THE INFLUENCE OF GAMMA-AMINOBUTYRIC ACID ON THE MOTOR RESPONSES OF THE NORMAL AND DENERVATED ADDUCTOR MUSCLE OF THE CRAYFISH

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It has been shown [5, 14, 15], that γ-aminobutyric acid (GABA) inhibits contraction of crayfish muscle. Just like factor J, its action resembles that of the inhibitory nerve of crustacea [7, 13]. This action increases the permeability of the muscular membranes, chiefly for Cl ions [4]. The inhibitory action of GABA has been confirmed by low concentrations of pyrotoxin. Its action was first demonstrated on the stretch receptors [3, 6], and on the crustacean heart [8]. Peripheral inhibition of the neuromuscular junctions in these animals was also blocked by pyrotoxin, which suppresses the action of the inhibitory axon; the action is also blocked by GABA [11, 12, 16, 17, 18], although it is not an inhibitor in the invertebrates in general [13].

In recent years, due to the great interest in γ-aminobutyric acid, valuable results have been obtained which are important for the study of the chemical transmission of nervous impulses. However, the mechanism of this transmission in invertebrates and, in particular, in the crustacea, continues to be neglected. To analyze this mechanism further, we have made a study of the influence of GABA on the motor responses of normal and of denervated crustacean muscle.

METHOD

The experiments were carried out between March and November on two species of crayfish—Astacus Astacus and Astacus Leptodactylus. The adductor muscle was removed from the isolated claw. The contractions of the adductor were recorded on a kymograph. The motor (thick) and inhibitory (thin) nerves were stimulated by square-wave impulses from two electronic stimulators. 0.1 ml GABA diluted by Van Harreveld's solution for crustacea [10] to a concentration of 10^{-5}–10^{-2}M was injected by syringe into the orifice in the fixed dactylopodite digit of the claw.

RESULTS

The influence of GABA on normal motor responses. We found that GABA exerted an inhibitory effect on muscular contraction, as has previously been described. Low concentrations of the GABA (10^{-4} M or less) caused a marked reduction in the excitability of the preparation, and of the amplitude of the muscular contractions, but did not produce a complete block. First the slow tonic response was abolished, and then the rapid reaction also. Figure 1, a shows the contractions of the adductor muscle in response to high- and low-frequency stimulation of the motor nerve. It can be seen that after the injection of GABA, there is a marked reduction in the contractions, the rhythmical activity is suppressed, and what was previously a prepessimal frequency for the muscle of 100 per second becomes pessimal.

GABA stimulates the action of the inhibitory nerve. We have previously demonstrated [1, 2] the two-fold action of the inhibitory nerve on the muscle. In some cases it could be inhibitory, and in others stimulatory. GABA reproduced only the inhibitory, and not the stimulatory effect. If, during a muscular contraction induced by stimulation of the motor nerve, stimulation is applied to the inhibitory nerve, then the muscular contractions either cease, or are decreased in amplitude. The same effect may be obtained without stimulating the inhibitory nerve, by injecting GABA (Fig. 1, b). After the injection, the effect of the inhibitory nerve is more marked (Fig. 1, c). It appears that GABA exerts a stimulating influence on the termination of the inhibitory nerve, facilitating the liberation of the inhibitory mediator. In such a case, after section of the inhibitory nerve and the degeneration of its endings, GABA would not be expected to inhibit muscular contraction.

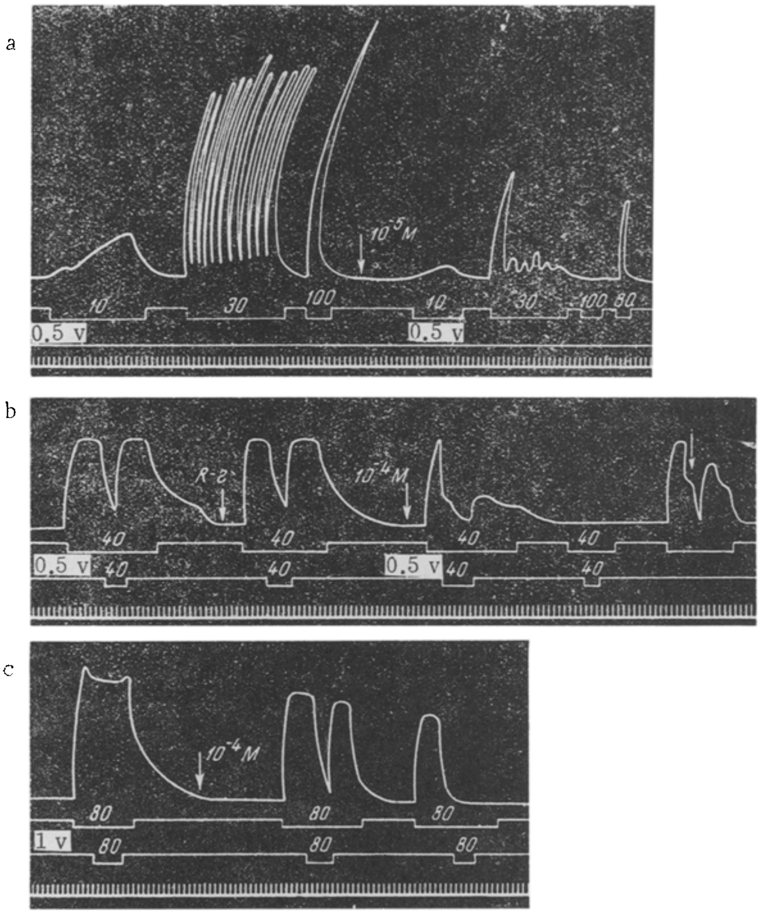

Fig. 1. Effect of GABA on the motor responses of a normal muscle – the adductor muscle of the crayfish claw. Curves, from above downwards: myogram; stimulus to motor nerve; baseline and marker for stimulus of inhibitory nerve; time marker (2 seconds). Figures – frequency of the impulses per second and potential (in volts). Arrows – time of injection of GABA. Explanation in text.

The influence of GABA on the motor responses of denervated muscle. In the intact crayfish, the nerves to the meropodite of the left claw were divided. We carried out three sets of experiments. In the first, we divided the two (thick) motor nerves, and in the second, only the (thin) inhibitory nerves; in the third, we completely denervated the muscle by section of both the inhibitory and motor nerves. The right intact claw was used as control. After denervation, and after 2, 5, 7, 10, 15, 20 and 30 days, we tested the effect of GABA on muscular contraction. The following results were obtained.

After division of the motor nerves, the inhibitory influence of GABA on contraction of denervated muscle was preserved. GABA acted in the same way on the response of the normal control claw.

Figure 2a shows the contraction of the adductor muscle in response to stimulation of the distal end (below the section) of the motor nerve, nine days after denervation. It can be seen that the injection of 10^{-4} M GABA suppresses the motor response of the muscle. Similar results were obtained at various times after denervation.

In highly sensitive preparations with rapid reactions, the inhibitory reaction did not appear until after repeated injections of GABA in high concentrations (10^{-3}M). In all cases, inhibition from GABA was eliminated by washing the preparation with Ringer's solution.

After section of the inhibitory nerve, there was a reduction or a complete loss of sensitivity to GABA. After high concentrations had been injected, there was no change in the reaction of the muscles, and in some cases GABA caused a weak stimulation of muscular contraction (Fig. 2, b). Before the injection, the muscle did not respond to stimuli applied to the motor nerve at a frequency of 180 cps, but after the injection it did. The pessimum frequency of stimulation changed from 180 to 190 cps. The time for complete loss of sensitivity to GABA varied from 3 to 5 days after section of the inhibitory nerve. Twenty or even 30 days after section, the sensitivity to GABA had not recovered. At this time, after the injection of GABA, the motor responses of the control right claw were suppressed (Fig. 2, c) as in a normal one.

Thus, the contraction of the adductor muscle of the claw after section of the inhibitory nerve was not changed by the injection of GABA. However, the sensitivity to GABA was partially preserved for a short time, up to two days after denervation. At this time, 10^{-3} and 10^{-4} M GABA suppressed the slow component of the muscular contraction, but did not influence the rapid component (Fig. 2, d). Similar results were obtained in experiments on the frog (Rana temporaria). In the mixed ileofibularis muscle, after the nerve-muscle preparation had been immersed in 10^{-3} M GABA solution, the slow responses disappeared immediately. In the tonic bundle of this muscle, after injection of the GABA, complete inhibition followed rapidly. In the m. sartorius inhibition developed very slowly. The rhythmical responses of this muscle after the injection of GABA appeared more markedly, while the "set" contraction vanished.

In the last set of experiments, the results of section of both the inhibitory and the motor nerves were the same as when the inhibitory nerve alone had been divided. After the GABA injection the motor responses of the denervated muscle showed no change.

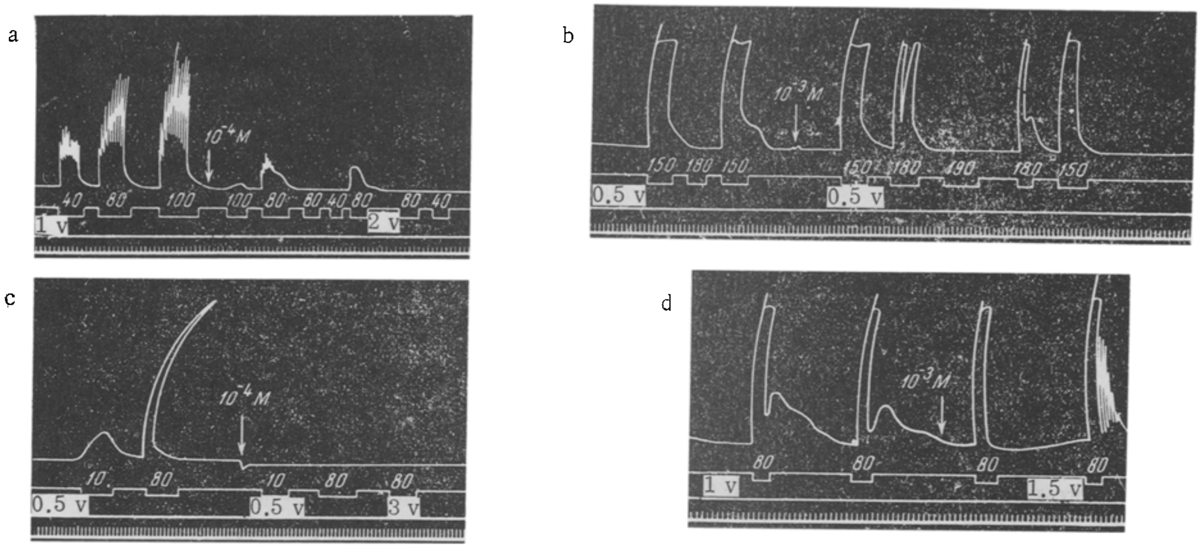

Fig. 2. Influence of GABA on motor responses of denervated adductor muscle of the crayfish: a) 9 days after section of the two motor (thick) nerves; b) 8 days after division of the inhibitory (thin) nerve; c) control right claw; d) 2 days after division of the inhibitory nerve. Remaining designations as in Fig. 1.

Figure 3 shows graphically the different influences of GABA on the contraction of the normal and the denervated adductor muscle. Contractions of the muscle with the divided motor nerve (Fig. 3, III) were inhibited after the injection of GABA, just as in a normal muscle (Fig. 3, II). After complete denervation (Fig. 3,IV), or after section of the inhibitory nerve alone (Fig. 3, V) sensitivity to GABA was lost. On stimulation of the motor nerve, these muscles responded just as did a normal muscle before GABA had been injected (Fig. 3, I). After the injection of GABA, the slow responses of normal and denervated muscles to stimuli applied at a slow rate to the motor nerve disappeared. Curve I, representing the responses of a normal muscle before the injection of GABA, begins on the ordinate, which corresponds to a low frequency as represented on the abscissa. The remaining curves, which illustrate the response of muscle after GABA, begin at some distance from the ordinate, which corresponds to a higher rate of stimulation (60 cps) to the motor nerve.

Our experiments indicate that GABA acts on the endings of the inhibitory nerve and facilitates the liberation of the inhibitory mediator. Possibly the GABA causes a liberation of the inhibitory mediator from the presynaptic nerve endings. The results obtained agree with those recently published by Van der Kloot [12]. If it is supposed [1, 2] that the inhibitory nerve is adrenergic, then GABA should act on the presynaptic membrane of adrenergic endings. In this case, apparently, sympathomimetic substances may also be a component of the inhibitory mediator—factor J [9, 10]—of the crustacea, because, according to our findings [2], their action, like that of the inhibitory nerve, may be suppressed by sympatholytin (Br-dibenamine). However, it is also possible that different nerve fibers in the crustacea are distinct in their chemical nature [9]. Perhaps the inhibitory nerve includes specific inhibitory and nonspecific adrenergic axons. The action of GABA on specific and nonspecific endings of the inhibitory nerve is not known, because there is no direct information indicating the presence of such endings. We may also suppose that in addition to its action on the inhibitory nerve endings, GABA also directly influences the post-synaptic membrane of the slow motor axon.

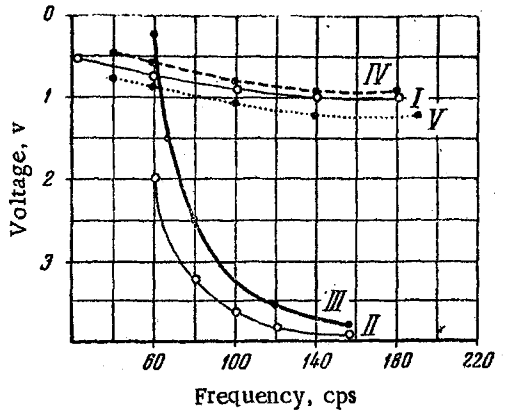

Fig. 3. Effect of GABA on motor responses of normal and denervated adductor muscles of the claw. I) Normal muscle; II) normal muscle after injection of GABA; III) muscle with motor denervation after injection of GABA; IV) muscle with complete denervation after injection of GABA; V) muscle with inhibitory denervation after injection of GABA.

A study of the chemical relationships between the inhibitory and motor innervation of the crustacea will be the subject of future investigations.

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SUMMARY

An investigation was made of the effect produced by gamma-aminobutyric acid (GABA) on the contraction of the normal and denervated adductor muscle of the claw of Astacus Astacus and of Astacus Leptodactylus. GABA was shown to exert an inhibitory effect on normal muscular contraction, a result which agreed with published reports. In simulating the action of the inhibitory nerve, GABA evidently acts on the nerve endings to promote the appearance of the mediator. There was no GABA effect on the muscular contraction after section of the inhibitory nerve alone, or after total denervation. The muscle was sensitive to GABA after division of the motor nerves and retention of the inhibitory innervation. Low GABA concentrations selectively blocked the slow component of this reaction, in both normal and in denervated muscle.

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