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Hydrostatic pressure reduces synaptic efficiency by inhibiting transmitter release

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Parmentier JL, Shrivastav BB, Bennett PB. Hydrostatic pressure reduces synaptic efficiency by inhibiting transmitter release. Undersea Biomed Res 1981; 8(3):175–183.—The amplitude of postsynaptic potentials at an identified synapse in the *Aplysia* central nervous system can be modulated by the application of hydrostatic pressure. Postsynaptically controlled functions, including responses to acetylcholine and the time course of decay of the synaptic responses, remain unaffected by compression. In contrast, frequency facilitation and posttetanic potentiation, which result from presynaptic processes, are altered by pressure in a manner similar to that of agents that block transmitter release. It is concluded that pressure reduces synaptic efficiency by interfering with certain presynaptic mechanisms associated with transmitter release.

pressure
presynaptic
synapse
transmitter release

Hydrostatic pressure is recognized as the basis of a serious clinical disorder, the high pressure nervous syndrome (HPNS), associated with deep diving in humans (1) and has been shown to produce hyperexcitability followed by convulsions in many intact vertebrate and invertebrate animals (2). Several groups have reported that pressure reduces the amplitude of postsynaptic responses in isolated neural preparations, but there is a disagreement about whether the pressure-sensitive site is presynaptic (3), postsynaptic (4, 5), or both (6, 7). We have used voltage clamp techniques to study the effects of high hydrostatic pressure on naturally evoked transmission across a single identified central synapse in the abdominal ganglion of the sea hare *Aplysia*. We report here that 100 atmospheres (ATA) of hydrostatic pressure reduces the amplitude of the synaptic response without changing its kinetics or affecting the response of the postsynaptic neuron to iontophoretically applied acetylcholine. These results support the hypothesis that pressure interferes primarily with presynaptic functions of the synapse.

MATERIALS AND METHODS

The abdominal ganglion of *Aplysia californica* was pinned in a wax-bottom dish filled with artificial seawater (494 mM Na^+, 10 mM K^+, 10 mM Ca^{2+}, 50 mM Mg^{2+}, 571 mM Cl^-, 30 mM

SO_4^{2-}, 2 mM HCO_3^-) adjusted to pH 7.8. The dish was placed inside a Wilson Universal High Pressure Chamber (8, 9), which consists of a series of bands that assemble vertically on clamp bolts and tighten from above to form metal-to-metal seals between each section. The chamber is fitted with two modified Narashige HMD-2 micromanipulators, one for intracellular recording and one for iontophoresis.

Neurosecretory cell R15 is a readily identifiable burst-firing neuron in the abdominal ganglion of *Aplysia* that has been the subject of previous investigations in our laboratory on the effects of hydrostatic pressure (9). Cell R15 receives a large, excitatory input from a cell in the pleural ganglion via the right cerebrovisceral connective (10). This synapse, termed RC1-R15, is thought to be cholinergic because the response can be blocked by *d*-tubocurarine, hexamethonium, and atropine. It has a fixed latency and firing threshold, indicating that this is a monosynaptic connection, and it requires Na^+ for its proper functioning. In addition the synapse exhibits a characteristic sequence of changes in its amplitude during and immediately after repetitive stimulation. Thus the RC1-R15 synapse is a useful model for investigating several different properties of synaptic function.

Neuron R15 was impaled with a glass microelectrode that had been jet-stream–beveled to 1 MΩ and filled with 1.5 M KCl. The preparation was allowed to stabilize under hyperpolarizing current for at least one hour before the experiments were begun. The single-electrode voltage clamp of Wilson and Goldner (11) was used to monitor synaptic currents. The holding potential was −80 mV in the cell body but was presumably less and indeterminate at the synapse. Clamping to this level produces large responses, since the synaptic event arises farther from its equilibrium potential and minimizes the possibility of contaminating the responses by any of several voltage-dependent membrane currents that are activated near threshold levels in this neuron. In addition, previous experiments (9) have shown that pressure does not affect the current-voltage relationship of R15 at this holding potential. The right connective was stimulated with a platinum hook electrode that raised the connective out of the seawater and into a covering layer of mineral oil. The synapse was activated at 1 Hz for the repetitive stimulation experiments, with the resulting postsynaptic potentials (PSPs) recorded on a Gould 220 chart recorder. All kinetic data were collected from isolated stimulations that were separated by at least 60 s. Current traces were photographed from a Tektronix 502 oscilloscope (Tektronix, Beaverton, OR) by a Nihon-Kohden camera for later analysis. Iontophorectic electrodes were filled with 1 M acetylcholine at pH 4.5. The chamber was filled with mineral oil, and compression was achieved by the controlled valving of helium gas into a feedline to the top of the chamber. Compression to 200 ATA with this system causes less than 2 mV piezoelectric offset in the electrode system, using a seawater and KCl agar bridge as the ground return. Temperature was continuously monitored by a thermocouple (Bailey Instruments, Saddle Brook, NJ) in the recording dish, and changes of less than 1.5°C were recorded during a standard compression to 100 ATA at a rate of 3–5 ATA/min. Stops were made at 33, 66, and 100 ATA for data collection. Experimental results were obtained from a total of 64 animals.

RESULTS

When neuron R15 is voltage clamped to −80 mV and the right connective is stimulated above its threshold value, the postsynaptic event is recorded as a rapid influx of current, which then decays as a first-order exponential with a time constant (t_D) of 15 to 20 ms.

Figure 1 demonstrates the effect of hydraulic pressure on both the naturally evoked excitatory postsynaptic potentials (EPSPs) and postsynaptic currents (EPSCs). Compression reduced the amplitude of the responses without altering either the synaptic delay, as measured

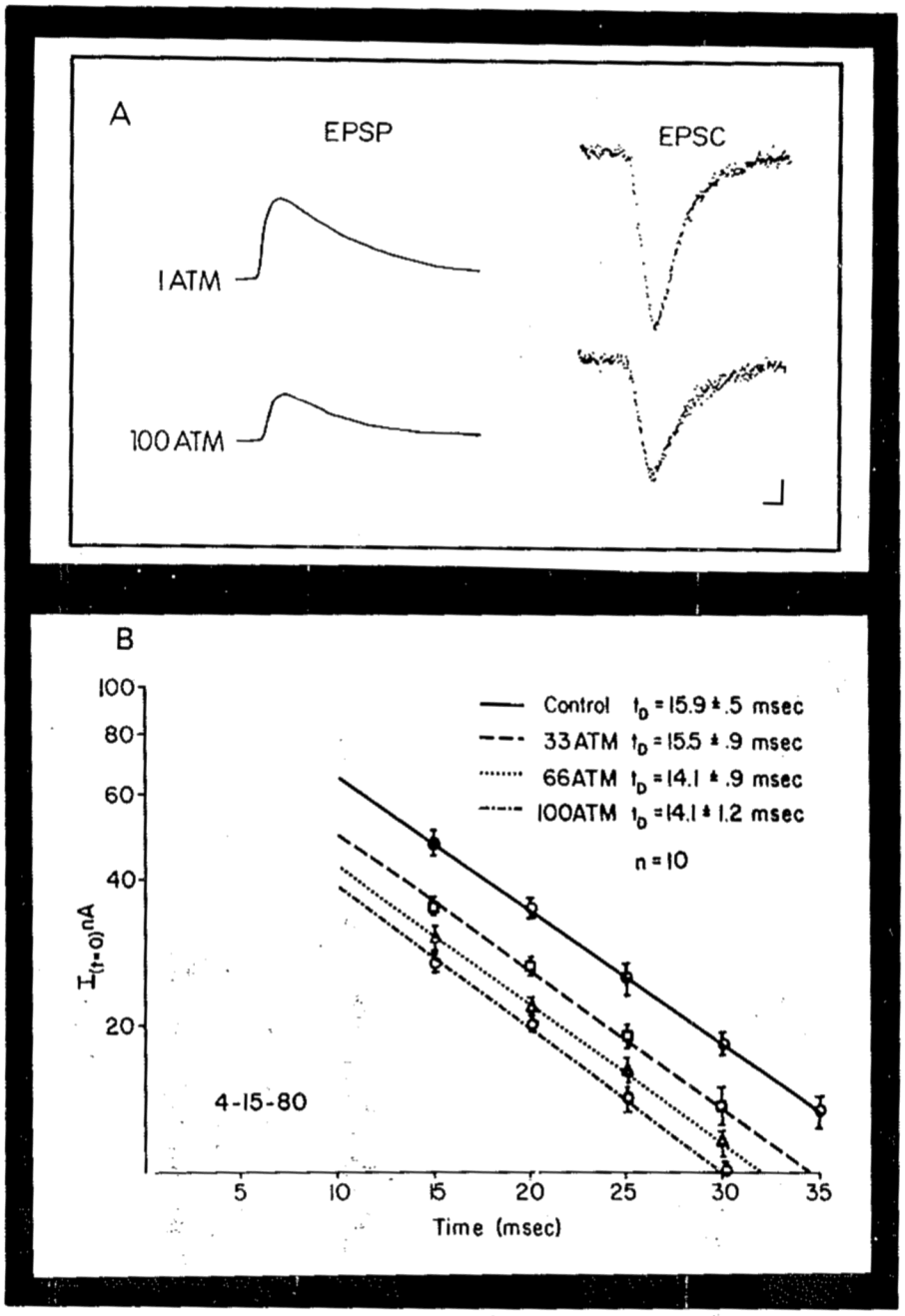

Fig. 1. *A:* hydraulic pressure at 100 ATA reduces amplitude of excitatory postsynaptic potentials (EPSPs) and excitatory postsynaptic currents (EPSCs) at RC1-R15 synapse without altering latency or growth phase of responses. Calibration for EPSP: vertical, 5 mV; horizontal, 50 ms. Calibration for EPSC: vertical, 10 nA; horizontal, 10 ms. *B:* mean amplitude (± SD) of EPSC decay phases of 10 synaptic events plotted against time shows that decay remains single-order exponential during compression to 100 ATA while the extrapolated $I_{(t=0)}$ is reduced. Table gives values for time constant of decay (t_D) each compression level.

from the stimulus artifact to the onset of the response, or the growth phase, as determined from time-to-peak measurements of the EPSC. The falling phase of the synaptic currents continued to follow an exponential time course at all pressure levels up to 100 ATA (Fig. 1*B*). Linear regression analysis of the decay phase was used to extrapolate both the maximum current at the start of each synaptic event ($I_{t=0}$) and the time constant of decay. These values were then normalized to control values for comparison among different preparations. Pooled results using averages of ten synaptic events at each compression level from seven different preparations are shown in Fig. 2. These results indicate that while 100 ATA linearly reduced the $I_{t=0}$ to 66.5 ± 7.1% of control (mean ± SD), there was no significant change in the latency or the time-to-peak measurements and only a slight tendency for reduction in the t_D values.

In order to examine the effect of pressure on postsynaptic receptor responses acetylcholine was iontophoresed onto the soma of neuron R15, alternating with naturally evoked activation of the RC1-R15 synapse. The actual location of the RC1-R15 postsynaptic membrane is unknown and presumably deep within the complex neuropil region of the ganglion. Thus it is not feasible to iontophoretically apply a putative transmitter directly to the postsynaptic membrane. There are chemoreceptors to several putative transmitters on the somatic membranes of *Aplysia* central neurons, however, and the properties of these receptors are considered to exemplify those found at the axoaxonic synapses of the same neuron. Three different responses to acetylcholine occur in these cells, depending on which current-carrying ion is activated. Iontophoresis of acetylcholine onto the soma of R15 produces a rapid excitatory response that disappears in Na^+-free seawater. Figure 3*A* shows that compression to 100 ATA did not alter the amplitude of the iontophoretic responses and only slightly lengthened their time course. The experiment was repeated in five different preparations. Seven additional

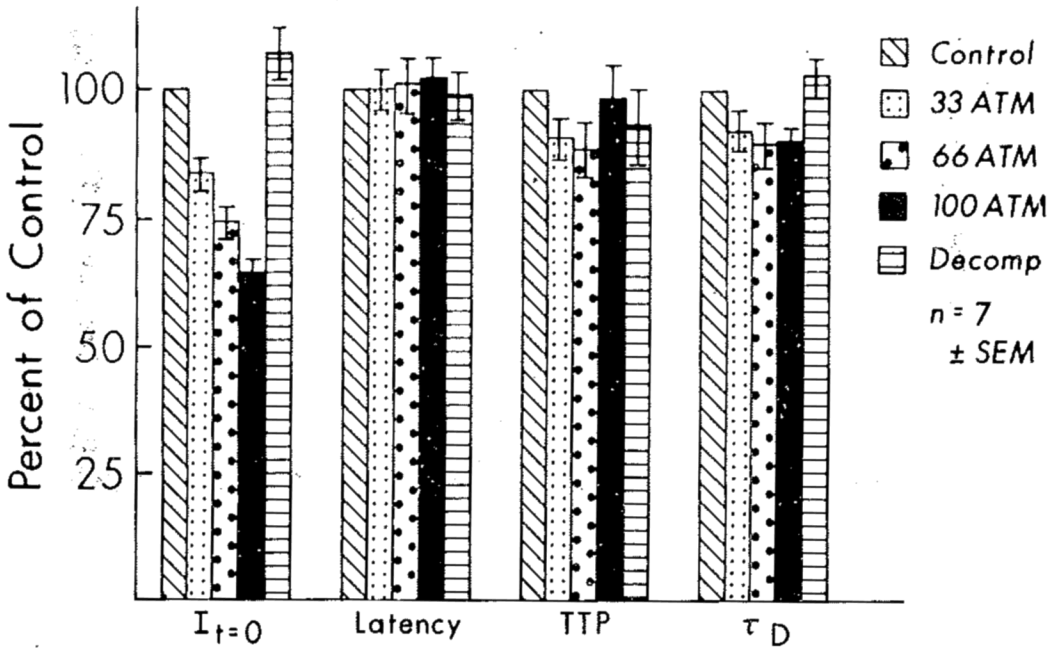

Fig. 2. Normalized results from 7 experiments, each averaged from 10 synaptic events at each compression level, show that $I_{t=0}$ is significantly reduced in amplitude by 100 ATA pressure while latency, growth phase or time-to-peak (TTP), and time constant of decay remain unaltered. Amplitude of $I_{t=0}$ recovers upon decompression.

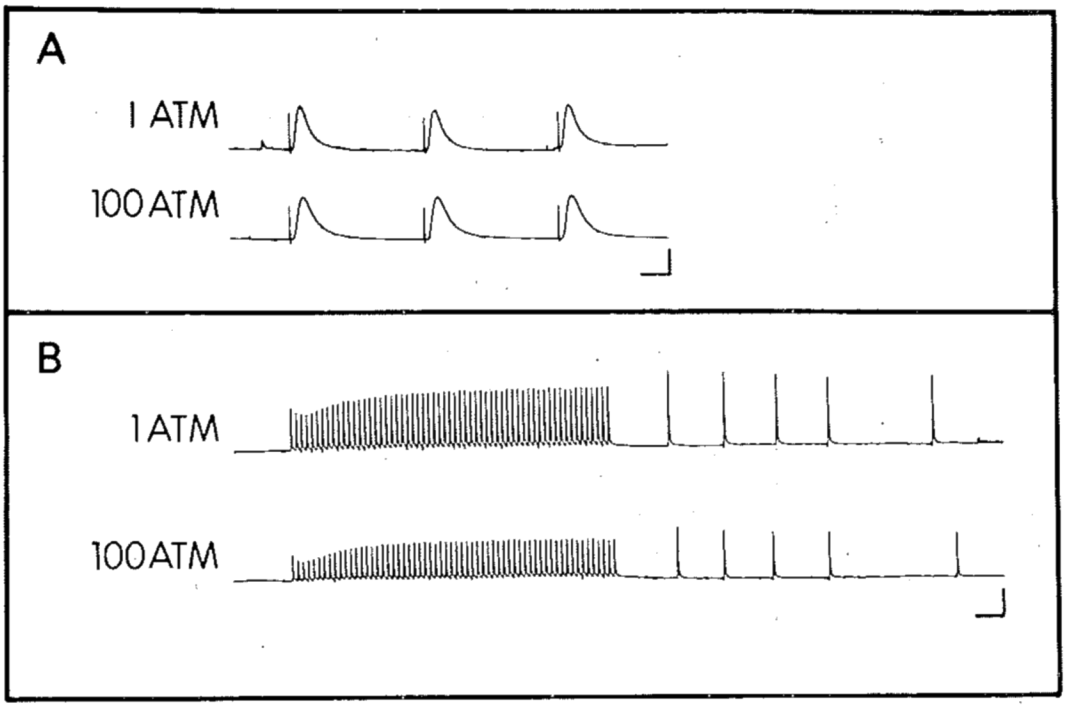

Fig. 3. *A:* pressure at 100 ATA does not reduce amplitude of responses to iontophoretically applied acetylcholine on R15. Calibration: vertical, 10 mV; horizontal, 10 s. *B:* pressure reduces amplitude of EPSPs in all phases of synaptic plasticity (synaptic depression, frequency facilitation, and posttetanic potentiation) that occur during and after repetitive stimulation of RC1-R15 synapse. However, its effect on $EPSP_1$ is greater than its effect on $EPSP_{60}$ or $EPSP_{PTP}$ (see text and Table 1). Calibration: vertical, 10 mV; horizontal, 5 s.

experiments on other *Aplysia* neurons showed that separate chloride-dependent and potassium-dependent hyperpolarizing iontophoretic responses to acetylcholine are also unaffected by pressure.

To study the effects of pressure on presynaptic facilitation of the RC1-R15 synapse the cell was taken out of voltage clamp and hyperpolarized to −80 mV. The right connective was stimulated repetitively at 1 Hz for 60 s; this was followed by a test pulse every 10 s for 1 min. Under these conditions the amplitude of the second EPSP in the train is smaller than that of the first (synaptic depression, or SD) but succeeding EPSPs facilitate to reach a steady-state amplitude of 50% to 100% larger than an isolated response (frequency facilitation, or FF). Following cessation of the stimulus train the test pulses show that the amplitude of the synaptic response continues to increase for a period of several seconds (posttetanic potentiation, or PTP) and then gradually declines to the amplitude of the initial EPSP. These phenomena have been observed at many synapses throughout the nervous system and, under physiological conditions, have usually been attributed to variations in the amount of transmitter released by the presynaptic terminal rather than to changes in the postsynaptic sensitivity to the transmitter substance. Synaptic depression is calculated as the ratio of the differences in amplitude of the first two EPSPs in the train to the initial EPSP as follows: $(EPSP_2-EPSP_1)/EPSP_1$. Frequency facilitation is calculated as the ratio between the amplitude of the last EPSP in the train and the initial EPSP as follows: $EPSP_{60}/EPSP_1$. Posttetanic potentiation is calculated as the ratio

between the amplitude of the potentiated EPSP measured 10 s after the cessation of the train and the initial EPSP as follows: $EPSP_{PTP}/EPSP_1$.

Figure 3*B* shows the effect of compression at 100 ATA on the amplitude changes following repetitive activation of the RC1-R15 synapse. It is apparent that while pressure reduced the size of each EPSP relative to its counterpart in control, the greatest effect was on the initial EPSP. This resulted in a rise of the calculated values of FF and PTP. Table 1 gives the values for frequency facilitation and posttetanic potentiation for 15 different preparations following compression to 100 ATA. It can be seen that both FF and PTP were increased by equal amounts (127.5% ± 31.2% and 127.0% ± 25.3%, respectively) over their control values. The same conditions reduced the amplitude of $EPSP_1$ to 65.8% ± 16.9% of its control value, a level virtually identical to that determined by calculating $I_{t=0}$ values by regression analysis of the current decay curves with the cell under voltage clamp.

An additional series of experiments was conducted to determine the degree of interaction between hydrostatic pressure and the concentration of divalent ions in the bathing medium. In Fig. 4 the Ca^{2+} level was raised to 15 mM from its normal value of 10 mM and the amplitude of the RC1-R15 synaptic event, as calculated from $I_{t=0}$ values, was increased to 203% ± 15.4% of control. One hundred ATA reduced the enhanced value to 123.8% ± 24% of control. Extrapolation of the curve in Fig. 4 suggests that the Ca^{2+} enhancement would be reversed at around 140 ATA. Ca^{2+} has been shown to affect the release of transmitter from preterminal endings. These results support the hypothesis of a presynaptic site for the effect of pressure.

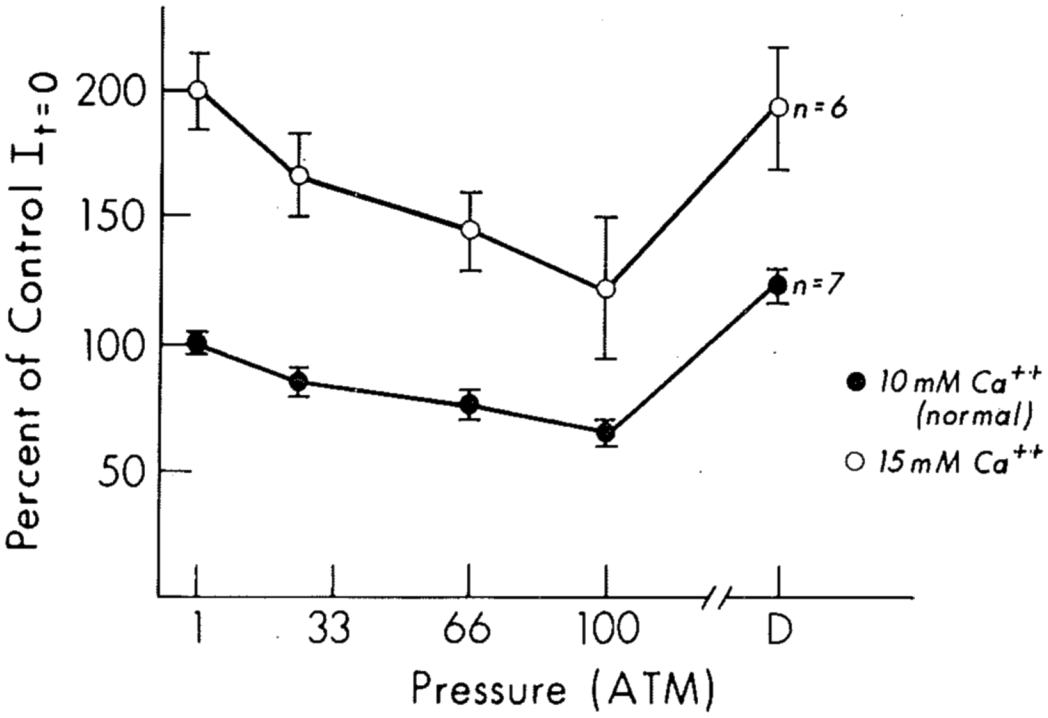

Fig. 4. Pressure opposes effect of Ca^{2+} on RC1-R15 synapse. Hydraulic pressure at 100 ATA reduces amplitude of $I_{t=0}$ values for EPSCs recorded in both normal (10 mM) and Ca^{2+}-enhanced (15 mM) seawater. The degree of reduction of the Ca^{2+}-enhanced response (from 203% to 122%) is almost identical to that seen in normal seawater (from 100% to 65%).

TABLE 1

EFFECT OF 100 ATA PRESSURE ON EPSPS, FREQUENCY FACILITATION, AND POSTTETANIC POTENTIATION AT RC1-R15 SYNAPSE

	Control			100 ATA Hydrostatic Pressure					
Preparation	$EPSP_1$, mV	Frequency facilitation*	Posttetanic potentiation†	$EPSP_1$, mV	Percent of control	Frequency facilitation*	Percent of control	Posttetanic potentiation†	Percent of control
1	10	1.35	1.8	6	60	1.58	117	2.12	117
2	6	2.79	3.79	4.75	79	4.42	158	5.36	141
3	4.25	2.75	2.82	4	94	2.76	100	3.75	132
4	23.5	1.14	1.23	9.5	40	2.10	184	2.3	186
5	20	1.2	1.79	12	60	1.66	138	1.79	100
6	14	1.35	1.22	11	78	1.31	97	1.27	98
7	13	1.88	1.82	12.1	93	1.84	98	1.77	97
8	10.5	1.74	1.72	8	76	1.6	91	1.62	94
9	16.5	2.08	2.87	8	48	2.87	138	2.93	153
10	10.5	1.9	1.92	6	57	2.29	120	2.41	125
11	9.1	1.8	1.81	5.75	63	2.0	111	2.05	113
12	14.2	1.7	1.76	10.8	76	1.75	103	1.85	105
13	10.5	1.60	1.54	7.5	71	1.61	100	1.66	108
14	10.75	1.39	1.76	5.5	51	1.72	123	2.5	142
15	16.5	1.24	1.36	7	42	1.64	132	1.64	120
					$\bar{X}$ = 65.8%		$\bar{X}$ = 127.5%		$\bar{X}$ = 127.0%
					SD = 16.9		SD = 31.2		SD = 25.3

*Calculated as ratio between amplitude of last EPSP in the train and the initial EPSP as follows: $EPSP_{60}/EPSP_1$. †Calculated as ratio between amplitude of the potentiated EPSP measured 10 s after the cessation of the train and the initial EPSP as follows: $EPSP_{PTP}/EPSP_1$.

DISCUSSION

Since synaptic latency was not altered by pressures of up to 100 ATA, it is unlikely that axonal conduction is impaired or that pressure has either altered diffusion time or caused changes in the dimensions of the synaptic cleft that might account for the reduced synaptic potentials. Similarly, compression did not alter the time constant of decay of the EPSPs or the iontophoretic responses to acetylcholine, both of which are properties of the postsynaptic side of the junction. The changes in EPSP amplitude following repetitive stimulation can be accounted for in terms of a material flow model of presynaptic neurotransmitter economics that presents rate constants for the depletion of transmitter from a readily releasable pool and for resupply into the pool by mobilization of transmitter from a secondary storage depot (12). Schlapfer et al. (13) have shown by ionic replacement studies on the RC1-R15 synapse that procedures that enhance transmitter release, such as raising the Ca^{2+} or lowering the Mg^{2+} levels in the seawater, result in a decreased level of FF and PTP. Conversely, the addition of Mg^{2+} or Co^{2+}, which decreases transmitter release presumably by reducing Ca^{2+} entry during the presynaptic action potential, causes the values of FF and PTP to exceed control levels. Since hydrostatic pressure causes the same effect as does the addition of Mg^{2+} or Co^{2+}, we suggest that pressure is exerting its effect on some aspect of the presynaptic processes that control transmitter release. Furthermore, since the enhancement of FF and PTP appear to result from a pressure reduction of the initial EPSP in the train, it is likely that pressure is affecting the membrane release process rather than subsequent mobilization.

It is not possible from the results presented here to identify a specific biological component of the excitation-secretion process that might be affected most directly by the effects of compression. Pressure may be directly affecting the rate of certain presynaptic reactions such as vesicle fusion to the preterminal membrane or the voltage-dependent entry of Ca^{2+}. Alternatively, pressure may be structurally disrupting the preterminal membrane (changes in "fluidity" or "microviscosity") in which the activating molecular components are embedded. Detailed studies of pressure effects on presynaptic rate processes may eventually allow more precise correlations to be made with the biological processes that are actually being affected.

Kendig and Cohen (5) were the first to report the synaptic depressant effect of high pressure on the polysynaptic rat superior cervical sympathetic ganglion preparation. Campenot (3) observed reductions in the amplitude of peripheral end plate potentials following compression of an arthropod neuromuscular junction and showed that the coefficient of variance for transmission across the synapse was affected by pressure. These results suggested that pressure was interfering with the quantal release of transmitter. Similarly, Wann et al. (6) have reported that pressures up to 100 ATA reduce the frequency of miniature end plate potentials recorded at frog neuromuscular junction. Their results suggest that compression does not damage or disrupt the release mechanism so much as alter the rate processes associated with quantal release. Peripheral neuromuscular synapses frequently display a high safety factor that allows transmission to be effective even if there has been some reduction in the size of the postsynaptic response. However, for synapses in the central nervous system a 30% reduction in transmitter release, as reported here following compression to 100 ATA, would severely impair postsynaptic activation and drastically compromise the subtle balance of membrane integrations that characterize interneuron function. These results point to the probability that the central synapses will be useful sites for further study of the cellular basis of pressure-induced neural disorders. In addition, the specificity shown by pressure in disrupting transmisssion at the RC1-R15 junction by affecting a presynaptic parameter demonstrates the potential value of

using compression as a dependent variable for the selective investigation of electrophysiological mechanisms.

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Parmentier JL, Shrivastav BB, Bennett PB. La pression hydrostatique reduit l'efficacité synaptique par l'inhibition du déclenchement de transmetteur. Undersea Biomed Res 1981; 8(3):175–183.— L'amplitude des potentiels postsynaptiques à une synapse identifiée dans l'*Aplysie* SNC peut être modifiée par l'application de la pression hydrostatique. Les fonctions contrôllées postsynaptiquement, les réponses à l'acétylcholine et le cours de temps de dépérissement des réponses synaptiques compris restent inaffectés de la compression. En contraste, la facilitation de fréquence et la potentiation posttétanique, qui résultent des procédés présynaptiques, sont altérées par la pression dans une manière pareille à celle des agents qui bloquent le déclenchement de transmitteur. On conclut que la pression réduit l'éfficacité synaptique par l'inhibition des certains mecanismes présynaptiques qui sont associés avec le déclenchement de transmitteur.

pression
présynaptique
synapse
déclenchement de transmitteur

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