Enhanced Excitability Compensates for High-Pressure-Induced Depression of Cortical Inputs to the Hippocampus

Adolfo E. Talpalar and Yoram Grossman

Department of Physiology, Faculty of Health Sciences, Zlotowski Center for Neuroscience, Ben-Gurion University of the Negev, Beer-Sheva 84105, Israel

Submitted 24 February 2004; accepted in final form 8 July 2004

Enhanced excitability compensates for high-pressure-induced depression of cortical inputs to the hippocampus. J Neurophysiol 92: 3309–3319, 2004. First published July 14, 2004; doi:10.1152/jn.00178.2004. High pressure (>1.0 MPa) induces the high-pressure neurological syndrome (HPNS) characterized by increased excitability of the CNS and cognitive impairments involving memory disorders. The perforant pathway transfer of cortical information to the hippocampal formation is important for memory acquisition. High pressure may alter information transfer in this connection. We used rat corticohippocampal slices for studying the effect of pressure on the transfer function between synaptic inputs from the medial perforant path (MPP) and spike generation by granule cells (GC) of the dentate gyrus. High pressure (10.1 MPa) reduced single MPP field excitatory postsynaptic potential (fEPSP) amplitude and slope by nearly 50%. Field antidromic action potentials (AAPs) elicited by stimulation of GC axons, and population spike (PS) generation by the pressure-depressed MPP fEPSP were not significantly altered at hyperbaric conditions. Nevertheless the relationship PS/fEPSP increased at high pressure, indicating dendritic hyperexcitability in the GC. PSs elicited by paired-pulse MPP fEPSPs at 10- to 200-ms interstimulus intervals and PS generated by trains of five fEPSPs at 25 Hz were also not affected in spite of severe pressure-induced synaptic depression. Similarly, trains of AAPs at 25–50 Hz were not significantly changed. Trains of fEPSPs at higher frequency (50 Hz), however, induced additional spikes at high pressure, indicating pressure disruption of the regular low-pass filter properties of the DG. Such effect was closely mimicked by partial blockade of GABAA inhibition. High pressure depresses synaptic activity while increases excitability in the neuronal dendrites but not in the axons. This mechanism, allowing neuronal communication at low input signals, may partially cope with pressure effects at the low frequency range (<50 Hz) but losses reliability at higher frequencies (>50 Hz).

INTRODUCTION

The high-pressure neurological syndrome (HPNS) affects depth divers exposed to pressures beyond 1.0 MPa (Rostain et al. 1983). Confusion, drowsiness, dizziness, and impairment of cognitive skills are frequent expressions of this disorder (for a review, see Jain 1994). Disturbances of memory and intellectual operation (Abraini 1997; Logue et al. 1986; Overman et al. 1989; Steevens et al. 1999; Vaernes et al. 1988) and of locomotor activity (Darbin et al. 2000; Tarasiuk and Grossman 1990) generate significant dysfunction and danger to humans working at high pressure. More severe manifestations, such as myoclonia (Darbin et al., 2000), convulsions, and death, were observed in experimental animals exposed to hyperbaric conditions (for review, see Bennett and Rostain 2003). The progressive occurrence of neurological signs is associated with increasing hyperexcitability in the electroencephalogram (EEG) (Bennett and Rostain 2003; Rostain et al. 1997; Vaernes et al. 1982) and evoked potentials (Harris et al. 1985; Lorenz et al. 1995; Vaernes and Hammerborg 1989). The mechanisms affecting human cognitive function at pressure are not yet clear but seemingly are generated by network dysfunction at respective cerebral areas (Abraini 1997). Learning and memory are related to the function of corticohippocampal areas in primates (Scoville and Milner 1957; Squire and Zola-Morgan 1991; Zola-Morgan et al. 1982) and in rats (Ferbinteanu et al. 1999; Quirk et al. 1992). Normal communication between the medial entorhinal cortex and the dentate gyrus (DG), the first station in the corticohippocampal circuit, is frequency dependent, displaying low-pass filter properties (Dreier and Heinemann 1991). The medial perforant pathway (MPP) conveys this connection (Swanson and Kohler 1986), and its synapses on granule cells (GC) show frequency-dependent depression (FDD) when stimulated at frequencies >1 Hz (Kilbride et al. 2001). Recently, we have demonstrated a significant depression in the performance of these synapses under high-pressure conditions. Single inputs of the MPP synapse (Talpalar and Grossman 2003) as well as the Shaffer collateral synapse at the CA1 area of the hippocampus (Fagni et al. 1987a), were severely depressed under high pressure. This depression of single synaptic events was associated with increased paired-pulse facilitation (Talpalar and Grossman 2003). Previous studies in invertebrate synapses showed increased facilitation (Campenot 1975; Golan and Grossman 1992) as well as enhanced tetanic and posttetanic potentiation (Grossman and Kendig 1988, 1990). In contrast, although high pressure increased facilitation in the MPP synapses, stimulation at frequencies (25–50 Hz) resulted, respectively, in conserved or even increased FDD (Talpalar and Grossman 2003). These synaptic modifications may alter the filter properties of the DG and consequently disrupt the normal decoding of entorhinal cortex firing by the GCs. This may result in altered memory acquisition (Moser and Andersen 1994) or even induce hippocampal seizures at the seizure-prone CA3 area (Scharfman and Schwartzkroin 1990), which is innervated by the GCs. Weakening of synaptic inputs seems paradoxical in view of the obvious signs of hyperexcitability during the HPNS. We used electrophysiological techniques in rat corticohippocampal slices to investigate the effect of high pressure of helium on the excitability of DG’s GCs as a model for HPNS. For this
purpose, we compared population spike generation in response to synaptic activation of the MPP and the antidromic stimulation of GCs axons during single and frequency stimulation.

**METHODS**

**Brain preparation**

Sprague-Dawley rats of both sexes (150–250 g) were killed (pentobarbital, 60 mg/kg); their brain was extracted (<1 min) and submerged in cold Ringer solution (4–6°C). Corticohippocampal slices (400 µm) were prepared as previously described (Drejer and Heinemann 1991; Talpalar and Grossman 2003). 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 CaCl₂, 2 MgSO₄H₂O, 1.25 NaH₂PO₄, 26 NaHCO₃, and 10 D-glucose. Solution was constantly bubbled with 95% O₂-5% CO₂ for a pH of 7.4.

**Pressure and compression**

Electrophysiological experiments were carried out in a pressure chamber (Canty Assoc.). The chamber was provided with an internal experimental bath containing a pair of stimulation electrodes and a temperature gauge. The slice, lying on the surface of the bath, was superfused with prewarmed Ringer solution. A remote-controlled manipulator allowed the placement of the recording pipette in different areas of the corticohippocampal slice (Talpalar and Grossman 2003). Hyperbaric pressure was attained by compressed helium, a gas that is chemically inert at these pressures (0.1–10.1 MPa). Some of the controls were taken at 0.2–0.4 MPa because these small pressures were more stable than at 0.1 MPa for further pressurization. Although a small population of medullary neurons was shown to be sensitive to these small pressures (Dean and Mulkey 2000), they did not significantly modified field potential recordings in corticohippocampal slices (Talpalar and Grossman 2003) and cerebellar preparations (Etzion and Grossman 2000). Rates of compression-decompression varied between 0.15 and 0.2 MPa/min. Samples were taken at control (0.1–0.4 MPa) and 5.1 and 10.1 MPa. Ringer solution (saturated at normal temperature) superfused with prewarmed Ringer solution (4–6°C). Corticohippocampal slices were more stable than at 0.1 MPa for further pressurization. Although small pressures of medullary neurons was shown to be sensitive to these small pressures (Dean and Mulkey 2000), they did not significantly modified field potential recordings in corticohippocampal slices (Talpalar and Grossman 2003) and cerebellar preparations (Etzion and Grossman 2000). Rates of compression-decompression varied between 0.15 and 0.2 MPa/min. Samples were taken at control (0.1–0.4 MPa) and 5.1 and 10.1 MPa. Ringer solution (saturated at normal pressure with 95% O₂-5% CO₂) was injected into the experimental bath by a fast high-pressure pump (LDC analytical minipump). To avoid transient effects of pressure (Grossman and Kendig 1984), samples were collected under strict conditions of temperature (30°C) and at least after 15–20 min of stable recording. This time excludes the time needed for stabilization of temperature transients of ±2°C during the periods of compression-decompression (~32 and 28°C, respectively). Decompression was routinely attempted to prove reversibility of high-pressure effects. Complete recovery was attained during decompression from 10.1 to 5.1 MPa in all the experiments, whereas almost complete recovery was observed in decompression from 5.1 to 0.4 MPa. Decompression <0.4 MPa was only successful in ~50% of the experiments (in which this final step of decompression was performed extremely slowly).

**Electrophysiological recordings**

Extracellular field potentials were recorded at the somatic (Fig. 1C) and at the inner dendritic area (Fig. 1B) of the DG using glass micropipettes (1.5–3 MΩ) filled with Ringer solution (Fig. 1A). Tungsten bipolar stimulating electrodes were placed either at the subiculum or the inner dendritic area of the DG for stimulating the MPP (Fig. 1A). Output/input curves were plotted using standardized stimulus-intensities (usually 4–5 steps, if not otherwise expressed) between the threshold intensity and the saturation level. The latter involved a set of experiments in which population spike (PS) amplitude was plotted as a function of field excitatory postsynaptic potential (fEPSP) of different amplitudes (6–10 intensities of stimulus) under control and experimental pressures.

**Data analysis**

Synaptic recordings of MPP fEPSPs at the inner dendritic region of the DG, its parameters, and their interpretation, were described elsewhere (Talpalar and Grossman 2003). In brief, fEPSP’s amplitude, indicating inward synaptic currents, and fEPSP’s slope expressing the rate of activation of synaptic receptors were used in these experiments for assessing synaptic activity. fEPSP’s slopes were commonly used because they are more reliable than fEPSP’s amplitude as a parameter for evaluating synaptic activation (Fagni et al. 1987a; Talpalar and Grossman 2003). GC excitability at high pressure was assessed in two ways: by generation of PS in response to MPP fEPSPs and by the induction of AAPs. The first describes the integrative capacity of the GC in response to synaptic activation; the second roughly delineates the general ability of GC to elicit spikes. Two different protocols were used for studying excitability at frequencies: paired-pulse stimulation
and multiple stimuli. Pairs of stimuli, with 10- to 100-ms interstimulus-interval (ISI), were delivered every 20 s. Slope of both fEPSPs in the pair, E1 and E2, and the PS generated were compared. Results were plotted at the normalized form E2/E1 and PS2/PS1 for each ISI. Paired-pulse depression (PPD) and paired-pulse facilitation (PPF) were used for describing respectively relative decrease or increase of E2 with respect to E1. The expression paired-pulse modulation (PPM; negative or positive) was used as a generic term. Trains of five impulses at frequency (25–50 Hz), delivered at a rate of one train per minute, were applied to the orthodromic afferent fibers (MPP) and antidromically to the mossy fibers. Analysis of these responses involved the measurement and comparison of each response in the train at each frequency and the comparison of the effect of frequency on amplitude, integral, or rates. Events were usually normalized with respect to the first event. We attempted to fit the observed patterns with mathematical functions (e.g., exponential decay) to be used for comparison under various conditions. Frequency-dependent potentiation (FDP), frequency-dependent depression (FDD) and the generic term frequency-dependent potentiation (FDP; positive or negative) were used for such synaptic behaviors.

The results of these experiments unless otherwise stated are expressed as means ± SE. The n expresses the number of slices successfully used in each experimental protocol. Given the time-consuming experiments at hyperbaric pressure usually just a single slice from each animal could be successfully used per experiment. Statistical tests were used for comparing the effect of various conditions on the electrophysiological signals (slope, amplitude, time constant, etc.). Student’s t-test for paired observations was used for comparing parameters at control and under experimental conditions in the same slice/experiment. ANOVA tests (1-way independent and for repetitive measurements) were used for comparing independent results and sets of stimuli at frequency. Degree of significance was indicated by values of P (results were considered statistically different for P < 0.05).

RESULTS

High pressure increases GCs excitability in response to MPP fEPSPs

The MPP input innervates the proximal dendrites of the GCs. Spike generation by the GCs depends on their threshold for action potential and on the amplitude of MPP synaptic potentials that activate them. Because MPP fEPSP’s slope is depressed by ~20% at 5.1 MPa and by ~55% at 10.1 MPa (Talpalar and Grossman 2003), we had to normalize the synaptic input to evaluate these cells’ excitability.

As a first approximation, we compared PS generation by fEPSPs the slopes of which at high pressure were matched to control fEPSP’s slope by increasing the stimulus intensity or duration. PS amplitude at pressure was significantly greater than that generated by equal fEPSPs at 0.1 MPa (Fig. 2A). For example, a just-suprathreshold fEPSP at 0.1 MPa generated a PS of 0.22 ± 0.21 mV, a fEPSP of the same slope induced PSs of 0.94 ± 0.29 mV at 5.1 MPa (n = 9; P < 0.002) and of 0.73 ± 0.21 mV at 10.1 MPa (n = 9, P < 0.05).

A second method was employed to normalize the stimulus intensity (i.e., the number of activated presynaptic axons). The MPP was stimulated with three different intensities calibrated at 0.1 MPa to evoke supramaximal, medium intensity (70% of maximal) and half-maximal (50%) fEPSPs (Fig. 2B).

Despite the pressure depression of the MPP fEPSPs (as in the preceding text), the PSs generated under these stimulus and pressure conditions were not significantly different from controls (see Table 1).

The amplitudes of PSs induced at high pressure were not statistically different from 0.1 MPa (except supramaximal fEPSP at 10.1 MPa that tended to be smaller). Nevertheless, PS’s amplitudes at 10.1 MPa were significantly smaller than at 5.1 MPa. Table 1 shows that this tendency was clearer for PS generated by submaximal fEPSPs. Extreme depression of MPP fEPSP input at 10.1 MPa may have reduced PS generation in spite of increased GC’s excitability. This effect, however, was accompanied with progressive broadening of the PS; this may mean that in spite of temporal dispersion the PS integral tended to remain the same. Because the PSs were induced by progressively depressed fEPSPs, which presumably were elicited by activation of equal number of MPP axons for each stimulus intensity (Talpalar and Grossman 2003), it seems that cortico-hippocampal transmission code for single response is kept “constant” due to some “compensation” mechanism. To char-

![FIG. 2.](http://jn.physiology.org/)

**High pressure depresses MPP fEPSPs but conserves GC’s PS firing.** MPP fEPSP recorded at the proximal dendritic area of the dentate gyrus showing PS generated at 0.1, 5.1, and 10.1 MPa. A: high pressure increased PS generation by control-matched fEPSPs. The intensity of the electric stimulus was increased at hyperbaric pressure to allow depressed fEPSPs to match control fEPSP’s slope by increasing the stimulus intensity or duration. PS amplitude at pressure was significantly greater than that generated by equal fEPSPs at 0.1 MPa (Fig. 2A). For example, a just-suprathreshold fEPSP at 0.1 MPa generated a PS of 0.22 ± 0.21 mV, a fEPSP of the same slope induced PSs of 0.94 ± 0.29 mV at 5.1 MPa (n = 9; P < 0.002) and of 0.73 ± 0.21 mV at 10.1 MPa (n = 9, P < 0.05).

A second method was employed to normalize the stimulus intensity (i.e., the number of activated presynaptic axons). The MPP was stimulated with three different intensities calibrated at 0.1 MPa to evoke supramaximal, medium intensity (70% of maximal) and half-maximal (50%) fEPSPs (Fig. 2B).

Despite the pressure depression of the MPP fEPSPs (as in the preceding text), the PSs generated under these stimulus and pressure conditions were not significantly different from controls (see Table 1).

The amplitudes of PSs induced at high pressure were not statistically different from 0.1 MPa (except supramaximal fEPSP at 10.1 MPa that tended to be smaller). Nevertheless, PS’s amplitudes at 10.1 MPa were significantly smaller than at 5.1 MPa. Table 1 shows that this tendency was clearer for PS generated by submaximal fEPSPs. Extreme depression of MPP fEPSP input at 10.1 MPa may have reduced PS generation in spite of increased GC’s excitability. This effect, however, was accompanied with progressive broadening of the PS; this may mean that in spite of temporal dispersion the PS integral tended to remain the same. Because the PSs were induced by progressively depressed fEPSPs, which presumably were elicited by activation of equal number of MPP axons for each stimulus intensity (Talpalar and Grossman 2003), it seems that cortico-hippocampal transmission code for single response is kept “constant” due to some “compensation” mechanism. To char-

![FIG. 2.](http://jn.physiology.org/)

**High pressure depresses MPP fEPSPs but conserves GC’s PS firing.** MPP fEPSP recorded at the proximal dendritic area of the dentate gyrus showing PS generated at 0.1, 5.1, and 10.1 MPa. A: high pressure increased PS generation by control-matched fEPSPs. The intensity of the electric stimulus was increased at hyperbaric pressure to allow depressed fEPSPs to match control fEPSP’s slope by increasing the stimulus intensity or duration. PS amplitude at pressure was significantly greater than that generated by equal fEPSPs at 0.1 MPa (Fig. 2A). For example, a just-suprathreshold fEPSP at 0.1 MPa generated a PS of 0.22 ± 0.21 mV, a fEPSP of the same slope induced PSs of 0.94 ± 0.29 mV at 5.1 MPa (n = 9; P < 0.002) and of 0.73 ± 0.21 mV at 10.1 MPa (n = 9, P < 0.05).

A second method was employed to normalize the stimulus intensity (i.e., the number of activated presynaptic axons). The MPP was stimulated with three different intensities calibrated at 0.1 MPa to evoke supramaximal, medium intensity (70% of maximal) and half-maximal (50%) fEPSPs (Fig. 2B).

Despite the pressure depression of the MPP fEPSPs (as in the preceding text), the PSs generated under these stimulus and pressure conditions were not significantly different from controls (see Table 1).

The amplitudes of PSs induced at high pressure were not statistically different from 0.1 MPa (except supramaximal fEPSP at 10.1 MPa that tended to be smaller). Nevertheless, PS’s amplitudes at 10.1 MPa were significantly smaller than at 5.1 MPa. Table 1 shows that this tendency was clearer for PS generated by submaximal fEPSPs. Extreme depression of MPP fEPSP input at 10.1 MPa may have reduced PS generation in spite of increased GC’s excitability. This effect, however, was accompanied with progressive broadening of the PS; this may mean that in spite of temporal dispersion the PS integral tended to remain the same. Because the PSs were induced by progressively depressed fEPSPs, which presumably were elicited by activation of equal number of MPP axons for each stimulus intensity (Talpalar and Grossman 2003), it seems that cortico-hippocampal transmission code for single response is kept “constant” due to some “compensation” mechanism. To char-
acterize this process more precisely, we stimulated the MPP with 10 different stimulus intensities (Fig. 3A) and normalized the synaptic transfer function by plotting PS amplitudes as a function of the fEPSPs’ slopes (Fig. 3B). This input/output plot shifted to the left at pressure, indicating hyperexcitability of the GCs. Namely, similar PSs could be elicited with smaller fEPSPs under pressure conditions.

High pressure does not change GCs antidromic action potentials

As suggested in the preceding text, the increase in the PS excitability may arise from changes in the action potential firing capability of the GCs. Therefore we examined the effects of pressure on antidromically evoked action potentials in these cells (Fig. 4A), a method that does not involve any synaptic activation. Pressure effects on the AAP are summarized in Fig. 4B. Increasing pressure from 0.1 to 5.1 MPa and to 10.1 MPa reduced AAP initial slope by 28 and 29%, respectively. The slope of decay of AAP was not statistically different at 5.1 MPa and showed a trend toward reduction by 16% at 10 MPa. AAP’s duration was prolonged by 21% at 5.1 MPa and by 20.4% at 10.1 MPa. The amplitude of the AAP at 5.1 MPa and at 10.1 MPa was not statistically different from controls. APP integral was almost constant at the three different pressures (Fig. 4). The small-amplitude reduction of AAPs under hyperbaric conditions seems to be a direct effect on the kinetics of the action potential. Reduction of the AAP’s initial slope may be interpreted as a slowing down of the kinetics of activation of the Na current responsible for the rising phase of the action potential, whereas the maintained or slightly increased decay rate suggests a small effect on the inactivation of Na currents and/or on the kinetics of K currents responsible for the decay of action potentials (Kendig 1984). The small depression of AAP amplitude and especially the lack of effect on AAP’s integral suggest that the effect of pressure on the amplitude of the action potential or on the total number of recruited fibers was minimal and, if at all, it was depressive.

These results, taken together with the depression of fEPSPs inputs, strongly indicate that the enhancement of PS firing by MPP fEPSPs at pressure stems from some boosting mechanism of the inputs generated at the dendritic region of these cells.

### Table 1. High-pressure effect on PS generated by fEPSPs of different intensities

<table>
<thead>
<tr>
<th>Stimulus intensity, (% maximal)</th>
<th>Pressure</th>
<th>PS Amplitude, mV</th>
</tr>
</thead>
<tbody>
<tr>
<td>50% (n = 8)</td>
<td>0.1 MPa</td>
<td>0.64 ± 0.26</td>
</tr>
<tr>
<td></td>
<td>0.1–5.1 MPa</td>
<td>0.26</td>
</tr>
<tr>
<td></td>
<td>5.1 MPa</td>
<td>0.87 ± 0.33</td>
</tr>
<tr>
<td></td>
<td>5.1–10.1 MPa</td>
<td>0.02</td>
</tr>
<tr>
<td></td>
<td>10.1 MPa</td>
<td>0.52 ± 0.24</td>
</tr>
<tr>
<td></td>
<td>0.1–10.1 MPa</td>
<td>0.58</td>
</tr>
<tr>
<td>70% (n = 9)</td>
<td>0.1 MPa</td>
<td>0.85 ± 0.11</td>
</tr>
<tr>
<td></td>
<td>0.1–5.1 MPa</td>
<td>0.33</td>
</tr>
<tr>
<td></td>
<td>5.1 MPa</td>
<td>1.28 ± 0.48</td>
</tr>
<tr>
<td></td>
<td>5.1–10.1 MPa</td>
<td>0.06</td>
</tr>
<tr>
<td></td>
<td>10.1 MPa</td>
<td>0.71 ± 0.27</td>
</tr>
<tr>
<td></td>
<td>0.1–10.1 MPa</td>
<td>0.49</td>
</tr>
<tr>
<td>&gt;100% (n = 13)</td>
<td>0.1 MPa</td>
<td>1.19 ± 0.29</td>
</tr>
<tr>
<td></td>
<td>0.1–5.1 MPa</td>
<td>0.81</td>
</tr>
<tr>
<td></td>
<td>5.1 MPa</td>
<td>1.13 ± 0.4</td>
</tr>
<tr>
<td></td>
<td>5.1–10.1 MPa</td>
<td>0.077</td>
</tr>
<tr>
<td></td>
<td>10.1 MPa</td>
<td>0.78 ± 0.26</td>
</tr>
<tr>
<td></td>
<td>0.1–10.1 MPa</td>
<td>0.06</td>
</tr>
</tbody>
</table>

Values are means ± SE. p, statistical significance for the indicated range; PS, population spike; fEPSP, field excitatory postsynaptic potential.
amplitude as a function of fEPSP’s initial slope resulted in a steeper slope of the fEPSP’s amplitude/slope relationship at high pressure (n/H11005 17). This suggests that synaptic inputs were either more efficiently conducted through the dendrites, or maybe even slightly amplified under such condition (Fig. 4C).

*Increased GC excitability maintains PS firing during MPP paired-stimulation at high pressure*

Paired-pulse stimulation to the MPP resulted in a couple of fEPSPs in the GCs (E1 and E2), the second one eliciting a PS with greater amplitude. We termed this behavior paired-pulse transfer (PPT). Under control conditions, PPT extended from ISIs of 10 to ~120 ms. The greatest PPT occurred at ISIs between 10 and 40 ms (Fig. 5, A and B). As we recently reported, high pressure depressed single E1 while increasing synaptic facilitation of E2, but the absolute size of synaptic inputs was smaller than the control (Talpalar and Grossman 2003). The time pattern of control PPT did not significantly change at 5.1–10.1 MPa pressure in spite of depression of synaptic inputs (Fig. 5C). Furthermore, two additional experiments extending the ISI range to 200 ms did not show significant difference in PPT (not shown).

The amplitudes of the second PS (PS2) at 0.1 MPa (10–40 ms ISI) tended to be larger than those at 10.1 MPa, but the difference did not reach statistical significance (Fig. 5C).

Increasing the stimulus intensity of E1 at 5.1 and 10.1 MPa to match the amplitude of control E1 produced a PS1 that was larger than controls (similar to that described for single stimulus), whereas the PS2 brought about during paired-pulse stimulation, for example at 20 ms ISI, was larger than that generated at 0.1 MPa (Fig. 5D).

*High pressure increases DG excitability during stimulation at frequency*

As we have recently shown (Talpalar and Grossman 2003), stimulation of the MPP with short trains at frequencies of 10–100 Hz elicited fEPSPs that showed a variable degree of facilitation of E2 and then followed by progressively decaying E3–5 as a function of frequency and use. This FDD of the fEPSPs was modulated by high pressure: at 25 Hz, FDD was not significantly altered, whereas at 50 Hz, it was increased. Similarly, under control conditions, stimulation at 25–50 Hz generated the largest PS at E2 (PPT, as described in the preceding text), whereas amplitudes of PSs induced by the following E5 decayed as a function of time and.

---

FIG. 4. High-pressure effects on GCs excitability: axonal-antidromic and synaptic-orthodromic stimuli. A: somatic recording of GC antidromic action potential (AAP) induced by stimulation of the mossy fibers at the hilus at 0.1, 5.1, and 10.1 MPa pressure. B: high-pressure effects on the different parameters of the AAP. Values were normalized with respect to equivalent parameters at control (0.1 MPa). Note that initial slope and duration of the AAP were very sensitive to pressure (n = 8, *P < 0.03, **P < 0.02). In contrast, AAP’s amplitude was not significantly affected by pressure, whereas the integral was the most constant parameter at all pressures. C: correlation between amplitude and slope in subthreshold fEPSPs at different pressures (n = 17). Linear regressions show that high pressure produced a steeper slope in this relationship (y = Bx). The regressions for 5.1 and 10.1 MPa have similar slopes and therefore were superimposed.
frequency (Fig. 6). We termed this phenomenon frequency-dependent attenuation (FDA) of the PS.

PS generation during trains at 25 Hz (Fig. 6A) was conserved. Although pressure depressed the fEPSPs (Fig. 6B), they still generated similar or just more intense PSs firing at this frequency (Fig. 6C; n = 11005). Maintenance of PSn’s amplitudes at high pressure was supported by increased excitability for all En in the train (Fig. 6D), which resulted in some tendency to hyperexcitability at E3 (Fig. 6C). This indicates that DG information transfer was roughly conserved at 25 Hz in spite of depressed MPP inputs. To assess if this mechanism was reliable at a higher frequency range, we studied this same system at 50 Hz. Figure 7 shows that high pressure severely depressed En inputs (fEPSPs) at 50 Hz (Fig. 7B). These inputs are able to initially maintain PS output, but then the system “loses control” allowing additional PS firing at later En in the train (Fig. 7, A, arrows, and C). High pressure (5.1 and 10.1 MPa) increased the absolute number of detectable PSs per train from 2.89 ± 0.48 at control to 3.56 ± 0.47 and to 3.56 ± 0.44 at 5.1 and 10.1 MPa, respectively (n = 11, P < 0.02 and P < 0.08, respectively). This was supported by progressively increasing excitability during the train (Fig. 7D), which seemed to begin to self-restrict at the end of the train under 10.1 MPa.

In contrast with increased excitability in response to synaptic stimulation, AAPs elicited by antidromic stimulation of the mossy fibers with trains at 25–50 Hz did not show relevant patterns of FDM at both control and high-pressure conditions (Fig. 8, A and B).

PPT during the first two responses at frequency is probably dependent on N-methyl-D-aspartate (NMDA) receptor activity (Joy and Albertson 1993), while later FDA of the following responses in the train is possibly the result of GABAergic inhibition (Golan et al. 1996; Buckmaster and Dudek 1997). We therefore tested whether the reduction of FDA at 50 Hz may result from decreased inhibition at the DG. Partial blockade of GABA\(_A\) inhibition with low-dose bicuculline methiodide (BMI, 0.3 \(\mu\)M) mimicked pressure effect on FDA (Fig. 9, A and B). Moreover, in presence of high dose of BMI (10 \(\mu\)M) high pressure did not further increase GC excitability during trains at 50 Hz but rather decreased it (n = 2; Fig. 9C). This suggests that hyperexcitability at high pressure partially involves synaptic depression of inhibitory pathways.

**Discussion**

**Effects of pressure on synaptic activity**

Pressure effects of synaptic inputs per se confirmed our recent report (Talpalar and Grossman 2003). In short, at 10.1...
MPa, single MPP fEPSPs were depressed by \(\sim 50\%\). These events displayed longer synaptic delay and prolonged time course. Paired-pulse protocols resulted in depression of the first fEPSP \((E_1)\) and in relative reduction of PPD at short ISIs (10 to 40 ms) and in some degree of PPF at ISIs of between 40 and 120 ms. In spite of less PPD and larger PPF, the absolute second fEPSP \((E_2)\) at high pressure remained smaller than controls. The effect of high pressure on short-term synaptic plasticity depended on the frequency of stimulation: trains of five stimuli at 25 Hz displayed the same rate of FDD, whereas stimulation at 50 Hz displayed greater FDD (Talpalar and Grossman 2003). This resulted in relatively conserved \(E_5\) at 25 Hz and in further depression of this response at 50 Hz. Regarding the efficacy of fEPSPs at high pressure, the present

FIG. 6. Effect of high pressure on GC firing during synaptic stimulation of the MPP with short trains at 25 Hz. A: field recordings at the granule cell layer of the DG. B: course of synaptic frequency-dependent modulation (FDM) of the fEPSP at 3 different pressures. Note that in spite of increased facilitation at high pressure, the fEPSPs the input \((E_1)\) does not reach control’s magnitude (means \(\pm\) SE, \(n = 9\)). C: PS generated by the train of fEPSPs at 25 Hz (means, \(n = 9\)). D: dynamics of excitability during stimulation at 25 Hz. Excitability \((PS/E_0)\) was calculated for each \(E_n\) in the train and normalized with respect to the excitability of \(E_1\) under control pressure condition.

FIG. 7. Effect of high pressure on GC firing during synaptic stimulation of the MPP with short trains at 50 Hz. A: field potentials recorded at the granule cells layer of the DG. B: course of FDM of the fEPSP at 0.1, 5.1, and 10.1 MPa. Note that in spite of the enhancement of facilitation on the synaptic input \((E_1)\) at high pressure, the absolute slope of the fEPSPs does not reach control’s magnitude (means \(\pm\) SE, \(n = 11\)). C: course of PS generation by fEPSPs at 50 Hz (means \(\pm\) SE, \(n = 11\)). D: dynamics of excitability during stimulation at 50 Hz. Excitability \((PS/E_0)\) was calculated for each \(E_n\) in the train and normalized with respect to the excitability of \(E_1\) under control pressure conditions.
experiments show that for single fEPSP the amplitude/slope relationship tended to increase at high pressure.

**GCs excitability**

GCs, in spite of the pressure depression of synaptic inputs, supported regular spike firing in response to a single MPP synaptic stimulus. Adjustment of the pressure-depressed synaptic inputs to control levels resulted in significant enhancement of population spike generation. These results indicate increased excitability of the GC. The maximal absolute amplitude of PS induced by any standardized fEPSP often occurred at 5.1 MPa. At 10.1 MPa, the amplitude of PS induced by a similar stimulus-intensity tended to be reduced presumably because of the extreme synaptic depression and the temporal dispersion of the PS. However, the PS/fEPSP relationship continued rising while increasing pressure (Fagni et al. 1987a). AAPs showed a pressure-resistant integral, a slightly reduced amplitude, and prolonged initial slope and duration. Stable integral suggests that the total number of recruited fibers was not changed. This means that changes in threshold and action potential amplitude may be minor. These findings are in accord with our recent report of minor effects of pressure in the main axons and presynaptic volley of MPP axons in the same system (Talpalar and Grossman 2003). Pressure-enhancement of excitability of CA1 pyramidal cells was also paradoxically correlated with depression of both synaptic inputs and antidromic action potentials (Fagni et al. 1987a). In addition, action potentials of CA1 pyramidal cells were also not significantly affected by pressure as shown by intracellular recordings (Southan and Wann 1991, 1996). Slow AAP rates may result from slowing down of the kinetics of individual action potentials and also by their unsynchronized recruitment. Differential effects on the conduction velocity of different kind of axons (Grossman and Kendig 1984) may result in unsynchronized AAPs. Slowing down of Na and K currents kinetics may induce these effects (Conti et al. 1982a,b; Grossman and Kendig 1984; Harper et al. 1981; Heinemann et al. 1987). Increased GC excitability in spite of synaptic depression and small changes in action potential suggests that pressure affects dendritic integrative properties. Hyperexcitability may be in-

FIG. 8. High pressure does not enhance AAPs generation at frequency. Single experiments showing trains of AAPs recorded in the GC’s layer of the dentate gyrus at 0.1, 5.1, and 10.1 MPa. AAPs were generated by stimulation at the hilus of the DG at 25 Hz (A) and at 50 Hz (B). Note that AAPs at pressure do not show hyperexcitability but rather slight progressive use- and time-dependent depression at 10.1 MPa.

FIG. 9. High-pressure effects on GC’s excitability during stimulation at frequency are mimicked by partial blockade of GABA<sub>A</sub>-mediated inhibition. A: recordings at the GC’s layer of the DG showing the effect of pressure on PS generation (↓) by a train in response to MPP synaptic stimulation at 50 Hz. B: similar recordings at atmospheric pressure show occurrence of later spikes at PS<sub>3–4</sub> after the progressive effect of bicuculline methiodide (BMI 0.3 μM). C: application of GABA<sub>A</sub> blocker (BMI 10 μM) significantly increases GC excitability during stimulation at 50 Hz (top). When this kind of inhibition has been blocked, high pressure not only does not increase (5.1 MPa) but also depresses (10.1 MPa) PS generation (↓).
duced by intrinsic “boosting” mechanism(s) or reduced tonic inhibition (see following text) at strategic regions of the dendrites (Faber and Korn 1986; Fagni et al. 1987a). The shift in the relationship between fEPSP’s slope and amplitude at high pressure may suggest boosting (Fagni et al. 1987a). Although lengthening of the decay time constant of EPSPs at pressure may reflect general effects on excitatory synapses (Ashford et al. 1982), the highly pressure-sensitive lengthening of fEPSP’s τ decay (Talpalar and Grossman 2003) may indicate increase of the NMDA receptor (NMDA-R) component (Fagni et al. 1987b; Roberts et al. 1996; Wardley-Smith and Wann 1989) or alternatively activation of voltage-dependent channels (Faber and Korn 1986; Harper et al. 1981; Stuart and Sakmann 1995). Action potential generation at the dendrites (Stuart and Sakmann 1994; Stuart et al. 1997a,b) and boosting of EPSPs by voltage-dependent currents have been observed in pyramidal cells of the neighboring CA1 (Spruston et al. 1995) and CA3 areas (Buzsaki et al. 1996) of the hippocampus as well as in other neurons (Hausser et al. 1995). Although these phenomena have not yet been studied in DG GCs, they are probably present in these cells and may influence the EPSP-to-spike transfer. Because the antidromic spikes (AALs) were unchanged or even depressed at high pressure, it is inferred that there were no changes toward hyperexcitability at the somata of these cells. Because the soma is the area where tonic inhibition was postulated to exert its most prominent effects (Soltész et al. 1995), it is also speculated that the effects of pressure on tonic inhibition were not very relevant.

Feedforward inhibition, affecting the efficacy of perforant path inputs at the GCs has also been described at the DG (Kneisler and Dingledine 1995). The GABA_A-mediated component of this input induces a shunt that decreases the late NMDA-dependent phase of the EPSP without affecting its early AMPA-mediated phase (Staley and Mody 1992). In spite of this effect, application of GABA_A blocker to the present preparation (see E1 in Fig. 9, B and C) failed to produce relevant effects on PS generation by a single stimulus. This lack of disinhibition may have resulted from disconnection of the MPP pathway that activates the inhibitory neurons due to the angle of slicing. Thus probably the maintenance of PS generation by a single fEPSP may be attributed to changes in the membrane-passive properties, the kinetics of voltage-dependent conductances, and/or NMDA-R activation. However, a more powerful feedforward inhibition, in the CA1 area of the hippocampus, was shown to decrease under high-pressure conditions (Zinebi et al. 1988). Because activation of feedforward inhibition at the DG requires recruitment of at least two synaptic inputs, it is likely that inhibition will be more susceptible to high-pressure effects than excitatory inputs. Therefore we cannot exclude some contribution of reduced feedforward inhibition to pressure-induced hyperexcitability.

Frequency response

Despite that pressure depressed paired fEPSPs, the PSs induced by them were similar to control indicating increased excitability of the GCs. Likewise, conserved spike generation was observed during stimulation with trains at 25 Hz. Trains of fEPSPs at higher frequency (50 Hz), however, showed roughly preserved PS1–2 but enhanced PS3–4, indicating hyperexcitability and disruption of the low-pass filter properties of the DG. This means that although this system copes quite well with the effects of high pressure at single stimulus and low-frequency responses, it fails to do so at high frequencies.

The regular mechanism for limitation of action potential generation by GC involves activation of recurrent inhibition by hilar interneurons (Gulyas et al. 1993). This mechanism was apparently altered at high pressure (Zinebi et al. 1988) probably as a result of general synaptic depression. This may allow PS generation also at later events (PS3–4) during the train. Partial blockade of GABA_A inhibition reproduced this effect, suggesting that pressure was partially blocking fast inhibitory function (Roberts et al. 1996; Zinebi et al. 1990). Furthermore, the fact that high pressure did not enhance PS generation at high frequency when fast inhibition was previously blocked suggests that enhanced excitability is, at least partially, due to GABA_A inhibition failure. This effect may result from proportional reduction of synaptic inputs (mostly involving activation of >2 synapses) necessary for the inhibitory function. Frequency dependence may be explained by slowed kinetics and conduction at high pressure leading to delayed inhibitory influences.

Increased excitability: an adaptive mechanism?

Changes induced by pressure in the PS/fEPSP relation at the low-frequency range seem to be an adaptive stabilizing mechanism. High pressure progressively depresses synaptic inputs while it increases excitability of the circuit. This is unlikely to induce the large hyperexcitability described in HPNS. The reliability of this mechanism may be challenged by extremes of intensity and frequency of neuronal activity. Transfer of regular activity from the cortex to the DG may be impaired if too depressed synaptic inputs cannot reach action potential threshold. However, highly synchronized activity and high frequency may easily spread through the DG to the CA3–CA1. Thus in some circumstances, pressure-induced changes may impair normal network function while favoring spread of anomalous activity. The postulated postsynaptic boosting of presynaptically depressed inputs may be interpreted as an adaptive strategy of the system for maintaining neural transmission under progressively lower incoming signals (Abraham and Bliss 1985).

GRANTS

This study was partially supported by a research grant from Ben-Gurion University to Y. Grossman.

REFERENCES


J Neurophysiol • VOL 92 • DECEMBER 2004 • www.jn.org


