TRANSMITTER RELEASE AT THE SQUID GIANT SYNAPSE IN THE PRESENCE OF TETRODOTOXIN

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IT is well known that depolarization of a motor nerve terminal, either by externally applied current or a raised concentration of potassium in the extracellular medium, leads to an acceleration of transmitter release[1]. At the squid giant synapse, where the size of the presynaptic axon permits the insertion of one or more electrodes, it has been shown that the amplitude of the excitatory postsynaptic potential (EPSP) is markedly affected by the size of the action potential of the presynaptic axon as well as by such factors as the rate of repetition of the stimulus and the ionic composition of the bathing medium[2]. The Puffer fish poison, tetrodotoxin, known to be a highly specific inhibitor of the sodium current system[3] and thus to block generation and propagation of the action potential in excitable tissue, does not prevent the effect of depolarization by potassium on transmitter secretion[4]. In its presence, small endplate potentials have been elicited by short pulses of depolarizing current applied to motor nerve terminals[5]. Because of the small size of the terminals it was not possible to determine the extent or time course of the membrane depolarization causing the transmitter release. The squid giant synapse was used in the present experiments in order to examine more critically the relationship between presynaptic depolarization and liberation of transmitter.

The experiments were performed at the Marine Biological Laboratory, Woods Hole, using the common squid, *Loligo pealii*. The method of dissection of the stellate ganglion was similar to that described by Bullock[6]. The stellate ganglion with giant axon and presynaptic nerve was mounted in a 'Lucite' chamber, at 8°–12° C in flowing sea-water previously aerated with oxygen. During the dissection, as much as possible of the ganglion tissue overlying the presynaptic axon was removed in order to insert the microelectrodes as close to the synapse as practicable. At the same time, great care was taken to avoid damage to this axon, as it was noted that even moderate manipulation resulted in a low resting potential.

Three microelectrodes were routinely used, two to record the voltages from the presynaptic and postsynaptic giant axons, and the third for passing current into the presynaptic axon. The methods of recording were conventional. A schematic diagram of the giant synapse with typical positioning of the microelectrodes is shown in Fig. 1 *A*. The microelectrodes were always inserted within 1·5 mm of the synapse.

Typical records obtained before adding tetrodotoxin are shown in Fig. 1 *B–E*. In response to a depolarizing pulse of 0·01 msec duration, an action potential was generated in the presynaptic axon followed in the postsynaptic axon by an EPSP which, when large enough, triggered an action potential. Fig. 1 *B* and *E* show records obtained with stimuli at 30 sec intervals; in Fig. 1 *C* can be seen the decline in EPSP amplitude which occurred during a short train of stimuli at 1/sec, while in Fig. 1 *D* the EPSP after an interval of 2 sec was still small. The effects of repetitive stimulation on the size of the EPSP can probably be attributed to depletion of the store of transmitter available for release; the amplitude of the presynaptic action potential remained constant throughout. This phenomenon was observed if the stimulus repetition rate was more than about 1/10 sec.

When the preparation was exposed to sea water containing tetrodotoxin ($2 \times 10^{-7} - 10^{-6}$ g/ml.), the presynaptic action potential could no longer be elicited after 5–30 min. At about the same time the postsynaptic action potential disappeared. Nevertheless, an EPSP could still be observed if the intensity and duration of the depolarizing current passed into the presynaptic axon were increased sufficiently. It was found that the EPSP could now be continuously graded, its amplitude varying with the presynaptic depolarization as the current pulse was altered. The latency of the EPSP measured from the peak of the presynaptic voltage transient was the same as from the peak of an action potential (1·5 msec at 10° C). Examples are shown in Fig. 2 *A–E*. These results and others obtained from the same synapse are shown in the graph Fig. 2 *G*, in which EPSP height is plotted against peak depolarization recorded from the presynaptic axon. Because the larger EPSPs were an appreciable fraction of the resting potential (70 mV) of the postsynaptic axon, correction was made for non-linear voltage response to a

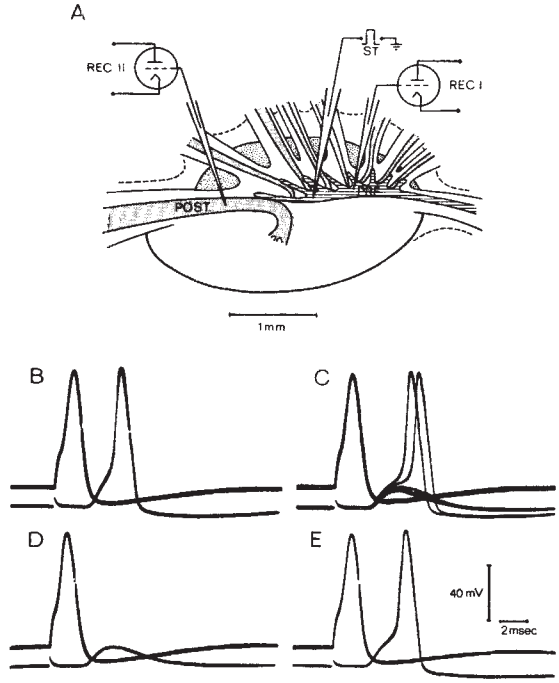

Fig. 1. *A*, Schematic diagram showing the giant synapse and typical placement of the electrodes. *B*, *C*, *D* and *E*, Records of potentials (positive upwards) in presynaptic axon (upper trace) and postsynaptic axon (lower trace) before tetrodotoxin. *B* shows a presynaptic action potential, EPSP and postsynaptic action potential, elicited 30 sec after a preceding stimulus. *C* shows superimposed records during stimulation at 1/sec. *D*, 2 sec later; *E*, 30 sec later. Voltage calibration: 40 mV for both traces. Time calibration: 2 msec.

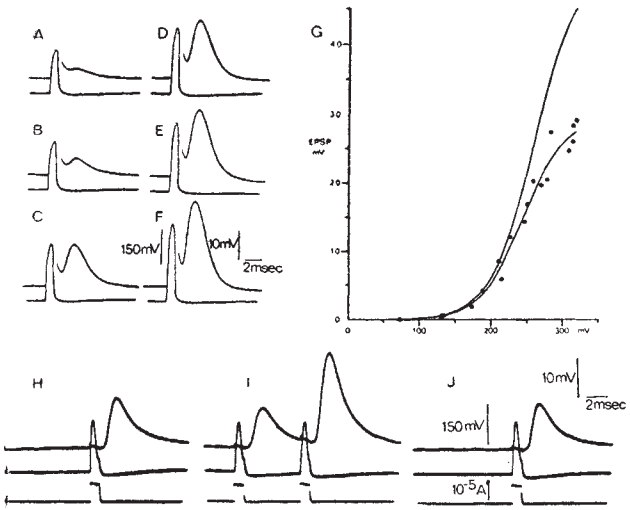

Fig. 2. After tetrodotoxin. *A–E*, Records of potentials (positive upwards) in presynaptic axon (lower trace) and postsynaptic axon (upper trace) with different pulses. Voltage calibrations: lower trace 150 mV, upper trace 10 mV. Time calibration: 2 msec. The resting potential in the presynaptic axon was −50 mV. *G*, Graph of EPSP amplitude (ordinate, mV) versus peak presynaptic depolarization (abscissa, mV). The upper curve was derived from the curve drawn by eye through the points by correcting for non-linearity of postsynaptic response (see text). In records *H–J*, the lowest traces show the current monitor (calibration 10^{-5} amp). *H*, Record of responses to a stimulus identical to the second of the pair in *I*. *I*, Responses to a pair of stimuli 4 msec apart. *J*, The responses to a stimulus identical to that in *H*, applied 1 sec after *I*.

postsynaptic conductance change according to the formula of Martin[7]. The resulting curve, also plotted in Fig. 2 *G*, should give a better picture of how transmitter release altered with presynaptic depolarization. The transmitter equilibrium potential was taken as zero[2]. These results closely resemble those relating presynaptic action potential amplitude and EPSP size[2], but it should be noted that at the time these records were obtained there was no indication of any presynaptic action potential or "local response". In general, however, the presynaptic voltage transient did not follow the time course which would be expected from a purely passive system, especially when the large depolarizing current pulses were applied. As seen in Fig. 2 *A–F* and *H–J*, the voltage transient fell significantly during a current pulse. This can be attributed, at least in part, to activation of the potassium current system, which is not blocked by tetrodotoxin[3].

One striking feature of these results is the magnitude of the presynaptic depolarization needed to evoke the EPSPs. A presynaptic depolarization of about 135 mV gave an EPSP of 0·5 mV; for a 10 mV EPSP about 225 mV was required. EPSPs of similar amplitude were observed by Takeuchi and Takeuchi with presynaptic action potentials of 50 and 90 mV respectively[2]. The difference becomes much smaller when one takes into account the decrement of the electrotonic potential which must have taken place along the presynaptic axon between the microelectrodes and the areas where transmitter was released (see Fig. 1 *A*). Assuming the specific resistance of the presynaptic axon membrane to be the same as that of the postsynaptic giant axon, a rough calculation indicates that the depolarization of the membrane in the synaptic region would be about 70 per cent of that recorded 1 mm from the synapse. It was evident, however, that with depolarizing current there was a decline of the input resistance of the presynaptic axon (estimated from the peak voltage response). It is therefore likely that under these conditions more decrement took place. For the same reason the true relationship between transmitter release and presynaptic depolarization must be steeper than appears from the graph (Fig. 2 *G*). Such intense depolarization as shown in Fig. 2 *A–G* was not always necessary to elicit an EPSP. In experiments in which the presynaptic axons had higher input resistances, 1 msec current pulses giving depolarizations as low as 90 mV were sometimes sufficient, perhaps because of a larger space constant.

It has been shown that at this synapse, when EPSPs are elicited at short intervals, the second is facilitated, and this has been related to the increase which occurs in presynaptic spike size[2], although at neuromuscular junctions under comparable conditions, such facilitation is not accompanied by an increase in the extra-cellularly recorded presynaptic spike[8]. Using the methods outlined, we have tested whether transmitter release evoked by the second of a pair of stimuli at short intervals would be potentiated. If, as suggested by Takeuchi and Takeuchi[2], facilitation at the squid giant synapse is secondary to the increase in spike size, no facilitation should appear when the second stimulus is a current pulse with neither its amplitude nor the consequent depolarization altered because of the preceding pulse. The results of such an experiment are shown in Fig. 2 *H–J*. Because the two pulses in the pair were from different stimulators, control pulses identical to the second were applied 1 sec before and 1 sec after the pair. It can be seen that the second stimulus was unchanged, but there was a clear potentiation of the EPSP, from 10·5 mV to 18·5 mV. Applying Martin's correction[7], the actual facilitation of the change in postsynaptic conductance, and presumably transmitter release, was to 210 per cent of the controls.

Several conclusions may be drawn from the results of these experiments. First, it is evident that transmitter release at the squid giant synapse is not dependent on the integrity of the sodium current system involved in generating the action potential. As at the neuromuscular junction, depolarization of the presynaptic nerve terminal is capable of stimulating transmitter liberation. It appears very unlikely from our results, however, that at the squid giant synapse an action potential which did not invade the nerve terminal could cause sufficient electrotonic depolarization to evoke a normal EPSP. It seems clear also that the facilitation phenomenon observed with pairs of stimuli cannot be attributed to alteration of the presynaptic action potential or action current; rather it would seem to reflect facilitation of the system which couples transmitter release to presynaptic depolarization. Virtually excluded also by our results is the possibility that this type of facilitation is normally secondary to the influx of sodium ions into the nerve terminal which presumably takes place during the action potential. The interesting question remains whether activation of the potassium current system is important, or essential, to the transmitter release mechanism. It should be emphasized that the results presented here also do not permit any discrimination between depolarization and current flow as the determining factor in evoking transmitter release.

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