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LOCALIZED ACTION OF GAMMA-AMINOBUTYRIC ACID ON THE CRAYFISH MUSCLE

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It has been observed that γ-aminobutyric acid (GABA) has an inhibitory action on the crayfish stretch receptor (Bazemore, Elliott & Florey, 1957; Kuffler & Edwards, 1958; Edwards & Kuffler, 1959; Hagiwara, Kusano & Saito, 1960), crustacean neuromuscular junction (Boistel & Fatt, 1958; Grundfest, Reuben & Rickles, 1959; Robbins, 1959; Kuffler, 1960; Dudel & Kuffler, 1961; Grundfest & Reuben, 1961), mammalian central nervous system (Elliott & Jasper, 1959; Curtis, Phillis & Watkins, 1959; Curtis & Watkins, 1960; Purpura, 1960; Krnjević & Phillis, 1963) and crayfish central nervous system (Furshpan & Potter, 1959). GABA occurs in high concentrations in the mammalian central nervous system (e.g. Bazemore *et al.* 1957; Roberts, 1960) and the crustacean central and peripheral nervous systems (Dudel, Gryder, Kaji, Kuffler & Potter, 1963; Kravitz, Kuffler, Potter & van Gelder, 1963). The importance of GABA has been emphasized by the recent discovery that GABA is found exclusively in the inhibitory fibres of the lobster, suggesting a specific role of GABA in inhibitory transmission (Kravitz, Kuffler & Potter, 1963). Enzymes which form and degrade GABA are also found in lobster axons (Hall, Kravitz & Potter, 1964). However, whether the site of action of GABA is confined to the subsynaptic membrane has not been determined. It is argued that neural inhibitory receptors are specifically involved (Van der Kloot & Robbins, 1959; Grundfest *et al.* 1959; Dudel & Kuffler, 1961). On the other hand GABA inhibits excitation in dendrites where no inhibitory synapses have been found (Kuffler & Edwards, 1958). In the mammalian central nervous system it has been suggested, from results of iontophoretic application of GABA, that GABA acts as a non-specific depressant (Curtis *et al.* 1959; Curtis & Watkins, 1960). In the Mauthner cell of goldfish, however, the action of GABA is similar to the neural transmitter and its action is confined to certain regions of the cell (Diamond, 1963).

The present experiments were designed to determine the site of action of GABA on crayfish muscle by using iontophoretic application from a

micropipette. Crustacean muscle is an adequate material for this purpose because its anatomy is relatively simple and the inhibitory mechanism in this muscle has been thoroughly investigated (Fatt & Katz, 1953; Boistel & Fatt, 1958; Hoyle & Wiersma, 1958; Kuffler, 1960; Dudel & Kuffler, 1961; Dudel, 1963). It will be shown that the action of GABA is confined to the inhibitory neuromuscular junction of the crayfish muscle (Takeuchi & Takeuchi, 1964*b*). A similar localization of action has already been shown in the case of L-glutamate (Takeuchi & Takeuchi, 1964*a*).

METHODS

Preparations and experimental procedures were similar to those used in the previous report (Takeuchi & Takeuchi, 1964*a*). The abductor of the dactylopodite in the walking leg of crayfish (*Cambarus clarkii*) was used. The composition of van Harreveld's solution was (mM): NaCl 207·5; KCl 5·4; $CaCl_2$ 13·5; $MgCl_2$ 5·3. Tris buffer was added to keep the pH at about 7·5. In some cases the concentrations of $CaCl_2$ and $MgCl_2$ were slightly modified. In particular, when the inhibitory nerve was stimulated, the concentration of $CaCl_2$ was increased to 18 mM to obtain larger responses. When the concentration of chloride in the outside solution was reduced, chloride was substituted by propionate.

For recording and for passing current, 3-M-K-propionate-filled micropipettes were used. In some cases in order to increase the inhibitory junctional potentials (i.j.p.s) the outside concentration of chloride was lowered and KCl-filled micropipettes were used.

GABA solution was prepared by adjusting the pH of γ-amino-*n*-butyric acid (Tokyo Kasei Kogyo, Co.) to pH 4 by adding HCl and a single or double-barrelled micropipette was filled with a solution of about 1 M. The isoelectric point of GABA is pH 7·3 and GABA was injected as cation by outward current through the micropipette. L-glutamate solution was adjusted to pH 8 by adding NaOH and glutamate was injected as anion. When GABA and glutamate were in a double-barrelled micropipette, the pipettes were prepared a few hours before each experiment in order to avoid possible mixing of drugs.

All experiments were performed at room temperature (21–23° C).

RESULTS

GABA-induced potential change

After the recording electrode was inserted, usually at the middle of the muscle fibre, the GABA-filled micropipette was moved along the surface of the fibre and GABA was applied iontophoretically by outward current through the pipette. After careful search along the muscle fibre, a place was found where momentary application of GABA produced a slow depolarization. In the experiment illustrated in Fig. 1 a GABA-filled micropipette was moved along a muscle fibre in 16μ steps and brief pulses of outward current were passed through the pipette. As the micropipette was moved from the focus of the sensitive area, the GABA-induced potential change (GABA potential) became smaller and slower, suggesting that the GABA-sensitive area was circumscribed.

After the approximate position of GABA-sensitive areas were determined, the tip of the GABA-filled micropipette was finely adjusted and

GABA-sensitive spots were more precisely located. The time course of the GABA potential was slow and it was relatively difficult to locate the GABA-sensitive spots critically. Figure 2 shows an example of GABA potential when GABA was applied near a focus. The potential reached its peak in 65 msec and its total duration was about 500 msec. The rise time

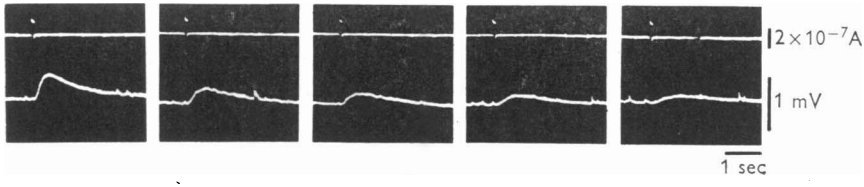

Fig. 1. GABA-induced depolarizations in a muscle fibre (lower traces). Upper traces, monitored current for iontophoretic application of GABA through a micropipette. Upward deflexion represents an outward current through the pipette. From left to right the GABA pipette was moved along the muscle fibre surface in 16μ steps.

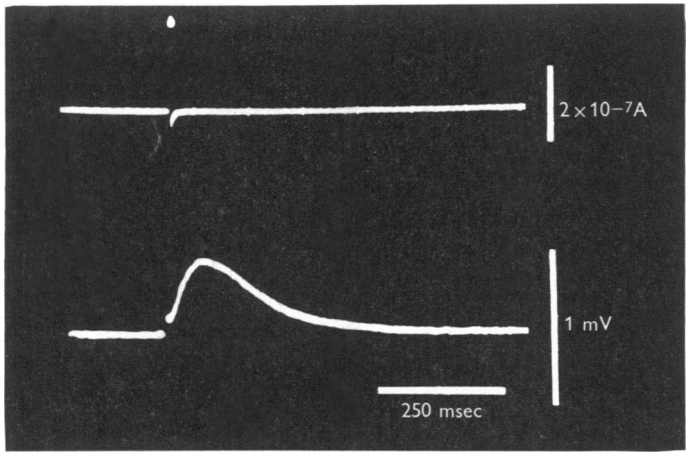

Fig. 2. An example of GABA potential recorded when the injection pipette was adjusted near a focus (lower trace). Upper trace, monitored injection current.

of the GABA potential was about 4 times slower than that of glutamate potential (compare with Fig. 3 in Takeuchi & Takeuchi, 1964*a*). The difference in the time courses between GABA potential and glutamate potential is clearly observed in Fig. 6*b*, where the GABA and glutamate receptors were close to each other and GABA and glutamate were applied from a double-barrelled micropipette. Since there seems to be little difference in diffusion constants between GABA and glutamate (cf. Longsworth, 1953), the difference in time courses between the GABA and glutamate potentials may be due either to a diffusion barrier around the

inhibitory junctional area or to a slower rate of reaction of the receptor to GABA than is the case with glutamate. Decomposition of compounds by enzymes or the desensitization of receptors may also have to be considered. At present one cannot decide between these alternatives. However, it should be noted that the time course of the inhibitory junctional

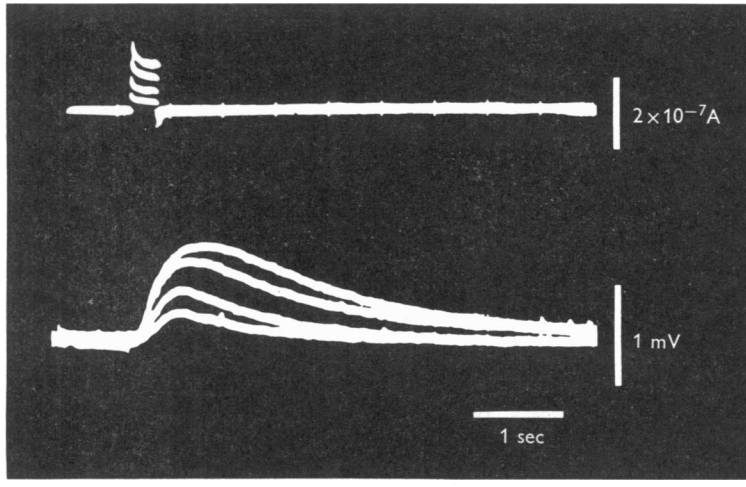

Fig. 3. GABA potentials superimposed, for different doses of GABA. Upper trace, monitored injection currents. The reversal potential was 6 mV more positive.

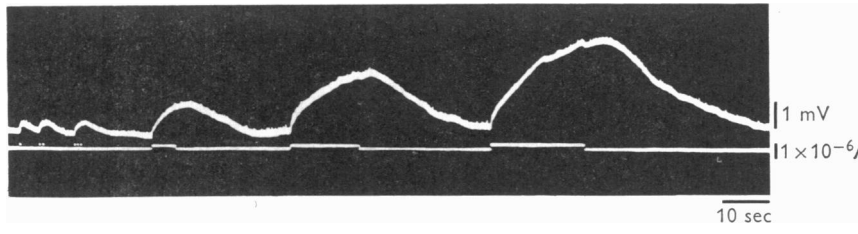

Fig. 4. GABA potentials recorded on moving film, changing the duration of injection. Lower trace, monitored injection current. The reversal potential was 6 mV more positive.

potentials (i.j.p.s) is also slower than that of the excitatory junctional potentials (e.j.p.s) (see Fig. 6*a*). In the case of Fig. 2, $8{\cdot}8 \times 10^{-10}$ C was passed through the GABA pipette. If the transport number is assumed as 0·5, about $4{\cdot}2 \times 10^{-15}$ mole of GABA produced a depolarization of 0·5 mV.

The time course of the GABA potential was dependent on the amount of GABA. In Fig. 3 are shown, superimposed, four traces recorded while the dose of GABA was changed. As the dose was increased the time course, especially its falling phase, was prolonged. Since in this case the reversal potential was 6 mV more positive than the resting potential and the

depolarization was about 1 mV, the prolongation of the time course was not due to the saturation of the potential.

When a steady dose of GABA was applied, the resultant GABA potential did not decline. This result differs from the glutamate potential which shows marked desensitization (Takeuchi & Takeuchi, 1964*a*). An example is shown in Fig. 4, where GABA was applied with different durations of application.

When a small conditioning dose of L-glutamate is applied, the test dose of L-glutamate produces a potentiated response (Takeuchi & Takeuchi, 1964*a*). In contrast, no appreciable potentiation was observed in the amplitude of the GABA potential when a small conditioning dose of GABA was applied. However, rather large doses of GABA were usually necessary to produce an appreciable potential change. Therefore, there still remains a possibility that with smaller doses a potentiating effect of GABA potential might be present.

Site of action of GABA

Previous studies have shown that L-glutamate-sensitive areas are localized on the crayfish-muscle surface and they coincide with the excitatory neuromuscular junctions (Takeuchi & Takeuchi, 1964*a*). The GABA-sensitive areas were also localized on the surface of the muscle. In this experiment the relation between the locations of glutamate-sensitive and GABA-sensitive areas has been investigated. A double-barrelled micropipette, one barrel being filled with L-glutamate and the other with GABA, was used to apply L-glutamate and GABA to the same area. The amplitudes and the time courses of glutamate and GABA potentials were measured when these drugs were applied at different points along the muscle fibre surface. An example is shown in Fig. 5, where open circles represent the rise time and filled circles the amplitude of the potentials. It is considered that the sensitive area is localized at the point where the largest and the most rapid response was obtained. In most cases, as in Fig. 5, GABA-sensitive areas coincided with glutamate-sensitive areas.

As a next step the correlation between GABA-sensitive spots and inhibitory junctions was studied. In the case of glutamate the sensitive areas coincided with the excitatory neuromuscular junction which was located by recording the e.j.p.s with a micro-electrode placed on the surface of the muscle fibre (Takeuchi & Takeuchi, 1964*a*). In the present experiments the same method was used to decide whether or not GABA-sensitive spots coincided with the inhibitory junctional areas. This proved somewhat difficult because the i.j.p.s were usually small and their time course relatively slow. It was found that GABA-sensitive areas coincided, in most cases, with glutamate-sensitive areas. In analogy, therefore, it

was expected that the inhibitory junctional area might be found near the excitatory junctional area. After an approximate position was located by recording the external e.j.p.s, the tip of the external recording microelectrode was finely adjusted while the inhibitory axon was repetitively stimulated. At a critical position of the tip of the electrode small downward deflexions could be recorded. In Fig. 6*A*, the upper trace in *a* shows the external record and the lower trace the internal i.j.p.s superimposed at a stimulation rate of 40/sec. In the external record sharp downward

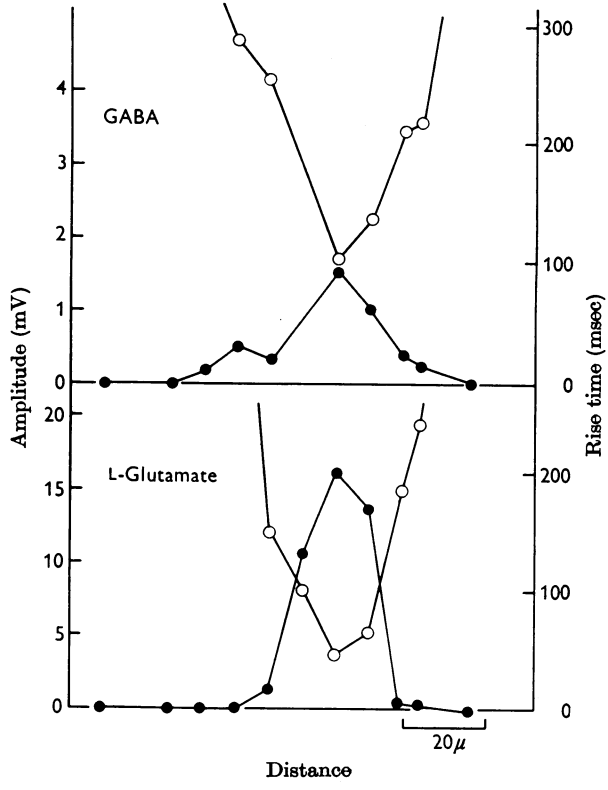

Fig. 5. Relation between the distribution of GABA potential and that of L-glutamate potential along a muscle fibre. ●, amplitude of GABA potentials and glutamate potentials obtained by injecting constant doses of drugs from a double-barrelled micropipette at different points on the muscle surface. ○, time to the peak of GABA and glutamate potentials. Abscissa, distance along the muscle surface.

deflexions of presynaptic nerve impulses are followed by the external i.j.p.s. A brief dose of GABA applied near the tip of the external electrode produced the GABA potential in the intracellular record (bottom trace in *b*) and the downward deflexion in the extracellular record (middle trace in *b*). At the same recording position the stimulation of the excitatory

axon produced external e.j.p.s as large as 1 mV (upper trace in Fig. 6*B*, *a*). The glutamate potential was obtained intracellularly as well as extracellularly by the application of L-glutamate to the same spot (Fig. 6*B*, *b*). This result indicates that the GABA-sensitive area is probably coincident with the inhibitory junctional area and that the inhibitory junctional area is also in close proximity (perhaps within a few μ) to the excitatory junctional area. In Fig. 6 it is noted that the variation in the amplitudes of both extracellular and intracellular i.j.p.s are smaller than in e.j.p.s. Although the mode of liberation of the inhibitory transmitter is not clear, the above result might be due to a large quantum content of i.j.p.s.

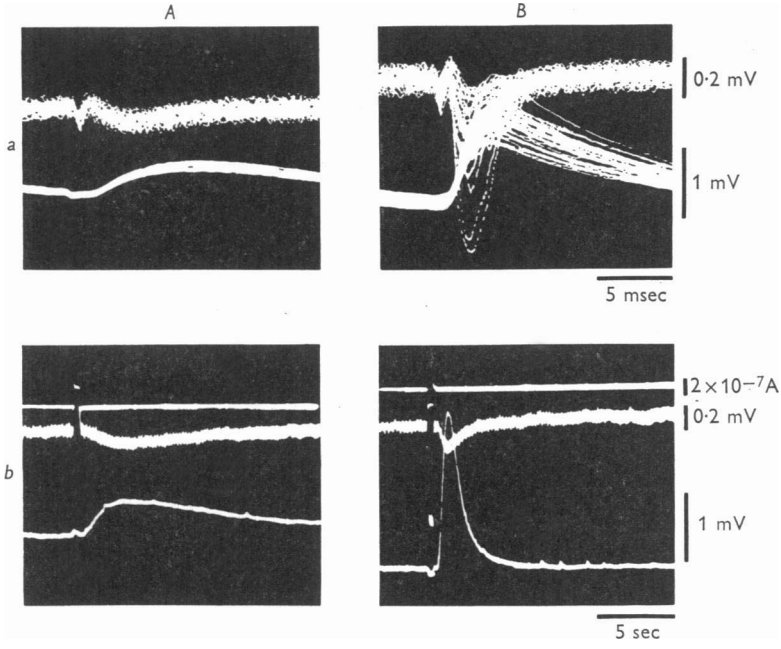

Fig. 6. An example of potential changes recorded at a junctional area. *a*: upper traces, extracellularly recorded potential changes obtained by stimulating the inhibitory nerve (*A*) and the excitatory nerve (*B*) at stimulation rate of 40/sec; lower traces, simultaneous intracellular i.j.p.s and e.j.p.s; about 40 sweeps were superimposed. *b*: upper traces, monitored injection currents, outward current being upward; middle traces, extracellularly recorded potential changes obtained by injection of GABA (*A*) and L-glutamate (*B*) from a double-barrelled pipette; lower traces, simultaneous intracellular records of GABA potential (*A*) and glutamate potential (*B*).

Since GABA and L-glutamate acted, in most cases, at the same area of muscle surface, it might be considered that both L-glutamate and GABA act at the same receptor site (Curtis & Watkins, 1960). It has been observed that the glutamate-sensitive receptor is desensitized by prolonged

application of L-glutamate (Takeuchi & Takeuchi, 1964*a*). If GABA acts at the same receptor, it might be expected that the GABA-sensitive receptor would also be desensitized by the prolonged application of L-glutamate. Brief test doses of L-glutamate and GABA were applied alternately from a double-barrelled micropipette to test sensitivity of the receptor. In Fig. 7 large rapid depolarizations are glutamate potentials and slow depolarizations GABA potentials. At the downward arrow a steady, conditioning application of L-glutamate started from a separate micropipette and marked depolarization was produced, declining gradually

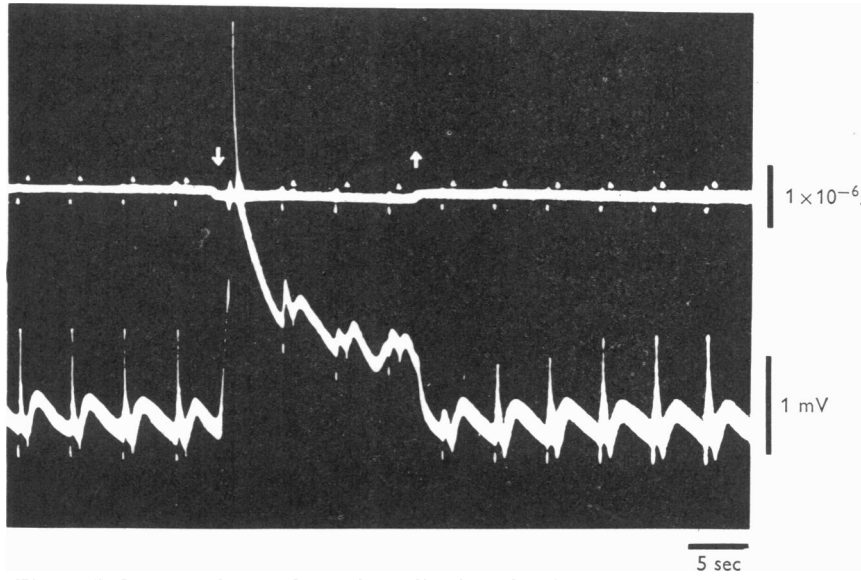

Fig. 7. Influences of a steady weak application of L-glutamate on glutamate and GABA potentials. Upper trace, monitored injection currents. Brief test doses of glutamate and GABA each through a double-barrelled micropipette induced large rapid depolarizations and slow depolarizations respectively (lower trace). During the period indicated by arrows steady conditioning dose of L-glutamate was injected through another pipette adjusted near the same point.

while the conditioning dose was maintained. During application of the conditioning dose the amplitude of superimposed glutamate potential produced by the first test pulse showed an increase and subsequent ones a decrease. After cessation of the conditioning dose of glutamate (at upward arrow), responses to test doses of glutamate were reduced for about 20 sec while no appreciable change was observed in the GABA potential. This result shows that even though the glutamate-sensitive receptor is desensitized after the conditioning dose, it has no influence on the GABA-sensitive receptor, suggesting that the GABA-sensitive receptor is different from the glutamate-sensitive receptor. The same

may also be true with the e.j.p.s and the i.j.p.s. In Fig. 8 the inhibitory and excitatory axons were stimulated separately by trains of stimuli at 30/sec and the i.j.p.s and e.j.p.s of about 0·8 mV and 2·5 mV respectively were produced. Bathing fluid was then changed to one containing L-glutamate (0·01 mM). In Fig. 8, *a* was obtained before and *b* about 1·5 min after changing the solution to L-glutamate solution, and *c* after returning it to the normal solution. In glutamate solution the amplitude of e.j.p.s was reduced to about 1 mV, while i.j.p.s showed no appreciable change in their amplitude.

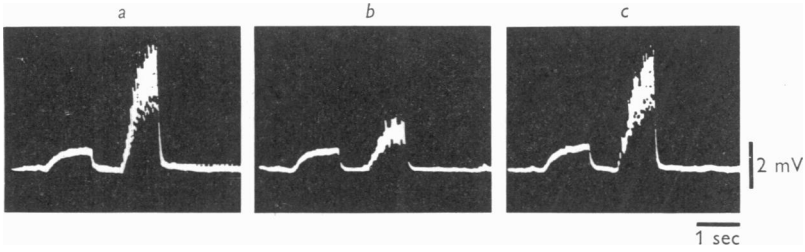

Fig. 8. Influence of L-glutamate contained in outside solution on i.j.p.s and e.j.p.s. I.j.p.s and e.j.p.s were produced successively at stimulation rate of 30/sec for about 1 sec. *a*, before and *b*, about 1·5 min after changing the outside solution to that containing 0·01 mM-L-glutamate. Marked decrease in amplitude of e.j.p.s is observed. *c*, after returning it to the normal solution.

When the tip of the GABA-filled micropipette penetrated the membrane, brief electrotonic depolarization was produced owing to the injection current pulse through the membrane and the slow GABA potential was not observed. This result indicates that the GABA-sensitive receptor is located on the outer surface of the muscle membrane as is the case for acetylcholine receptors in frog striated muscle (del Castillo & Katz, 1955).

Reversal potential

The reversal potentials of i.j.p.s and for GABA potentials were measured on single muscle fibres. In this case 3-M-K-propionate-filled micropipettes were used for both recording and polarizing electrodes. The reversal potential for the GABA potential and the i.j.p.s coincided within half a mV of each other. In Fig. 9 the i.j.p.s and the GABA potential were recorded at varying membrane potentials. At *N*, a brief train of i.j.p.s was produced at 30/sec followed by the GABA potential, the injection current being monitored on the uppermost trace. The membrane potential was reduced from the resting potential of 70 mV (bottom trace) by cathodic current. Both i.j.p.s and GABA potential reversed their signs at about 66 mV. This result confirms the report by Kuffler (1960).

When the recording and/or polarizing electrodes were filled with KCl,

more depolarization was usually necessary to reverse the GABA potential than to reverse the i.j.p.s. The difference in the reversal potentials between GABA action and i.j.p.s was marked when the GABA-sensitive spot was found near the KCl electrode. When the sensitive spot was found at a point far from the KCl electrode, the difference in the reversal potentials was not observed. The explanation of this result is that when the GABA-sensitive spot is near the KCl electrode, the Cl^- concentration in the muscle fibre under the sensitive spot is increased, thus the reversal potential of

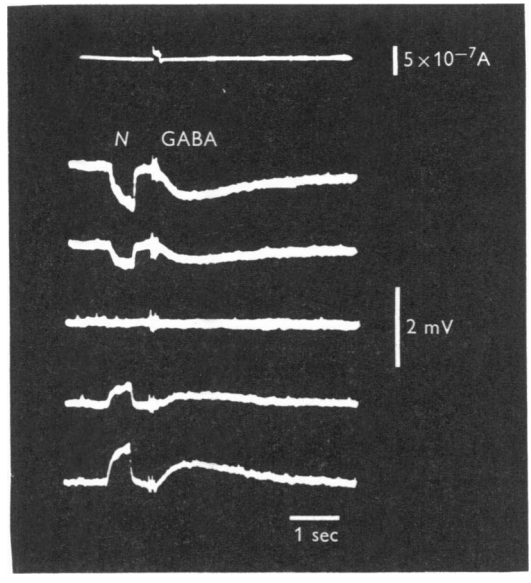

Fig. 9. Reversed potential of i.j.p.s and GABA potential. *N*, i.j.p.s produced by stimulating the inhibitory nerve at 30/sec; GABA, potentials produced by iontophoretic injection of GABA on the sensitive area of the same muscle fibre. Upper trace, monitored injection current. Bottom trace was obtained at the resting potential (−70 mV), and the muscle fibre was depolarized in 2 mV steps. The reversal potential, in this case, was about −66 mV.

GABA action is changed to a more depolarized level. On the other hand i.j.p.s are produced by widely distributed synapses on the muscle fibre (van Harreveld, 1939), so that an increase in Cl^- concentration at a localized point of muscle fibre has less influence on the reversal potential of intracellularly recorded i.j.p.s than on the GABA potential. When Cl^- in van Harreveld's solution was substituted by propionate, the reversal potential of both GABA potential and i.j.p.s changed to a more depolarized level.

The reversal potential of i.j.p.s obtained here may not indicate exactly the true inhibitory equilibrium potential, because a polarizing current

does not depolarize the whole fibre uniformly (Burke & Ginsborg, 1956). However, this introduces only a small error, because the depolarization used is small, usually a few mV, and the muscle fibre is short compared to the length constant.

The reversal potential was usually a few mV more positive than the resting potential. This value was susceptible to changes in the membrane potential. If the membrane was maintained for some time at a depolarized level by a polarizing current, the reversal potential became more positive and vice versa.

DISCUSSION

The present results indicate that GABA has a specific action on the inhibitory neuromuscular junction and that the reversal potentials of the i.j.p.s and the GABA potential were the same. These results suggest that the GABA potential is produced by the same ionic mechanisms at the junctional membrane as the i.j.p.s (Boistel & Fatt, 1958).

The minimum dose of GABA which produced a GABA potential of approximately the same size as i.j.p.s was about 4×10^{-15} mole. This amount is large compared to that for the glutamate potential ($4{\cdot}5 \times 10^{-16}$ mole, Takeuchi & Takeuchi, 1964*a*). But, since the dose of GABA necessary to evoke a response is very sensitive to the position of the tip of the micropipette, and GABA-sensitive spots were relatively difficult to locate precisely, the difference in doses may be less.

The time course of i.j.p.s is very much slower than that of e.j.p.s (see Fig. 6 and Fatt & Katz, 1953). In this connexion it is interesting to note that the time course of the GABA potential is slower than that of the glutamate potential. It was observed that L-glutamate imitates the excitatory transmitter in crayfish muscle (Takeuchi & Takeuchi, 1964*a*) and that glutamate is the only excitatory compound extracted from the lobster nerve (Kravitz, Kuffler, Potter & van Gelder, 1963). Then there is a parallelism in the time course between the action of the excitatory and inhibitory amino acids and the neural transmitters.

If one considers these results together with those by Dudel *et al.* (1963), Kravitz, Kuffler, Potter & van Gelder (1963) and Kravitz, Kuffler & Potter (1963), they suggest that GABA and L-glutamate are promising candidates for the inhibitory and excitatory transmitters at crustacean neuromuscular junctions. However, more criteria should be fulfilled to decide whether these substances are the neural transmitters (cf. Kravitz, Kuffler & Potter, 1963; Takeuchi & Takeuchi, 1964*a*).

In the mammalian central nervous system it was argued from the relation between the activities and the molecular structures of the excitatory and the depressant amino acids that the depressant and the excitant

amino acids act at the same receptor site (Curtis & Watkins, 1960). In the crayfish muscle, however, after the desensitization of glutamate-sensitive receptor by prolonged application of L-glutamate, the application of GABA still produced normal responses. This observation suggests that different receptors are involved for the GABA and glutamate actions. Specific actions of picrotoxin on the GABA action and the i.j.p.s have also been observed (Robbins & Van der Kloot, 1958; Van der Kloot & Robbins, 1959; Grundfest *et al.* 1959).

In the present experiments the inhibitory junctional areas were found in most cases very close to the excitatory junctional areas. This finding coincides with the histological observations on the same preparation that the two nerve fibres run parallel and often end almost at the same place, although sometimes one of them continues for a short distance apparently unaccompanied by the other (van Harreveld, 1939).

SUMMARY

1. The effect of γ-aminobutyric acid (GABA) on the abductor of the dactylopodite in the walking leg of crayfish was investigated. The drug was applied iontophoretically and the resulting potential changes were recorded extracellularly and intracellularly with glass micro-electrodes.

2. Application of GABA to well-circumscribed regions on the muscle surface produced a transient slow depolarization. These GABA-sensitive spots as a rule were identical with areas that were sensitive to L-glutamate.

3. The focus of GABA-sensitive spots coincided with the inhibitory neuromuscular junctions localized by recording the extracellular inhibitory junctional potentials (i.j.p.s). Injection of GABA into the interior of muscle fibres produced no appreciable depolarization.

4. Following a steady conditioning dose of glutamate the sensitivity of the GABA receptor was unchanged, while marked desensitization was observed on the glutamate receptor. When L-glutamate (0·01 mM) was added to the outside solution, a remarkable decrease in the size of excitatory junctional potentials (e.j.p.s) was observed with no change in amplitude of i.j.p.s.

5. Reversal potentials of i.j.p.s and GABA potential coincided within half a mV of each other.

6. The results indicate that GABA has a localized action at the inhibitory junctional area and mimics the action of the neural inhibitory transmitter.

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REFERENCES

BAZEMORE, A. W., ELLIOTT, K. A. C. & FLOREY, E. (1957). Isolation of factor I. *J. Neurochem.* **1**, 334–339.

BOISTEL, J. & FATT, P. (1958). Membrane permeability change during inhibitory transmitter action in crustacean muscle. *J. Physiol.* **144**, 176–191.

BURKE, W. & GINSBORG, B. L. (1956). The action of the neuromuscular transmission on the slow fibre membrane. *J. Physiol.* **132**, 599–610.

CURTIS, D. R., PHILLIS, J. W. & WATKINS, J. C. (1959). The depression of spinal neurones by γ-amino-*n*-butyric acid and β-alanine. *J. Physiol.* **146**, 185–203.

CURTIS, D. R. & WATKINS, J. C. (1960). The excitation and depression of spinal neurones by structurally related amino acids. *J. Neurochem.* **6**, 117–141.

DEL CASTILLO, J. & KATZ, B. (1955). On the localization of acetylcholine receptors. *J. Physiol.* **128**, 157-181.

DIAMOND, J. (1963). Variation in the sensitivity to gamma-amino-butyric acid of different regions of the Mauthner neurone. *Nature, Lond.*, **199**, 773–775.

DUDEL, J. (1963). Presynaptic inhibition of the excitatory nerve terminal in the neuromuscular junction of the crayfish. *Pflüg. Arch. ges. Physiol.* **277**, 537–557.

DUDEL, J., GRYDER, R., KAJI, A., KUFFLER, S. W. & POTTER, D. D. (1963). Gamma-aminobutyric acid and other blocking compounds in crustacea. I. Central nervous system. *J. Neurophysiol.* **26**, 721–728.

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

EDWARDS, C. & KUFFLER, S. W. (1959). The blocking effect of γ-aminobutyric acid (GABA) and the action of related compounds on single nerve cells. *J. Neurochem.* **4**, 19–30.

ELLIOTT, K. A. C. & JASPER, H. H. (1959). Gamma-aminobutyric acid. *Physiol. Rev.* **39**, 383–406.

FATT, P. & KATZ, B. (1953). The effect of inhibitory nerve impulses on a crustacean muscle fibre. *J. Physiol.* **121**, 374–389.

FURSHPAN, E. J. & POTTER, D. D. (1959). Slow post-synaptic potentials recorded from the giant motor fibre of the crayfish. *J. Physiol.* **145**, 326–335.

GRUNDFEST, H. & REUBEN, J. P. (1961). Neuromuscular synaptic activity in lobster. In *Nervous Inhibition*, ed. FLOREY, E., pp. 92–104. Oxford: Pergamon Press.

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

HAGIWARA, S., KUSANO, K. & SAITO, S. (1960). Membrane changes in crayfish stretch receptor neuron during synaptic inhibition and under action of gamma-aminobutyric acid. *J. Neurophysiol.* **23**, 505–515.

HALL, Z., KRAVITZ, E. A. & POTTER, D. D. (1964). Enzymes of γ-amino-butyrate (GABA) metabolism in isolated excitatory and inhibitory axons of the lobster. At *The Symposium on Neurophysiology, Tokyo.*

HOYLE, G. & WIERSMA, C. A. G. (1958). Inhibition at neuromuscular junctions in crustacea. *J. Physiol.* **143**, 426–440.

KRAVITZ, E. A., KUFFLER, S. W. & POTTER, D. D. (1963). Gamma-aminobutyric acid and other blocking compounds in crustacea. III. Their relative concentrations in separated motor and inhibitory axons. *J. Neurophysiol.* **26**, 739–751.

KRAVITZ, E. A., KUFFLER, S. W., POTTER, D. D. & VAN GELDER, N. M. (1963). Gamma-aminobutyric acid and other blocking compounds in crustacea. II. Peripheral nervous system. *J. Neurophysiol.* **26**, 729–738.

KRNJEVIĆ, K. & PHILLIS, J. W. (1963). Iontophoretic studies of neurones in the mammalian cerebral cortex. *J. Physiol.* **165**, 274–304.

KUFFLER, S. W. (1960). Excitation and inhibition in single nerve cells. *Harvey Lect.* 1958–1959, pp. 176–218.

KUFFLER, S. W. & EDWARDS, C. (1958). Mechanism of gamma aminobutyric acid (GABA) action and its relation to synaptic inhibition. *J. Neurophysiol.* **21**, 589–610.

LONGSWORTH, L. G. (1953). Diffusion measurements, at 25°, of aqueous solutions of amino acids, peptides and sugars. *J. Amer. chem. Soc.* **75**, 5705–5709.

PURPURA, D. P. (1960). Pharmacological actions of γ-amino acid drugs on different cortical synaptic organizations. In *Inhibition in the Nervous System and Gamma-Aminobutyric Acid*, ed. ROBERTS, E., pp. 495–514. Oxford: Pergamon Press.

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.

ROBERTS, E. (1960). Free amino acids of nervous tissue: some aspects of metabolism of gamma-aminobutyric acid. In *Inhibition in the Nervous System and Gamma-Aminobutyric Acid*, ed. ROBERTS, E., pp. 144–158. Oxford: Pergamon Press.

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

TAKEUCHI, A. & TAKEUCHI, N. (1964*b*). Iontophoretic application of gamma-aminobutyric acid on crayfish muscle. *Nature, Lond.*, **203**, 1074–1075.

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

VAN HARREVELD, A. (1939). The nerve supply of doubly and triply innervated crayfish muscles related to their function. *J. comp. Neurol.* **70**, 267–284.