Electrical Changes in Pre- and Postsynaptic Axons of the Giant Synapse of *Loligo*

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ABSTRACT Potential changes both in pre- and postsynaptic axons were recorded from the giant synapse of squid with intracellular electrodes. Synaptic current was also recorded by a voltage clamp method. Facilitation of postsynaptic potential caused by applying two stimuli several milliseconds apart was accompanied by an increase in the amplitude of the presynaptic action potential. Depression of the postsynaptic potential occurred without changes in the presynaptic action potential. Increase in the concentration of Ca in sea water caused an increase in amplitude of the synaptic current. On the other hand increase in Mg concentration decreased the amplitude of the synaptic current. In these cases no appreciable change in the presynaptic action potential was observed. Extracellularly recorded potential changes of the presynaptic axon showed mainly a positive deflexion at the synaptic region and a negative deflexion in the more proximal part of the presynaptic axon. Mechanism of synaptic transmission is discussed.

At synapses where chemical transmission occurs, the arrival of a nerve impulse at the presynaptic terminal is considered to cause release of the transmitter which, in turn, produces a depolarization of the postsynaptic membrane. Several investigators have attempted to correlate electrical changes in the presynaptic structures with those occurring in the postsynaptic structures during transmission. Thus, del Castillo and Katz (1954 *d*) observed that hyperpolarizing current applied to the presynaptic nerve fiber at the neuromuscular junction augmented the amplitude of the end-plate potential. Also, during post-tetanic potentiation of the monosynaptic reflex of spinal motoneurons, hyperpolarization of primary afferent fibers was observed (Eccles and Krnjević, 1959). In both these synapses, the postsynaptic structure is relatively large and its electrical changes may be easily measured but the

presynaptic elements are comparatively small and their membrane potentials are not easily measured directly.

In the squid stellate ganglion a large presynaptic axon divides into smaller branches, each of which ends blindly on the surface of one of the third order giant axons, where it makes synaptic contact (Young, 1939). Both the pre- and postsynaptic axons are large enough for the insertion of intracellular micropipettes, so that electrical changes in both axons can be recorded (Hagiwara and Tasaki, 1958). Recently it has been confirmed that transmission across this synapse is associated with the release of a transmitting agent and also that small increases in the amplitude of the presynaptic action potential appreciably augmented the size of the postsynaptic potential (Hagiwara and Tasaki, 1958).

The purpose of the present investigation was to study the relationship between electrical changes in the presynaptic axon and the postsynaptic potential (p.s.p.) under various conditions.

METHODS

The experiments were performed at the Marine Biological Laboratory, Woods Hole. The stellate ganglion with its presynaptic fiber and the last stellar nerve were dissected from *Loligo pealii* as described by Bullock (1948). Connective tissue and nerve fibers covering the pre- and postsynaptic axons were removed under a dissecting microscope until the contact of the presynaptic axon with the postsynaptic axon could be observed. Experiments were done on the distal synapse and to avoid the influence of the proximal synapse, in most cases the presynaptic fibers were cut or crushed except for the largest presynaptic axon (Bryant, 1959). Experiments were performed in cold running sea water (10–15°C) bubbled with air. Transmission was usually maintained for about 6 hours under these conditions. When the concentration of calcium or magnesium in sea water was changed, crystalline $CaCl_2$ or $MgCl_2$ was added to the sea water.

Wire electrodes were made from enameled nichrome wire of about 25 μ diameter by scraping off the insulating enamel for a length of about 1 mm. This electrode was pushed longitudinally into the postsynaptic axon through a hole made about 15 mm from the synapse, until the tip of the electrode reached the synaptic region. A wire electrode was also used to pass current when the voltage clamp method was applied to the postsynaptic axon. A 3 M KCl-filled microelectrode was used occasionally to record potential changes across the postsynaptic membrane and as potential and current electrodes for the presynaptic axon. Reference electrodes were silver wires or occasionally 3 M KCl-filled microelectrodes. Potential changes were recorded through high input impedance preamplifiers with negative capacity and conventional equipment. The method of voltage clamping of the postsynaptic axon was similar to that of Hagiwara and Tasaki (1958).

RESULTS

Facilitation and Depression

In many synapses or neuromuscular junctions facilitation and depression of the postsynaptic potential are observed. It has been suggested that certain processes of potentiation are due to an increased amplitude of the presynaptic spikes (Lloyd, 1949; del Castillo and Katz, 1954 *c;* Eccles and Krnjević, 1959; Wall and Johnson, 1958). In the present study this possibility was tested by simultaneously recording the presynaptic spike and the p.s.p.

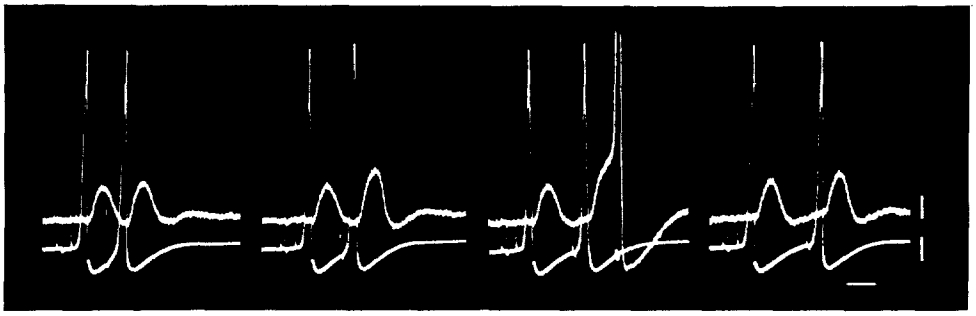

FIGURE 1. Facilitation of p.s.p. demonstrated by double shock method. Upper beam, intracellularly recorded p.s.p. Lower beam, intracellularly recorded presynaptic action potential. Time scale, 5 msec. Voltage scale, 5 mv for upper beam and 10 mv for lower beam.

Double stimuli, at intervals of up to several milliseconds, were applied to the presynaptic axon at a frequency of 2 per second. In some preparations, after the beginning of such stimulation, the postsynaptic fiber failed to produce an action potential, and only the p.s.p. remained. In such preparations, the amplitudes of the p.s.p. produced by the first and by the second stimuli could be compared (Fig. 1). At certain stimulus intervals, the amplitude of the second p.s.p. (upper traces) was larger than that of the first, and sometimes reached threshold for production of an action potential. Similar results were also observed even though the amplitude of the p.s.p. was smaller than those in Fig. 1, the possible contribution of a local response being excluded under this condition. In Fig. 2 the amplitude of presynaptic action potential and that of the p.s.p. are plotted as a function of stimulus interval. The time courses of the two curves are similar. An increase of 5 per cent in the presynaptic spike produced an augmentation in amplitude of the p.s.p. of 50 per cent. The facilitation of the p.s.p. seems to be due mainly to the change in amplitude of the presynaptic action potential.

When stimuli were applied to the presynaptic axon at a low rate, the amplitude of the p.s.p. decreased gradually and in some cases transmission was blocked. This effect could also be seen by recording the synaptic current in the postsynaptic axon under conditions of voltage clamping (Fig. 3). Stimuli were applied to the presynaptic axon near its cut end at a frequency of 2/sec. The amplitude of the synaptic current decreased gradually and after six to

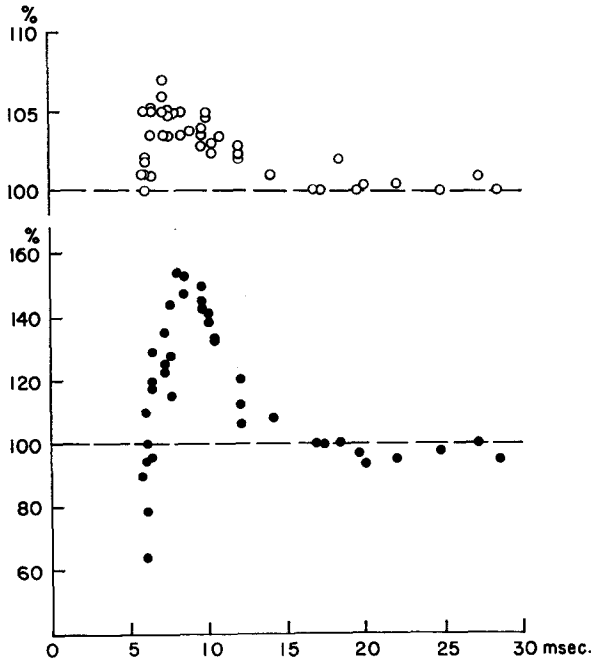

FIGURE 2. Facilitation caused by single conditioning stimulus. Relative amplitude of p.s.p. (filled circles) and that of presynaptic action potential (open circles) are plotted against stimulus interval.

seven stimuli reached almost constant amplitude, while no change was detected in the amplitude of the presynaptic action potential.

Eccles and Krnjević (1959) observed that post-tetanic potentiation of the monosynaptic reflex of spinal motor neurons is associated with an increase in the amplitude of the action potential of presynaptic fibers. Also in the squid giant synapse, after tetanic stimulation of the presynaptic axon (100/sec.), post-tetanic potentiation of p.s.p. was observed, accompanied by an increase in the amplitude of the presynaptic action potential. Similar tetanic stimulation applied to the postsynaptic axon did not change the amplitude of the action potential.

Effect of Calcium and Magnesium

It is known that calcium and magnesium have marked effects on the release of transmitter from nerve endings at the neuromuscular junction and in sympathetic ganglia (Hutter and Kostial, 1954; del Castillo and Katz, 1954 *a*, *b;* Boyd and Martin, 1956). In the giant synapse of squid, calcium has been found to facilitate and magnesium to depress transmission (Bryant, 1958).

In the experiments to be described, the postsynaptic membrane was clamped at the resting potential to avoid disturbances which might be produced by action potentials or local responses in the postsynaptic axon, as well as the effects of changes in membrane resistance which might occur after

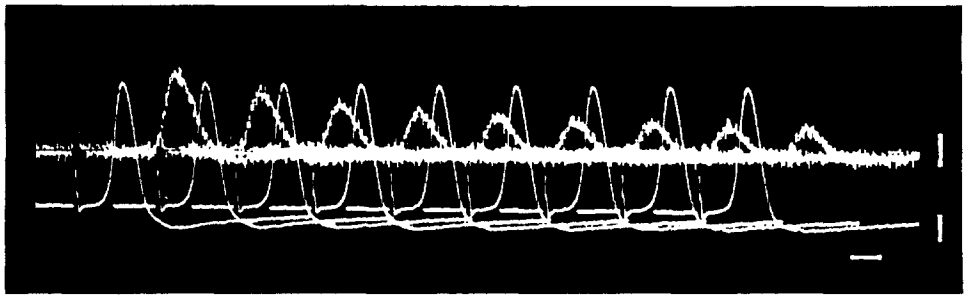

FIGURE 3. Depression of synaptic current by repetitive stimulation. Upper beams, synaptic current recorded by voltage clamping. Lower beams, intracellularly recorded presynaptic action potential. Stimulus was applied at a frequency of 2/sec. Time scale, 1 msec. Current scale, 1 × 10^{-6} A. Voltage scale, 20 mv.

changing the outside concentration of Ca or Mg. Changes in the membrane resistance alter the amplitude of the p.s.p., even though the conductance change of the postsynaptic membrane by the transmitter remains the same (Katz and Thesleff, 1957; Takeuchi and Takeuchi, 1960).

In Fig. 4, the lower traces show the presynaptic action potential and the upper traces the synaptic current. The left hand figures of both *A* and *B* were obtained in sea water. In the right hand figure of *A*, the concentration of Mg in the sea water was doubled (106.8 mM), while in the right hand figure of *B* the concentration of Ca was increased to three times its normal value (27 mM). The increase in Ca concentration increased the amplitude of the synaptic current while increasing the Mg concentration decreased this amplitude. In both cases, the time course of the synaptic current was not appreciably altered. In Mg-treated preparations random fluctuations in amplitude of the synaptic current, such as have been observed at the neuromuscular junction, were not detected. It may also be noted that no ap-

preciable changes in the amplitude of the presynaptic potential occurred following these changes in Ca or Mg concentration. In squid synapse each postsynaptic axon is in contact with a single branch of the presynaptic fiber. Therefore the effects of Ca and Mg on the synaptic transmission are not likely to be due to an action on branching points in the presynaptic axon.

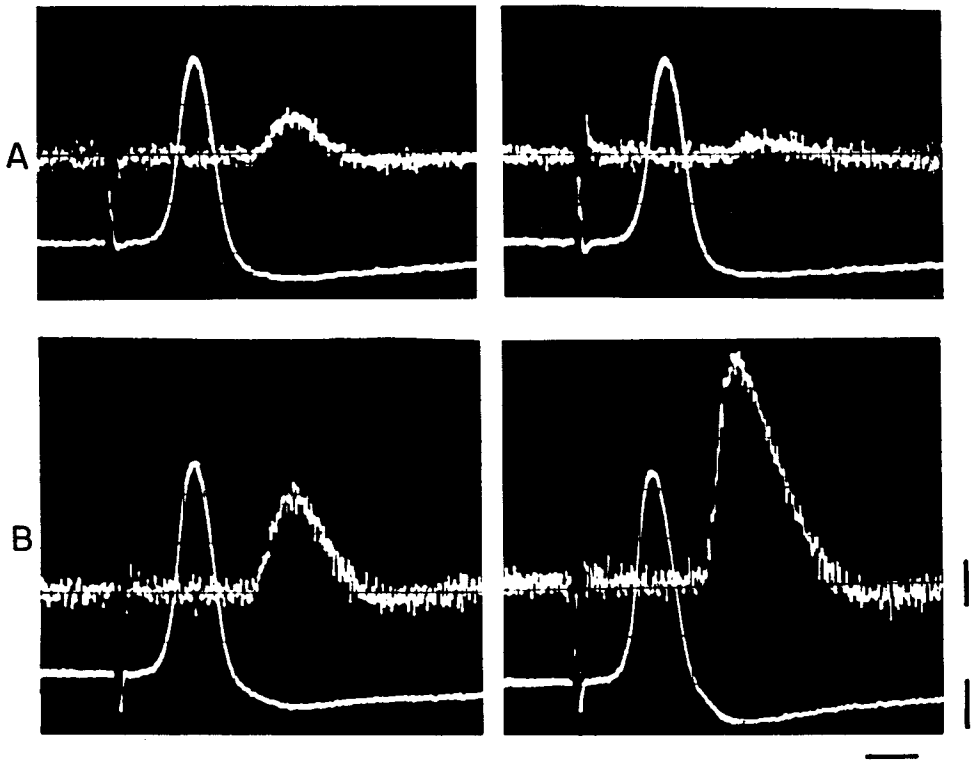

FIGURE 4. Effect of Ca and Mg on synaptic current. Upper beams, synaptic current recorded by voltage clamping. Lower beams, intracellularly recorded presynaptic action potential. *A* left, in normal sea water. *A* right, in high Mg sea water (total concentration 106.8 mM). *B* left, in normal sea water. *B* right, in high Ca sea water (total concentration 27 mM). Time scale, 1 msec. Current scale, 1×10^{-6} A. Voltage scale, 20 mv.

Externally Recorded Potential Changes

During the course of these experiments, it was noted that the potential changes recorded from an external microelectrode placed close to the presynaptic axon at the synaptic region differed from those recorded at some distance away from the synapse. In Fig. 5 the upper trace in record *A* shows the potential changes recorded from the synaptic region while that in *B* shows the potentials recorded from the same presynaptic axon about 2 mm proximal to the synapse. In these records upward deflection indicates positivity of the

microelectrode relative to the bath electrode. The lower traces show the postsynaptic potential changes recorded with a wire electrode.

In Fig. 5 *A*, about 1 msec. after the stimulus artefact, the electrode external to the presynaptic axon recorded a small positive and negative deflexion. About 1 msec. later there appeared a large negative deflexion with intermittent positive deflexion arising from it. Simultaneously recorded potential changes in the postsynaptic axon showed that the large negative deflexion corresponded to the p.s.p., while the subsequent occasional positive deflexions were associated with postsynaptic action potentials. When the postsynaptic

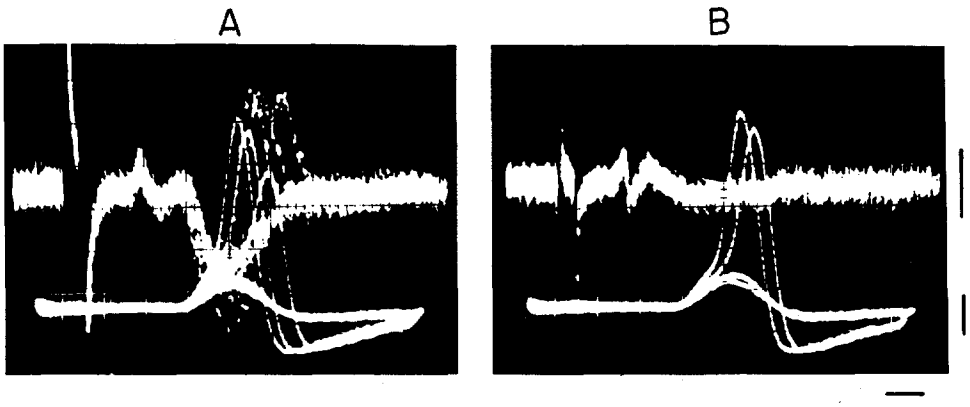

FIGURE 5. Extracellularly recorded presynaptic spike. Upper beams, potential changes recorded by extracellular microelectrode placed on the presynaptic axon. Lower beams, postsynaptic potential changes recorded by intracellular wire electrode. Approximately five traces were superimposed at 8/sec. *A*, in synaptic region. *B*, at a point about 2 mm proximal to the synapse. Time scale, 1 msec. Voltage scale, 0.5 mv for upper beams and 20 mv for lower beams.

action potential was blocked by repetitive stimulation, only the negative deflexion was observed external to the presynaptic axon and its time course was very similar to that of synaptic current recorded during voltage clamping (Fig. 3). The delayed negative-positive deflexion appears to be generated by the synaptic current and action current flowing through the postsynaptic membrane, while the small initial positive and negative deflexion may be attributed to action currents in the presynaptic axon. The external potential changes recorded from the presynaptic axon at a point about 2 mm proximal to the synaptic region show a steep negative going deflexion superimposed on the positive deflexion (Fig. 5 *B*) and little or no potential change was observed corresponding to the synaptic current in the postsynaptic axon.

In Fig. 6 *B* the upper beam shows the potential change recorded externally and the lower beam the p.s.p. at the synaptic region. When the microelectrode was pushed forward slightly, the presynaptic potential changed abruptly to a

more negative level indicating that the tip of the electrode had penetrated the presynaptic axon, and stimulation produced a large positive spike (Fig. 6 *A*). The peak of the externally recorded positive deflexion fell on the

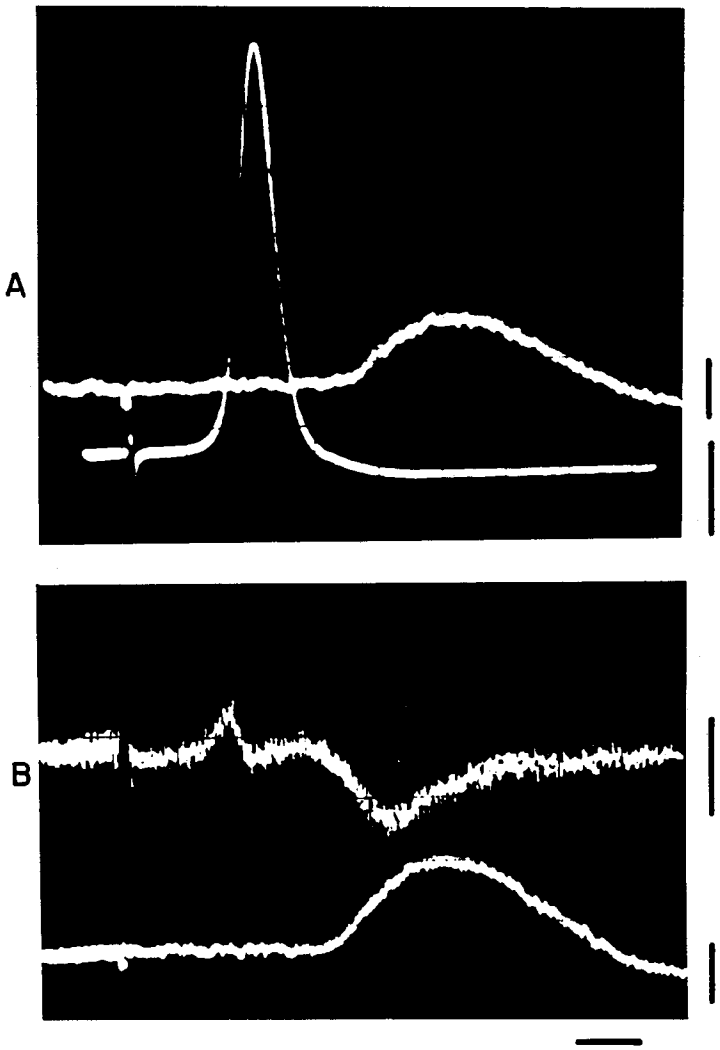

FIGURE 6. Potential changes of presynaptic axon recorded at synaptic region. *B*, upper beam, potential changes recorded from just outside of the presynaptic axon; lower beam, intracellularly recorded p.s.p. *A*, upper beam, intracellularly recorded p.s.p.; lower beam, after impaling the presynaptic axon with the microelectrode. Time scale, 1 msec. Voltage scale, 2 mv for p.s.p.; 20 mv for lower beam of A and 1 mv for upper beam of B.

rising phase of the internally recorded action potential (compare *A* and *B*). When a recording electrode was inserted in the presynaptic axon while another electrode was placed just external to the axon, internal and external potential

changes of the presynaptic axon were recorded simultaneously. At the synaptic region the external electrode recorded a positive deflexion and at the more proximal point the external electrode recorded a negative one. The peak of the positive deflexion recorded externally at the synapse and the peak of the negative deflexion recorded externally at the more proximal point fell on the rising phase of the internally recorded action potential.

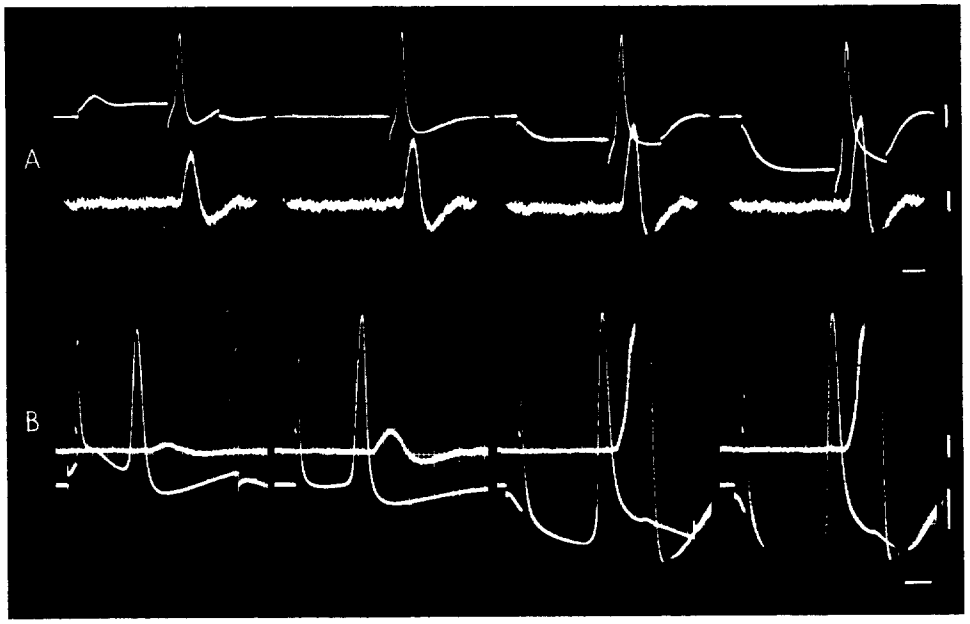

FIGURE 7. Effect of polarization of presynaptic axon on the p.s.p. and on the presynaptic action potential. *A*, recorded at synaptic region; upper beam, presynaptic action potential. Lower beam, p.s.p. *B*, recorded at a point about 2 mm proximal to synaptic region; upper beam, p.s.p.; lower beam, presynaptic action potential. Time scale, 5 msec. for *A*, 2 msec. for *B*. Voltage scale, *A* upper, 20 mv; *A* lower, 1 mv; *B* upper, 5 mv; *B* lower, 20 mv.

When the tip of the electrode is close to the membrane, externally recorded potential changes may be considered to be proportional to the current flow through the membrane; a positive deflexion indicates outwardly directed current while negativity indicates inwardly directed current through the membrane.

The synaptic delay measured from the peak of the presynaptic action current to the postsynaptic current was about 1.2 msec. at 15°C.

Polarization of the Presynaptic Axon

In these experiments, both current and recording electrodes were inserted in the presynaptic axon at the synaptic region. Stimuli were applied to the

central end of the presynaptic axon. When the presynaptic membrane was hyperpolarized the amplitude of the action potential increased and at the same time, the amplitude of the p.s.p. was augmented (Fig. 7). Conversely, under depolarization (first sweep), the decrease in amplitude of the presynaptic action potential was accompanied by a decrease in amplitude of the p.s.p. The

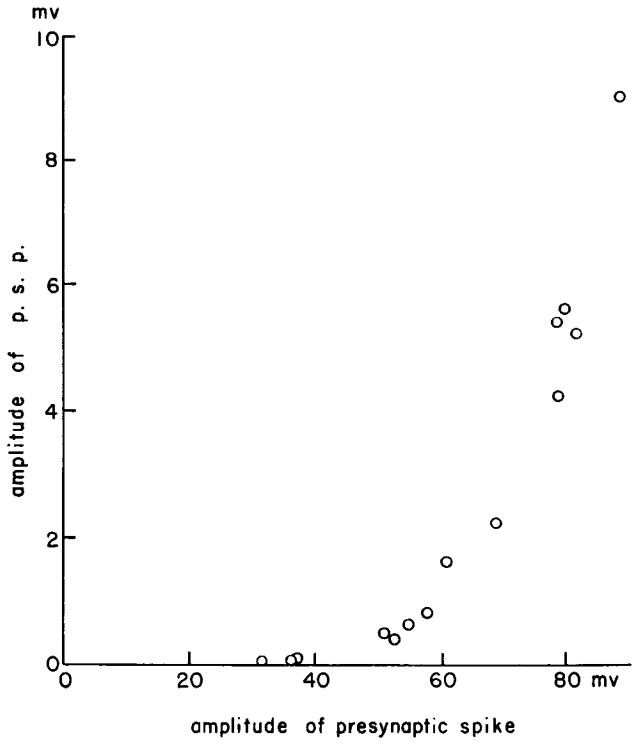

FIGURE 8. Relationship between the amplitude of p.s.p. and that of presynaptic action potential recorded at synaptic region. Abscissa, amplitude of presynaptic action potential. Ordinate, amplitude of p.s.p.

relationship between amplitude of presynaptic action potential and the size of the p.s.p. is shown in Fig. 8. The results are similar to those reported by Hagiwara and Tasaki (1958). When the amplitude of the presynaptic action potential was decreased to about 40 mv, no appreciable potential change could be recorded in the postsynaptic axon. For higher amplitudes small increases in the presynaptic action potential caused very large changes in the amplitude of the p.s.p. Similar results were also observed when the amplitude of the presynaptic action potential was changed by applying procaine.

When the membrane was strongly hyperpolarized, the absolute level attained by the presynaptic action potential decreased although its amplitude became larger (Fig. 7 *A*). These findings may be contrasted with the action potentials obtained from a more proximal region of the presynaptic axon,

where the levels attained by the action potentials starting from various resting potentials are almost the same except when the membrane was depolarized (Fig. 7 *B*). This suggests that the membrane conductance was relatively high in this region at the peak of the action potential. The observed decrease in the peak value of the action potential in the depolarized condition may be due to inactivation of the ion-carrying system of the membrane.

It should be noted here that when the presynaptic fiber membrane was strongly hyperpolarized in the synaptic region, the p.s.p. size became larger, even though the absolute peak level attained by the presynaptic spike was lower.

DISCUSSION

In the present experiments an inwardly directed current coincided with the rising phase of the action potential recorded internally in the proximal portion of the presynaptic axon, suggesting active change at this point; however, at the synaptic region no inward current was recorded during the rising phase of the presynaptic spike. One possible explanation for this is that the nerve terminal became active but no inward current was observed because of the special condition of the nerve terminal; *i.e.*, the terminal is a blind end. Another is that the nerve impulse did not invade fully the nerve terminal. At present we cannot decide between these possibilities, but the result that strong hyperpolarization in the synaptic region of the presynaptic fiber reduced the absolute level attained by the presynaptic action potential seems to favor the latter possibility. The present results, however, do not exclude the possibilities that active changes are weak or take place in small areas of the terminal presynaptic axon membrane. The presynaptic axon seems sensitive to injury, and the inability of the nerve ending to exhibit active change might be due to some unphysiological condition. Most preparations, however, maintained transmission in these conditions.

The present results provide only indirect evidence about the mechanisms of release of transmitter and, hence, the following considerations must be regarded as speculative. The amplitude of the p.s.p. depended on the amplitude of the presynaptic action potential but not on the peak value of the presynaptic spike. Thus the amount of the transmitter released might be determined by the amount of the current rather than by the absolute level of the depolarization. However, the mechanisms of the release of the transmitter may not be so simple, since Ca and Mg had marked effects on the amplitude of p.s.p. without appreciable changes in the presynaptic action potential. At present there is no evidence which shows that Ca and Mg change the amount of the transmitter released from the presynaptic nerve ending. Mg and Ca have effects not only on the cholinergic neuromuscular junction and sympathetic ganglion

(Hutter and Kostial, 1954; del Castillo and Katz, 1954 *a*, *b*; Boyd and Martin, 1956) but also on the non-cholinergic neuromuscular junction of insects (Hoyle, 1955). If it is assumed that Ca and Mg have a similar effect on the giant synapse, the present results suggest that the release of the transmitter is determined not only by the electrical changes at the presynaptic axon but also by some intermediate processes. The mechanism of Ca and Mg action on the neuromuscular junction was discussed by del Castillo and Katz (1954 *a*), and they suggested that calcium and magnesium combine with the carrier of transmitter.

In the squid giant synapse spontaneous postsynaptic potential changes could not be detected. Furthermore appreciable random variations in amplitude of the p.s.p. were not observed, even in media with high magnesium. This might suggest that the mechanism for the release of transmitter in the squid giant synapse differs from that in the neuromuscular junctions of vertebrates and crustacea (del Castillo and Katz, 1954 *b*; Boyd and Martin, 1956; Liley, 1956; Dudel and Kuffler, 1961). However, there are factors which may obscure the presence of a quantal release mechanism. The effective membrane resistance of the postsynaptic axon was of the order of 5 K ohm, and if the conductance change were of the same magnitude as that of the spontaneous miniature e.p.p. of the frog, the potential change produced by such a conductance change would be of the order of 5 μv (*cf.* Katz and Thesleff, 1957). Therefore the conductance change would have to be larger than that of the miniature e.p.p. for the potential change to be detected. The amplitude of the synaptic current was of the order of 5 μa in normal sea water. If the driving EMF were assumed to be 60 mv, the resistance change produced by the transmitter at the postsynaptic membrane might be of the order of 10 K ohm. If the resistance inserted by the miniature discharge were assumed to be 7 M ohm, as it is at the neuromuscular junction (del Castillo and Katz, 1956; Takeuchi and Takeuchi, 1960), the quantum content of this synaptic potential would be 700. This value is much larger than that of the neuromuscular junction. Therefore while the release of the transmitter may not be quantal, it is possible that the release is quantal and that the quantum content is large and the potential change produced by each quantum is too small to be detected.

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