J. Physiol. (1969), **205**, *pp.* 377–391
With 10 *text-figures*
Printed in Great Britain

A STUDY OF THE ACTION OF PICROTOXIN ON THE INHIBITORY NEUROMUSCULAR JUNCTION OF THE CRAYFISH

BY A. TAKEUCHI AND NORIKO TAKEUCHI

From the Department of Physiology, School of Medicine, Juntendo University, Hongo, Tokyo, Japan

(*Received* 12 *May* 1969)

SUMMARY

1. The effect of picrotoxin on the neuromuscular junction of the crayfish (*Cambarus clarkii*) was investigated. The potential changes were recorded intracellularly and extracellularly with micro-electrodes. The membrane conductance of the muscle fibre was also measured.

2. Picrotoxin depressed the amplitudes of the inhibitory junctional potentials and the potential changes produced by iontophoretically applied γ-aminobutyric acid (GABA), but had no appreciable effect on the excitatory junctional potentials and the potential changes produced by L-glutamate.

3. The presynaptic action of GABA and the neural transmitter was depressed by picrotoxin. The presynaptic action of β-guanidinopropionic acid was also depressed by picrotoxin.

4. The increase in the membrane conductance produced by the addition of GABA in the bath fluid was depressed by picrotoxin. The dose–response relation showed that picrotoxin depressed the conductance increase produced by GABA in a non-competitive manner. The action of picrotoxin on the conductance increase produced by GABA was more effective in low Cl^- solution.

5. The analysis of the dose–response curves showed that the action of picrotoxin was well expressed by the Michaelis–Menten equation, but the slope of the dose–response curve of GABA was steeper than this relation. It is proposed that the conductance of the junctional membrane was increased by the combination of two molecules of GABA with a receptor, and the attachment of one molecule of picrotoxin to a specific site depressed the conductance increase.

INTRODUCTION

It is known that picrotoxin has a strong convulsant action in vertebrates and invertebrates. Neurophysiological investigations have shown that picrotoxin blocks several types of inhibitory synapses in crustacea (Elliott & Florey, 1956; Robbins & Van der Kloot, 1958; Robbins, 1959; Van der Kloot & Robbins, 1959; Grundfest, Reuben & Rickles, 1959; Kuffler, 1960; Van der Kloot, 1960) and in insects (Usherwood & Grundfest, 1965) and that it depresses the presynaptic inhibition in the mammalian central nervous system (Eccles, Schmidt & Willis, 1963). Recent investigations have demonstrated that γ-aminobutyric acid (GABA) is released by inhibitory stimulation at the lobster neuromuscular junction (Otsuka, Iversen, Hall & Kravitz, 1966) and acts at the inhibitory neuromuscular junction of the crayfish (Takeuchi & Takeuchi, 1965), and that the action of GABA on the crustacean synapse is blocked by picrotoxin. Since GABA is a possible inhibitory transmitter in some synapses of the mammalian central nervous system (Obata, 1965; Krnjević & Schwartz, 1967), the action of picrotoxin and GABA on the crustacean inhibitory synapses may be of some general application.

It has been postulated that picrotoxin acts as a competitive antagonist of both the inhibitory transmitter substances and GABA by combining reversibly at inhibitory receptor sites on the post-synaptic membrane (Robbins & Van der Kloot, 1958; Grundfest *et al.* 1959; Van der Kloot, 1960). Experimental work demonstrating the mode of action of picrotoxin is lacking. The present study was undertaken to obtain quantitative information concerning the action of picrotoxin by measuring the membrane conductance of the crayfish muscle fibre. It was observed that picrotoxin acts as a non-competitive antagonist at the crayfish inhibitory neuromuscular junction.

METHODS

Experimental procedures were similar to those reported previously (Takeuchi & Takeuchi, 1964, 1965). The abductor muscle of the dactyl in the first walking leg of the crayfish (*Cambarus clarkii*) was used. Potential changes produced by nerve stimulation and by the iontophoretic applications of GABA and L-glutamate were recorded intracellularly with a 3 M-KCl-filled micro-electrode and extracellularly with a 3 M-NaCl-filled micro-electrode. Muscle membrane conductance was measured as described previously (Takeuchi & Takeuchi, 1967), using 3 M-K-propionate- or 0·6 M-K_2SO_4-filled micro-electrodes. The normal concentration of Cl^- in the bathing solution was 246·5 mM and, as the Cl^- concentration was decreased, it was replaced with methyl sulphate (Tokyo Kasei) or methanesulphonate (Tokyo Kasei). In order to increase Cl^- concentration NaCl or choline-Cl was added to the solution. The pH of the solution was adjusted to 7·2 by Tris maleate buffer. The pH and osmolarity of the solutions were checked before each experimental run. Picrotoxin solution was specially prepared for each experiment. The bath fluid was replaced with the new

solution continuously. When the solution was changed to one containing picrotoxin, the action of picrotoxin reached 90 % of its maximal value in 2–3 min. (The action of GABA applied under the same condition reached the 90 % maximal value in 20–50 sec.)

RESULTS

Effect on the post-synaptic inhibition

The excitatory and inhibitory axons were stimulated repetitively by short trains of stimuli. The excitatory (e.j.p.s) and inhibitory junctional potentials (i.j.p.s) recorded intracellularly are shown in Fig. 1*a*. When

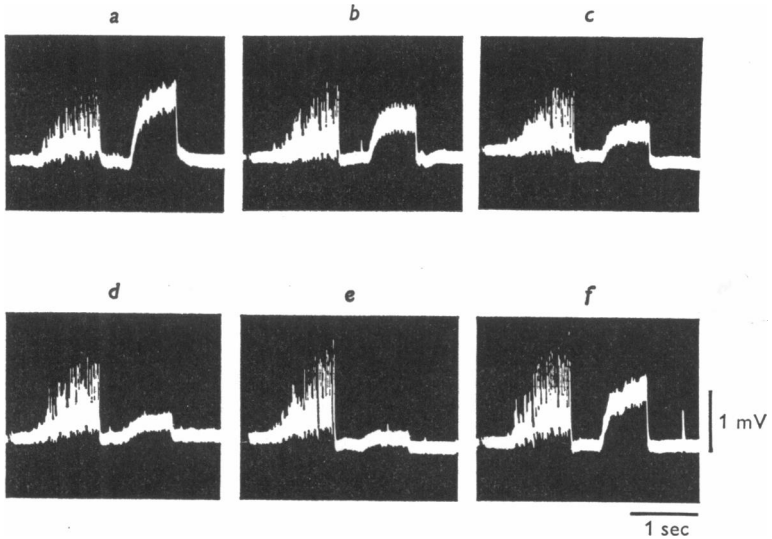

Fig. 1. Effect of picrotoxin on the e.j.p.s and i.j.p.s recorded intracellularly from a muscle fibre. E.j.p.s and i.j.p.s were produced successively by trains of stimuli (30/sec for 1 sec and 50/sec for 0·7 sec respectively). *a*, recorded in normal solution; *b*, in 1×10^{-6} M; *c*, 5×10^{-6} M; *d*, 1×10^{-5} M; *e*, 3×10^{-5} M picrotoxin; *f*, 20 min after washing with normal solution.

the bath fluid was changed to one containing picrotoxin, the amplitude of the i.j.p.s decreased, while no appreciable change was observed in the e.j.p.s (Fig. 1*b* to *e*). The preparation was soaked in each solution for 10–15 min. The amplitude of the i.j.p.s recovered about 20 min after washing the preparation with normal saline solution (Fig. 1*f*). The concentration of picrotoxin used in the present experiment produced no appreciable changes in the reversal potential of the i.j.p.s, the resting membrane conductance and potential.

The relationship between the concentration of picrotoxin and the amplitudes of e.j.p.s and i.j.p.s is shown in Fig. 2. Since the reversal potential of the i.j.p.s was close to the resting potential (in this case at 3·5 mV depolarized level), the non-linearity of the i.j.p. size was corrected (Martin,

1955) (filled circles in Fig. 2). The open circles represent the mean amplitudes of the last five e.j.p.s in each train of stimuli (the stimulation rate of 30/sec and duration of 1 sec). While there were some variations in amplitude, a marked decrease was observed in the i.j.p. size, but little change in the e.j.p. The curve of Fig. 2 is drawn according to eqn. (1), assuming $K_b = 3 \times 10^{-6}$ M, $m = 1$ and $\beta = 0{\cdot}9$. These constants are of the same range as those obtained from the conductance measurements of

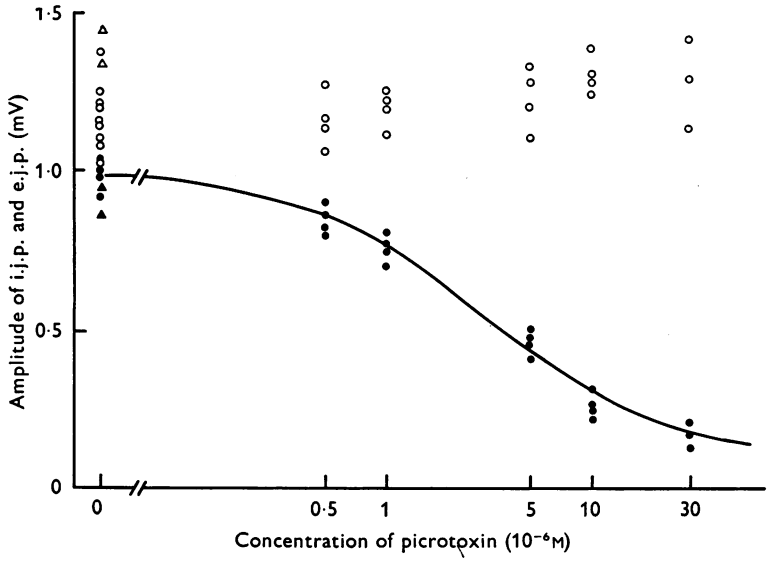

Fig. 2. Relationship between the concentration of picrotoxin and the amplitudes of e.j.p.s and i.j.p.s. ○, mean amplitude of last five e.j.p.s produced at stimulation rate of 30/sec for 1 sec. ●, maximal amplitude of i.j.p.'s at the end of stimulation (stimulation rate of 50/sec and duration of 0·7 sec). The amplitude of i.j.p.s was corrected by method of Martin (1955). Theoretical curve was drawn according to eqn. (1), assuming $K_b = 3 \times 10^{-6}$ M, $m = 1$ and $\beta = 0{\cdot}9$. △ and ▲, control amplitudes of e.j.p.s and i.j.p.s after washing with normal solution.

GABA action (see p. 9). In Fig. 2, the amplitude of e.j.p.s had a tendency to increase at higher concentrations of picrotoxin. However, the increase in the e.j.p. size was not always observed and the control e.j.p.s obtained after washing the preparation with normal solution (open triangles in Fig. 2) was of the same amplitude as that obtained with high concentrations of picrotoxin. Therefore, the increase in the e.j.p. size may not be due to the action of picrotoxin, but to the facilitation of e.j.p. after repeated stimulations.

It has been shown that L-glutamate and GABA activate the excitatory and inhibitory junctional membranes respectively (Takeuchi & Takeuchi,

1964, 1965). Glutamate and GABA were applied iontophoretically to a junctional area from a double-barrelled micropipette and potentials were recorded intracellularly (Fig. 3*a*). A drop of 1×10^{-3} M picrotoxin was applied near the muscle fibre. The amplitude of GABA potentials decreased, while no change in the glutamate potential was observed (Fig. 3*b*). After washing the preparation with normal solution, the GABA potential recovered (Fig. 3*c*). In this case, a high concentration of picrotoxin was applied and the recovery of the GABA potential was incomplete.

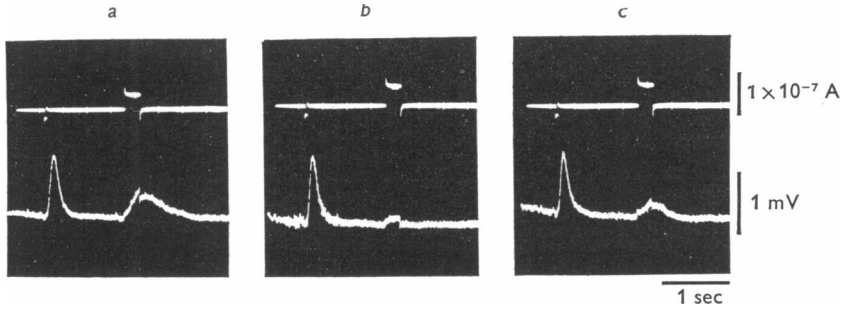

Fig. 3. Effect of picrotoxin on the glutamate and GABA potentials. Upper trace, monitored injection current. Lower trace, glutamate and GABA potentials produced successively by the iontophoretic injection of L-glutamate and GABA from a double-barrelled micropipette. *a*, before; *b*, a drop of 1×10^{-3} M picrotoxin was applied near the muscle fibre. *c*, after washing with normal solution.

Effect on the presynaptic inhibition

The excitatory axon was stimulated repetitively and the e.j.p.s were recorded extracellularly with the recording micro-electrode placed on the surface of the muscle at a junctional area. The effect of GABA on the presynaptic terminal was investigated by the iontophoretic application of GABA to this junction. The effect of iontophoretically applied GABA was altered by a small change in the position of the tip of a GABA-filled micropipette. In order to decrease this error the micropipette was placed at a point a small distance away from the junction and the relatively larger doses of GABA were applied. The bath solution was kept flowing and the solution was replaced alternately with the normal and the picrotoxin solutions. An example is shown in Fig. 4. The middle traces show the extracellular e.j.p.s and the bottom traces the intracellular e.j.p.s. The injection currents of GABA applications are monitored on the upper traces. The extracellular e.j.p.s were abolished by the application of GABA in normal solution (Fig. 4*a*, *c*) and a little change was observed in picrotoxin solution (1×10^{-5} M) (Fig. 4*b*). Since the depression of the extracellular e.j.p.s by the application of GABA is its action on the

presynaptic nerve terminal (cf. Takeuchi & Takeuchi, 1966), picrotoxin is considered to have depressed the presynaptic action of GABA.

The action of inhibitory stimulation on the e.j.p.'s is presented in Fig. 5. In Fig. 5*A*, 1 and 3 show the e.j.p.s and i.j.p.s produced at a stimulation rate of 20/sec (about twenty traces are superimposed). Records in *a* were obtained in normal solution and those in *b* were recorded from the same fibre

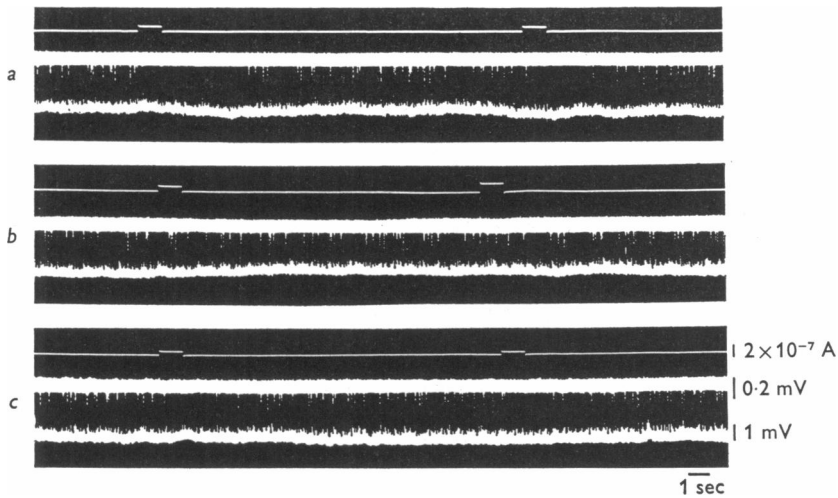

Fig. 4. Effect of picrotoxin on the extracellular e.j.p.s. Lower trace, intracellular e.j.p.s produced at stimulation rate of 20/sec. Middle trace, extracellularly recorded e.j.p.s recorded at a junctional area. Upper trace, monitored current for injection of GABA. *a*, recorded in normal solution before application of picrotoxin; *b*, in 1×10^{-5} M picrotoxin; *c*, after washing with normal solution.

in a solution containing 1×10^{-5} M picrotoxin. When the inhibitory axon was stimulated a few msec preceding an excitatory stimulation, the amplitude of e.j.p.s was markedly decreased in the normal solution (*a* 2) and a little change was observed in the presence of picrotoxin (*b* 2). The amplitude of e.j.p.s recorded intracellularly was measured at various intervals between excitatory and inhibitory stimulations (Fig. 5*B*). The abscissa shows the time interval between the inhibitory and the excitatory nerve spikes as recorded in Fig. 5*A*. Open and filled circles represent the amplitudes of the intracellular e.j.p.s recorded in normal solution. Crosses are those obtained in the solution containing 1×10^{-5} M picrotoxin. The amplitude of the e.j.p.'s at the peak inhibitory action was about 30% of the control in normal solution but was about 78% in 1×10^{-5} M picrotoxin (Fig. 5*B*). If the inhibitory action of the neural transmitter is expressed by a decrease in the amplitude of the e.j.p.s, it was depressed

to about 70 % of the control by addition of 1×10^{-6} M picrotoxin, 30 % by 1×10^{-5} M and 20 % by 3×10^{-5} M picrotoxin. It has been observed that the inhibitory action of the neural transmitter in the abductor muscle is attributable mainly to its action on the presynaptic terminal (Dudel & Kuffler, 1961). Present results indicate that picrotoxin depressed the presynaptic action of the transmitter.

It has been shown that β-guanidinopropionic acid has a presynaptic

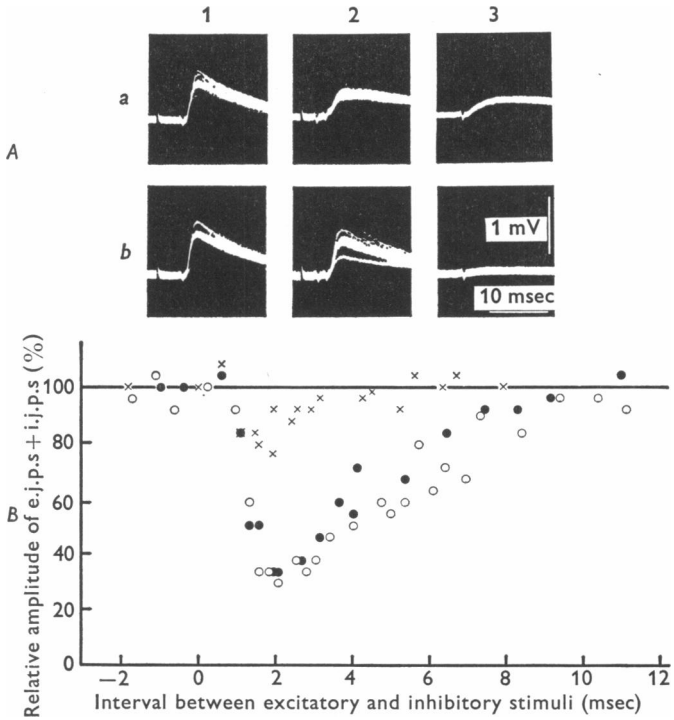

Fig. 5. *A*: Effect of picrotoxin on the action of inhibitory nerve stimulation on the amplitude of e.j.p.s. *a*, obtained in normal solution. *b*, in 1×10^{-5} M picrotoxin. 1, e.j.p.s and, 3, i.j.p.s produced at stimulation rate of 20/sec and recorded intracellularly. 2, the inhibitory axon was stimulated about 2 msec before excitatory stimulation. E.j.p.s are markedly reduced in size in normal solution (*a* 2), but a little changed in the solution containing picrotoxin (*b* 2). About twenty traces were superimposed. The excitatory and inhibitory nerve spikes are seen preceding e.j.p.s and i.j.p.s.

B: Effect of inhibitory stimulations on the amplitude of e.j.p.s. Abscissa, interval between the excitatory and inhibitory stimuli (the interval was measured from the excitatory and the inhibitory nerve spikes recorded as in Fig. 5*A*); it is expressed as minus when i.j.p. follows e.j.p. and plus when i.j.p. precedes e.j.p. Ordinate, relative size of e.j.p.; 100 % is the mean amplitude of control. ○, recorded in normal solution before application of picrotoxin. ×, in 1×10^{-5} M picrotoxin. ●, after returning the preparation to the normal solution.

inhibitory action but does not increase the post-synaptic membrane conductance (Kuffler, 1960; Dudel, 1965). The amplitude of the e.j.p.s was decreased to about 40% of the control by the addition of 2×10^{-4} M-β-guanidinopropionic acid. Picrotoxin in a concentration of 2×10^{-5} M restored the e.j.p. size to about 80% of its original value.

Pentylenetetrazol in concentrations of up to 10 mM showed no appreciable action on e.j.p.s, i.j.p.s or presynaptic inhibition.

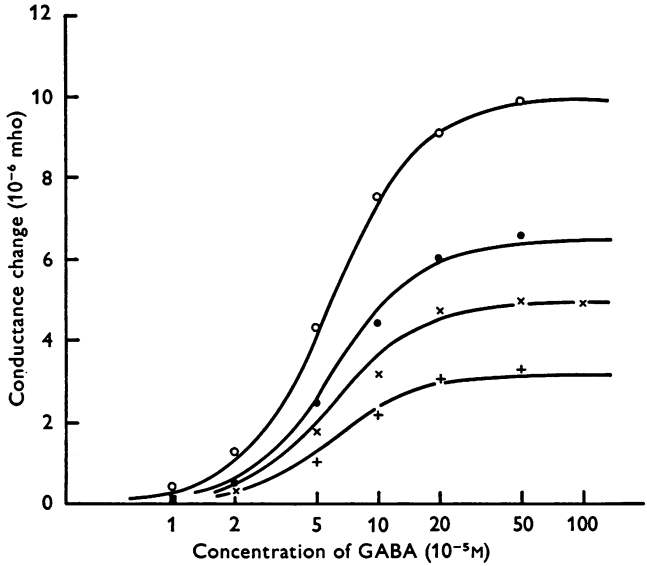

Fig. 6. Dose–response curve of the GABA action. Abscissa, log concentration of GABA. Ordinate, conductance increase of the muscle membrane produced by GABA ($g_m L$). ○, measured in normal solution; ●, in 1×10^{-6} M; ×, 2×10^{-6} M; +, 5×10^{-6} M picrotoxin. Curves were drawn according to eqns. (1) and (2), assuming $K_a = 3{\cdot}6 \times 10^{-9}$ M^2; $K_b = 1{\cdot}5 \times 10^{-6}$ M, $n = 2$, $m = 1$ and $\beta = 0{\cdot}88$.

Effect of picrotoxin on the membrane conductance change produced by GABA

The membrane conductance of the muscle ($g_m L$) was measured as described previously, g_m being the membrane conductance per unit length and L, the half-length of the muscle fibre (Takeuchi & Takeuchi, 1967). The increase in the membrane conductance ($g_m L$) produced by GABA was plotted against the log concentration of GABA (Fig. 6). When picrotoxin was added to the solution, the dose–response curve declined. This result suggests that picrotoxin depressed the action of GABA in a non-competitive manner (cf. Barlow, 1964).

Non-competitive antagonism is expressed by eqn. (1). It is assumed that picrotoxin binds at the sites and the formation of the complex with

picrotoxin depends on the proportion of the sites not occupied by picrotoxin. The picrotoxin-receptor complex does not result in a conductance change but interferes with the GABA-receptors so that the available number of GABA-receptors is decreased. If the conductance change is assumed to be linearly proportional to the number of receptors occupied by GABA, the relative conductance change is expressed by

$$y' = y\left(1-\frac{\beta}{1+K_b/B^m}\right), \tag{1}$$

$$y = \frac{1}{1+K_a/A^n}, \tag{2}$$

where y' is the relative conductance change produced by GABA in the presence of picrotoxin and y is that produced without picrotoxin. A and B are the concentrations of GABA and picrotoxin respectively. K_a and K_b are dissociation constants for GABA- and picrotoxin-receptor complexes. m and n are the number of molecules of picrotoxin and GABA respectively attached to receptors and β is a constant. The curves in Fig. 7 are drawn according to eqns. (1) and (2), assuming $n = 2$, $m = 1$, $K_a = 3{\cdot}6\times10^{-9}$ M^2, $K_b = 1{\cdot}5\times10^{-6}$ M and $\beta = 0{\cdot}88$. The curves fit the experimental values in general. However, it was observed sometimes that the action of picrotoxin was greater at lower concentrations of GABA.

The non-competitive antagonism may be tested by plotting the log of $y'/(y'_{max}-y')$ against the log concentration of GABA, y'_{max} being the maximal value of y'. This relation would be linear. If picrotoxin is a non-competitive antagonist, the relations obtained in different concentrations of picrotoxin would fall on the same line (Matsumoto & Kumoi, 1958). An example in Fig. 7 indicates that this is the case.

The relative effect of picrotoxin (z) may be expressed by $1-y'/y$. This was plotted against the concentration of picrotoxin in Fig. 8, where circles represent mean values of six to twelve experiments and bars represent $\pm$ S.D. This may be expressed as

$$z = 1-\frac{y'}{y} = \frac{\beta}{1+K_b/B^m}. \tag{3}$$

When the concentration of picrotoxin was increased, z did not approach unity but became saturated at β (cf. Ariëns, Simonis & Van Rossum, 1964). This value was estimated from the asymptote of the curve drawn as in Fig. 8. When $\log\{z/(\beta-z)\}$ was plotted against the log concentration of picrotoxin, the relation would be linear (Fig. 9). The slope of the line would be m and the concentration of picrotoxin at which $z/(\beta-z)$ is unity would correspond to K_b. Mean value ($\pm$ S.E.) of $m = 1{\cdot}04$ ($\pm 0{\cdot}02$) (nine

experiments), $K_b = 3{\cdot}3(\pm 0{\cdot}49) \times 10^{-6}$ M (eight experiments) and $\beta = 0{\cdot}89(\pm 0{\cdot}01)$ (nine experiments). The curve of Fig. 8 was drawn according to eqn. (3), assuming $m = 1$, $\beta = 0{\cdot}88$ and $K_b = 2{\cdot}6 \times 10^{-6}$ M.

Mean values of n and K_a may be calculated in a similar way by plotting $\log \{y/(1-y)\}$ against the log concentration of GABA (see Fig. 7). The mean value ($\pm$S.E.) of n was 1·9 ($\pm 0{\cdot}03$) (fourteen experiments) and the concentration of GABA at which $y/(1-y)$ was unity was $4{\cdot}1(\pm 0{\cdot}22) \times 10^{-5}$ M (fourteen experiments).

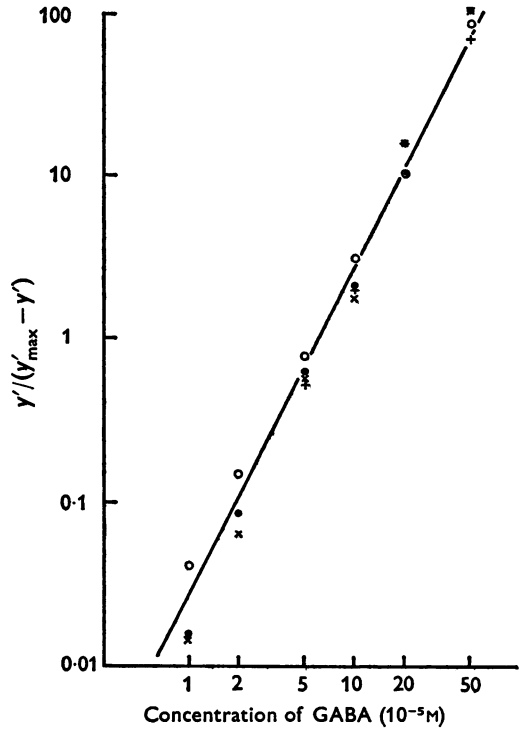

Fig. 7. Relationship between the concentration of GABA and $y'/(y'_{max}-y')$ in various concentrations of picrotoxin. y' is the conductance increase produced by GABA and y'_{max} is the maximal value of y'. ○, measured in normal solution; ●, in 1×10^{-6} M; ×, 2×10^{-6} M; +, 5×10^{-6} M picrotoxin. The slope of the line is 2.

Concentration of Cl^- and action of picrotoxin

The action of picrotoxin was tested in solutions containing various concentrations of Cl^-. A fixed concentration of GABA was added to the solution and the increase in membrane conductance was measured. The membrane conductance was measured under the condition in which the addition of GABA produced little or no potential change (cf. Takeuchi &

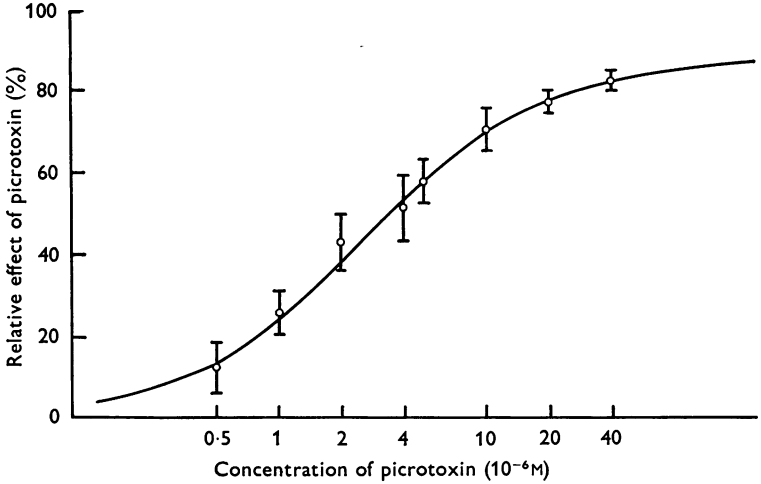

Fig. 8. Relationship between the relative effect of picrotoxin and the concentration of picrotoxin. ○, mean value of six to twelve experiments. Bars indicate ± S.D. The curve is drawn according to eqn. (3) ($m = 1, \beta = 0{\cdot}88$ and $K_b = 2{\cdot}6 \times 10^{-6}$ M).

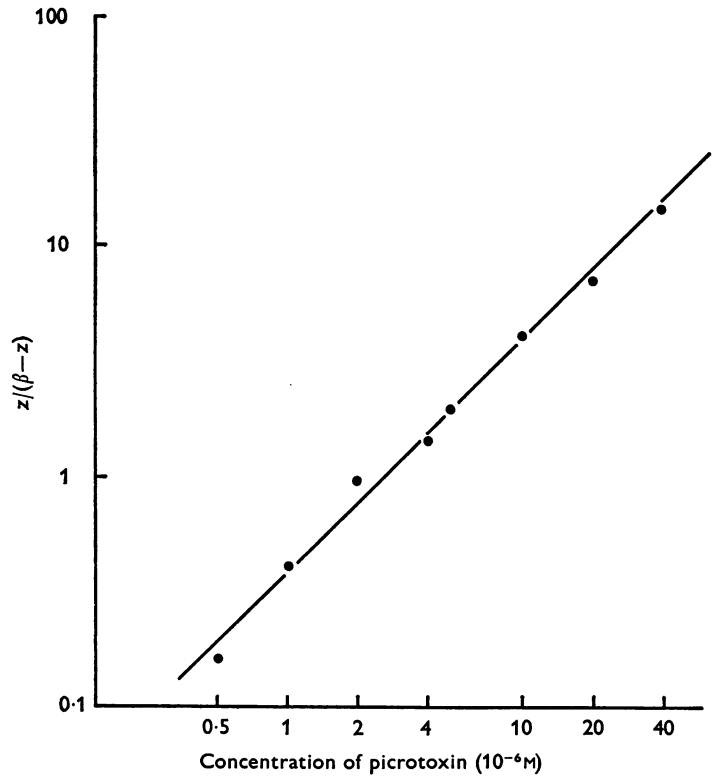

Fig. 9. Relationship between the concentration of picrotoxin and $z/(\beta-z)$. z is the relative effect of picrotoxin and β is a constant which corresponds to the maximal value of z. The slope of the line is 1.

Takeuchi, 1967). Picrotoxin was then added to the solution and the conductance increase produced by GABA was compared with that measured in the solution without picrotoxin. The relative effect of picrotoxin $(1-y'/y)$ was plotted against the concentration of Cl^- (Fig. 10), where y' is the conductance change produced by 2×10^{-4} M-GABA in the presence of picrotoxin and y is that in the solution without picrotoxin. In Fig. 10, the concentration of Cl^- was changed from 95 to 295 mM. It was observed that the effect of picrotoxin was enhanced at lower Cl^- concentrations and depressed at higher concentrations of Cl^-.

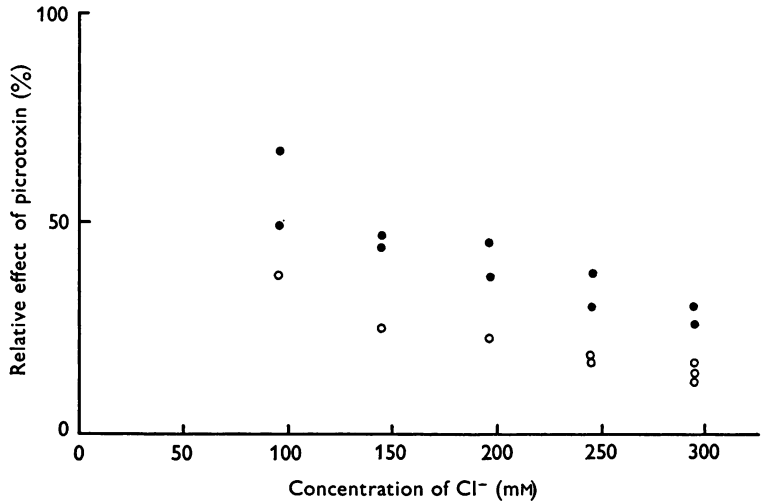

Fig. 10. The relative effect of picrotoxin in various concentrations of Cl^-, substituted with methylsulphate. Concentration of Cl^- was increased to 295 mM by adding NaCl. ○, measured in 2×10^{-6} M picrotoxin; ●, 4×10^{-6} M picrotoxin. The concentration of GABA applied was 2×10^{-4} M.

DISCUSSION

Present experiments indicate that picrotoxin depresses the increase of the membrane conductance produced by GABA in a non-competitive manner. The action of picrotoxin on the i.j.p.s may also be explained by non-competitive antagonism, because the depression of the i.j.p. size by picrotoxin was expressed by eqn. (1) (Fig. 2). The neural inhibition of the e.j.p.s produced by the stimulation of the inhibitory axon was depressed by picrotoxin of the same concentrations. This suggests that picrotoxin depresses the presynaptic inhibition in a similar way.

The non-competitive antagonism of picrotoxin may be explained in several ways; e.g. it may be due to the fact that picrotoxin combines with the GABA-receptor irreversibly, causing a decrease in the number of

receptors available. The action of picrotoxin was abolished reversibly by washing the muscle with normal solution. Therefore it seems unlikely that the non-competitive nature of the action of picrotoxin is caused by its irreversible binding with the GABA-receptor. Alternatively picrotoxin may prevent GABA from binding with the GABA-receptor by combining at a site different from the GABA-receptor. Another possibility is that picrotoxin interferes with the ionic gating process at the junctional membrane. The observation that the effectiveness of picrotoxin on the conductance increase produced by GABA was influenced by the Cl^- concentration might be related to the latter possibility. It has been reported that picrotoxin depressed the Cl^- conductance of the crustacean muscle membrane (Grundfest, 1961). However, the concentration of picrotoxin used in his experiment was about 100 times as high as that necessary to depress the i.j.p.s and the mechanisms of the drug action might be different in these cases.

It was observed that the dose–action curve of picrotoxin could be reasonably well explained by the Michaelis–Menten equation, while the slope of the dose–response curve of GABA was steeper than this. It is suggested that two molecules of GABA combine with a receptor and produce the conductance increase (Takeuchi & Takeuchi, 1967). It is also considered that the combination of GABA molecules with a receptor does not take place at one instant but it occurs stepwise, as

$$(\text{GABA}) + \text{Receptor} \rightleftharpoons (\text{GABA})\,(\text{Receptor})$$
$$(\text{GABA}) + (\text{GABA})\,(\text{Receptor}) \rightleftharpoons (\text{GABA})_2\,(\text{Receptor}) \rightarrow \text{Conductance increase}$$

The relative response (y) could be expressed by the following relation,

$$y = \frac{1}{1 + K_2/A + K_1K_2/A^2}, \tag{4}$$

where K_1 is the dissociation constant for the first reaction and K_2 is that for the second reaction. In order to fit this relation to the data, K_1/K_2 would be about 100, which suggests that the rate of conductance increase is limited by the first reaction (approximate values of K_1 and K_2 would be 5×10^{-4} and 5×10^{-6} M respectively). At present, no evidence is available to determine whether the complex formation occurs in one step or in stages. It was observed that the dose–response curves of substances which have molecular structures related to GABA could also be explained by eqn. (2) or (4) (A. Takeuchi & N. Takeuchi, unpublished). Therefore it is proposed that the combination of two molecules of a substance with a receptor is necessary to produce the conductance increase of the crayfish junctional membrane.

In the present experiment, it was observed that picrotoxin did not abolish the i.j.p.'s or GABA action, but about 10% of the response remained even with high concentrations of picrotoxin; i.e. β was less than 1. This result may correspond to the observation that, in the mammalian central nervous system, picrotoxin depressed the presynaptic inhibition, but even large doses did not abolish the presynaptic inhibition (Eccles *et al.* 1963).

The authors wish to express their thanks to Professors S. W. Kuffler and M. Otsuka for helpful comments on the manuscript. The work was supported in part by grants from the Ministry of Education.

REFERENCES

ARIËNS, E. J., SIMONIS, A. M. & VAN ROSSUM, J. M. (1964). Drug–receptor interaction: Interaction of one or more drugs with different receptor systems. *Molecular Pharmacology*, ed. ARIËNS, E. J., vol. 1, pp. 287–393. New York: Academic Press.

BARLOW, R. B. (1964). *Introduction to Chemical Pharmacology*, 2nd edn., pp. 14–16. New York: Wiley.

DUDEL, J. (1965). Presynaptic and postsynaptic effects of inhibitory drugs on the crayfish neuromuscular junction. *Pflügers Arch. ges. Physiol.* **283**, 104–118.

DUDEL, J. & KUFFLER, S. W. (1961). Presynaptic inhibition at the crayfish neuromuscular junction. *J. Physiol.* **155**, 543–562.

ECCLES, J. C., SCHMIDT, R. & WILLIS, W. D. (1963). Pharmacological studies on presynaptic inhibition. *J. Physiol.* **168**, 500–530.

ELLIOTT, K. A. C. & FLOREY, E. (1956). Factor I—Inhibitory factor from brain. *J. Neurochem.* **1**, 181–192.

GRUNDFEST, H. (1961). General physiology and pharmacology of junctional transmission. *Biophysics of Physiological and Pharmacological Actions*, ed. SHANES, A. M., pp. 329–389. Washington D.C.: American Association for the Advancement of Sciences.

GRUNDFEST, H., REUBEN, J. P. & RICKLES, W. H. Jr. (1959). The electrophysiology and pharmacology of lobster neuromuscular synapses. *J. gen. Physiol.* **42**, 1301–1323.

KRNJEVIĆ, K. & SCHWARTZ, S. (1967). The action of γ-aminobutyric acid on cortical neurones. *Expl Brain Res.* **3**, 320–336.

KUFFLER, S. W. (1960). Excitation and inhibition in single nerve cells. *Harvey Lect.* **54**, 176–218.

MARTIN, A. R. (1955). A further study of the statistical composition of the end-plate potential. *J. Physiol.* **130**, 114–122.

MATSUMOTO, H. & KUMOI, T. (1958). 'Dose–action curves' of various pharmacological agents and a mathematical analysis of mechanism of antagonism. *Kobe J. Med. Sci.* **4**, 139–162.

OBATA, K. (1965). Pharmacological study on postsynaptic inhibition of Deiter's neurones. *Abstr. XXIII Int. Physiol. Congr. Tokyo*, p. 406.

OTSUKA, M., IVERSEN, L. L., HALL, Z. W. & KRAVITZ, E. A. (1966). Release of gamma-amino butyric acid from inhibitory nerves of lobster. *Proc. natn. Acad. Sci. U.S.A.* **56**, 1110–1115.

ROBBINS, J. (1959). The excitation and inhibition of crustacean muscle by amino acids. *J. Physiol.* **148**, 39–50.

ROBBINS, J. & VAN DER KLOOT, W. G. (1958). The effect of picrotoxin on peripheral inhibition in the crayfish. *J. Physiol.* **143**, 541–552.

TAKEUCHI, A. & TAKEUCHI, N. (1964). The effect on crayfish muscle of iontophoretically applied glutamate. *J. Physiol.* **170**, 296–317.

TAKEUCHI, A. & TAKEUCHI, N. (1965). Localized action of gamma-aminobutyric acid on the crayfish muscle. *J. Physiol.* **177**, 225–238.

TAKEUCHI, A. & TAKEUCHI, N. (1966). A study of the inhibitory action of γ-aminobutyric acid on neuromuscular transmission in the crayfish. *J. Physiol.* **183**, 418–432.

TAKEUCHI, A. & TAKEUCHI, N. (1967). Anion permeability of the inhibitory postsynaptic membrane of the crayfish neuromuscular junction. *J. Physiol.* **191**, 575–590.

USHERWOOD, P. N. R. & GRUNDFEST, H. (1965). Peripheral inhibition in skeletal muscle of insects. *J. Neurophysiol.* **28**, 497–518.

VAN DER KLOOT, W. G. (1960). Picrotoxin and the inhibitory system of crayfish muscle. *Inhibition in the Nervous System and Gamma-aminobutyric Acid*, ed. ROBERTS, E., pp. 409–412. New York: Pergamon.

VAN DER KLOOT, W. G. & ROBBINS, J. (1959). The effects of γ-aminobutyric acid and picrotoxin on the junctional potential and the contraction of crayfish muscle. *Experientia* **15**, 35–36.