Modulation of Rat Corticohippocampal Synaptic Activity by High Pressure and Extracellular Calcium: Single and Frequency Responses

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INTRODUCTION

High pressure is a thermodynamic variable affecting the kinetics of processes. Since the volume of the system is related to the effects of pressure, pressure-induced changes in the kinetics will reveal volume changes. This kind of analysis is applicable to cell membranes, ion channels, and transmitter release (Conti et al. 1987; Heinemann et al. 1987a). Hyperbaric environment affects the normal function of the nervous system, inducing the high-pressure neurological syndrome (HPNS). HPNS is characterized in humans by confusion, drowsiness, dizziness, impairment of cognitive performance, and tremor at pressures above 1.5 MPa. These are associated with signs of CNS hyperexcitability in the EEG (Bennett and Rostain 2003). In animals, higher pressures result in myoclonia, convulsions, and ultimately death. Some of pressure effects on the CNS presumably involve changes in the performance of corticohippocampal structures. These brain structures are involved in primate learning and memory (Scoville and Milner 1957; Squire and Zola-Morgan 1991; Zola-Morgan et al. 1982), as well as spatial learning tasks in rats (Ferbinteanu et al. 1999; Quirk et al. 1992).

The cellular mechanisms underlying the deleterious effects of high pressure on human cognitive function remain unclear. They have been partially attributed to narcosis (Abraini 1997), but our preliminary experiments showed, for the first time, relevant changes in the function of corticohippocampal structures under high pressure of helium (Talpalar and Grossman 1994). We have studied the medial perforant pathway (MPP) that originates from spiny stellate neurons at the medial entorhinal cortex. This is the principal track conveying cortical information into the hippocampal formation (Swanson and Kohler 1986). Axons of the MPP establish synapses onto the inner dendritic area of the granule cells at the dentate gyrus (DG). These and other CNS synapses such as the Shaffer collateral at the CA1 area of the hippocampus (Fagni et al., 1987a) and the climbing (Etzion 2001) and parallel (Etzion and Grossman 2000) fibers at the cerebellum were depressed at high pressure. Whereas studies of crustacean neuromuscular synapses have shown depression of single synaptic events, associated with increased facilitation (Campenot 1975; Golan and Grossman 1992; Grossman and Kendig 1988, 1990) and tetanic and post-tetanic potentiation (Grossman and Kendig 1988, 1990), little is known about high-pressure modulation of CNS synaptic activity at high frequencies. The MPP under normal conditions usually exhibits a small degree of paired-pulse facilitation (PPF), but net frequency-dependent depression (FDD; low-pass filter property) occurs at high-frequency trains (Dreier and Heinemann 1991; Kilbride et al. 2001). The effect of high pressure on FDD, however, has not yet been studied at any synapse. If high pressure diminishes FDD or switches it into frequency-dependent potentiation, the filter properties of the MPP-dentate gyrus may be disrupted. Inputs generated at the entorhinal cortex may penetrate the hippocampal formation without restrictions or may even be synthetically amplified. This, in turn, may induce hyperactivity at the sei-
pressure-prone CA3 area of the hippocampus (Scharfman and Schwartzkroin 1990). While the effect of hyperbaric pressure on invertebrate single synaptic events was partially antagonized by increasing [Ca\(^{2+}\)]\(_o\), high-pressure effects on synaptic activity were partially mimicked by reducing [Ca\(^{2+}\)]\(_i\) (Golan and Grossman 1992; Golan et al. 1994; Grossman and Kendig 1990). These experiments, together with the recording of presynaptic currents at the frog neuromuscular junction (Grossman et al. 1991), suggested that high pressure might have reduced Ca entry into the terminals. This hypothesis was additionally reinforced by the high-pressure reduction of Ca\(^{2+}\) uptake (Gilman et al. 1986) and transmitter release (Gilman et al. 1988a,b) by synaptosomes. Assuming a similar rationale, we studied the effects of high pressure and reduced [Ca\(^{2+}\)]\(_o\) on mammalian CNS synapse during single and frequency responses. We suggest that additional mechanisms may be involved in pressure effects on central synapses that exhibit FDD: if FDD is a consequence of depletion of synaptic releasable quanta (Stevens and Wang 1995), reduction of synaptic release by high pressure may leave behind intact quanta, which may be released by later stimuli. However, if high pressure not only reduces synaptic release but also depresses the rate of refilling of synaptic sites, FDD may be maintained or even enhanced.

**METHODS**

**Brain preparation**

Sprague-Dawley rats of both sexes (150–250 g) were killed under deep anesthesia (pentobarbital, 60 mg/kg). The brain was extracted in \(<1\) min and submerged in cold Ringer solution (4–6°C). Corticohippocampal slices (400 \(\mu\)m) were prepared as described by Dreier and Heinemann (1991). Slices were cut in a horizontal vibratome (Campden Instruments) and conserved in an incubation chamber at 25°C for later utilization. Regular Ringer solution contained (in mM) 124 NaCl, 3 KCl, 2 CaCl\(_2\), 2 MgSO\(_4\)H, 1.25 NaH\(_2\)PO\(_4\), 26 NaHCO\(_3\), and 10 n-glucose. Solution was constantly bubbled with 95% O\(_2\)-5% CO\(_2\) for a pH of 7.4.

**High pressure and pressurization**

Experiments at high pressure were carried out in a pressure chamber (Canty), which was provided with a remote control manipulator allowing the displacement of the recording pipette (Etzion and Grossman 2000; Grossman and Kendig 1988). Pressurization was attained by compressed helium, which is chemically inert at 0.1–10.1 MPa. Some controls were taken at 0.2–0.4 MPa since they were more stable than at 0.1 MPa. Dean and Mulkey (2000) have shown that a specific, although small, population of medullar neurons is sensitive to these relatively small pressures. Therefore the effects of these small pressures were thoroughly investigated. They did not induce any detectable effect on the electric recordings of corticohippocampal and cerebellar preparations (Etzion and Grossman 2000; Talpalar 2002). However, they greatly improved the chance for successful stable compression. The compression/decompression rate reached 0.15–0.2 MPa/min. Pressure stops were at 5.1 and 10.1 MPa. Oxygenated (95% O\(_2\)-5% CO\(_2\) at normal pressure) prewarmed normal Ringer solution was provided by a fast high-pressure pump (LDC analytical minipump). At every pressure-step, recordings were taken under strict conditions of temperature (30°C) and after a 15-min minimal stabilization time. This was necessary for avoiding transient effects (Grossman and Kendig 1984) including the temperature transients of approximately 2°C occurring during compression/decompression (≤32 and 28°C, respectively). In all the experiments, decompression was attempted to demonstrate reversibility of pressure effects.

Reversibility varied under different conditions: from 10.1 to 5.1 MPa, reversibility was complete; between 5.1 and 0.5 MPa recovery was often complete; and decompression below 0.4 MPa only succeeded in approximately 50% of the cases. Although signs of initial recovery were observed in all experiments, full synaptic recovery was observed in only approximately 50% of them.

**Electrophysiological recordings**

Extracellular field excitation postsynaptic potentials (fEPSPs) were recorded at the inner dendritic area of the DG using glass micropipettes (1.5–3 MΩ) filled with Ringer solution (Fig. 1A). Tungsten bipolar electrodes were placed either at the subiculum, the entorhinal cortex, or the inner dendritic area of the DG for stimulating the MPP (Fig. 1, C and D). Various stimulus intensities (usually 5–10 steps between the threshold intensity and the saturation level) were used for inducing orthodromic responses.

![FIG. 1. Parameters of field potentials recorded at the dendritic area of the dentate gyrus after stimulation of the medial perforant path. A: field excitatory postsynaptic potentials (fEPSPs): initial slope (I), amplitude (2), delay (3), and decay time (4). B: presynaptic input volley (IV): initial slope (I) and amplitude (2). C: schematic diagram of the preparation: CA, cornu ammonis; DG, dentate gyrus; EC, entorhinal cortex; Sub, subiculum; AB, angular bundle; D: infrared photography of the hippocampus and dentate gyrus areas in the preparation showing the location of recording electrode and the alternative place of a proximal stimulus electrode.](downloaded from http://jn.physiology.org/ by 10.220.33.5 on October 20, 2017)
Data analysis

The parameters for the analysis of individual fEPSPs and presynaptic input volleys are shown in Fig. 1, A and B, respectively. Both the fEPSP’s amplitude, indicating synaptic current, and the fEPSP’s slope, indicating rate of activation of synaptic receptors, were used for the evaluation of synaptic field potential. We measured both parameters in all the protocols, but for the sake of clarity, we show only fEPSPs’ slopes in the results under paired-pulses and frequency stimulation. This is because fEPSPs’ slopes are more reliable than fEPSPs’ amplitudes as pure synaptic parameters (they are less contaminated by the initiation of population spikes; Fagni et al. 1987a).

Two different approaches were used for studying frequency response of the synapse. 1) Pairs of stimuli, with 10- to 100-ms inter-stimulus intervals (ISIs), were delivered every 20 s. Slope of both fEPSPs in the pair, $E_1$ and $E_2$, were compared. Results were usually plotted as the normalized form $E_2/E_1$ for each ISI. Paired-pulse depression (PPD) and PPF were used for describing respectively relative depression or increase of $E_2$ with respect to $E_1$. The term paired-pulse modulation (PPM; positive or negative) was eventually used for generically referring to depression and facilitation. 2) Five-impulse train stimulation (1/min) at a frequency range of 25–50 Hz was used. Analysis of the responses was carried out in two ways: 1) individual measurement and comparison of each response in the train for each frequency and 2) comparison of the effect of frequency on certain relevant fEPSPs in the train (usually $E_2$ and $E_3$ normalized to $E_1$). In addition, when a pattern of modulation involving the whole set of events was suspected (e.g., exponential decay), experimental results were fitted with respect to a presumed typical function. Frequency-dependent potentiation (FDP), FDD, and the generic term frequency-dependent modulation (FDM; positive or negative) were used for describing such synaptic tendencies.

The results of the following experiments are expressed as mean ± SE. The n expresses the number of successful experiments for each experimental protocol, which equals the number of slices exposed at the various specified conditions. Since high-pressure experiments were often long-lasting, they were usually carried out using a single slice from each animal. The performed statistical tests compare the effect of various pressures/calcium concentrations on electrophysiological events (slope, amplitude, time constant, etc.). When the comparison was made between signals in the same slice under control and experimental conditions, significance of difference between means was assessed using Student’s t-test for paired observations. ANOVA tests for repetitive measurements were used for comparing sets of stimuli at frequency. The degree of significance was denoted by the values of P. Results are considered statistically different when $P < 0.05$.

RESULTS

High pressure depresses single fEPSPs

MPP synapses display both N-methyl-D-aspartate (NMDA) and non-NMDA receptors components (Staley and Mody 1992). The EPSPs induced by activation of these receptors may be additionally amplified by the recruitment of voltage-dependent channels (Urban et al. 1998). MPP synaptic strength increases in direct proportion to the stimulus intensity (Fig. 2A), reflecting the progressive recruitment of presynaptic fibers and presumably the additional NMDA and voltage-dependent components. Amplitude and slope of fEPSPs often exhibit a saturation curve in response to increasing stimulus intensities (Fig. 2B and C). To estimate pressure effects in the presence of these multiple components, MPP synaptic activity was assessed at various stimulus intensities. High pressure depressed fEPSP amplitude and slope at all stimulus intensities, but pressure effects were more evident at the lowest intensity.

Systematical study of the MPP synapses was performed using half-maximal-intensity fEPSPs (Fig. 3A). This was a compromise between low-stimulus fEPSPs that were considered unreliable because they may disappear when depressed at high pressure and high stimulus intensities that may contaminate pure synaptic events by generating population spikes.

The average amplitude of single half-maximal fEPSPs was depressed by 31 and 47% at 5.1 and 10.1 MPa, respectively (Fig. 3B). The slope of the same fEPSPs was depressed by 21 and 55%, respectively, at these pressures (Fig. 3C). Direct correlation was found between the depression of fEPSP’s amplitude and slope under hyperbaric conditions. Pressure prolonged the fEPSP’s delay ≥31% at 10.1 MPa (Fig. 3, D and E). The change at pressure for each experiment was directly proportional to the duration of the delay at control (linearly correlated $B = 1.09, R = 0.85, P < 0.0003$; data not shown). This indicates reduced conduction velocity of MPP axons in addition to increased synaptic delay. Decay time of single fEPSP was prolonged by 58 ± 18% at 10.1 MPa. Figure 4 summarizes the effect of 10.1 MPa (normalized) on the various parameters of MPP fEPSPs.

The input volley (IV) reflects presynaptic currents at MPP axonal terminals. At control temperature (30°C), IVs were fast and relatively small. They often occurred tightly coupled to fEPSPs, which impeded reliable measurements in most of the experiments. The following results were obtained from experiments that displayed measurable clear-cut IVs at all pressure-steps. IV amplitude was depressed by 32.2 ± 9.7% at 10.1 MPa (from 0.34 ± 0.06 to 0.24 ± 0.07 mV, $P < 0.03, n = 5$, Fig. 5A). The reduction to 0.28 ± 0.08 mV at 5.1 MPa was not significantly different from control ($n = 5$). High pressure also increased the IV’s duration by 26.2% at 10.1 MPa (from 1.07 ± 0.04 to 1.35 ± 0.05 ms, $n = 3, P < 0.07$) and reduced its initial slope by 20.7% at 10.1 MPa (from 0.29 to 0.23
mV/ms, n = 3, P < 0.02). The changes induced by high pressure on the amplitude and kinetics of the IV were small but consistent. Change in IVs may secondarily affect the induction of fEPSPs. In contrast with the depression of IVs, high pressure (10.1 MPa) did not affect MPP field action potentials recorded at the hippocampal fissure following stimulation of the medial entorhinal cortex (Fig. 5B, n = 2).

High pressure shifts PPM toward facilitation

Examples of paired MPP fEPSPs at 20- and 40-ms ISIs under control and 10.1 MPa pressure are shown in Fig. 6, A and B, respectively. Control PPM showed maximal PPD (30%) at 10-ms ISI and almost plateau-phase of weak PPF (approximately 5%) at ISIs of 35–80 ms. Figure 6C shows the course of the PPM under control and high-pressure conditions. High pressure shifted the whole range of PPM toward PPF. Maximal effect was observed at ISIs 30 and 40 ms, at which PPM increased by 22 and 26%, respectively, at 10.1 MPa. The plateau phase of steady PPF (calculated by curve linearization at ISIs of 40–120 ms) increased from 1.05 ± 0.02 at control to 1.17 ± 0.04 at 5.1 MPa and to 1.2 ± 0.05 at 10.1 MPa (ANOVA, P < 0.001 and P < 0.0003, respectively). PPFs at 5.1 and 10.1 MPa were not significantly different.

Despite the PPF enhancement at high pressure, E2 at pressure was smaller than E1 or E2 at 0.1 MPa (Fig. 6A). At the 40-ms ISI, normalization of E2 at the various pressures with respect to E1 at 0.1 MPa resulted in the following values: 1.05, 0.58 (P < 0.005), and 0.42 (P < 0.0002) for 0.1, 5.1, and 10.1 MPa, respectively. Similar calculation of E2 at the 10-ms ISI resulted in 0.7, 0.37, and 0.28 for the respective pressures. This indicates that increased facilitation is relative and does not reflect absolute increase in quantal content of E2. Plotting each E2 PPF (40-ms ISI) as a function of the remaining fraction of E1 at 10.1 MPa resulted in an inverse linear relationship (data not shown). This supports the hypothesis that the enhancement of PPM at pressure is due to the depression of E1.

FIG. 4. Effects of high pressure on the different parameters of the fEPSPs: initial slope, amplitude, decay time, and delay. Values were normalized with respect to control (n = 9).

FIG. 5. Effects of high pressure on the potentials generated by MPP presynaptic fibers. A: effects of 5.1 and 10.1 MPa on the input volley (IV). B: effect of 10.1 MPa pressure on MPP axonic potentials. This compound field potential, which is generated by pure axonic currents, was recorded close to the hippocampal fissure while stimulating at the medial entorhinal cortex.
High pressure enhances FDD

fEPSPs evoked by trains of five stimuli at various frequencies were compared under control and high-pressure conditions. Examples at 25 and 50 Hz are shown in Fig. 7, A and B.

In addition to the depression of the slope of $E_1$ and increased PPM of $E_2$ (as described above in the two previous sections), high pressure (10.1 MPa) increased the FDD of the rest of the responses in the train. Since the second responses were initially potentiated and successive events were depressed, the analysis of the time course of FDD was performed between $E_2$ and $E_5$ responses. FDD build-up was defined by the exponential decay of fEPSPs’ slopes in the train. The decay time constant of this exponent is $\tau_D$, and the asymptotic level was estimated by $E_5/E_2$. We assumed that the decay was exponential based on previous experimental data in which longer trains of fEPSPs displayed such a relationship (Kilbride et al. 2001; Talpalar 2002). FDD of fEPSPs’ slopes at 25 Hz tended to be maintained under 5.1 and 10.1 MPa (Fig. 7, A and C), while FDD at 50 Hz was progressively increased at 5.1 and 10.1 MPa (Fig. 7, B and D).

Table 1 compares in detail the effects of pressure on FDD during the trains ($n = 7$ for each frequency). The development of FDD, expressed by $\tau_D$, was apparently accelerated as pressure was increased. Statistical analysis of the difference ($E_n - E_{n+1}$) between events displaying pure FDD ($E_2 - E_3$) indicated that FDD was not significantly different at 25 Hz, but significantly steeper at 50 Hz ($n = 21, P < 0.02$). Concomitantly, $\tau_D$s computed for each experiment indicated that only the change for 50 Hz at 10.1 MPa was significant ($n = 7, P < 0.01$). The estimated asymptotic level of the exponent ($E_5/E_2$)
TABLE 1. Frequency response at high pressure

<table>
<thead>
<tr>
<th>Pressure</th>
<th>Asymptotic Level ($E_a/E_x$)</th>
<th>$\tau_0$ (ms)</th>
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<tbody>
<tr>
<td></td>
<td>25 Hz</td>
<td>50 Hz</td>
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<tr>
<td>0.1 MPa</td>
<td>0.66 ± 0.05</td>
<td>0.61 ± 0.06</td>
</tr>
<tr>
<td>5.1 MPa</td>
<td>0.6 ± 0.07</td>
<td>0.55 ± 0.08</td>
</tr>
<tr>
<td>10.1 MPa</td>
<td>0.62 ± 0.06</td>
<td>0.42 ± 0.07</td>
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Values are mean ± SD.

at the two frequencies was not significantly changed by pressure.

Low $[Ca^{2+}]_o$ reproduces pressure depression of single fEPSPs

Lowering $[Ca^{2+}]_o$ from 2 to 1 mM at atmospheric pressure (Fig. 8) depressed the fEPSP’s slope and amplitude by 51 ± 8% ($n = 7$) and 56 ± 7% ($n = 7$), respectively. The decay time of single fEPSPs was prolonged by 51 ± 14% ($n = 5$). These effects were roughly similar to the effects of 10.1 MPa on the same parameters. The delay between the stimulus artifact and the initiation of the fEPSP was 2.41 ± 0.14 and 2.55 ± 0.18 ms for 2 and 1 mM $[Ca^{2+}]_o$, respectively. Thus low $[Ca^{2+}]_o$ prolonged the delay by only 5 ± 2% ($n = 7, P < 0.04$), unlike pressure (10.1 MPa), which increased the delay by 31%.

Low $[Ca^{2+}]_o$ partially reproduces pressure effects on PPM

Lowering $[Ca^{2+}]_o$ from 2 to 1 mM (Fig. 9) shifted fEPSPs PPM at ISIs between 20 and 100 ms toward PPF (Rausche et al. 1988). The maximal effect of $[Ca^{2+}]_o$ was observed at the 100-ms ISI, which showed an increase in PPM from 0.99 ± 0.04 to 1.73 ± 0.26 ($n = 4$). This PPF was significantly greater than the PPF reached at 2 mM $[Ca^{2+}]_o$ under 10.1-MPa conditions (Fig. 6: 1.18 ± 0.1, P < 0.05).

Low $[Ca^{2+}]_o$ unlike high pressure, turns FDD into net FDP

As shown above, stimulation with trains at 50 Hz under 2 mM $[Ca^{2+}]_o$ resulted in pure fEPSP FDD (Fig. 7, B and D). This was the case for the controls in another set of experiments (Fig. 10, A and B), in which $[Ca^{2+}]_o$ was reduced to 1 mM. This resulted in a considerable net FDP (Fig. 10B). Stimulation with 25-Hz trains at 2 mM $[Ca^{2+}]_o$ elicited a variable degree of PPM for $E_x$, followed by FDD for successive fEPSPs (Fig. 10C). Lowering $[Ca^{2+}]_o$ to 1 mM resulted in even greater net FDP, since control FDD was smaller. Under control conditions, the decay rate of FDD between $E_x$ and $E_1$ was $-0.13 ± 0.003$ per fEPSP. Whereas at 1 mM $[Ca^{2+}]_o$, maximal FDP was usually observed at $E_2$, during the rest of the train, FDP decayed only slightly. Low $[Ca^{2+}]_o$ significantly increased FDP (ANOVA, $P < 0.003$). This effect was significantly different from the effect of 10.1 MPa pressure on FDM (ANOVA, $P < 0.02$).

It is important to note that, although pressure and low $[Ca^{2+}]_o$ identically decreased $E_1$ amplitude and slope and similarly increased PPF, they differ in their effect on frequency response. Examination of responses normalized with respect to $E_1$ under normal conditions (Fig. 11) clearly shows that neither low $[Ca^{2+}]_o$ nor pressure restored the absolute value of $E_2$, although PPF is relatively increased. Furthermore, successive responses continued to decline at high pressure, whereas under 1 mM $[Ca^{2+}]_o$, they maintained the facilitated level that may equal that of normal conditions at the end of the train.

DISCUSSION

Pressure modulation of single fEPSPs

High pressure depressed single MPP fEPSPs. This is similar to the high-pressure effect on Schaffer collateral synapses at the CA1 area of the hippocampus (Fagni et al. 1987a) and parallel fibers synapse on Purkinje cells in the cerebellum (Etzion and Grossman 2000). High pressure depressed invertebrate excitatory synapses by decreasing quantal content, $m$, as a result of reduction of release probability, $p$, and the number of active release sites, $n$, without changing the quantal size, $q$ (Golan et al. 1995). A similar quantal mechanism may explain the effect of pressure on MPP synapses. In addition to the decrease in $m$, reduction of IV and prolongation of synaptic delays, which are also observed in invertebrate synapses (Golan and Grossman 1992), suggest involvement of presynaptic factors. On the other hand, postsynaptic factors, like changes in maximal conductance of synaptic receptors, seem unlikely. Cholinergic receptors are resistant at pressures higher than the presently applied (Heinemann et al. 1987b). Data reporting glutamate non-NMDA receptor activity suggest that these receptors are also largely pressure resistant: kinetics of kainate receptors are also largely pressure resistant: kinetics of kainate (Shelton et al. 1993) and quisqualate receptors (Macdonald et al. 1993) were definitely stable at very high pressures. In

FIG. 8. Effect of low $[Ca^{2+}]_o$ on single MPP fEPSPs under normobaric conditions (0.1 MPa). A: dendritic recording of MPP fEPSPs at the inner molecular layer of the dentate gyrus at 1 and 2 mM $[Ca^{2+}]_o$. B: statistical analysis of the effect of low $[Ca^{2+}]_o$ on the different parameters of single fEPSPs ($n = 7$). Values were normalized to controls at 2 mM $[Ca^{2+}]_o$. 
contrast, pressure effect on NMDA receptors (NMDA-R) is still controversial. The increased NMDA-R fraction in hippocampal CA1 fEPSPs (Fagni et al. 1987b; Zinebi et al. 1990) may result directly from an increase in specific NMDA-R activity (Daniels et al. 1998) or indirectly due to dendritic depolarization and/or reduced GABAergic inhibition (Zinebi et al. 1990). It is worth noticing that no significant change in membrane potential and input resistance was reported for CA1 pyramidal cells recorded somatically at pressure (Southan and Wann 1996). Although a GABA<sub>α</sub> receptor was reported as insensitive to pressure (Daniels et al. 1998), it is possible that other subunits combinations would be found susceptible to pressure by reduced affinity to its neurotransmitter, as reported for glycine receptors (Roberts et al. 1996; Shelton et al. 1993). Reduction of membranal GABA<sub>α</sub> shunt may enhance the NMDA-R fraction (Staley and Mody 1992).

Lowering [Ca<sup>2+</sup>]<sub>i</sub> from 2 to 1 mM mimicked the effect of 10.1 MPa on single MPP fEPSPs, suggesting that both responses result from reduced Ca<sup>2+</sup> entry through Ca channels into MPP terminals (Etzion and Grossman 2000; Golani et al. 1994; Grossman and Kendig 1990; Grossman et al. 1991). The parallel reduction of fEPSP amplitude and slope is attributed to the decrease in release probability due to smaller Ca<sup>2+</sup> entry. Increased fEPSP decay time may result from less synchronization of synaptic release at different terminals due to the increased synaptic delay per se (Golan and Grossman 1992). However, the considerable prolongation of total delay at high pressure suggests that axonal conduction velocity at the terminals is also affected (Grossman and Kendig 1984).

**Pressure modulation of frequency response**

High pressure increased PPF and diminished PPD. The increase in PPF was most prominent at ISIs between 30 and 80 ms. PPF presumably results from accumulation of residual Ca<sup>2+</sup> at MPP terminals whenever synaptic release is not yet saturated. PPD at similar ISIs is usually attributed to the transient depletion of the releasable quanta at synaptic terminals (Stevens and Wang 1995). Therefore during vesicle depletion, the temporal summation of residual Ca<sup>2+</sup> cannot elicit additional release. If high pressure is reducing the quantal content of fEPSPs at a level below saturation, releasable vesicles...
high pressure reduces Ca$_2^+$ accumulation (or slower replenishment of the releasable pool) under pressure conditions. Simple decrease of Ca$_2^+$ accumulation may reduce facilitation, but lack of late facilitation may result from a more specific impairment in Ca kinetics like slow recovery or prolonged inactivation of a Ca-channel type (Forsythe et al. 1998; McNaughton et al. 1998). The effects of high pressure and low [Ca$_2^+$]_o on trains of fEPSPs at relatively high frequencies (25, 50 Hz) were even more divergent: 1) high pressure increased both PPF and FDD, which means that it reduced the m of E$_1$ and also p or n of E$_{n>2}$ (Golan et al. 1995); and 2) low [Ca$_2^+$]_o reduced the m of E$_1$ (presumably by reducing p) but thereafter increased the m of E$_{n>1}$. This suggests that the depression of synaptic release by high pressure is more complex than that of low [Ca$_2^+$]_o alone. Two possible mechanisms may explain this difference: either high pressure slows the rate of replenishment of active zones or it reduces the rate of Ca$_2^+$ accumulation by prolonging inactivation of certain Ca channels responsible for late facilitation. These MPP experiments partially support the hypothesis that high pressure reduces Ca$_2^+$ entry into synaptic terminals. The frequency response of the synapses raises the possibility that additional mechanism(s) involved in the replenishing of the releasable pool of quanta is slowed by exposure to pressure. Further experiments are needed to sort out which of the steps in the release mechanism, such as docking, priming, fusion (Heinemann et al. 1987a), or emptying (Rahamimoff and Fernandez 1997), is involved with pressure depression of synaptic release. Depression of single synaptic events may interrupt or even suppress the transmission of relevant information normally generated at low frequency. Enhancement of synaptic facilitation may switch the characteristic low-pass filter properties of the dentate gyrus to a higher frequency band. These changes may disrupt corticohippocampal communication in two ways: 1) by turning normal low-frequency information unreliable and 2) by allowing larger high-frequency background noise. This kind of activity may impair adequate memory task performance during HPNS (Abraini 1997). In addition, increased frequency-pass band of corticohippocampal synapses may ease the spread of a focal cortical seizure into the hippocampus (Dreier and Heinemann 1991). Indeed, high pressure induced changes in primate cortical activity. Mild EEG effects were typically characterized by increased theta waves; more severe manifestations involved focal or generalized seizures (for a review see Bennett and Rostain 2003). However, conserved FDD at the dentate gyrus will limit the spread of such activity into the hippocampal structures by just allowing the transfer of short “raids” of filtered high-frequency activity.

**Pressure and [Ca$_2^+$]_o effects**

There is consensus about the depressive effect of pressure and low [Ca$_2^+$]_o on a variety of synapses (Etzion and Grossman 2000; Golan and Grossman 1992; Grossmann and Kendig 1988, 1990). Single crustacean synapses revealed that high pressure and low [Ca$_2^+$]_o diminish the number of available active zones and slightly increased probability of release (Golan et al. 1994). Not only could pressure effects be mimicked by reduction of [Ca$_2^+$]_o, but they can also be partially compensated by an increase in [Ca$_2^+$]_o (Golan and Grossman 1992). Both high pressure and low [Ca$_2^+$]_o increased facilitation at short ISIs (10–20 ms), but results are more controversial at longer intervals (Golan and Grossman 1992; Grossman and Kendig 1988, 1990). Tetanic potentiation, a phenomenon that also depends partially on Ca$_2^+$ accumulation (Atwood and Wojtowicz 1986), was enhanced by both high pressure and low [Ca$_2^+$]_o (Grossman and Kendig 1988, 1990). Similarly, high pressure and low [Ca$_2^+$]_o increased post-tetanic potentiation (PTP) (Grossman and Kendig 1988, 1990). The effect of low [Ca$_2^+$]_o on PTP was qualitatively comparable to the effect of high pressure, but quantitatively smaller. PTP enhancement may suggest that Ca$_2^+$ removal from synaptic terminals was impaired at high pressure; however, the difference in the recovery time was not significant (Grossman and Kendig 1990). If MPP synapses accumulate Ca$_2^+$ during frequency stimulation, maintained FDD may arise from malfunction of vesicular refilling or be secondary to impaired terminal invasion by the action potential.

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**REFERENCES**


Southan AP and Wann KT. Effects of high helium pressure on intracellular and field potential responses in the CA1 region of the in vitro rat hippocampus. Eur J Neurosci 8: 2571–2581, 1996.


