

# Desensitization of Gamma Aminobutyric Acid (GABA) Receptors in Muscle Fibers of the Crab *Cancer borealis*

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**ABSTRACT** *Carcinus* muscle fibers respond to  $\gamma$ -aminobutyric acid (GABA) with a conductance increase that subsides rather rapidly. In the larger fibers which have low input resistance the decrease may disappear within 2 min. The inhibition of the excitatory postsynaptic potentials (EPSP's) by GABA nevertheless persists as long as the drug is applied. The subsidence of the increased conductance indicates that the membrane of the inhibitory synapses has become desensitized to GABA. The persistence of inhibition of the EPSP's appears to be due to an action of the drug on the presynaptic terminals of the excitatory axons which reduces or blocks the secretory activity that releases the excitatory transmitter.

## INTRODUCTION

Some pharmacological differences appear to exist in the neuromuscular transmission of different crustaceans. The inhibitory synaptic membrane of muscle fibers of crayfish (Boistel and Fatt, 1958; Girardier et al., 1962; Dudel, 1965; Takeuchi and Takeuchi, 1965, 1966 *a*; Ozeki et al., 1966), and lobster (Grundfest et al., 1959; Florey and Hoyle, 1961; Gainer et al., 1967; Motokizawa et al., 1969) is activated by  $\gamma$ -aminobutyric acid (GABA) and this is reflected in an increased conductance for Cl as well as in the diminution of concurrent excitatory postsynaptic potentials (EPSP's). The effects of GABA mimic fully the effects of stimulation of the inhibitory axon and there is now good evidence that GABA may itself be the inhibitory transmitter (Kravitz et al., 1963; Otsuka et al., 1966).

However, while application of GABA caused diminution of the EPSP's of muscle fibers of the crab, *Cancer magister* (Florey and Hoyle, 1961), the

effective resistance ( $R_{eff}$ ) of the fibers seemed to be little affected by the agent, giving rise to questions regarding the mechanism of the inhibition. Similar data were also obtained for *C. borealis* (Reuben and Grundfest, unpublished; Aljure et al., 1962; cf. Fig. 25 in Grundfest, 1961, and Fig. 45 in Grundfest, 1966). Furthermore, and also in *C. borealis*, maximal stimulation of the inhibitor axon caused a decrease of  $R_{eff}$  that did not exceed about 30% of the resting resistance (Aljure et al., 1962). This change appeared to be inadequate to account for the observed large inhibitory effects on the EPSP's. It was therefore suggested that the inhibitory transmitter and GABA exerted some of their action by depressing the release of excitatory transmitters from the presynaptic terminals of the exciter axon; i.e., by a mechanism of presynaptic inhibition. However, Eisenberg and Hamilton (1963) reported a rather substantial decrease in  $R_{eff}$  on application of GABA to muscle fibers of *C. borealis*. Atwood (see his reviews of 1967, 1968), studying a variety of crab species, concluded that only a portion of the muscle fibers are innervated by an inhibitor axon. He suggested that the various discrepant experimental observations might be explained if only the muscle fibers that are innervated by the inhibitory axon are responsive to GABA. This situation has indeed been found in insect muscles. Only those fibers that respond with IPSP's to stimulation of the inhibitor axon respond to GABA with a decrease in  $R_{eff}$  (Usherwood and Grundfest, 1965). However, the explanation seemed to be inadequate for the muscles of *C. magister* or *C. borealis*, since the lack of decrease in  $R_{eff}$  during inhibition of EPSP's by GABA was obtained with intracellular recordings in one and the same fiber (cf. Fig. 45 in Grundfest, 1966).

The mode of action of GABA as an inhibitory agent in crab neuromuscular transmission was therefore reexamined in the present work. As will be shown, GABA does diminish  $R_{eff}$ , but only to a rather small degree and only transiently, while the EPSP's are inhibited as long as the agent is applied. Thus the inhibitory synaptic membrane becomes desensitized to GABA much as the vertebrate muscle fiber (Katz and Thesleff, 1957), marine electroplaques (Bennett et al., 1961; Bennett and Grundfest, 1961), and molluscan neurons (Tauc and Bruner, 1963) become desensitized to acetylcholine. GABA itself also exerts presynaptic inhibition, presumably by diminishing the secretory activity in the nerve terminals of the exciter axon. The latter action of GABA has also been found in neuromuscular preparations of lobster (Grundfest and Reuben, 1961) and crayfish (Dudel, 1965). A preliminary account of the present work has been published (Epstein and Grundfest, 1968).

#### METHODS AND MATERIAL

Neuromuscular preparations of the closer muscle (adductor of the dactylopodite) of the walking leg of the crab *C. borealis* were used. The muscle is innervated by three axons of relatively large diameter, two exciters and one inhibitor axon. The exo-

skeleton of the distal portion of the propodite was removed and the most superficial 6 to 10 muscle fibers were exposed between their points of insertion at the propodite and the distal tendon. The nerve to the leg was exposed at the level of the meropodite. Usually it was quite simple to divide the nerve into two bundles so as to have the inhibitory axon for the closer separated from the two excitatory axons. In order to obtain independent activation of each of the latter the bundle containing them had to be dissected in most cases almost to the level of the single isolated fiber.

The preparation was bathed in a saline containing (in mM/liter) 470 NaCl, 8 KCl, 20 CaCl<sub>2</sub>, and 10 MgCl<sub>2</sub>. The pH was adjusted to 7.6–7.8 by adding Tris buffer. In some experiments in which the CaCl<sub>2</sub> was changed, the osmolarity of the medium was kept constant by adding or subtracting stoichiometrically corresponding amounts of NaCl.

The experiments were carried out at temperatures between 15° and 18°C maintained by means of a water bath surrounding the chamber. In any given experiment the temperature changes were kept within a range of less than 1°C, since appreciable variations in the membrane potential ( $E_M$ ) and  $R_{eff}$  (ca. 5% of the latter) of the muscle fibers were seen with changes in temperature of 1°C or more.

The system for recording and stimulating was standard for this laboratory. Fine bipolar platinum wire electrodes were used for stimulation of the nerve fibers. The intracellular microelectrodes were filled with 3 M KCl. One, for recording potentials, was connected to a neutralized capacity amplifier. A second, positioned some 50–150  $\mu$  from the first, was used for applying current to change the membrane potential. It has been shown in lobster muscle fibers (Motokizawa et al., 1969) that the small and relatively brief currents applied through such an electrode do not measurably affect the reversal potential for the IPSP ( $E_{IPSP}$ ), presumably an indication that they do not alter intracellular Cl concentration significantly. Still smaller effects are to be expected in crab, since the muscle fibers are even larger than in the lobster. For extracellular recording of synaptic currents, microelectrodes filled with 3 M NaCl, or sometimes with 3 M KCl, were used. As a rule their resistance was less than 5 M  $\Omega$ . The greatly amplified signal was AC-coupled to the oscilloscope.

Drugs were applied dissolved in the standard saline and their presence did not appreciably change the pH. The solutions were circulated through the rectangular chamber, the inlet being at the end in which was placed the distal portion of the crab's leg with the exposed muscle fibers. Continuous suction was applied at the other end of the chamber. Different volumes of the new bathing solution were used according to the procedure of a given experiment. As noted in the Results section, the agent was sometimes applied at one time. In other cases the new medium flowed continuously into the chamber. However, the change in the bath always involved circulating seven to eight times the volume of the chamber in the first 30–45 sec.

The experiments described were performed during the period July, 1967–January, 1968, and some 50 preparations were examined.

## RESULTS

As in various muscles of other species of crabs (Dorai Raj, 1964; Atwood, 1963, 1965, 1967; Hoyle, 1967) the fibers of the closer muscle in the walking

legs of *C. borealis* form a heterogeneous population with respect to diameter. The diameters ranged between 120  $\mu$  and 400  $\mu$  in some 300 superficial fibers that were seen during the present work. Within a given muscle the range was related to the size of the animal. The resting potential ( $E_M$ ) varied between  $-58$  mv and  $-71$  mv.  $R_{eff}$ , calculated from the hyperpolarizations induced by small or moderate inward currents, ranged between 15 and 300 k $\Omega$ . The two exciter axons induced somewhat different responses in the muscle fibers (Fig. 1 A, A'). Usually the first few EPSP's evoked by repetitive stimulation of one ("slow") axon were small, but their amplitude increased rapidly, denoting a considerable degree of facilitation. The EPSP evoked by a single stimulus to the other ("fast") axon was maximal or nearly so. The subsequent

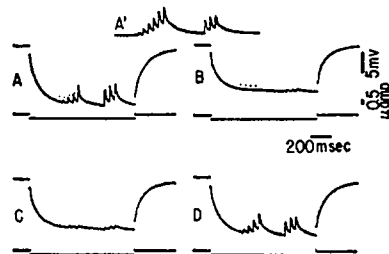


FIGURE 1. Similar effects of GABA on responses to stimulation of fast and slow exciter axons. A', trains of slow and fast EPSP's were evoked consecutively in a muscle fiber having a diameter of 300  $\mu$  and with  $R_{eff}$  about  $3 \times 10^4$  ohms. The volley of six stimuli to the slow axon evoked slowly increasing facilitation. A, another control during injection of a hyperpolarizing current. The volley to the slow axon was reduced to four stimuli. B,  $R_{eff}$  was reduced and both EPSP types were inhibited on applying GABA ( $3 \times 10^{-6}$  w/v). C,  $R_{eff}$  had returned practically to its initial value, but the EPSP's were still inhibited. D, after washing out the GABA.

EPSP's in a train showed little or no facilitation, the growth of the depolarization occurring mainly or entirely by summation.

*Effects of GABA on  $R_{eff}$  and EPSP's Evoked by Stimulating the Two Axons*  
When GABA was applied to the preparation (Fig. 1 B)  $R_{eff}$  decreased somewhat, but it returned within a few minutes nearly to its initial value (C). Both the fast and the slow EPSP's were almost completely inhibited throughout the time of application of GABA and both recovered fully when GABA was washed out (D). Thus, these data confirm the findings of earlier workers. GABA can induce inhibition of EPSP's when there is little or no effect of the agent on  $R_{eff}$ . However, there does appear to be a rather small but transient decrease in  $R_{eff}$  immediately after GABA is applied.

*Comparison of the Effects of GABA and IPSP's on the Membrane Potential and  $R_{eff}$*   
The IPSP's evoked by single stimuli to the inhibitor axon were small.

In the present work the IPSP's studied were the maximal responses evoked by stimulating the axon at some 60–100/sec. The reversal potential varied within only a narrow range (–62 to –64 mv; Figs. 2–4). Thus, the maximal IPSP's were depolarizing when  $E_M$  was in the range most frequently observed (–65 to –70 mv; Figs. 2–4). During the maximal IPSP's  $R_{eff}$  decreased more in the smaller than in the larger fibers (Figs. 2 and 3). In the 400  $\mu$  fiber (Fig. 2)  $R_{eff}$  decreased by some 25% whereas in the 120  $\mu$  fiber of Fig. 3  $R_{eff}$  decreased by about 35%.

When GABA was applied in concentrations  $>10^{-6}$  w/v there was a change in membrane potential similar to, but somewhat larger than that during the maximal IPSP's (Figs. 2 and 3). The reversal potentials in the presence of

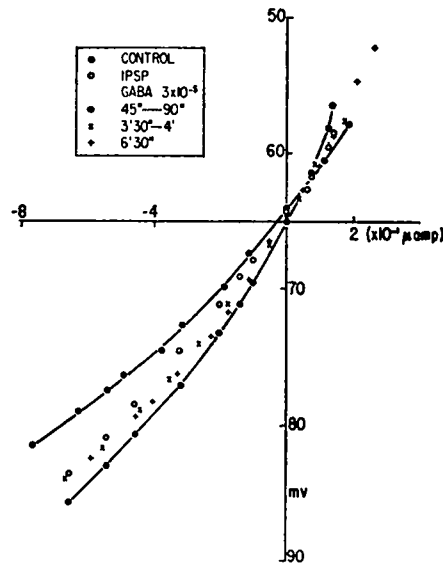


FIGURE 2. Changes in the current-voltage characteristic and in  $R_{eff}$  in a fiber of 400  $\mu$  diameter (initial  $R_{eff}$  50 k $\Omega$ ) that were induced by stimulation of the inhibitor axon at 80/sec (open circles) and at various times after applying GABA, as indicated in inset box. GABA was applied in a continuously flowing saline bathing medium. After exposure to GABA for more than 2 min  $R_{eff}$  was higher than during the IPSP's and approached the initial value.

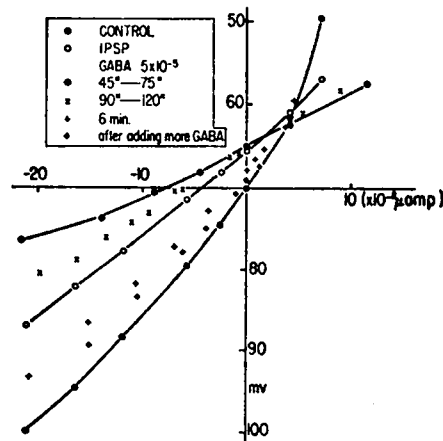


FIGURE 3. Similar data on a fiber of 120  $\mu$  diameter ( $R_{eff}$  about 160 k $\Omega$ ). The inhibitor axon was stimulated at 6/sec. The IPSP's were among the largest seen causing a depolarization of about 5 mv and  $R_{eff}$  decreased very markedly. It decreased still more immediately after applying GABA but within 6 min had returned to nearly the initial value. Further addition of GABA to the bath had no effect.

GABA were very close to those obtained for  $E_{IPSP}$ . The decrease in  $R_{eff}$  observed within the first few minutes after the addition of GABA was larger than that observed during the IPSP's. However, during continued application of GABA the slope of the current-voltage characteristic returned toward the control value as did the change in membrane potential (+ symbols, Figs. 2 and 3). Further addition of GABA to the bath did not restore the decrease in  $R_{eff}$  or the change in  $E_M$  that had been observed on the initial application of GABA (Fig. 3). This finding indicates that the return of  $R_{eff}$  toward the control value was not due to inactivation of the GABA, as has been found in crayfish stretch receptors (Kuffler and Edwards, 1958; Edwards and Kuffler, 1959). Rather, the return of  $R_{eff}$  indicates desensitization of the inhibitory postsynaptic membrane.

*Time Course of Desensitization* The time course of the effects of GABA on  $R_{eff}$  and on the EPSP's is shown in the two simultaneous experiments of Fig. 4 which were done on a fiber 200  $\mu$  in diameter (upper graph) and on

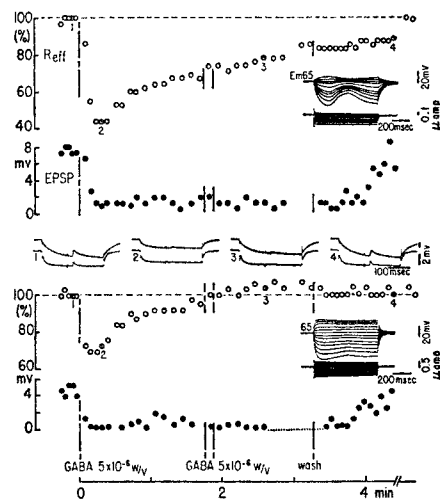


FIGURE 4. Effects of GABA ( $5 \times 10^{-6}$  w/v) on  $R_{eff}$  (open circles, changes expressed relative to the initial values) and on the fast EPSP's in two muscle fibers of 200  $\mu$  diameter (upper set) and 300  $\mu$  (lower set) from the same preparation. Four records showing examples of the measurements, the numbers on the records corresponding to numbers and center-dotted circles on  $R_{eff}$  graphs.  $R_{eff}$  decreased more in the smaller fiber and some of the decrease persisted until GABA was washed out (procedures are indicated above the abscissa). In the larger fiber  $R_{eff}$  returned to the initial value after 2 min and was not reduced by further addition of GABA. The EPSP's, however, remained depressed in both muscle fibers. The effect of GABA was reversed rapidly on washing with a GABA-free medium. Inset records show superimposed sweeps during which the inhibitor axon was stimulated repetitively while currents were injected into the muscle fibers. The IPSP's were small at the resting potentials ( $-65$  mv in both fibers). The effects of the injected currents were larger in the smaller fiber, as is to be expected. The IPSP's in this fiber reversed sign when the membrane was depolarized about 5 mv.

another 300  $\mu$  diameter. Initially, following application of  $5 \times 10^{-6}$  w/v GABA there was a sharp drop in  $R_{eff}$  which was greater, however, in the smaller fiber. In both fibers the EPSP's were almost abolished. However, while the EPSP's remained depressed, the change in  $R_{eff}$  began to reverse within about 30 sec. In the fiber of larger diameter in which  $R_{eff}$  had decreased by only about 30% the reversal to the resting value was almost complete within less than 2 min, and further addition of GABA did not restore the decrease in  $R_{eff}$ . In the smaller fiber in which  $R_{eff}$  had initially diminished to about 40% of the resting value the return to higher values was slower and recovery of  $R_{eff}$  to the control value was incomplete. The effects on both  $R_{eff}$  and the EPSP's were reversed on washing out the GABA.

Further data are shown in Fig. 5 from another 200  $\mu$  fiber. The EPSP was a compound response elicited by stimulation of both exciter axons and its duration was consequently longer than that of the EPSP's registered in Fig. 4. As in Fig. 4, the EPSP's remained depressed throughout the period that GABA ( $10^{-6}$  w/v) was applied. The fiber depolarized about 2 mv on introducing GABA. This was reduced to about 1 mv during continued action of GABA and was increased transiently when more GABA was added to the bath.  $R_{eff}$ , which had decreased to about 50% of the control on adding GABA the first time, gradually returned to higher values and this return was not appreciably affected by further addition of GABA. However, the addition of  $5 \times 10^{-5}$  w/v picrotoxin rapidly abolished the change in  $R_{eff}$ , the diminution of the EPSP's and the depolarization that had persisted during application

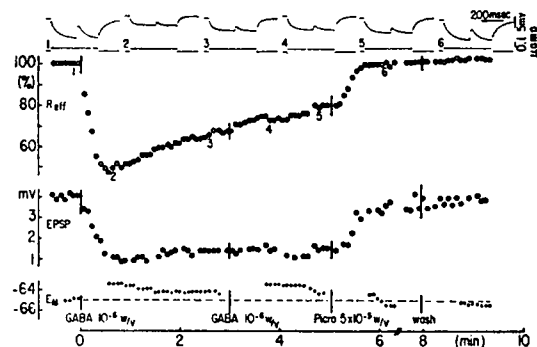


FIGURE 5. Similar measurements on another 200  $\mu$  fiber. EPSP's shown in the records above the graphs were evoked by stimulating both exciter axons simultaneously. Numbers on records correspond to center-dotted and numbered circles on  $R_{eff}$  graphs. The slow increase in  $R_{eff}$  was not reversed by adding more GABA, but addition of picrotoxin ( $5 \times 10^{-5}$  w/v) rapidly returned both  $R_{eff}$  and EPSP's to their initial values. GABA caused a depolarization of the fiber, indicating that  $E_{IPSP}$  was positive to the resting potential ( $E_M -65$  mv). The depolarization which was subsiding with time after initial exposure to GABA increased briefly on further addition of GABA, but this was reflected only slightly in  $R_{eff}$  and EPSP. The fiber repolarized on addition of picrotoxin.

of GABA. All values returned to the control levels and were not altered further on washing out the drugs.

Desensitization is accelerated in the cholinergic neuromuscular synapses of the frog by increasing the level of Ca in the bathing medium (Manthey, 1966). In four crab preparations no change was observed in the rate of return of  $R_{eff}$  toward the control value when Ca was varied over a 30-fold range, from 2 mM/liter to 60 mM/liter.

*Effects of GABA on the Synaptic Currents Associated with EPSP's.* The synaptic current was depressed on application of GABA (Fig. 6). In this experiment the fiber was relatively large ( $R_{eff}$  about  $1.6 \times 10^4$  ohms) and the maximum decrease in  $R_{eff}$  caused by GABA was only about 25%. After some 3 min exposure to GABA  $R_{eff}$  returned to the control values (C, D, E), but the EPSP's were still small. These data do not exclude the possibility that GABA is itself a weak curariform inactivator of the excitatory postsynaptic membrane.

*Changes in Presynaptic Responses* More direct evidence for a presynaptic action of GABA was sought by recording the current from the presynaptic terminals. These recordings proved to be rather difficult in the *C. borealis* preparations. The most successful experiment is shown in Fig. 7. The upper trace of A shows superposed recordings of the intracellularly recorded EPSP's

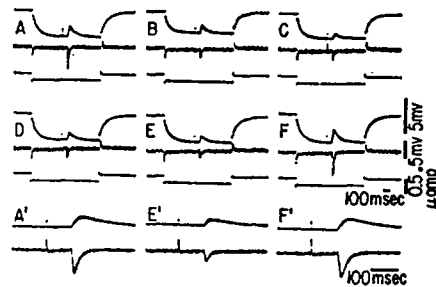


FIGURE 6. Simultaneous recordings of the EPSP (intracellular registration, upper traces) and of the synaptic current (extracellular registration at 20 times higher gain, middle traces) as well as measurements of the changes in  $R_{eff}$  (injected current shown on lowest traces). Two sweeps are superimposed in all the records. The EPSP's were evoked by stimulating both exciter axons. They and the synaptic currents are shown registered on an expanded sweep in A', E', and F' which correspond to A, E, and F above. The fiber was large but was partly hidden by adjacent fibers and its diameter could not be measured accurately.  $R_{eff}$  was about 16 k $\Omega$  initially (A).  $R_{eff}$ , the EPSP, and the synaptic current decreased (B) 1 min after application of GABA ( $8 \times 10^{-6}$  w/v). After 3 min (C)  $R_{eff}$  had returned nearly to the initial value but the EPSP and the synaptic current remained small. Both remained small at 9 min (D) and 13 min (E) while GABA was circulated in the bath.  $R_{eff}$  was essentially back to its initial value, since there was little or no change in this parameter when the GABA was washed out (F). The EPSP and the synaptic current returned to their control value.



evoked by repetitive stimulation of an exciter axon at low frequency. The externally recorded potential (lower trace) shows an early diphasic component which precedes the registration of the postsynaptic current flow and which represents activity in the presynaptic terminals. Both registrations were clearly diminished when GABA was applied so as to depress the EPSP (B), the presynaptic component less than the postsynaptic. Much clearer evidence of inhibition of the presynaptic impulse in an exciter axon by a preceding inhibitory volley has been obtained by Atwood (1967) in another crab, *Pachygrapsus*.

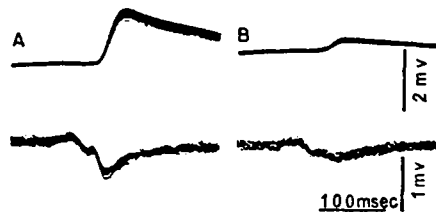


FIGURE 7. Presynaptic action of GABA. Simultaneous registration (repetitive sweeps) of an intracellularly recorded EPSP (top traces) and of the extracellularly recorded potentials (lower traces). A, before and B, after applying GABA to the preparation. The diphasic deflection preceding that caused by the synaptic current is due to the action potential of the nerve terminals. It was reduced somewhat when the EPSP and the synaptic current were reduced by GABA.

#### DISCUSSION

The foregoing data show that GABA does activate the inhibitory neuromuscular synapses of *C. borealis*. However, the inhibitory postsynaptic membrane rapidly becomes desensitized to GABA, since the initial decrease in  $R_{eff}$  is reversed within a few minutes and  $R_{eff}$  returns toward its control value. The sustained inhibitory effect of GABA on excitatory transmission is caused by presynaptic inhibition, GABA reducing the activity of the terminals of the two excitatory axons.

Applications of GABA evoke an electrogenesis of the same sign, magnitude, and reversal potential as that elicited by neural stimulation. In *C. magister*, the inhibitory axons contain about 100 times as much GABA as do the exciter axons (M. G. Sorenson, personal communication) and it is thus likely that GABA is the inhibitory transmitter in crab, as it probably is in other crustacean systems (Kravitz et al., 1963; Otsuka et al., 1966). It is also likely that GABA is the inhibitory transmitter at insect neuromuscular junctions (Usherwood and Grundfest, 1965). The inhibitory synapses in *Limulus* eye are also activated by GABA (Behrens and Wulff, 1969), suggesting that the inhibitory synapses of these diverse groups of arthropods may possess common phar-

macological characteristics. Like the inhibitory synapses of lobster, crayfish, and insect, those of the crab are blocked by picrotoxin.

Several factors probably account for the discrepancies in earlier work on crab muscle fibers. At least some of the work was done at room temperatures, in the range of 20°C (Reuben and Grundfest, unpublished; Aljure et al., 1962). Atwood et al. (1967) reported that the effectiveness of GABA in reducing resistance is decreased with increasing temperature. This effect may be due, at least in part, to the decrease of  $R_{eff}$  that is observed in the muscle fibers on raising the temperature (Fatt and Katz, 1953). In the present work  $R_{eff}$  decreased about 5% when the temperature was raised 1°C, and as shown in Figs. 2-5, when  $R_{eff}$  is low the change induced by GABA is smaller. Furthermore, the change in  $R_{eff}$  induced on activation of the inhibitory synapse by GABA is smaller in fibers of *C. borealis* than it is in muscle fibers of crayfish (Boistel and Fatt, 1958; Girardier et al., 1962; Ozeki et al., 1966; Takeuchi and Takeuchi, 1966 *b*), lobster (Grundfest et al., 1959; Gainer et al., 1967), and grasshopper (Usherwood and Grundfest, 1965). The largest reduction in  $R_{eff}$  observed in the present work was by about 50%. In the other preparations the decrease may be as large as fourfold. Above all, however, the maximum effect of GABA on  $R_{eff}$  is short-lived in *C. borealis*, whereas it is persistent in the other preparations. In the crab  $R_{eff}$  returns rapidly toward its resting value and in the larger fibers  $R_{eff}$  closely approaches the resting value even in the presence of high concentrations of GABA.

The return of  $R_{eff}$  toward its higher resting value indicates that GABA, as well as activating, also desensitizes the inhibitory postsynaptic membrane in crab muscle fibers. To our knowledge, there have been no previous reports of the desensitization of inhibitory membrane by GABA. The inhibitory synaptic membrane remains activated as long as GABA is applied in muscle fibers of lobster (Grundfest et al., 1959), crayfish (Boistel and Fatt, 1958), or insects (Usherwood and Grundfest, 1965). Desensitization to agents related to GABA probably occurs in crustacean stretch receptors, but was not observed for GABA itself (Kuffler and Edwards, 1958; Edwards and Kuffler, 1959).

The persistent depression of EPSP's after the inhibitory synapses had become desensitized indicates that GABA acts upon the presynaptic terminals of the exciter axon as well as upon the inhibitory postsynaptic membrane. Inhibitory effects on presynaptic terminals by GABA have been observed in lobster (Bergmann et al., 1959; Grundfest and Reuben, 1961) and in crayfish (Dudel, 1965; Takeuchi and Takeuchi, 1966 *b*). These effects, like those on the postsynaptic membrane, probably involve Cl activation and are reversed by picrotoxin. Direct evidence of presynaptic inhibition can be provided by recording the changes in the response of the presynaptic terminals (Dudel and Kuffler, 1961; Dudel, 1965). Our own data (Fig. 7) are only indicative of the presynaptic action of GABA, but Atwood (1967) has recorded in *Pachygrapsus*

a clear-cut diminution in the response of the exciter axon when an inhibitory volley precedes the excitatory.

The data of Fig. 5 also provide evidence for a presynaptic inhibitory action of GABA. The conductance increase produced by GABA had fallen from its maximum (50%) to about 20% within 5 min. Inhibition of the EPSP's was still maximal. Picrotoxin eliminated the inhibition as well as the remaining decrease in  $R_{eff}$ . Thus, the elimination by picrotoxin of the inhibition must be due, at least in part, to an effect that is unrelated to the change of conductance of the inhibitory postsynaptic membrane. Reuben and Grundfest (unpublished, cf. Fig. 45 in Grundfest, 1966) found that picrotoxin eliminated inhibition of EPSP's by GABA even when  $R_{eff}$  was not lower than in the resting cell.

The occurrence of presynaptic inhibitory effects of GABA in crab as well as in crayfish and lobster neuromuscular systems leads to the conclusion that the excitatory presynaptic terminals in all three forms possess GABA receptors. Furthermore, these receptors also react to picrotoxin. Thus, the GABA receptors of the presynaptic terminals probably have chemical structures that are similar to those of the postsynaptic GABA receptors. The present data yield an additional finding, that the postsynaptic GABA receptors of crab become desensitized to this agent, while neither those of the presynaptic terminals or of the postsynaptic membrane in crayfish and lobster exhibit this phenomenon. Dudel (1965) has reported another variety of difference between presynaptic and postsynaptic GABA receptors in crayfish. Presynaptic inhibition is also induced by  $\beta$ -guanidino propionic acid, whereas this agent is a competitive inhibitor of GABA at the postsynaptic sites.

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