Helium pressure alteration of function in squid giant synapse

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Henderson, J. V., M. T. Lowenhaupt, and D. L. Gilbert. 1977. Helium pressure alteration of function in squid giant synapse. Undersea Biomed. Res. 4(1):19–26.—The squid giant synapse, which permits intracellular electrical measurements in a single, excitatory synapse, was exposed to helium pressures up to 204 atm. By stimulating presynaptically and recording postsynaptically with an intracellular electrode it was found that pressure alters, but does not prevent, synaptic transmission of action potentials. Synaptically transmitted action potentials are prolonged in the same way as in the directly stimulated axon. However, slowing of the excitatory postsynaptic potential and marked increases in synaptic fatigue were observed at pressures as low as 35 atm. These changes may contribute to high pressure nervous effects by interfering with information transfer within the nervous system.

Effects of pressure on the nervous system have been observed at several levels, from molecular events (Henderson and Gilbert 1975) to the behavior of human divers (Brauer 1968; Hunter and Bennett 1974). Although several studies have reported the effects of pressure in isolated nerves (Ebbecke and Schaefer 1935; Grundfest 1936; Spyropoulos 1957a,b; Henderson and Gilbert 1975), the synapse has until recently been neglected. Campenot (1975) reported that hydrostatic pressures of 59 to 200 atm decreased the amplitude of intracellularly recorded lobster neuromuscular junctional potentials. His analysis of this postsynaptic response, a single depolarization proportional to the frequency of presynaptic stimulation, suggested that pressure may act presynaptically. In the rat superior cervical ganglion (Kendig, Trudell, and Cohen 1975) helium or hydrostatic pressure to 137 atm decreased the amplitude of the compound action potential recorded extracellularly from the synaptically stimulated postganglionic nerve. Kendig and Cohen (1976) also found that pressure reversibly depressed electromyogram amplitude in the synaptically stimulated rat diaphragm if calcium is reduced. To define the influence of pressure on synaptic function further, we studied pressure effects in a classic synaptic preparation.

The squid giant synapse offers several advantages for high pressure studies. The postsynaptic nerve is the well-characterized squid giant axon, already studied at elevated hydrostatic
(Spyropoulos 1957a) and helium (Henderson and Gilbert 1975) pressures. The synapse has itself been intensely studied at normal pressure and has one-to-one chemical transmission of action potentials. Finally, the large size of the synapse enables intracellular placement of electrodes in the synaptic region, thus permitting recording of electrical activity in a single, excitatory synapse.

In these experiments we studied only the response of the postsynaptic membrane to presynaptic stimulation. Although we could not distinguish between pre- and postsynaptic sites of pressure action, we observed notable changes in synaptic function at only moderately increased pressures. These changes may contribute to high pressure neurological effects.

METHODS

Experiments were carried out in a pressure chamber (Henderson, Morin, and Laplancher 1975) constructed from a commercially available pipercross. In addition to providing means for continuous flow of tissue bathing solution and microscopic observation while under pressure, the chamber’s modular design adapts for use with a variety of preparations. A module for squid giant synapse was constructed incorporating a Plexiglas tissue bath (after Manalis 1974) and micromanipulator for electrode placement. Temperature was controlled at 15°C ± 0.2 with a Pellicor thermoelectric unit (Cambion, Cambridge, Mass.) mounted under the tissue bath and placed in a feedback loop with a pressure-insensitive thermistor.

Preparations were bathed in flowing, filtered natural seawater having a pH at 15°C of about 7.8 and pre-equilibrated with 100% oxygen. Oxygen (100%) was also used to purge the pressure chamber before pressurization. Synapses were exposed to helium pressure up to 204 atm at a rate of about 7 atm/min.

The schematic of Fig. 1 shows the experimental arrangement. Following dissection of the stellate ganglion of the squid, Loligo pealei (Bullock 1948), the tissue was mounted in the bath so that the postsynaptic axon passed through a slot in one of the bath walls. The internal electrode, a 50-μm insulated platinum wire, was advanced intracellularly down the length of the postsynaptic axon until its bare tip rested near the synaptic region. The presynaptic nerve was then stimulated extracellularly (0.2 ms square wave pulses, 5 times threshold) either singly or repetitively at 20 to 80 Hz. A silver-silver chloride wire served as reference electrode. Responses were recorded on Polaroid film using a Tektronix storage oscilloscope and magnetic tape using a Nicolet digital oscilloscope. The latter method provided very accurate measurement of voltage-time coordinates.

A possible role of oxygen in our observations deserves consideration since hypoxia is known to accelerate synaptic fatigue (Bryant 1958). Though we did not measure $P_{O_2}$ directly, we began each experiment with 1 atm $O_2$ in both the bathing solution and the ambient gas. At maximum pressure this should have remained unchanged. If observed effects are due to hypoxia, by returning to lower pressure, and thus reducing the $P_{O_2}$, we would expect to observe an increased effect. This did not occur and the observed effects were fully reversible, even on return to 1 atm. We conclude hypoxia played no significant role in our measurements.

RESULTS

We studied effects of pressure in six synapses. Results were generally in agreement and records obtained at normal pressure compare well with those published in the literature. Preparatory to our synapse studies we examined the response of the directly stimulated axon.
Fig. 1. Schematic of experimental arrangement. Insulated platinum wire with bared tip is advanced intracellularly down length of postsynaptic squid giant axon. Axon is stimulated synaptically by extracellular stimulation of presynaptic nerve.

As seen in Fig. 2, the most striking effect is prolongation of the action potential. There is also a decrease in action potential amplitude of about 10%.

Figure 3 shows action potentials elicited by presynaptic stimulation at normal (left) and high pressure (right). Even at maximum pressure of 204 atm no synaptic block of single action potentials occurred. The influence of pressure on action potential shape is very similar to

Fig. 2. Tracings of action potentials in directly stimulated squid giant axon at 1 and 158 atm, space-clamped action potentials (entire length depolarized simultaneously). Em is resting membrane potential.
isolated nerve; prolongation and decreased amplitude are seen. However, the part of the record preceding the action potential demonstrates two additional effects: slowing of the excitatory postsynaptic potential (EPSP) and changes in action potential threshold. The part of each curve of Fig. 3 between the beginning of the response at far left and the initiation of the action potential (arrows) shows the EPSP. Here the postsynaptic membrane generates an action potential by bringing the adjacent axonal membrane to threshold. At 204 atm the rate of rise of the EPSP is greatly decreased and the axon is brought more slowly to threshold. The second effect is a change in threshold potential to a more depolarized level, i.e., threshold has increased.

Table 1 compares the relative contributions to total synaptic delay of presynaptic-synaptic events (axonal conduction velocity, chemical transmission) and postsynaptic events (EPSP slowing, threshold change). Values are from the experiment of Fig. 4; since some synaptic fatigue was observed even at very low stimulus frequencies, intervals shown are from the first record obtained at each pressure. The measurements from column 1 are from the onset of the stimulus artifact (see Fig. 4) to the onset of the EPSP, and the measurements of column 2 are from the onset of the EPSP to the threshold, threshold being taken as the point of maximum curvature at the beginning of the action potential. While the first column intervals change by a maximum of only 15%, those of the second column increased 50% at 35 atm and about 5-fold at 204 atm. Thus pressure appears to affect the postsynaptic response primarily, since the effect on this is disproportionately greater than the effect on conduction-transmission.

Although the directly stimulated axon responds to many thousands of stimuli without fatigue, fatigue of synaptic transmission with repetitive stimulation at normal pressure has been widely reported (Hagiwara and Tasaki 1958; Kusano and Landau 1975). Figure 4 summarizes an experiment in which the synapse was stimulated repetitively at several pressures.
TABLE 1
Contributions to synaptic delay

<table>
<thead>
<tr>
<th>Pressure, atm</th>
<th>Interval measured, ms</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>(1) 2.61 (2) 0.42</td>
</tr>
<tr>
<td>35</td>
<td>2.82 0.63</td>
</tr>
<tr>
<td>102</td>
<td>2.64 1.74</td>
</tr>
<tr>
<td>204</td>
<td>3.01 2.23</td>
</tr>
</tbody>
</table>

Column 1: stimulus artifact to onset of EPSP; Column 2: onset of EPSP to threshold. Values based on experiment shown in Fig. 4; intervals are from first record obtained at each pressure, since some fatigue occurred even at very low stimulation frequencies.

Fig. 4. Tracings from Polaroid records showing failure of synaptic transmission with repetitive stimulation at different pressures (psi) and frequencies. Pressure increases synaptic fatigue and also slows EPSP. Right lower frame shows reversibility of these effects. Square-waves at left of each tracing are stimulus artifacts. Note that rising phase of action potentials exceeded storage ability of oscilloscope.
Each frame is a tracing from Polaroid film of superimposed action potentials elicited until the synapse failed. Decreased density of the tracing reflects decreased numbers of action potentials before failure of synaptic transmission occurred. Stimulation frequencies were 80 Hz in the left column, and 40 Hz in the right. Note that the left column begins with a record obtained at 1 atm and the right column begins with one obtained at 500 psi. This was done to include the right lower frame, obtained after returning to 1500 psi, which demonstrates the reversibility of the effects. Pressure decreased the number of action potentials obtained before failure occurred, as quantified in Fig. 5. We see that the synapse fatigued about 4 times more easily at only 35 atm, while at maximum pressure synaptic fatigue increased over 20-fold.

![Graph](image)

**Fig. 5.** Semi-log plot of number of action potentials prior to failure in repetitively stimulated synapse as a function of pressure. Based on experiment of Fig. 4.

**DISCUSSION**

In this preliminary study we found that helium pressure alters, but does not prevent, transmission in a single, excitatory synapse. Three major effects were seen: 1) slowing of the EPSP; 2) changes in threshold; and 3) increased synaptic fatigue. Considering these effects, we feel that pressure acts principally to slow the EPSP; the other two effects may be secondary to this. Slowing of the EPSP can account for the change in threshold potential, i.e., increased threshold, since a slower rate of axonal depolarization can increase threshold (Khodorov 1974). Changes in threshold potential similar to those we observed at high pressure are seen in previously published records obtained at normal pressure: as the synapse fatigues the EPSP slows and threshold potential moves toward zero potential (e.g., Hagiwara and Tatsuki 1958). A careful study of the effect of pressure on threshold in the directly stimulated axon has yet to be made; previously published reports are conflicting (compare Spyropoulos 1957a,b).
The striking increase in synaptic fatigue at high pressure might similarly be a consequence of a slowed EPSP in that the normal process of fatigue, leading to slowing and decreased amplitude of the EPSP (Bullock 1948; Hagiwara and Tasaki 1958; Kusano and Landau 1975), is superimposed.

It must be emphasized that our results have not distinguished the site of pressure action. Whether alteration of the postsynaptic potential or increased fatigue are the result of pressure action of pressure has been implicated by Campenot (1975). Kusano and Landau (1975) and others believe synaptic fatigue at normal pressure is caused by presynaptic depletion of neurotransmitter. However, a variety of other mechanisms are consistent with our results: pressure may slow gating in ionic channels of the presynaptic (e.g., calcium channels) or postsynaptic membrane, as occurs in squid axon (Henderson and Gilbert 1975); desensitization of the postsynaptic membrane may occur (Katz and Thesleff 1957; Gardner and Kandel 1972); or pressure may interfere with the release of neurotransmitter by disaggregating microtubules (compare Salmon 1975; Gray 1976).

The neurological changes seen in divers and animals at high pressure may relate directly to our observations. Under natural conditions synapses transmit rapidly modulating trains of action potentials at relatively high frequencies. By increasing synaptic delay and fatigability, pressure could interfere profoundly with this function. The result in the intact animal or human diver would be a disorganization of information transfer in the nervous system at a basic level.

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REFERENCES


