

## Effect of Black Widow Spider Venom on the Lobster Neuromuscular Junctions

NOBUFUMI KAWAI, ALEXANDER MAURO, and  
HARRY GRUNDFEST

From the Laboratory of Neurophysiology, Department of Neurology, College of Physicians and Surgeons, Columbia University, New York 10032 and The Rockefeller University, New York 10021. Dr. Kawai's present address is the Metropolitan Institute for Neurosciences, Fuchu-City, Tokyo, Japan.

**ABSTRACT** The effect of black widow spider venom (BWSV) on the junctions of the lobster nerve-muscle preparation was studied by intracellular recordings. After application of BWSV both excitatory and inhibitory postsynaptic potentials (epsp and ipsp) were augmented then suppressed. The frequency of miniature potentials was markedly increased by BWSV. Summated postsynaptic conductance changes appeared to be responsible for the membrane depolarization and the decrease in effective membrane resistance seen in the early stages of the venom action. In the later stages both excitatory and inhibitory "giant miniature potentials" were evoked. No discernible changes were found in the reversal potential of the epsp and ipsp and in the sensitivity of the postsynaptic membrane. The results indicate that BWSV has a presynaptic action at crustacean neuromuscular junctions.

### INTRODUCTION

The electrophysiological effects of black widow spider venom (BWSV) in the arthropod nervous system were first studied on the cockroach nerve cord (Neri et al., 1965) where it was established that BWSV in small concentrations induced an increase in spontaneous firing followed by a total cessation of activity. Shortly thereafter it was observed that the stretch receptor neuron of the crayfish was equally sensitive to the venom with a pattern of behavior mimicking that of the nerve cord (Grasso and Paggi, 1967). Intracellular recording subsequently showed that introduction of the venom into the bathing solution induced, within several minutes, depolarization of the cell body and concomitant firing which ceased when the membrane potential fell to the level sufficient to block repetitive firing (Obara and Mauro, unpublished data). In the 6th abdominal ganglion-cercal nerve preparation of the cockroach, extracellular recordings of the presynaptic and postsynaptic

pathways demonstrated that block in transmission occurred with the application of BWSV without impairment of axonal transmission (D'Ajello et al., 1969). Thus in the arthropods, cockroach and crayfish, these experiments indicated that either presynaptic terminals or soma-dendritic regions of a neuron may be implicated in the action of BWSV.

Intracellular recordings from cell bodies in the cockroach 6th abdominal ganglion have shown depolarization with application of BWSV but unfortunately they have not resolved whether the depolarization is due to the venom acting directly on the postsynaptic membrane as in the crayfish stretch receptor or to release of transmitter which thereby depolarizes the cell body (D'Ajello et al., 1971).

Recently electrophysiological evidence has been obtained in the frog nerve-muscle synapse which indicates clearly that the venom induces a marked increase in the rate of miniature end-plate potentials (Longenecker et al., 1970) and block of transmission. Moreover, ultrastructural studies of the nerve terminal have provided evidence for massive release of transmitter substance as inferred from the disappearance of synaptic vesicles after prolonged action of the venom (Clark et al., 1972). Similar evidence pointing to presynaptic action of the venom has been obtained in the cat soleus and tenuissimus muscles (Okamoto et al., 1971) and in the rat diaphragm (Ceccarelli and Mauro, manuscript in preparation). And recent experiments on the rat superior cervical ganglion indicate that here again the venom exerts its effect at the presynaptic endings (Paggi and Rossi, 1971; Paggi and Toschi, 1972). That the venom does not act exclusively at cholinergic terminals is seen in the very recent findings showing complete release of catecholamine from the nerve terminals in the rat iris (Frontali, 1972).

The present work was undertaken to study the effect of BWSV on the arthropod nerve-muscle preparation which is especially interesting in that it contains both excitatory and inhibitory synapses and, moreover, neither synapse is cholinergic. The crustacean nerve-muscle preparation is ideally suited for such an investigation since the inhibitory and excitatory nerves are readily accessible for independent stimulation.

#### METHODS

Most of the experiments were performed on the stretcher muscle of the walking legs of the lobster (*Homarus americanus*). The excitatory and the inhibitory nerves to the stretcher muscle were isolated for external stimulation as described previously (Grundfest et al., 1959; Motokizawa et al., 1969). The nerve-muscle preparation was immersed in a saline solution containing 468 mM NaCl, 10 mM KCl, 22 mM CaCl<sub>2</sub>, and 8 mM MgCl<sub>2</sub>. The pH was adjusted to  $7.5 \pm 0.5$  with tris(hydroxymethyl)amino-methane (Tris) buffer. Experiments were carried out at room temperature (20°–25°C). Glass microelectrodes with electrical resistance of 3–7 M $\Omega$  were filled with 3 M

KCl for intracellular recording from the muscle fibers and with 2 M K-citrate for applying current. Pairs of fine silver wires served as stimulating electrodes for the axons. The electrophysiological equipment was conventional.

The solution containing black widow spider venom was prepared from four pairs of glands (eight poison glands) which were extracted from thawed cephalothoraces (stored at  $-30^{\circ}\text{C}$ ) and macerated in 1.0 ml of saline. Solutions kept at  $3^{\circ}\text{C}$  were potent for about 2 wk. In most of the experiments 0.05–0.1 ml of venom solution was applied to the muscle surface which was covered with a thin layer of solution (1.0 ml). Doses of venom solution less than 0.02 ml produced no effect. A dose of 0.05 ml appeared to be maximal. The pharmacological characteristics of BWSV are described by Frontali et al. (1972). See also paper in press by Granada et al. (1972).

## RESULTS

### *Effect of BWSV on epsp and ipsp*

Upon application of BWSV the first observable change after a latency of a few minutes was an augmentation of both excitatory and inhibitory post-synaptic potentials (epsp's and ipsp's) accompanied by depolarization of the membrane (Figs. 1, 2). The augmentation of psp's was greatest in the first response so that facilitation of subsequent psp's became less prominent. Thus, as depicted in Fig. 2 B, the ratio of the amplitude of epsp's ( $e_2/e_1$ ) elicited by the second and first stimulus, which is normally about 2, decreased considerably under the action of BWSV. Frequently the amplitude ratio reversed so that  $e_2/e_1$  became less than 1. Decrease in facilitation was also noted in the ipsp's. This was clearly seen when the membrane was depolarized by outward currents to enhance the ipsp (Fig. 3, see responses marked by arrows in second column).

The depolarization induced by BWSV, which reflects summation of miniature potentials as described later, showed a gradual increase and attained an amplitude of nearly 10 mv 5 min after applying venom (Fig. 2, filled

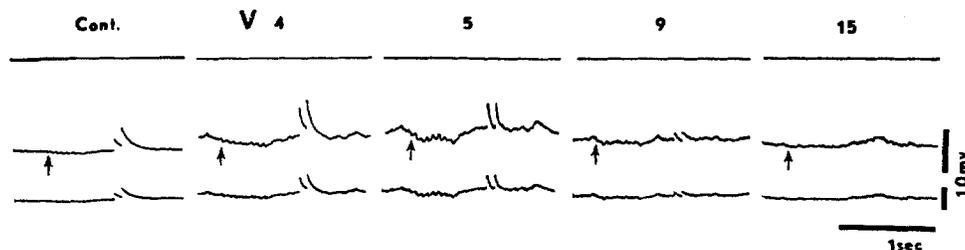


FIGURE 1. Augmentation and suppression of epsp's and ipsp's by BWSV. 25/sec stimulation of the inhibitory axon begins at the arrows and summated ipsp's are followed by two epsp's evoked by stimulating the excitatory axon. The upper trace is zero reference for the lowest trace. The middle trace was recorded at higher amplification. *Cont.*, before applying BWSV;  $V_{4-15}$ , 4–15 min after applying BWSV (0.05 ml of the venom solution). Further description is given in text.

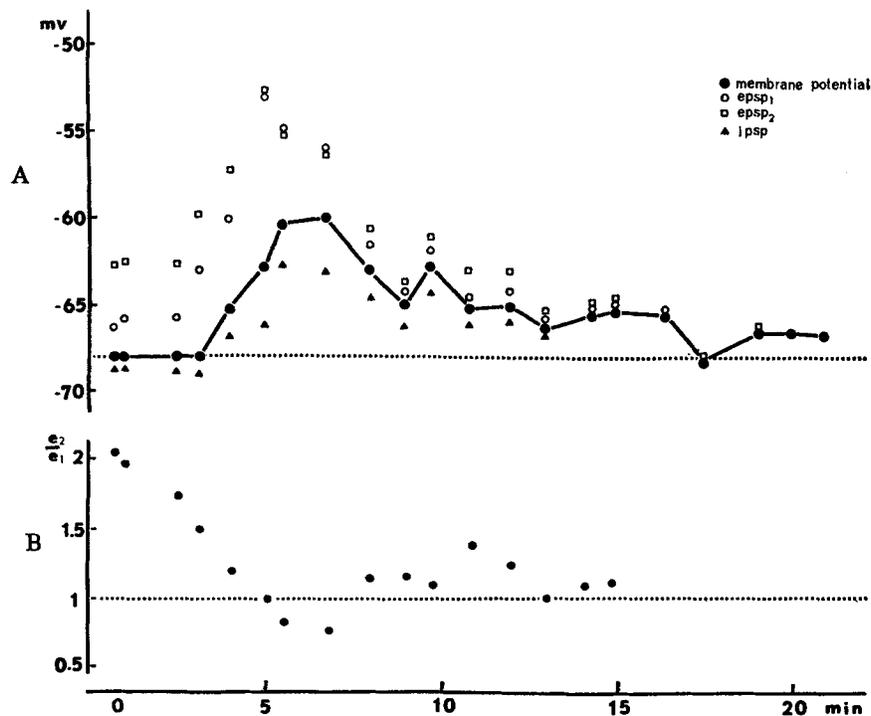


FIGURE 2. Time-course of changes in membrane potentials and psp's taken from the experiment shown in Fig. 1. BWSV was applied at time 0. (A) Ordinate, recorded potential. The solid line shows the membrane potential. Resting potential is given by the dotted line. Open circles and squares indicate peak potentials of the epsp elicited by the first and second stimulus, respectively. Triangles indicate peak potentials of ipsp's. (B) Ordinate, ratio between the second and the first epsp ( $e_2/e_1$ ).

circles). About the same time as the depolarization reached its peak the amplitudes of both psp's started to decline (open circles and squares in Fig. 2). Small psp's occurred at 9 min; 15 min after applying venom the psp's had almost disappeared and only spontaneous potentials were seen (Fig. 1). Thereafter membrane depolarization subsided gradually. In most cases both epsp's and ipsp's disappeared between 10 and 20 min after application of BWSV. We could not see any significant difference in the rate of disappearance between epsp's and ipsp's. In 14 experiments, five preparations showed that ipsp's disappeared earlier than epsp's, in four preparations ipsp's persisted longer, and in five cases both psp's disappeared at about the same time. In any case, the time difference was less than 5 min.

The effects of BWSV were not reversible even after washing the preparation for more than 2 hr. However, when antivenin obtained from horse serum (200–300 units Lyovac; Merck, Sharp, & Dohme, West Point, Pa.) was applied a short time after the effect of BWSV began, the action of venom was

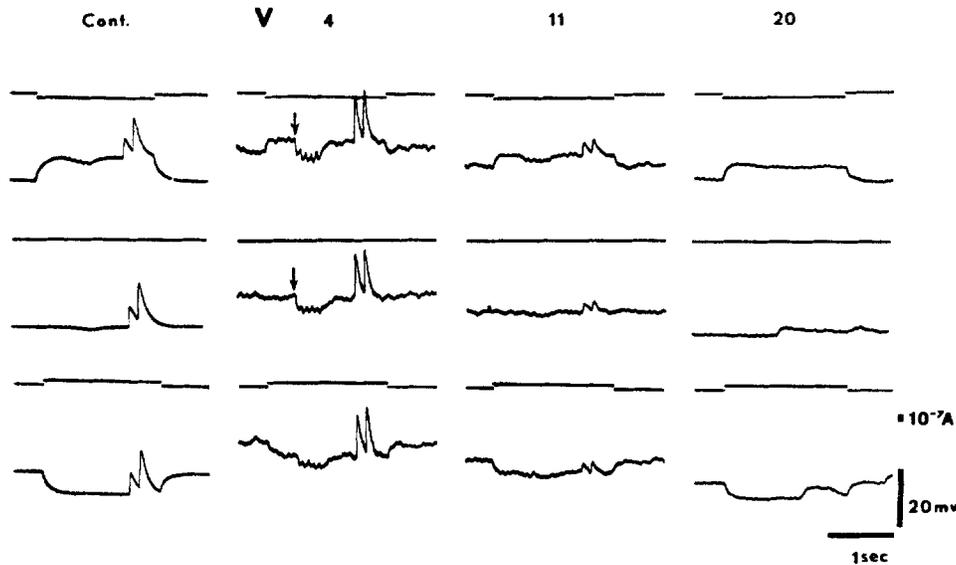


FIGURE 3. Changes in effective resistance and psp's after applying BWSV. Upper traces monitor duration of intracellularly applied currents. During this interval a train of ipsp's and a subsequent train of two epsp's were evoked by repetitive stimuli. Arrows in two of the series indicate the start of trains of stimuli applied to inhibitory axons. Left column: *Cont.*, controls. Other columns designated  $V_{4-20}$  refer to 4, 11, or 20 min after introducing the venom. Upper row, responses during application of outward currents, shown here as downward deflections; middle row, no current; lowest row, inward currents. Further description is given in text.

blocked; that is, psp's persisted and spontaneous potentials were not prominent. The successful block of the presynaptic action of black widow venom by the antivenin is consistent with the results obtained in the frog nerve terminal (Longenecker et al., 1970).

#### *Effect of BWSV on Effective Resistance and Reversal Potential of psp's*

A shortening of the falling phase of individual epsp's under the action of venom (Fig. 1) suggests a decrease in effective resistance. Results shown in Fig. 3 verified this expectation. Effective resistance calculated by applying small hyperpolarizing currents was 88 kohm in the control saline. After addition of venom, the resistance fell to 58 kohm at 4 min and was 64 kohm at 11 min. At 20 min after the addition of venom, when psp's had disappeared and membrane depolarization had subsided, the resistance returned to 72 kohm. Data shown in Fig. 4 are from an experiment in which current-voltage ( $I-V$ ) characteristics were studied to obtain the reversal potentials of the psp's. In control saline (Fig. 4 A) the estimated values of the reversal potential for the ipsp and the epsp (Grundfest, 1961; Grundfest and Reuben,

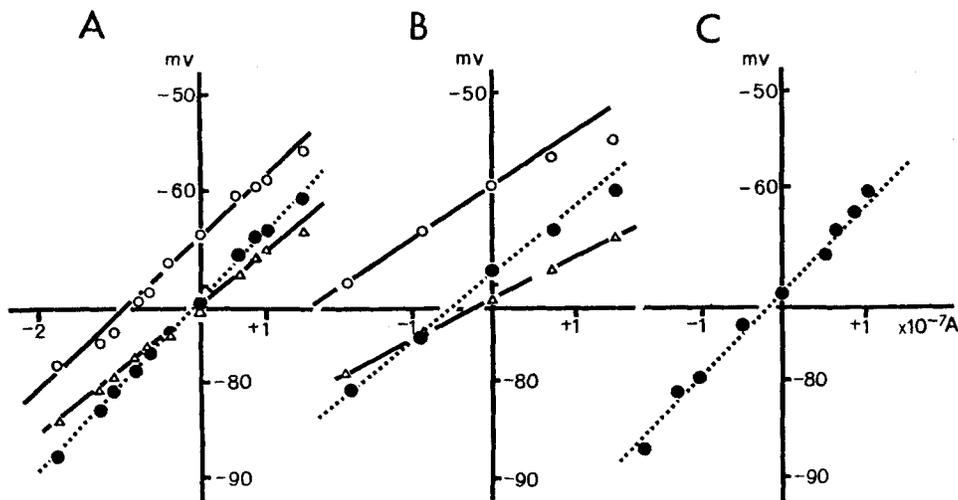


FIGURE 4.  $I$ - $V$  characteristics and determination of reversal potentials of epsp and ipsp under the action of BWSV. Ordinate: recorded potential. In each graph the membrane potential (filled circles), the peak of epsp (open circles), and the peak of ipsp (triangles) is plotted against applied current (abscissa). (A) Control  $I$ - $V$  relations before application of the venom. The current axis intersects the potential axis at  $-72$  mv to indicate resting membrane potential. The membrane potential line and ipsp line cross at the ipsp reversal potential ( $-76$  mv). The membrane potential line and epsp line cross at  $-20$  mv (not shown in graph), the epsp reversal potential. Effective resistance of the resting fiber is  $82$  k $\Omega$ . (B)  $I$ - $V$  relations obtained between 2.5 and 3 min after application of venom. The resting resistance dropped to  $64$  k $\Omega$ . Reversal potentials determined as in (A) are  $-75$  mv for ipsp and  $-16$  mv for epsp. (C) 30 min after introducing venom. psp's are abolished. Resistance increased to  $78$  k $\Omega$ .

1961; Motokizawa et al., 1969) were  $-76$  and  $-20$  mv, respectively. As noted above, an unsteady depolarization developed after venom was applied, making it difficult to obtain accurate  $I$ - $V$  relations. Therefore, for the data given in Fig. 4 B measurements were made in the early stages of venom action (2.5–3 min) when depolarization was not fully developed and membrane potentials were relatively stable. Under this condition, despite the decrease in the effective resistance approximate values of the reversal potentials were  $-75$  mv for the ipsp and  $-16$  mv for the epsp, showing that BWSV made little or no change in the electromotive force (emf) of the synaptic battery.

#### *Miniature Potentials*

An application of BWSV produces an enormous discharge of miniature end-plate potentials in frog neuromuscular junctions (Longenecker et al., 1970) and also in mammalian preparations (Okamoto et al., 1971; Ceccarelli and Mauro, manuscript in preparation). The present experiments proved

that BWSV also induces a huge increase in the number of miniature potentials in crustacean muscles. As shown in Fig. 5, the frequency of miniature potentials, which was about 0.5/sec in the control, rose progressively after application of venom and reached a peak of more than 200/sec within 10

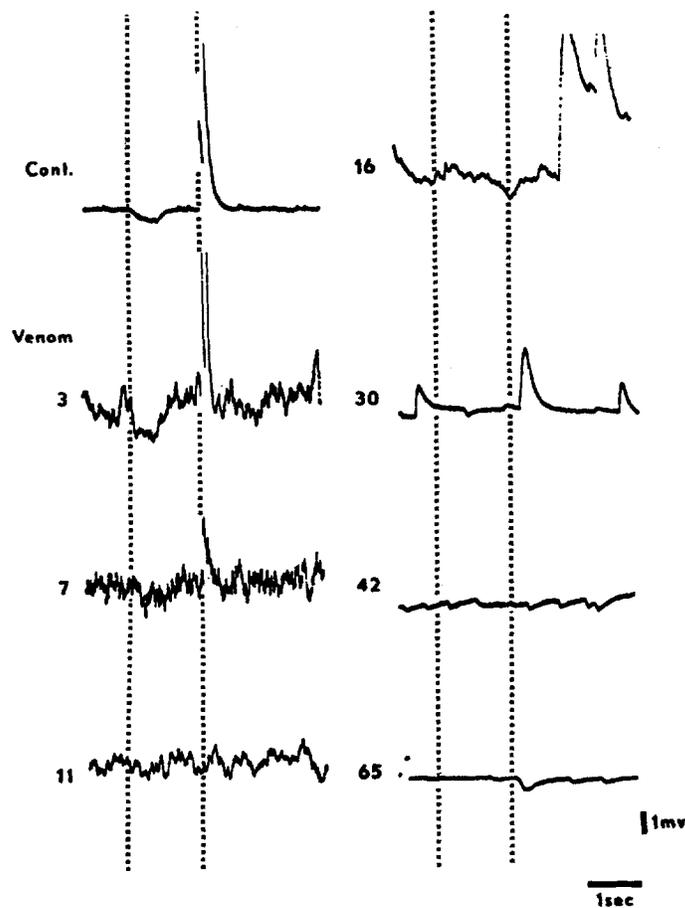


FIGURE 5. Effects of BWSV on spontaneous miniature potentials. In each record repetitive stimuli to the inhibitory axon and two stimuli to the excitatory nerve were applied. The dotted lines indicate start of stimulation of each nerve. *Cont.*, control record before applying the venom. Summated ipsp's and subsequent two epsp's and miniature activity are seen. 3 min after applying venom, both ipsp's and epsp's were augmented. The peaks of epsp's are off the scale and not registered. 7 min after applying venom the frequency of miniature potentials greatly increased while psp's decreased. Decrease in facilitation of epsp's is marked. 11 min later psp's are small or absent. Frequency of miniature potentials decreased. 16 and 30 min after applying venom, high frequency miniature activity subsided and large prolonged giant miniature potentials appeared. 42 and 65 min after venom, inhibitory giant miniature potentials are dominant. Further description is given in text.

min. The peak level lasted for a few minutes whereupon the frequency gradually declined. Owing to the extremely high frequency of the discharges the amplitude of miniature potentials could not be determined exactly. However, the average amplitude of miniature potentials appeared unaffected by venom provided it was measured at the very onset of venom action before the membrane had depolarized markedly and before the accompanying decrease in input resistance had appeared. After the frequency of miniature potentials began to decline, large and prolonged spontaneous potentials were invariably seen superimposed on small miniature activity (Fig. 5, 16 and 30 min after venom). The amplitude of these "giant miniature potentials" was sometimes greater than 10 mv and they lasted for several seconds. 30 min after venom application, high frequency miniature potentials almost vanished and only giant miniature potentials appeared sporadically. Spontaneous inhibitory giant miniature potentials were frequently seen during late venom action (Fig. 5, 42 and 65 min). It is not certain whether these ipsp's appeared exclusively in the late stages of the venom action or were already present earlier but not discernible due to the relatively large magnitude of the excitatory spontaneous potentials. These inhibitory giant miniature potentials are not observed in a preparation treated with picrotoxin (Fig. 6), whereas excitatory giant miniature potentials persist after the addition of tetrodotoxin (Fig. 6, 36 min). It was shown in other experiments that tetrodotoxin is also ineffective for inhibitory giant miniature potentials.

#### *Effect of BWSV in Ca-Free Solutions*

In vertebrate neuromuscular preparations calcium is considered to be essential for transmitter action (Katz and Miledi, 1965). The dependence of transmission on external calcium is also found in crustacean neuromuscular synapses (Reuben and Gainer, 1962; Otsuka et al., 1966; Bracho and Orkand, 1970; April and Reuben, 1971). In order to test the effect of BWSV in the absence of calcium in the external solution, the preparation was soaked in calcium-free saline containing 10 mM ethylene glycol bis( $\beta$ -aminoethyl ether)*N,N,N',N'*-tetraacetic acid (EGTA) and 20 mM  $MgCl_2$  for 15 min (Fig. 7). Epsp's were abolished and miniature potentials were lost in base-line noise. This was probably due to decrease in input resistance. When added to this solution BWSV caused bursts of miniature potentials and later giant miniature potentials of both excitatory and inhibitory types appeared as in normal saline. The increase in miniature synaptic potentials induced by depolarization of the nerve terminals is dependent upon the presence of Ca and is blocked by high concentrations of Mg (Liley, 1956). It may be concluded therefore that, as in the frog (Longenecker et al., 1970), the effect of venom is not due to the depolarization of the nerve terminals per se.

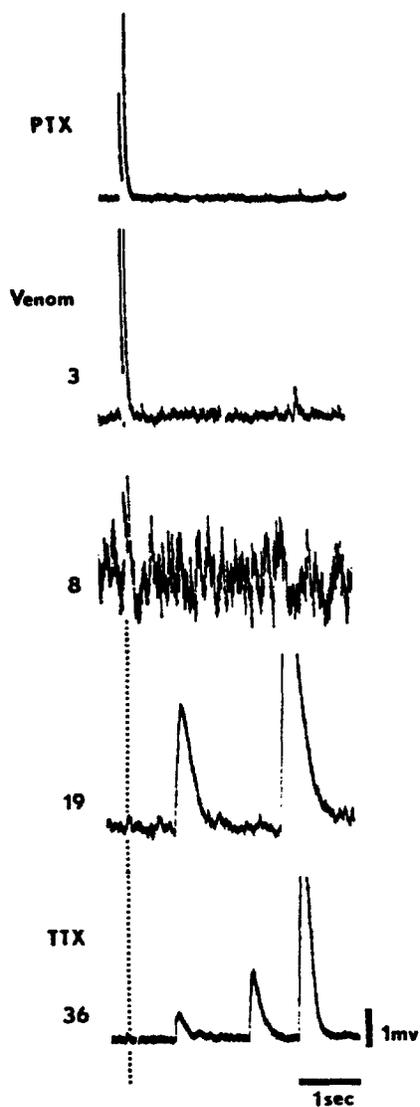


FIGURE 6. BWSV action on picrotoxin (PTX)-treated preparation. Top record was obtained from the muscle 20 min after addition of picrotoxin ( $2 \times 10^{-5}$  g/ml). No ipsp's are seen in this and all other records. The amplitude of evoked epsp's decreased greatly in the record made at 8 min while the spontaneous miniature activity showed a marked increase. At 19 min giant miniature potentials appeared while evoked epsp's were abolished. The bottom record was made 5 min after an addition of tetrodotoxin (TTX) ( $1 \times 10^{-5}$  g/ml). The dotted line indicates the start of the excitatory nerve stimulation.

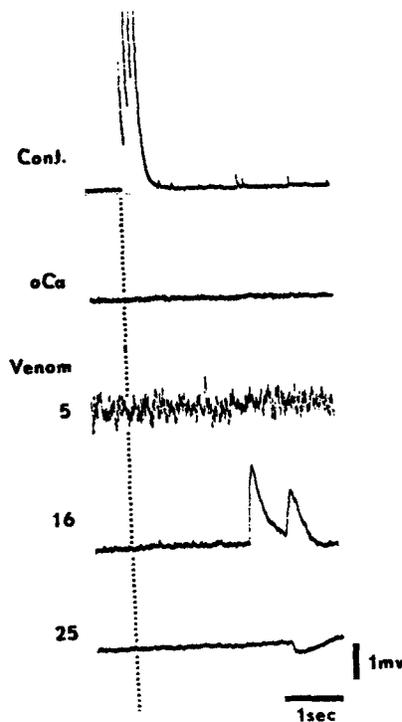


FIGURE 7 Effect of BWSV in calcium-free solution. Top record shows train of four epsp's and miniature potentials in normal solution. Resting potential was  $-70$  mv. The second record was taken at 15 min after soaking in Ca-free solution. Resting potential decreased to  $-64$  mv. Stimuli applied to the excitatory nerve (dotted line) failed to evoke epsp's but BWSV induced miniature activity and both hyperpolarizing and depolarizing giant miniature potentials.

#### *Effect of BWSV on Cesium-Treated Muscles*

After soaking in saline in which cesium was substituted for potassium, both the epsp's and ipsp's evoked in lobster muscle are markedly augmented (Grundfest and Reuben, 1961). This augmentation is due to a presynaptic action of Cs (Gainer et al., 1967). In Fig. 8 the preparation soaked 5 hr in saline containing 10 mM Cs (replacing K) produced large single ipsp's and epsp's. After addition of BWSV, both the ipsp's and epsp's were initially enhanced but soon diminished. At 7 min after the venom was applied only very small psp's can be seen. Thus, blockade of transmission appeared to take place more rapidly in Cs-containing saline than in the normal medium. In three experiments with Cs-treated muscle the mean time of disappearance of epsp's was  $8.7 \pm 0.9$  min, whereas in normal saline it was  $16.8 \pm 2.5$  min (in 28 preparations). The action of BWSV on spontaneous miniature potentials appeared to be unaffected by Cs treatment. This is in accord with

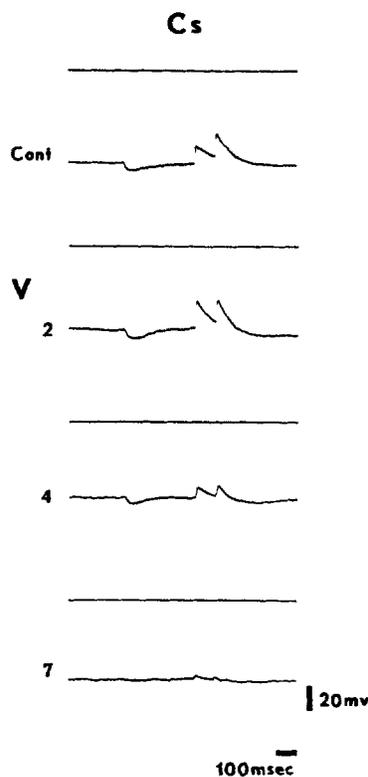


FIGURE 8. Effect of BWSV in cesium-treated preparation. Records are taken from muscle soaked 5 hr in the saline containing 10 mM CsCl substituted for KCl. Note the large single ipsp and two esp's in the control record and the earlier disappearance of psp's after applying venom. In each set of records, the upper trace was set at  $-18$  mv.

the findings that Cs does not affect either the amplitude or the frequency of the miniature potentials (Gainer et al., 1967).

#### *Sensitivity of Postsynaptic Membrane*

The above results suggest that, under the action of BWSV, the initial augmentation and ensuing suppression of psp's are not due to the change in the characteristics of the postsynaptic membrane. This was confirmed for the inhibitory component by studying the sensitivity of the postsynaptic membrane to  $\gamma$ -aminobutyric acid (GABA) which is known as a specific activator of inhibitory membranes in lobster (Grundfest et al., 1959). As shown in Fig. 9, GABA activated the inhibitory subsynaptic membrane and reduced effective resistance from a control value of 140 to 45 k $\Omega$ . The resting potential shifted from  $-76$  to  $-80$  mv. After washing, the resting potential as well as resistance returned to the original level. At 60 min after application of BWSV,

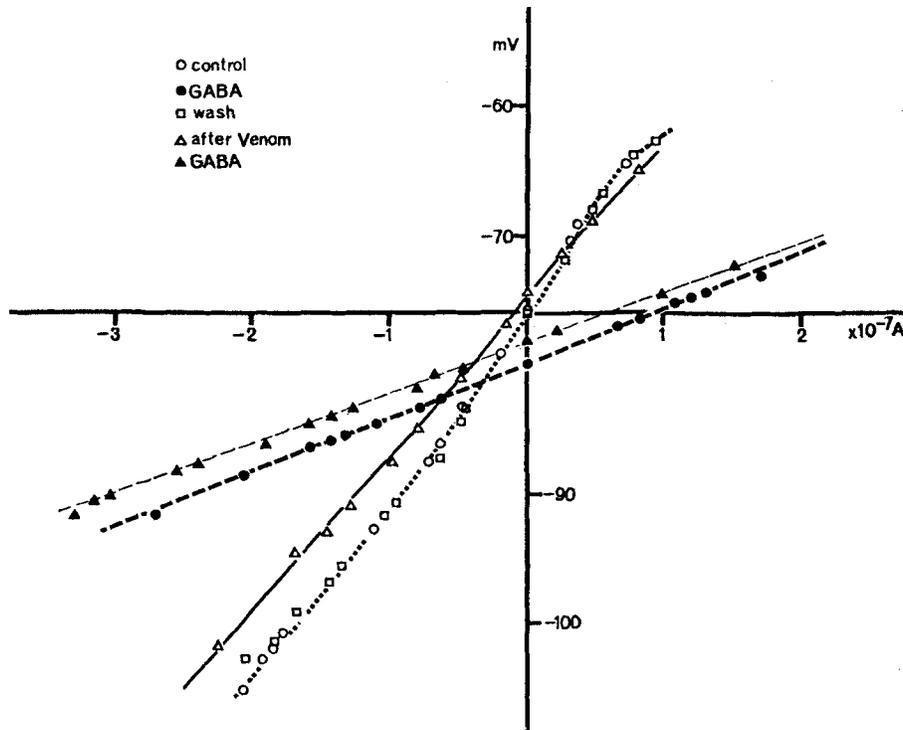


FIGURE 9. Effect of GABA as a test of the sensitivity change of the postsynaptic membrane after BWSV.  $I$ - $V$  characteristics obtained from control solution (open circles) show input resistance as  $140\text{ k}\Omega$ . Addition of GABA ( $1 \times 10^{-8}\text{ M}$ ) hyperpolarized the membrane from  $-76$  to  $-80\text{ mV}$  and the resistance decreased to  $45\text{ k}\Omega$  (filled circles). At 60 min after application of BWSV (open triangles) potential and resistance are  $-74\text{ mV}$  and  $130\text{ k}\Omega$ , respectively. GABA caused membrane hyperpolarization to  $-78\text{ mV}$  and the resistance decreased to  $45\text{ k}\Omega$  (filled triangles).

when sporadic spontaneous miniature potentials were present, addition of GABA gave almost the same change in resistance.

#### *Other Species*

A number of experiments were done using nerve-muscle preparations of crayfish claw opener muscle and also crab closer muscle. The results obtained were essentially the same as in the lobster preparation.

#### DISCUSSION

The present data indicate that BWSV initially caused an augmentation of both epsp's and ipsp's of crustacean muscle fibers accompanied by an increase in the rate of miniature potentials. Later both psp's were blocked. These data, along with the recent report by Frontali (1972) that BWSV

induced virtually complete release of the catecholamine content of the nerve terminals in the rat iris, indicate clearly that the venom can act at noncholinergic as well as at cholinergic synapses. The avalanche of miniature potentials and eventual block of neuromuscular transmission are likely to be an action of BWSV common to all nerve-muscle preparations. In this respect, it is of interest that recently Piek et al. (1971) reported that wasp venom blocked both excitatory and inhibitory neuromuscular transmission in insects and caused a decrease in miniature potentials without initial enhancement.

As in other nerve-muscle preparations studied, the action of BWSV on the lobster neuromuscular junction appears to be solely presynaptic. The recorded changes in membrane potential upon application of the venom do not implicate direct action of the venom on the postsynaptic membrane for several reasons. Firstly, decrease in effective resistance with membrane depolarization can be ascribed to the summation of conductance changes induced by the action of the neurotransmitter on the postsynaptic membrane. It is true that significant membrane depolarization was not observed in the vertebrate neuromuscular preparations (Longenecker et al., 1970; Okamoto et al., 1971). However, this is not in contradiction with the present results since in crustacean muscle the motor innervation is widely distributed over the muscle fiber surface (Wiersma, 1941) in contrast to the mono- or bi-terminal innervation in vertebrate muscle. Therefore, if BWSV acts on each nerve terminal it will cause transmitter release over the whole length of the muscle fiber and the resulting conductance change in postsynaptic regions will then produce concomitantly a decrease in membrane potential and effective membrane resistance. Secondly, neither the reversal potential of the e<sub>psp</sub> nor the reversal potential of the i<sub>psp</sub> is affected by the venom (Fig. 4). Furthermore, the sensitivity of either postsynaptic membrane is not changed by the venom as was shown by comparing two postsynaptic properties before and after the application of venom, namely, the amplitude of the response of the inhibitory postsynaptic membrane to GABA (Fig. 9) and the amplitude of the excitatory miniature potentials.

The progressive diminution of facilitation in the early stage of venom action that is indicated by the decrease in the ratio  $e_2/e_1$  (Fig. 2 B) occurred while both p<sub>sp</sub>'s were augmented. Then, the first e<sub>psp</sub> of Fig. 2 A increased from about 2 to about 8 mv, and the second e<sub>psp</sub> increased from about 5 to 8 mv (Fig. 2 A). Since the first nerve impulse must have released more transmitter in the venom-treated preparation, less transmitter should have been available for the secretory activity induced by the second response of the nerve. The decrease in facilitation suggests that the amount of transmitter released is to some degree dependent upon the amount available.

The decrease in amplitude of the individual responses during the later

stages may be due either to depletion of transmitter in the terminal or to impairment of conduction down to the nerve terminal as a consequence of depolarization induced by the action of the venom which presumably occurs as has been clearly seen in the frog terminal (Longenecker et al., 1970). The inversion of the amplitude ratio  $e_2/e_1$  to values less than unity, occurring at a much later stage, has been observed in many other preparations and is usually interpreted as "fatigue" of the phasic responses of the nerve terminal.

Large and prolonged giant miniature potentials usually appeared at about the same time as the evoked psp's disappeared, and they persisted thereafter for as long as 1–2 hr. Similar spontaneous potentials were observed in serotonin and in  $\text{NH}_4\text{Cl}$ -treated muscle (Grundfest and Reuben, 1961; April and Reuben, 1971) and in BWSV-treated frog muscle (Hurlbut, Longenecker, and Mauro, unpublished data). Liley (1957) also reported giant potentials in the rat neuromuscular junction and suggested that these potential are produced by multiterminal release of transmitter. The giant potentials reported by Liley, however, were a few millivolts in amplitude and about 10 msec in duration and occurred with the normal saline bathing the preparation. They occurred along with the relatively smaller miniature potentials. The giant miniature potentials of the present work developed after the major miniature activity had subsided.

The discharge evoked by BWSV of both small and giant miniature potentials persisted in the presence of tetrodotoxin (TTX) (Fig. 6). This behavior is consistent with the venom acting presynaptically to cause release of transmitter. Spontaneous miniature activity also persists in lobster muscle treated with saxitoxin (Reuben and Grundfest, 1960) and in crayfish (Ozeki et al., 1966) and vertebrate muscle (Elmqvist and Feldman, 1965) treated with TTX. Moreover, TTX does not abolish psp's evoked by local stimulation of the axon terminals (Katz and Miledi, 1967; Ozeki et al., 1966).

Work in the Laboratory of Neurophysiology is supported in part by grants from the Muscular Dystrophy Associations of America, by Public Health Service Research Grants (NB 03728 and Training Grant NB 05328) from the National Institute of Neurological Diseases and Stroke, and by a grant from the National Science Foundation (GB-6988X).

We wish to thank Dr. M. Sorenson for helpful discussion and Dr. S. P. April for assistance in the experiments on the crayfish preparation.

We are deeply grateful to Dr. Nora Frontali for her assistance in obtaining venom.

*Received for publication 1 May 1972.*

#### REFERENCES

- APRIL, S. P., and J. P. REUBEN. 1971. The effect of divalent cations upon the evoked and spontaneous release of excitatory transmitter at the crustacean neuromuscular junction. *Fed. Proc.* **30**:557.
- BRACHO, H., and R. K. ORKAND. 1970. Effect of calcium on excitatory neuromuscular transmission in the crayfish. *J. Physiol. (Lond)*. **206**:61.
- CLARK, A. W., W. P. HURLBUT, and A. MAURO. 1972. Changes in the fine structure of the neuromuscular junction of the frog caused by BWSV. *J. Cell. Biol.* **52**:1.

- D'AJELLO, V., A. MAURO, and S. BETTINI. 1969. Effect of the venom of the black widow spider, *Latrodectus mactans tredecimguttatus*, on evoked action potentials in the isolated nerve cord of *Periplaneta americana*. *Toxicon*. 7:139.
- D'AJELLO, V., F. MAGNI, and S. BETTINI. 1971. The effect of the venom of the black widow spider *Latrodectus mactans tredecimguttatus* on the giant neurones of *Periplaneta americana*. *Toxicon*. 9:103.
- ELMQVIST, D., and D. S. FELDMAN. 1965. Spontaneous activity at a mammalian neuromuscular junction in tetrodotoxin. *Acta Physiol. Scand.* 64:475.
- FRONTALI, N. 1972. Catecholamine-depleting effect of black widow spider venom on iris nerve fibres. *Brain Res.* 37:146.
- FRONTALI, N., F. GRANATA, and P. PARISI. 1972. Effects on black widow spider venom on acetylcholine release from rat cerebral cortex slices *in vitro*. *Biochem. Pharmacol.* 21:969.
- GAINER, H., J. P. REUBEN, and H. GRUNDFEST. 1967. The augmentation of postsynaptic potentials in crustacean muscle fibers by cesium. A presynaptic mechanism. *Comp. Biochem. Physiol.* 20:877.
- GRANATA, F., P. PATTI, and N. FRONTALI. 1972. Effects of chromatographic fractions of black widow spider venom on the *in vitro* biological systems. *Toxicon*. In press.
- GRASSO, A., and P. PAGGI. 1967. Effect of *Latrodectus mactans tredecimguttatus* venom on the crayfish stretch receptor neurone. *Toxicon*. 5:1.
- GRUNDFEST, H. 1961. Ionic mechanism in electrogenesis. *Ann. N. Y. Acad. Sci.* 94:405.
- GRUNDFEST, H., and J. P. REUBEN. 1961. Neuromuscular synaptic activity in lobster. In *Nervous Inhibition*. E. Florey, editor. Pergamon Press Ltd., London. 92.
- GRUNDFEST, H., J. P. REUBEN, and W. H. RICKLES, JR. 1959. The electrophysiology and pharmacology of lobster neuromuscular synapses. *J. Gen. Physiol.* 42:1301.
- KATZ, B., and R. MILEDI. 1965. The effect of calcium on acetylcholine release from motor nerve terminals. *Proc. R. Soc. Lond. B. Biol. Sci.* 161:496.
- KATZ, B., and R. MILEDI. 1967. Tetrodotoxin and neuromuscular transmission. *Proc. R. Soc. Lond. B. Biol. Sci.* 167:8.
- LILEY, A. W. 1956. The effects of presynaptic polarization on the spontaneous activity at the mammalian neuromuscular junction. *J. Physiol. (Lond.)*. 134:427.
- LILEY, A. W. 1957. Spontaneous release of transmitter substance in multiquantal units. *J. Physiol. (Lond.)*. 136:595.
- LONGENECKER, H. E., W. P. HURLBUT, A. MAURO, and A. W. CLARK. 1970. Effects of black widow spider venom on the frog neuromuscular junction. *Nature (Lond.)*. 225:701.
- MOTOKIZAWA, F., J. P. REUBEN, and H. GRUNDFEST. 1969. Ionic permeability of the inhibitory postsynaptic membrane of lobster muscle fibers. *J. Gen. Physiol.* 54:437.
- NERI, L., S. BETTINI, and M. FRANK. 1965. The effect of *Latrodectus mactans tredecimguttatus* venom on the endogenous activity of *Periplaneta americana* nerve cord. *Toxicon*. 3:95.
- OKAMOTO, M., H. E. LONGENECKER, and W. F. RIKER. 1971. Destruction of mammalian motor nerve terminals by black widow spider venom. *Science (Wash, D. C.)*. 172:733.
- OTSUKA, M., L. L. IVERSEN, S. W. HALL, and E. S. KRAVITZ. 1966. Release of gamma-aminobutyric acid from inhibitory nerves of lobster. *Proc. Natl. Acad. Sci. U.S.A.* 56:1110.
- OZEKI, M., A. R. FREEMAN, and H. GRUNDFEST. 1966. The membrane components of crustacean neuromuscular system. I. Immunity of different electrogenic components to tetrodotoxin and saxitoxin. *J. Gen. Physiol.* 49:1319.
- PAGGI, P., and A. ROSSI. 1971. Effect of *Latrodectus mactans tredecimguttatus* venom on sympathetic ganglion isolated *in vitro*. *Toxicon*. 9:265.
- PAGGI, P., and G. TOSCHI. 1972. Effects of denervation and lack of calcium on the action of *Latrodectus* venom on rat sympathetic ganglion. *Life Sci.* 11:413.
- PIEK, P., P. MANTEL, and E. ENGELS. 1971. Neuromuscular block in insects caused by the venom of the digger wasp *Philanthus triangulum* F. *Comp. Gen. Pharmacol.* 2:317.
- REUBEN, J. P., and H. GAINER. 1962. Membrane conductance during depolarizing postsynaptic potentials of cryafish muscle fiber. *Nature (Lond.)*. 193:142.
- REUBEN, J. P., and H. GRUNDFEST. 1960. Inhibitory and excitatory miniature postsynaptic potentials in lobster muscle fibers. *Biol. Bull.* 119:335.
- WIERSMA, C. A. G. 1941. The efferent innervation of muscle. *Biol. Symp.* 3:259.