M picrotoxin. If one estimates the relative antagonistic potencies of the two compounds by comparing the reduction in maximal conductance increase, then it would appear that picrotoxin was 40–50 times more potent, on a molar basis, than bicuculline in this preparation.

DISCUSSION

The electrophysiological properties of the post-synaptic membrane of the hermit crab neuromuscular junction are characteristic of fibres that have a poorly developed electrical excitability. The membrane constants suggest that the membrane behaves as a highly leaky and capacitative cable. The fibres typically have a low membrane resistance, many of the values for R_m being $< 600\ \Omega\ cm^2$. These values are in the range found by Fatt & Katz (1953), using infinite cable theory, for *Portunus* and *Carcinus*, although their values for *Eupagurus* were higher ($\simeq 2000\ \Omega\ cm^2$). However, the time constants that we observed using infinite cable theory were similar to those of Fatt & Katz and consequently our C_m values were very high – outside the usual range for biological tissues. Muscles of many crustacean species are known to possess deep invaginations of the surface and furthermore the transverse tubular system arises not only from the outer surface but also from the sarcolemma of these folds. This morphology contributes largely to the apparent high C_m and is responsible for the low R_m (see Keynes, Rojas, Taylor & Vergara, 1973).

The hermit crab inhibitory neuromuscular junction was found to be a suitable system for studying GABA-mediated inhibition in the following ways: (1) neurally evoked inhibition; (2) e.j.p. attenuation on addition of GABA; and (3) increase in post-synaptic membrane conductance after addition of GABA. In considering the relative contributions of pre- and post-synaptic components of GABA-mediated inhibition, it was interesting to find that the threshold and maximal concentrations of GABA for e.j.p. attenuation and increase in membrane conductance were very similar. Moreover, the quantitative findings indicated that the post-synaptic action of GABA (5×10^{-5} and 1×10^{-4} M) could indeed explain the major part of its ability to attenuate e.j.p.s. Thus it would appear that in these concentrations exogenous GABA has relatively little influence on excita-

tory transmitter release, since if it were having a potent presynaptic action in addition to its effect on muscle membrane conductance, then one might expect a proportionally greater reduction in e.j.p. amplitude than in membrane resistance, as was found with higher concentrations of GABA ($2–8 \times 10^{-4}$ M). This, however, does not diminish the role of presynaptic mechanisms during neurally evoked inhibition. Indeed, one feature of neural inhibition was that it was nearly always possible to completely block the e.j.p. with a sufficiently high frequency of inhibitory axon stimulation, even when there was no accompanying hyperpolarization, thus demonstrating the efficiency of the physiological inhibitory mechanism. In addition the experiments with picrotoxin further emphasize the importance of the presynaptic component of neural inhibition since higher concentrations of picrotoxin were required to block this effect than were required to antagonize the post-synaptic conductance increases on addition of GABA. Therefore our findings suggest that the access of exogenous compounds, either GABA or picrotoxin, to the presynaptic sites is in some way impaired.

We have confirmed the results of Takeuchi & Onodera (1972) that bicuculline was considerably less potent than picrotoxin in antagonizing the post-synaptic action of GABA. Moreover, since bicuculline is also ineffective against neurally evoked inhibition, it cannot be considered to block the presynaptic site either. Thus we suggest that picrotoxin is the better tool for studying GABA-mediated inhibition in crustacea.

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