

THE ELECTROPHYSIOLOGY AND PHARMACOLOGY OF LOBSTER NEUROMUSCULAR SYNAPSES*

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ABSTRACT

Effects of drugs on resting potential, membrane resistance, and excitatory and inhibitory postsynaptic potentials (e.p.s.p.'s and i.p.s.p.'s) of lobster muscle fibers were studied using intracellular microelectrodes. Acetylcholine, *d*-tubocurarine, strychnine, and other drugs of respectively related actions on vertebrate synapses were without effects even in 1 per cent solutions (10^{-2} *w/v*). Gamma-aminobutyric acid (GABA) acted powerfully and nearly maximally at 10^{-7} to 10^{-6} *w/v*. Membrane resistance fell two- to tenfold, the resting potential usually increasing slightly. This combination of effects, which indicates activation of inhibitory synaptic membrane, was also produced by other short chain ω -amino acids and related compounds that inactivate depolarizing axodendritic synapses of cat. The conductance change, involving increased permeability to Cl^- , by its clamping action on membrane potential shortened as well as decreased individual e.p.s.p.'s.

Picrotoxin in low concentration (*ca.* 10^{-7} *w/v*) and guanidine in higher (*ca.* 10^{-3} *w/v*) specifically inactivate inhibitory synapses. GABA and picrotoxin are competitive antagonists. The longer chain ω -amino acids which inactivate hyperpolarizing axodendritic synapses of cat are without effect on lobster neuromuscular synapse. However, one member of this group, carnitine (β -OH-GABA betaine), activated the excitatory synapses, a decreased membrane resistance being associated with depolarization. The pharmacological properties of lobster neuromuscular synapses and probably also of other crustacean inhibitory synapses appear to stand in a doubly inverted relation to axodendritic synapses of cat.

INTRODUCTION

The inhibitory effect of γ -aminobutyric acid (GABA or C_4) on crayfish stretch receptors (3, 29) and muscle fibers (33) is apparently produced in a

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fundamentally different manner than is its action, and that of many other related substances (37-40), on mammalian cortex. In the latter, the short chain aliphatic ω -amino acids (glycine, C₂; β -alanine, C₃; GABA; δ -aminovaleric acid, C₆) are members of a group of synapse inactivators (16, 17, 20) which selectively block the depolarizing (excitatory) axodendritic synapses of the cat brain. In the bullfrog spinal cord GABA probably has the same action (42). Another group, of which the longer chain ω -amino acids (ϵ -aminocaproic acid, C₆; ω -aminocaprylic acid, C₈; ω -aminononanoic acid, C₉) are examples, are also inactivating drugs, but of the hyperpolarizing (inhibitory) synapses of the dendrites (40). Accordingly, they exhibit many similarities to strychnine, which is a specific inactivator of inhibitory synapses in motoneurons of cat (cf. reference 9) and toad (31). In the crayfish, on the other hand, GABA appears to activate the inhibitory synapses of the stretch receptors (29). This conclusion, based on extracellular recordings, has been confirmed with intracellular measurements in the present work on lobster neuromuscular synapses¹.

The apparently divergent actions of GABA in the vertebrate and invertebrate synapses seemed to merit further investigation as an essay in comparative pharmacology in experiments designed not only to record the possible effects on membrane potentials, but also to measure actions on the membrane conductance produced by a number of drugs. If synapses are activated by a drug, the conductance should increase (17, 20, 21). Activation of excitatory synapses should cause depolarization, but activation of the inhibitory synapses may lead to only small changes in resting potential. In the case of crustacean cells (14, 30) the change might be of either sign depending upon whether the resting potential is more positive or more negative than the "equilibrium potential" of the inhibitory postsynaptic potential (i.p.s.p.). If the latter equals the resting potential, the transducer action (19) of the inhibitory membrane should change the membrane conductance without affecting the membrane potential. On the other hand, inactivator drugs should block postsynaptic potentials (p.s.p.'s) selectively or not (17, 20) without affecting the membrane potential. The resistance of the resting fiber might or might not change when the responsiveness of the synaptic membrane is blocked.

The neuromuscular synapses of the lobster (*Homarus americanus*) were chosen for these experiments because the muscles of the walking legs have large fibers (100 to 300 μ diameter) that can be readily penetrated by two micro-electrodes, as required by the experimental design; they are supplied with inhibitory and excitatory nerve fibers, and the axons of these different functions

¹ This work was carried out during the tenure of Grass Foundation Fellowships at the Marine Biological Laboratory in the summer of 1958 by J. P. Reuben and W. H. Rickles, Jr. It was reported at the Electrobiological Seminars of the Marine Biological Laboratory and has appeared in abstract form (22).

can be separated for individual stimulation. In the study, a large variety of drugs was examined in the attempt to delineate a pattern of comparative pharmacology in relation to that of vertebrate synapses. The effects of ions and of their interactions with drugs were also studied, but these data will be reported elsewhere².

Methods

The extensor (stretcher; *cf.* reference 23) muscle of the propodite of the walking legs was used because it could be exposed without damage on opening the carapace. The leg was removed after pinching it off with a hemostat proximal to the ischiopodite. A minimum amount of blood was lost with this procedure and the lobsters survived well successive amputations of the legs.

The exoskeleton was removed from the lateral and ventral sides of the meropodite. The extensor and flexor muscles were cut away, leaving the nerve bundle intact. The chitin was next removed from the lateral aspect of the carpopodite, care being taken not to injure the membrane which surrounds the extensor muscle. Further dissection was carried out under a binocular microscope, after the leg had been placed in a bathing chamber, fixed to prevent movement, the extensor muscle facing upward and the meropodite perpendicular to the propodite and carpopodite. The level of the muscle was slightly higher than that of the base of the leg.

The nerve trunk was separated into bundles and each was stimulated one at a time until a contraction was obtained in the extensor muscle. Fine platinum wires served as electrodes and as supports to raise the nerves to the air-solution interface for stimulation. The bundle containing the excitatory fiber was further divided until the single intact axon, about 60 to 80 μ in diameter, was obtained. The inhibitory fiber, also a large axon, was then found and separated from smaller fibers. The two pairs of stimulating electrodes were carried on independent manipulators. From time to time the axons were lowered into the bathing solution and with this precaution they survived for many hours. The membrane over the extensor muscle was then removed in order to aid penetration of the muscle fibers by microelectrodes.

Two KCl-filled microelectrodes (34) were inserted into a muscle fiber as routine, one for recording the potential, the other for applying known polarizing currents. The distance between the electrodes was 200 to 400 μ or 15 to 50 per cent of the length constant of the muscle fibers which ranged from 0.8 to 1.7 mm. The current applied, as well as the membrane voltage, was recorded on a dual trace oscillograph. The stimulating, amplifying, and recording equipment was standard for the laboratory (2, 15). In most of the records shown in the figures, the pulses applied to the polarizing microelectrode were 1 sec. in duration. The stimuli to the axons were delivered at different frequencies during a 0.5 sec. interval.

² A publication by Boistel and Fatt (3 *a*) which appeared after the present paper was submitted deals primarily with these ionic aspects. As in the present work, they concluded that increased Cl^- conductance is involved in producing inhibitory activity. While their study of pharmacological properties was not as extensive as ours, these authors also report similar findings regarding the action of GABA.

More than 50 preparations were used for the present experiments which were done at room temperature ranging from about 20–26°C. As will be described elsewhere, the electrical responses of the muscle fibers and their membrane resistance were markedly changed by different ions (*cf.* also Figs. 8 and 11). *Homarus* physiological solution (7) was used in most of the present experiments. The solution level in the bath was adjusted so that about 50 per cent of the extensor muscle was above the fluid surface. Test solutions were applied with a syringe or pipette and the action of most of the drugs was attained very rapidly, usually within 1 to 3 seconds. In many cases drugs were tested on a number of muscle fibers in each experiment. This was possible because the effects of most of the active agents were rapidly reversible. The drugs were added to the muscle in concentrations³ which ranged from 10⁻¹² to 10⁻² w/v. However, in the present work, only the order of the activity of the agents is of interest and quantitative dose-effect data will not be reported.

RESULTS

The Potentials of the Muscle Fiber.—Most of the fibers had resting potentials of 60 to 80 mv., but lower or higher values were occasionally observed. Stimulation of the exciter axon at low frequency evoked summative and facilitating e.p.s.p.'s (Fig. 1 *a*), that were augmented by hyperpolarizing the muscle fiber. Stimulating at frequencies above 50/sec. often produced spike-like responses (13) which represent graded electrically excitable activity (1, 19, 21) similar to that found in insect muscle fibers (5, 6, 32). These responses were associated with strong contractions of the muscle which tended to break the electrodes or damage the muscle fibers. In the experiments reported here, therefore, the excitatory axon was stimulated only at low frequency (5 to 25/sec.). Stimulation of the inhibitory axon at low frequency usually produced very small i.p.s.p.'s (*c*). These could be of either sign, depending on the resting potential (14, 30) and their sign could be changed by polarizing the muscle fiber (*d, e*). For the present experiments, however, the inhibitor axon was stimulated at high frequencies (50 to 200/sec.). This usually produced a considerable potential, in most cases hyperpolarizing. It always became apparently depolarizing and was augmented, by hyperpolarizing the muscle fiber (*b*). The changes in the p.s.p.'s produced by the applied currents indicate that these responses are electrically inexcitable (17). The p.s.p.'s were not evoked by the intracellularly applied depolarizing and hyperpolarizing currents used in the course of the present work.

The inhibitory action of the i.p.s.p. upon the e.p.s.p. is shown in Fig. 2. Stimulation of the inhibitory axon at the resting potential of the muscle fiber produced no potential sign of the inhibitory activity (*D*). However, on hyperpolarizing the muscle fiber a "depolarizing" i.p.s.p. was produced, larger with

³ W/v units represent the ratio of the weight in grams of the compound tested to the volume of solution in cubic centimeters. Accordingly, a 1 per cent solution is 10⁻² w/v.

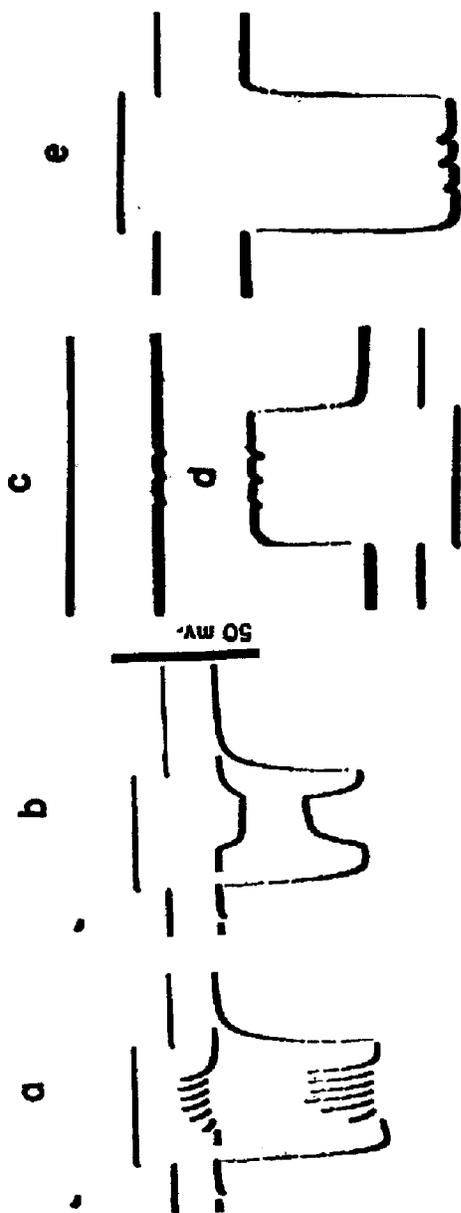


FIG. 1. Excitatory and inhibitory postsynaptic potentials recorded in a lobster muscle fiber. *a*, the e.p.s.p.'s to stimulation of the excitatory axon at 10/sec.; at the resting membrane potential (inset) and on hyperpolarizing the membrane with a current (recorded as an upward deflection on the upper trace) through another intracellular electrode. The current pulse in this and all subsequent figures except Fig. 2 *B*, *C*, lasted 1 sec. Amplifier calibration (50 mV.) is shown by the 100 msec. pulse at the beginning of the voltage trace. *b*, the responses under the same conditions to stimulating the inhibitory axon of the same muscle fiber at 100/sec. *c*, *d*, *e*, another muscle fiber, responding to stimulation of the inhibitory axon at 5/sec. The i.p.s.p.'s (*c*) were unusually large in this fiber. They were augmented by depolarization (*d*) and inverted by hyperpolarization (*e*). The absolute value of the resting potential is not shown in this or other figures.

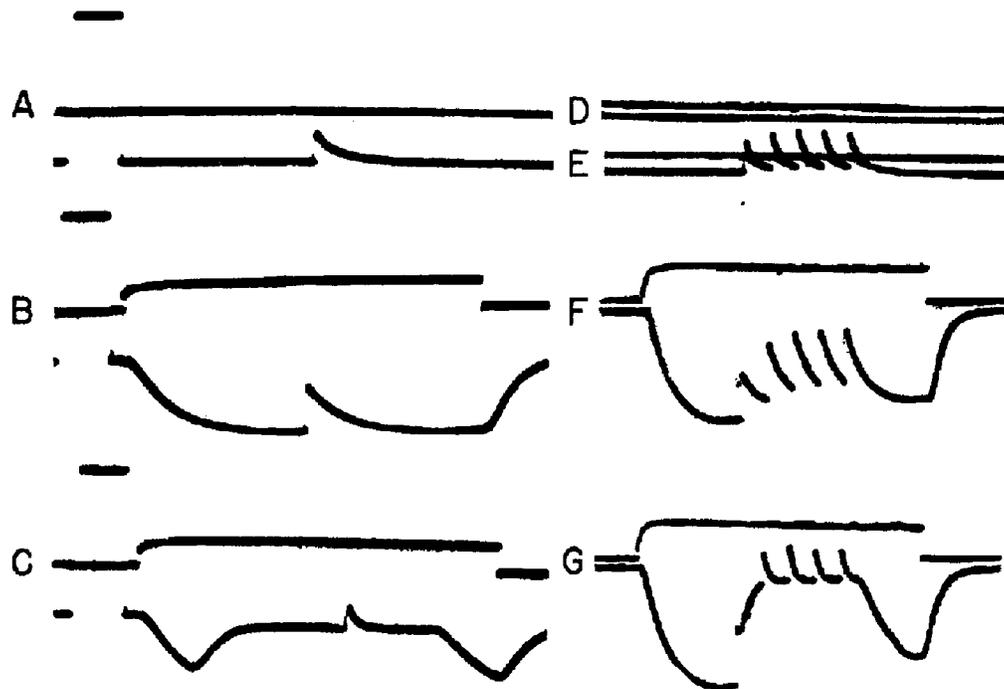


FIG. 2. Effects of i.p.s.p. on e.p.s.p. The preparation had been treated with 5-HT (10^{-3} *w/v*), but this had not yet affected the resting potential which was 75 mv. in the muscle fiber of the records. A single stimulus to the exciter axon evoked a large e.p.s.p. (A). When produced during hyperpolarization of the muscle fiber, the decay of the e.p.s.p. had about the same time constant as did the onset of the hyperpolarizing potential (B). Although at the resting potential stimulation (100/sec.) of the inhibitor axon produced no i.p.s.p.'s (D), during hyperpolarization the activity of the inhibitory synapses was disclosed by a clamping action which drove the membrane potential almost back to the resting level (C, G). The e.p.s.p. evoked during this inhibitory activity was not only smaller, but also briefer (C). When a train of e.p.s.p.'s was produced during the i.p.s.p. the summation that was prominent on stimulating the exciter axon at 10/sec. (E, F) was eliminated (G). The first e.p.s.p. of the train, produced at the onset of the i.p.s.p., was not affected. The amplitudes of the shortened e.p.s.p.'s were about the same size as in the unpolarized muscle fiber (E), but much smaller than in the hyperpolarized (F). Fluctuations in the size of the individual e.p.s.p.'s (G), that were more marked in other records, were probably due to different phase relations of the individual excitatory and inhibitory p.s.p.'s. The 50 mv. calibrating square pulses represent 100 msec. for A-C and 200 msec. in D-G.

larger hyperpolarizations (*C*, *G*), the potential in each case returning almost to the resting level. The e.p.s.p.'s produced during the i.p.s.p. were not only smaller but were also briefer, and because of this, the successive e.p.s.p.'s evoked at 10/sec. (*G*) did not summate.

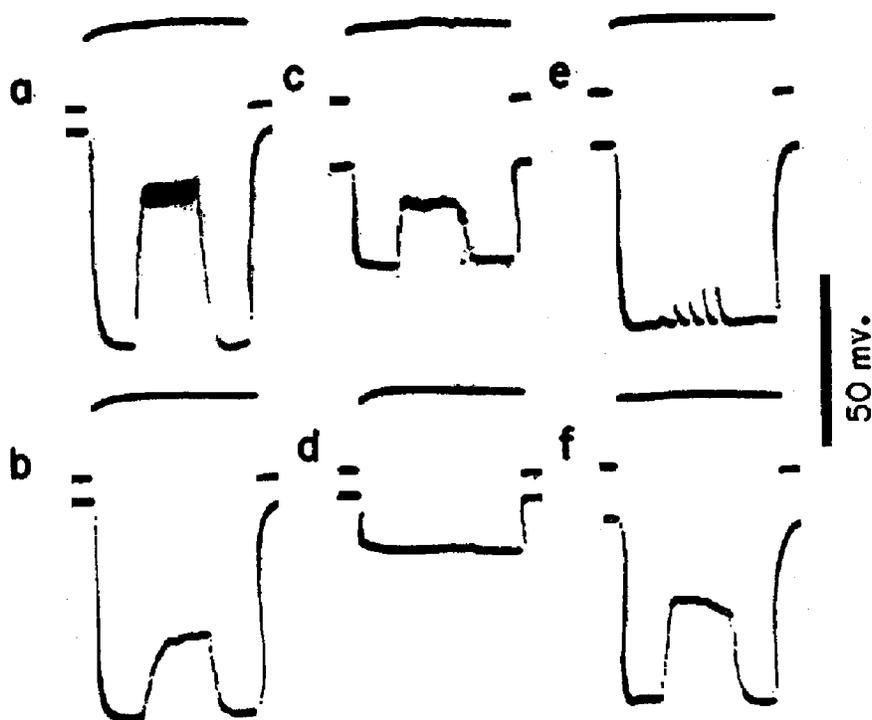


FIG. 3. The effects of GABA (10^{-7} w/v) on the p.s.p.'s of a muscle fiber. The responses are shown during the hyperpolarization of the membrane by an applied current which was the same for all the records, as shown by the current trace. *a*, e.p.s.p.'s at 25/sec.; *b*, i.p.s.p.'s at 100/sec.; *c*, *d*, about 4 and 15 sec. respectively after applying GABA; *e*, *f*, about 1 min. after washing out the GABA. The stimulation frequency for the excitatory axon was decreased to 10/sec. The smaller hyperpolarizations produced by the current in *c* and *d* denote the lowered membrane resistance of the muscle fiber. The effect of the GABA was greater in the later recording (*d*).

The duration of the individual p.s.p.'s depended upon the membrane resistance, the time constant of the falling phase being about the same as that of the rise or fall of the membrane voltage during and after a polarizing pulse (*B*). The decay of the summated p.s.p.'s also took place with the time constant of the membrane, about 80 msec. (*F*). The time constant of the falling phase

of the e.p.s.p.'s decreased to about one-third during the i.p.s.p. (*C, G*) indicating that the membrane resistance had decreased correspondingly.

Effects of GABA.—The maximal effects of GABA were produced by a concentration of about 10^{-4} *w/v*, but minimal actions were observed with application of 10^{-12} *w/v* of the drug. The major portion of the action, however, occurred when solutions at concentrations of 10^{-7} or 10^{-8} *w/v* were used (Fig. 3). Within 2 to 5 seconds after applying the drug the membrane resistance fell (*c, d*) two- to tenfold in different preparations, as denoted by the smaller membrane hyperpolarization produced by a polarizing pulse of constant amplitude. The time constant of the membrane also decreased. Both p.s.p.'s evoked during hyperpolarization of the fiber (*a, b*) were decreased, the i.p.s.p. (*d*) much more markedly than the e.p.s.p. (*c*; cf. Fig. 6). The effects of the drug were reversible (*e, f*). The effect on the resting potential was less marked, but this usually increased by a few millivolts. This change was also rapid, occurring within a few seconds. It was particularly marked when the GABA was applied to muscle fibers depolarized by excess external K^+ . The continued application of GABA, even in concentrations as high as 10^{-2} *w/v* had no obvious effect on the membrane potential within the limit of stability of the recording system, which was approximately ± 0.5 mv./min.

The changes in membrane resistance obtained during the experiment of Fig. 3 are shown graphically in Fig. 4. Other compounds which in cat cortex act as does GABA (40) were also tested in the present work. All these substances (β -alanine, β -OH-GABA, γ -aminocrotonic acid, guanidinoacetic acid) produced the same effects as did GABA. The results indicate that these compounds activate the inhibitory synapses of the lobster muscle fiber, since they all increase membrane conductance. That the effect is on the inhibitory synaptic membrane rather than on the excitatory is indicated by the absence of depolarization.

Effect of Picrotoxin.—This drug, which in the vertebrate activates excitatory synapses (cf. reference 9) and antagonizes the action of GABA (37, 40), also antagonizes the action of the "inhibitory factor" on the stretch receptor (10) and blocks the action of the inhibitory nerve on crayfish muscles (41). The present experiments demonstrate that picrotoxin is a selective inactivator of the inhibitory synapses in lobster muscle fibers (Fig. 5). The e.p.s.p. (*a*), augmented by hyperpolarization (*b*), was not affected by applying picrotoxin 10^{-6} *w/v* (*c*). The i.p.s.p. in this experiment was small (*d*), but was augmented with increasing hyperpolarization (*e, f*). Application of the picrotoxin almost entirely eliminated the i.p.s.p. (*g-i*), even during strong hyperpolarization (*i*). In these records it may be noted that the blockade of the inhibitory synapses occurred with no change in the membrane resistance. This is further shown in Fig. 6, which presents graphically data from the same experiment. The absence of change in membrane conductance (or a slight decrease in the latter) indicates

that the drug inactivated the inhibitory synaptic membrane. This confirms, with data at the cellular level, the conclusion of Robbins and Van der Kloot

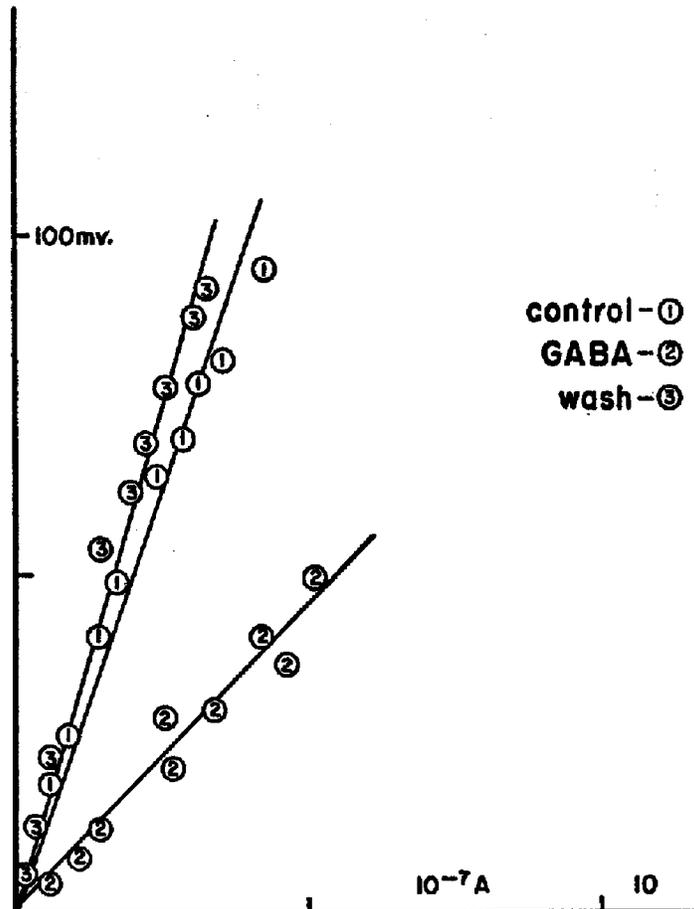


FIG. 4. The reversible change in membrane resistance produced by GABA. Same muscle fiber as in Fig. 3. Decreased resistance (higher conductance) is denoted by the lower slope of the line fitted to the experimental data. In this and the subsequent graphs the ordinate represents the membrane voltage (in millivolts) produced by a hyperpolarizing pulse as given on the abscissa in 10^{-7} A units. The numbers indicate the order in which the procedures were carried out.

(41). Guanidine also blocked the i.p.s.p.'s selectively, but at 10^{-3} w/v only. It was not effective at 10^{-4} w/v.

Interaction of Picrotoxin and GABA.—Drugs of opposite effects confined to a

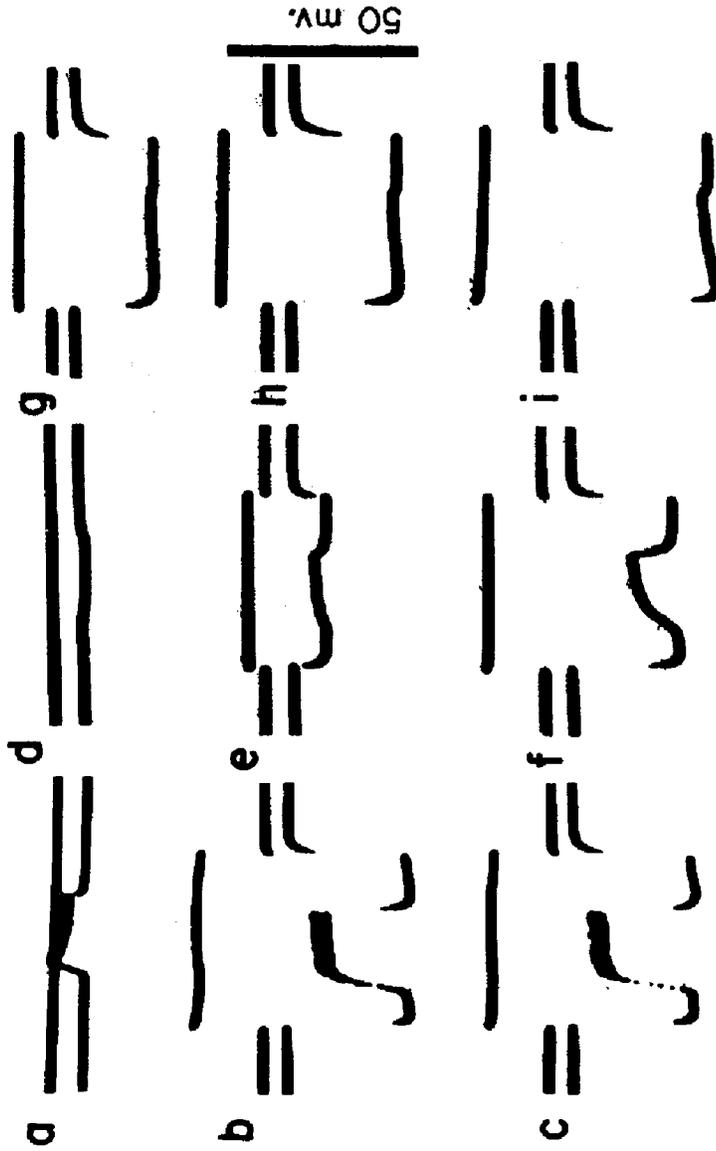


FIG. 5. Selective blockade of i.p.s.p.'s by picrotoxin. *a, b*, e.p.s.p.'s at the resting membrane potential and during hyperpolarization. *c*, the e.p.s.p.'s were not affected by picrotoxin (10^{-6} *w/v*). *d, e, f*, the i.p.s.p.'s of the untreated unpolarized muscle fiber (*d*) and at different values of the membrane potential (*e, f*). *g, h, i*, the i.p.s.p.'s were blocked almost completely by the picrotoxin. The membrane resistance was not affected by the drug.

specific variety of synapse are likely to be competitive antagonists (16, 20). This type of interaction, but with a modification which will be discussed below, was manifested by picrotoxin and GABA (Fig. 7). As in Fig. 5, the e.p.s.p.'s

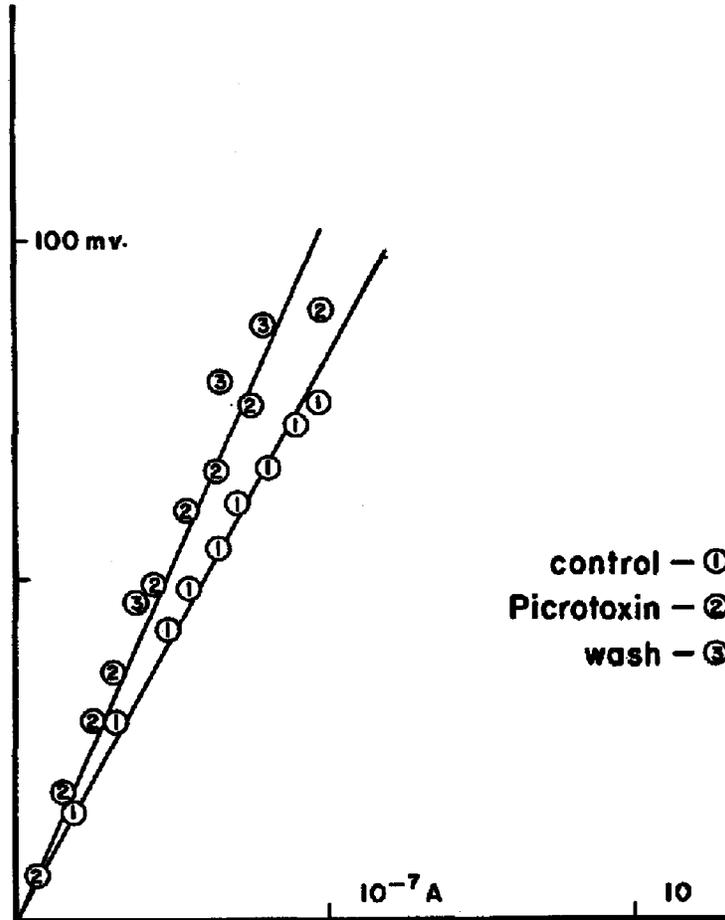


FIG. 6. The effect of picrotoxin on membrane resistance. Same muscle fiber as in Fig. 5. The small increase in membrane resistance on applying the drug was absent in other experiments.

(*a*) were not affected (*b*) by picrotoxin (10^{-6} *w/v*) while the i.p.s.p.'s (*a'*) were markedly diminished (*b'*). Application of 10^{-7} *w/v* GABA immediately decreased the membrane resistance and also the e.p.s.p. (*c*). The i.p.s.p.'s were not visible even when the membrane was hyperpolarized very strongly (*c'*). The competitive effects with respect to the e.p.s.p. and membrane conductance

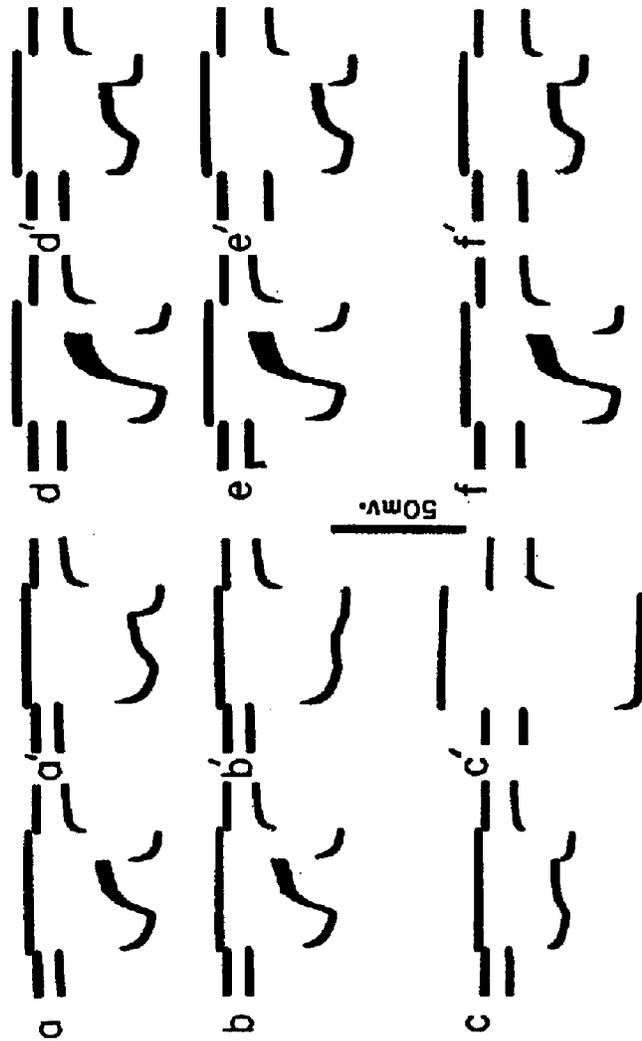


FIG. 7. Competitive interactions of picrotoxin and GABA. *a, a'*, the e.p.s.p.'s and i.p.s.p.'s of a hyperpolarized muscle fiber. *b, b'*, the differential blockade of the latter (*b'*) by picrotoxin (10^{-6} w/v) was not accompanied by a change in membrane resistance. *c*, addition of GABA (10^{-7} w/v) decreased the membrane resistance and the e.p.s.p. *c'*, the i.p.s.p. was not evident even in the strongly hyperpolarized fiber. *d, d'*, *etc.*, the e.p.s.p.'s and membrane resistance during action of picrotoxin (left column) and GABA (right). They are respectively the 3rd, 4th, and 5th in a series of alternating applications, first of picrotoxin (10^{-6} w/v) then of GABA (10^{-7} w/v) to the muscle fiber. Note the marked diminution of the e.p.s.p.'s by GABA.

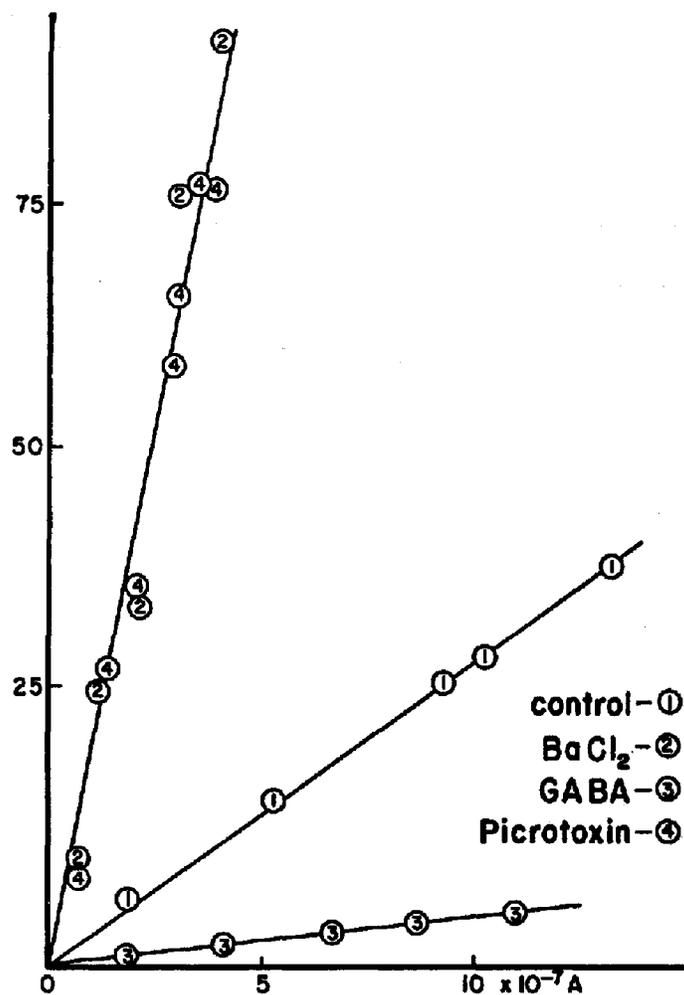


FIG. 8. Interaction of GABA and picrotoxin. GABA was applied in this experiment after the membrane resistance had been increased about eightfold by substituting Ba⁺⁺ for half the Na⁺ in the bathing solution. GABA decreased the membrane resistance about 50-fold. Picrotoxin reversed the action of GABA. The concentrations (10⁻³ w/v) of the respective drugs were 10⁴ and 10³ higher in this experiment than in that of Fig. 7. The qualitative actions of the drugs nevertheless were the same.

were repeatedly reversible from one action to the other by alternately applying picrotoxin (*d, e, f*) and GABA (*d', e', f'*). Another experiment (Fig. 8) shows the full scope of the competitive action on the membrane conductance. The membrane resistance of the muscle fiber was first increased by treating it with

a solution containing BaCl_2 substituting for half the sodium. As in crayfish muscles (12) and in those of *Romalea* (32), the membrane resistance rose about eightfold in this experiment. GABA (10^{-8} w/v) then decreased the resistance

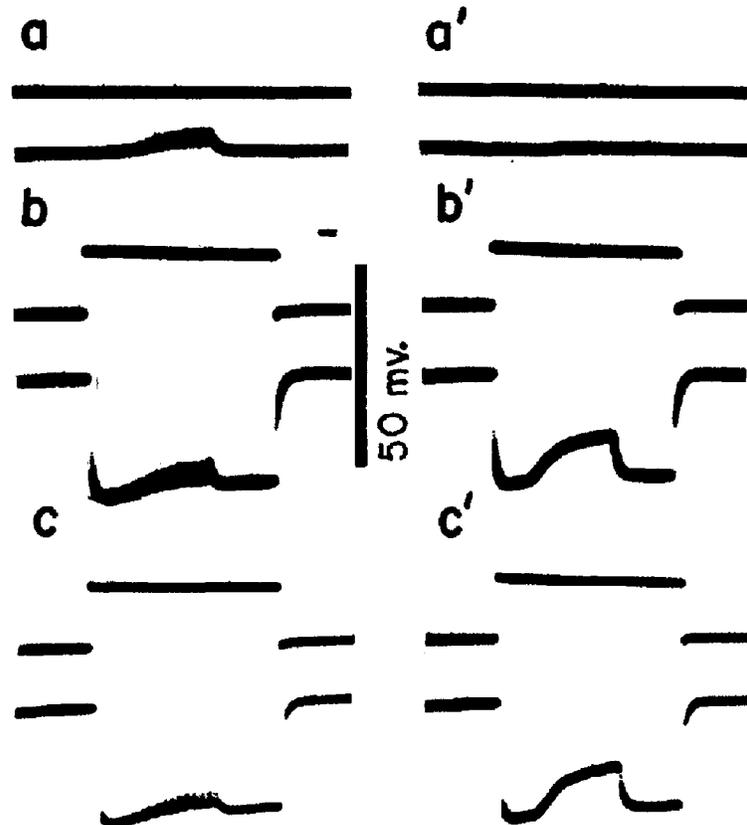


FIG. 9. Lack of effect of strychnine. *Left*, e.p.s.p.'s and *right*, i.p.s.p.'s *a*, *b*, before, and *c*, after applying strychnine (10^{-2} w/v). The change in membrane potential produced by the current pulses was not affected by the strychnine.

almost by 50 times from this elevated value and about sixfold from the initial resistance. Picrotoxin (10^{-3} w/v) restored the previous high resistance.

Effects of Other Synaptic Agents.—A large number of compounds that are synaptic agents in vertebrates had no effect upon the neuromuscular synapses of the lobster. The more significant representatives of these agents which showed no actions, even in concentrations of 10^{-2} w/v, were acetylcholine, the anticholinesterases, eserine and prostigmine, decamethonium and hexametho-

relatively high concentrations (*ca.* 10^{-3} *w/v*). Therefore, the significance of their effects is not as yet clear.

No agent was found that was an inactivator of the excitatory synapses in the same selective way that picrotoxin is for the inhibitory. The dinoflagellate-

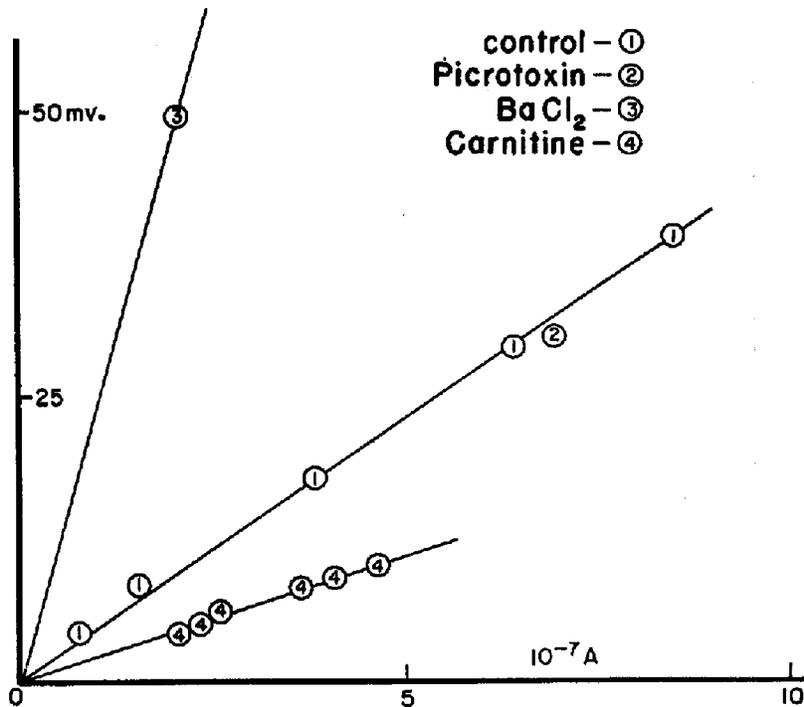


FIG. 11. Effect of carnitine on membrane resistance. The initial membrane resistance was not affected by strong picrotoxin (10^{-2} *w/v*), and the drug did not prevent increase of the membrane resistance by substituting Ba^{++} for half the Na^{+} of the bathing fluid. The decreased membrane resistance caused by carnitine was accompanied by depolarization of the fiber and contraction of the muscle.

produced "mussel venom" (8) is a very powerful blockader of both kinds of p.s.p.'s, effective in concentrations as low as 10^{-10} *w/v*. It blocked the e.p.s.p.'s slightly more rapidly than it did the i.p.s.p.'s, but it is not yet clear whether the action was on the presynaptic fibers or on the synaptic membrane. In the cat brain blockade of directly excited afferent fibers has been found (Purpura and Grundfest, unpublished data).

DISCUSSION

The effects of GABA on the lobster muscle fiber are clearly those of an activator of the inhibitory synaptic membrane (17). Crustacean muscle and stretch

receptors apparently have resting potentials close to the equilibrium potential of these synapses. Accordingly, the i.p.s.p.'s that are produced by activity of the membrane may be small and of either sign (14, 30). Activation of the membrane by GABA causes only similar small changes in the membrane potential. However, the membrane conductance increases and the high degree of this change exerts a "clamping" action, resisting change of the membrane potential by other means. Thus, a given applied current produces less change in membrane voltage in the muscle fibers after GABA than before (Figs. 3 and 7). Likewise, the depolarizing electrogenic activity of the e.p.s.p.'s now produces a smaller IR drop across the membrane, and this accounts in part for the inhibitory action of both GABA and the i.p.s.p.'s. The inhibitory effects seen in Figs. 3 and 7 were exerted in the face of an apparent absence of i.p.s.p.'s, which is explained by the strong activation of the inhibitory synapses by GABA. The large conductance change was added to only slightly by stimulating the inhibitory axon and only a small change of the membrane potential then resulted. For this reason the relatively high concentration of GABA used in the experiment of Fig. 7 (*c'*) did not restore the i.p.s.p.'s blocked by picrotoxin. Hoyle and Wiersma (24) could not obtain an inhibitory action by GABA. In the light of the present data and those of McLennan (33), their results cannot be explained at this time.

The clamping action of the i.p.s.p. on the size of the e.p.s.p. was described by Fatt and Katz (14). They also noted the resultant decrease in the duration of the e.p.s.p., and the absence of summation caused by the shortening of the e.p.s.p.'s. In the case of gradedly responsive muscles, in which the different levels of depolarization may result in graded mechanical responses (1, 21, 23), abolition of summation of the e.p.s.p.'s (Fig. 2 *G*) by the i.p.s.p. would reduce their excitatory effectiveness perhaps even more than would the reduction in the height of the individual e.p.s.p.'s.

Blocking of access of the excitatory transmitter to a common receptor site, as was postulated by Fatt and Katz (13; *cf.* also reference 11) may be a secondary effect (*cf.* reference 16). However, as a primary action, it seems to be ruled out in a number of ways by the present experiments. Transmitters of both varieties are synapse activators whose effects are always to increase the conductance of the synaptic membrane, as is the case also for GABA (Figs. 3 and 4) and carnitine (Fig. 11). They operate on different membrane components as demonstrated by the selective blockade of only the inhibitory synapses by picrotoxin. The different electrogenic actions of GABA and carnitine, the repolarization produced by GABA in KCl-depolarized muscles, and the addition of conductance changes produced independently by GABA and carnitine (unpublished data) are other evidence that the synaptic membranes (or receptors) for excitation and inhibition are independent.

Hoyle and Wiersma (24, 25) suggest the possibility of an inhibitory mechanism independent of the conductance changes of the synaptic transducers and

of the effects of these on the potentials. It seems advisable, however, to explore more fully the implications of the demonstrably strong clamping actions of the i.p.s.p. before seeking other explanations. For example, the clamping action not evidenced by a change in the resting potential could also be exerted on the graded pulsatile activity of the gradedly responsive muscle fibers and this would diminish mechanical responses.

The falling phase of the e.p.s.p. was shortened to at least one-third by the i.p.s.p. (Fig. 2 C) or to about the degree to which the membrane conductance was increased by maximal activation of the inhibitory synapses (Figs. 4 and 8). Fatt and Katz (14) also reported a similar decrease in the time constant, from 27 to 10 msec. The dependence of the duration of the e.p.s.p. on the time constant of the membrane suggests that the transmitter action for the e.p.s.p.'s must be relatively short (*cf.* reference 9). When the membrane resistance was increased (as in Figs. 8 and 11) or decreased, the i.p.s.p.'s, as well as the e.p.s.p.'s were both prolonged or shortened, as will be described elsewhere. Thus, both transmitters probably have short action relative to the time constant of the membrane. The decreased membrane time constant observed during activation of the inhibitory synapses was also obtained with GABA (Figs. 3 and 7) and the individual e.p.s.p.'s were likewise affected. Carnitine also decreased the time constant.

The time constant of the fibers in the stretcher muscle of the lobster is larger than in other crustacean muscle fibers (13). However, the same authors reported (14) that the falling phase of an e.p.s.p. decayed with a time constant which varied from 2 to 3 msec. up to 10 or 20 times higher. The lobster muscle fibers have been found to be very sensitive to ions (unpublished data) and this perhaps also accounts for the variations in e.p.s.p.'s of other crustacean muscles.

The characteristics of the ionic changes involved in the transducer action of the inhibitory membrane are at present under analysis. The repolarization by GABA of a muscle fiber depolarized by excess K^+ in the external medium indicates that another ionic mobility is involved during activity, the "hyperpolarizing" sign of the latter denoting that it is that of Cl^- . However, the participation of enhanced K^+ conductance is not ruled out by these experiments. The data in Figs. 8 to 11 suggest that full activation of the inhibitory synapses is probably more effective in producing a conductance change than is the activation of the excitatory synapses by strong carnitine. However, analysis of the relative conductance changes produced by activity of the "inhibitory" and "excitatory" membranes awaits more extensive experiments.

It is unlikely that the action of GABA is an effect on the electrically excitable membrane of the muscle fiber. A rigorous test would involve determining the ionic mobilities, as in voltage clamp experiments, which has not yet been done. However, other data militate against this possibility. Certainly, the effects of picrotoxin are on the inhibitory synaptic membrane, since this drug does not

affect the membrane resistance nor the e.p.s.p. The competitive antagonism of GABA and picrotoxin therefore indicates that GABA also acts on the inhibitory synaptic membrane. The relative absence of changes in resting potential when the membrane conductance was greatly modified by GABA also is evidence that the electrically excitable membrane is not affected. Further evidence of this type is the repolarization by GABA of fibers depolarized by excess K^+ . As noted above, this bespeaks a specific ionic effect, probably enhanced Cl^- permeability rather than the generally augmented permeability which might be expected if GABA had increased the conductance of the electrically excitable membrane. The depolarization caused by carnitine might be more readily ascribed to a direct action on the electrically excitable membrane. However, drugs in general are much more effective on synaptic membranes, although they may exert secondary actions on the electrically excitable membrane (16, 18, 20).

The data on the neuromuscular synapses of lobster support a theoretical view on the nature of neuropharmacological effects in terms of electrophysiological phenomena (16, 17, 20). Several aspects are particularly well illustrated in the present work. The classification of drugs as activators or inactivators, operating unspecifically, or specifically, on various synaptic membranes is borne out. The activators excite the response of the membrane. The inactivators hinder or block the response independently of whether the excitation derives from neural stimulation or from applied chemical agents. The relatively simple reversible antagonistic actions of GABA and picrotoxin (Figs. 7 and 8) are examples of the uncomplicated interplay that is to be expected if the agents do not produce "side effects" of various kinds which may affect the properties of the synaptic membrane. The lobster synaptic membranes appear to be played upon independently by the agents which act specifically on one or the other variety of synapse. Thus, the effects on the membrane conductance of the activators GABA and carnitine are additive (data to be published). As a result of the uncomplicated actions of the drugs, the effects of very high concentrations were qualitatively similar to those of low concentrations, differing only in degree. Kuffler and Edwards (29) report that high concentrations of GABA depolarized the crayfish stretch receptor, as judged by external potential recordings.

In addition to delineating a method for quantitative determination of pharmacological effects at the cellular level, the work reported here also emphasizes the possibility for classifying synaptic membranes in structural and comparative terms (*cf.* reference 40). The method, study of various strategic molecular configurations which do or do not affect synaptic membrane in specific ways, is essentially that used to deduce structures of other complex surfaces, as in antibody-hapten kinetics (*cf.* reference 35) or protein-dye combination (*cf.* reference 28).

The actions related to these configurations in the case of synaptic drugs are effects on membrane permeability which may be studied in a variety of ways; by changes in potential or resistance, as described in the present work; by the effects of ions on these membrane properties (data to be published); or by other methods, such as the effects on kinetics of ion movements. Since permeability changes are probably important aspects of all cellular activity the information gleaned from the study of synaptic structures and effects of drugs may eventually relate to the general physiology of cellular membranes.

The availability of comparative data on synaptic pharmacology is of primary importance for these purposes, the relative ineffectiveness as well as the effectiveness of drugs contributing information. Thus, the two kinds of synaptic

TABLE I

Doubly Inverted Pharmacological Relations of Lobster Neuromuscular Synapses and of Cat Axodendritic Cortical Synapses, with Respect to Activator and Inactivator Drugs

Action	Animal	Synapses	
		Excitatory	Inhibitory
Activator	Lobster	Carnitine	GABA
	Cat	Picrotoxin	?
Inactivator	Lobster	?	Picrotoxin
	Cat	GABA	Carnitine

membranes of lobster muscle are inert to strychnine and to C_6 and C_8 , while they respond respectively with activation to GABA and carnitine. This indicates that the molecular structures of the synapses, while bearing some similarity to those of vertebrate synapses are not identical with the latter. This is not a surprising conclusion, but the present data add specificity to it. However, at this time, it is not fruitful to attempt detailed deductions regarding comparative structural properties of the membranes on the basis of the specific types of action of only a few drugs.

Nevertheless, some broader conclusions may be indicated. The synaptic membranes in lobster muscle, probably in other crustacean neuromuscular synapses as well, and in the crayfish stretch receptor, stand in a doubly inverted relationship to the axodendritic synapses of cat (Table I). Only one of the drugs which act like strychnine in cat has been found to affect the neuromuscular synapses of the lobster, but in the latter carnitine is an activator of excitatory synapses, while in the cat it inactivates the inhibitory axodendritic synapses. Likewise, GABA and its relatives are activators of inhibitory lobster synapses, whereas in cat they inactivate the excitatory. Picrotoxin like metrazol, activates excitatory synapses in the cat brain. It is an inactivator of the inhibitory neuromuscular synapses of the lobster.

A relationship which may have some evolutionary significance lies in the fact that in the vertebrate the amino acid drugs do not act on skeletal muscle synapses nor on the axosomatic, whereas those that act in the crustaceans affect the peripheral synapses.⁴ Kuffler and Edwards report (29, note added in proof) that Furshpan and Potter found a "large conductance change during GABA action" in crayfish spinal cord synapses. Furthermore, "GABA and the 'inhibitory' synaptic potentials had a similar equilibrium level near the resting potential." Although hyperpolarizing p.s.p.'s were not found in the earlier work which had shown the occurrence of p.s.p.'s in crayfish and earthworm cord giant axons (26, 27; and unpublished data), inhibitory effects were at that time observed. The new data indicate that the crustacean inhibitory synapses of the nerve cord and of the periphery have similar pharmacological properties. There is some indication that the amino acid drugs also act on autonomic synapses in mammals (*cf.* reference 40).

Another correlation may, perhaps, also have evolutionary meaning. The neuromuscular synapses of the grasshopper, *Romalea microptera*, are not affected by the amino acid drugs that have been tested (32; and unpublished data). Since the arthropod classes probably derive from different phyletic stems (43) it may be possible to utilize the potentialities of functional relations of pharmacological data to aid phylogenetic classifications. In this connection it may be noted that the giant fiber synapse of the squid is relatively inert to most of the available pharmacological agents (4), but other invertebrate systems may be exquisitely sensitive to drugs which also act on vertebrate synapses (*cf.* reference 36).

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