Amino Acids (1991) 1: 47–56

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Excitatory and inhibitory amino acids involved in the high pressure nervous syndrome: epileptic activity and hyperexcitability

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Summary. Epileptic-like activities are observed in mammals exposed to ambient pressures higher than 20 atm. These symptoms are part of the so called "high pressure nervous syndrome". In the search of the cellular mechanisms of this syndrome, we examined synaptic and intrinsic pressure-induced changes in the in vitro hippocampal slice preparation in the rat. We found that pressure (80 atm) depresses the efficiency of excitatory amino acidergic and inhibitory GABA synaptic transmissions, while it increases the intrinsic excitability of the CA1 pyramidal cells and induced multiple population spikes. The changes were associated with a selective increase in the effects of NMDA and *L*-homocysteate, while the postsynaptic effects of GABA was unchanged. NMDA antagonists and GABA synergistic drugs antagonized the pressure-induced hyperexcitability and multiple population spikes. These results suggest that pressure would decrease transmitter release at the tested excitatory and inhibitory synapses and would facilitate NMDA postsynaptic mechanisms. Thus, changes in both NMDA and GABA processes might be involved in the development of the high pressure nervous syndrome.

Keywords: Pressure – Excitatory amino acids – GABA – Hippocampal slice – Synaptic transmission – Excitability

Introduction

Helium pressure induces various neurological disturbances in animals and man: the high pressure nervous syndrome [2, 3, 14, 18]. This syndrome includes tremor, myoclonies, convulsions and epileptic seizures when animals are exposed to pressure higher than 20 atm. High pressure also induces changes in

EEGraphic activity [24]. Nevertheless, these pressure effects on the central nervous system are still not understood, possibly because of the relative complexity of the integrated systems usually studied. In vivo studies revealed that central nervous structures are pressure sensitive. For example, pressure-induced paroxystic activities have been reported in animals at cortical [23], mesencephalic, bulbar and spinal levels [4, 12]. It has also been shown that high pressure induces changes in hippocampal EEGraphic activities [11].

In the present study, we examined pressure effects on the hippocampal activity of the CA1 pyramidal cells in vitro in the rat. The laminar organization of the hippocampus provides a substantial advantage in the study of synaptic transmission [1]. Thus, a monosynaptic pathway can be activated by electrical stimulation of the Schaffer/commissural fibers which evokes a population excitatory postsynaptic potential (EPSP) in the CA1 region. This synapse is aminoacidergic; probably glutamavergic of aspartavergic [8, 21, 25]. In addition to this input, CA1 pyramidal cells are under control of NMDA and GABA neuromodulatory processes. The aim of the present study was to examine the effect of pressure on these excitatory and inhibitory aminoacidergic mechanisms controling CA1 pyramidal cell activity.

Materials and methods

In vitro hippocampal slices were prepared from adult male Sprague-Dawley rats and incubated as described by Dunwiddie and Lynch [9]. Briefly, rat were decapitated and the brain was rapidly removed and placed in ice-cold medium. Hippocampi were dissected, cut into 350 μm transverse slices and placed in a recording chamber at a temperature of 34 to 35°C. The medium in the chamber had the following composition (mM): NaCl (124), KCl (3), MgSO4 (2.5), $CaCl_2$(2), KH_2PO_4(1.25), $NaHCO_3$(26), D-glucose (10) and *L*-ascorbic acid (3). The solution was continuously bubbled with 95% O_2, 5% CO_2. The recording chamber was placed in a high pressure vessel and pressure was applied by means of pure helium as previously described by Fagni et al. [13]. Under pressure, the preparation was continuously perfused (submerged). The medium bubbled outside the high pressure vessel supplied the preparation with oxygen and carbon dioxide (P_{O2} = 500 mb, P_{CO2} = 65 mb in the incubation bath).

Bipolar stimulating electrodes were positioned in the stratum radiatum and in the alveus in order to activate the Schaffer/commisural afferents of CA1 pyramidal cells and the axons of these cells, respectively (Fig. 1A). Micropipettes filled with 2 M NaCl (1 to 5 MegOhm impedance) were positioned in the apical dendritic zone of the CA1 pyramidal cells to record the synaptic potential (EPSP) and in the cell body layer of these cells to record the population spike (PS) and antidromic potential (AP). Electrical stimulation consisted of 0.1 ms pulse of 0 to 200 μA delivered at a frequency of 0.04 Hz. The amplitude of the EPSP, PS and AP were continuously measured on a digital storage oscilloscope and were compared under normal and high pressures, in absence and in presence of drug.

The efficiency of the recurrent and feed-forward GABA inhibitions were tested by paired-pulse stimulations under normal and high pressures. To test recurrent inhibition, a recording electrode was placed in the dendritic layer, one stimulating electrode was positioned in the stratum radiatum to evoke EPSP and another one in the alveus to evoke AP (Fig. 3A). Paired-pulse stimulations were delivered with interpulse intervals of 30 to 70 ms, according to the following paradigms: a conditioning pulse delivered to the alvear electrode

was followed by a test pulse delivered in the stratum radiatum (antidromic-orthodromic stimulation paradigm; Fig. 3B). To test the feed-forward inhibition, two stimulating electrodes were positioned in the stratum radiatum, placed equidistant on both sides of a recording electrode located in the CA1 pyramidal layer. This allowed activation of different populations of Schaffer/commissural fibers according to the procedure described by Dunwiddie and Lynch [9].

Drug solutions were adjusted to pH 7.4 and were added to the perfusion medium. Drug-containing perfusion was started after stable evoked responses were observed for a period of at least 10 min.

Results

Changes in synaptic and dendro-somatic transfers under pressure

High pressure of helium (80 atm) decreased (50%) the evoked field potentials (EPSP, PS and AP) studied in the absence of drug. The change observed in the PS was concomitant with the decline observed in the amplitude of the EPSP. To test the transfer of currents from the dendrites to the soma of CA1 pyramidal

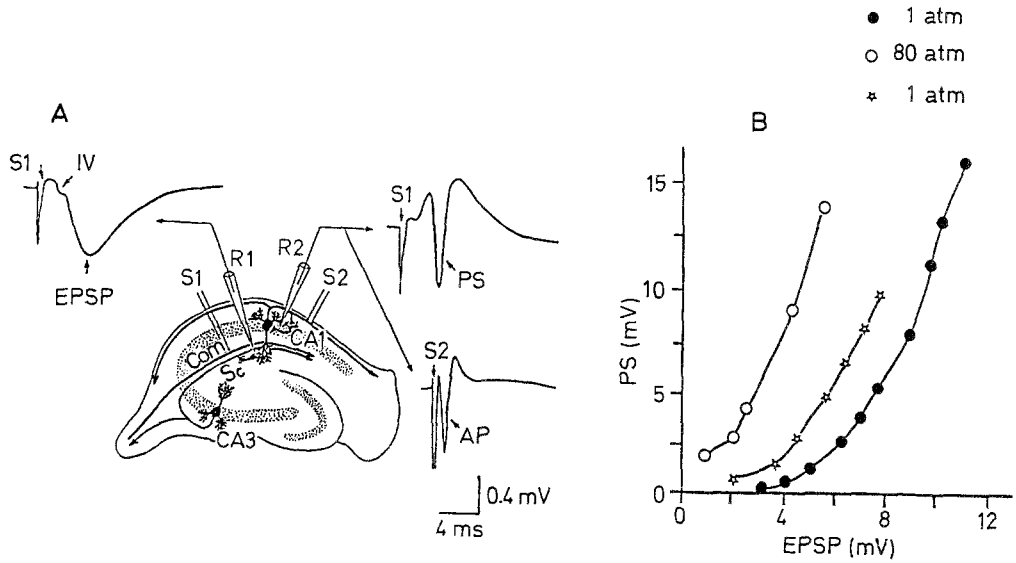

Fig 1. Effect of pressure on the intrinsic excitability of CA1 pyramidal cells. **A** Diagram showing the arrangement of stimulating (*S1* and *S2*) and recording (*R1* and *R2*) electrodes. S1 was positioned in the stratum radiatum to activate Schaffer/commissural afferents (Sc-Com) and S2 in the alveus to activate the axons of CA1 pyramidal cells. R1 was positioned in the apical dendrite zone to record the field excitatory postsynaptic potential (*EPSP*) and input volley (*IV*) and R2 in the cell body layer to record the population spike (*PS*) and field antidromic potential (*AP*). **B** Amplitude of the PS expressed as a function of the amplitude of the EPSP before compression (filled circles), at 80 atm (open circles), and after decompression (asterisks). Note that high pressure facilitated the transfer curve relating EPSP to PS

cells, the amplitude of the PS was plotted against the amplitude of the EPSP (index of cell excitability; Fig. 1B). This curve shows that for a given size of the EPSP, the amplitude of the PS was facilitated under pressure. This facilitation was significant at $p < 0.05$ ($n = 8$, Mann-Whitney non-parametric test). This pressure effect was reversible after decompression.

Effects of excitatory amino acids under pressure

Under normal pressure, brief application of excitatory amino acids (*L*-glutamate, *L*-aspartate, quisqualate, NMDA, kainate, *D*- and *L*-homocysteate) depressed the field AP and EPSP. These effects were dose and time dependent and were fully reversible. The effects of amino acids were quantified under normal and

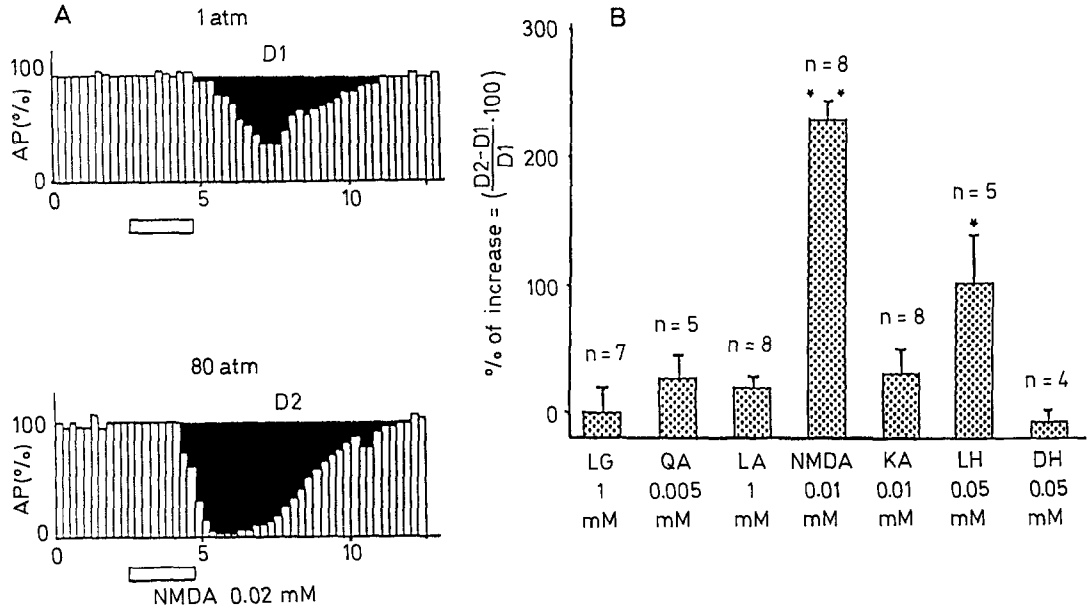

Fig. 2. Pressure selectively increased the effects of NMDA and *L*-homocysteate on CA1 field potentials. **A** Quantification of drug effects. The amplitude of the antidromic (*AP*) was expressed as percentage of the control (predrug) period. The histograms show an example of the effect of a brief application of NMDA (horizontal bar) before compression (1 atm) and under helium pressure (80 atm) in the same hippocampal slice. The filled area of each histogram represents the integration with time of the decrease in the height of the AP induced by the NMDA application. This filled area was taken as an index of the drug effect. **B** The effects of different excitatory amino acids, quantified as described in (*A*), were measured under high pressure and expressed as percentage of control values measured under 1 atm before compression, in the same slices. Each vertical bar represents the mean ± SEM of the indicated number of experiments. *significant change with $p < 0.1$ (Mann-Whitney U-test). **significant change with $p < 0.05$. Abbreviations: *L*-glutamate (*LG*), quisqualic acid (*QA*), *L*-aspartate (*LA*), *N*-methyl-*D*-aspartate (*NMDA*), kainic acid (*KA*), *L*-(*LH*) and *D*-homocysteic acid (*DH*). Similar results were obtained when population EPSP was used to quantify excitatory amino acid effects

high pressures by calculating the sum of the percentage decreases in the amplitude of the EPSP and AP from the time at which the potentials began to decrease until the time of complete recovery ([10]; Fig. 2A). Helium pressure did not qualitatively modify the effect of the tested amino acids measured on the field potentials. However, it enhanced the action of NMDA and to a lesser extent the effect of *L*-homocysteate (Fig. 2B). On the other hand, high pressure did not significantly affect the action of *L*-glutamate, *L*-aspartate, kainate quisqualate and *D*-homocysteate at the concentrations indicated in the Fig. 2B.

Effect of pressure on the GABA inhibition of CA1 pyramidal cells

Figure 3B shows that when the EPSP (test response) was preceded by an AP (conditioning response), the test EPSP decreased in amplitude (recurrent inhibition). A similar result was found with feed-forward inhibition where the test

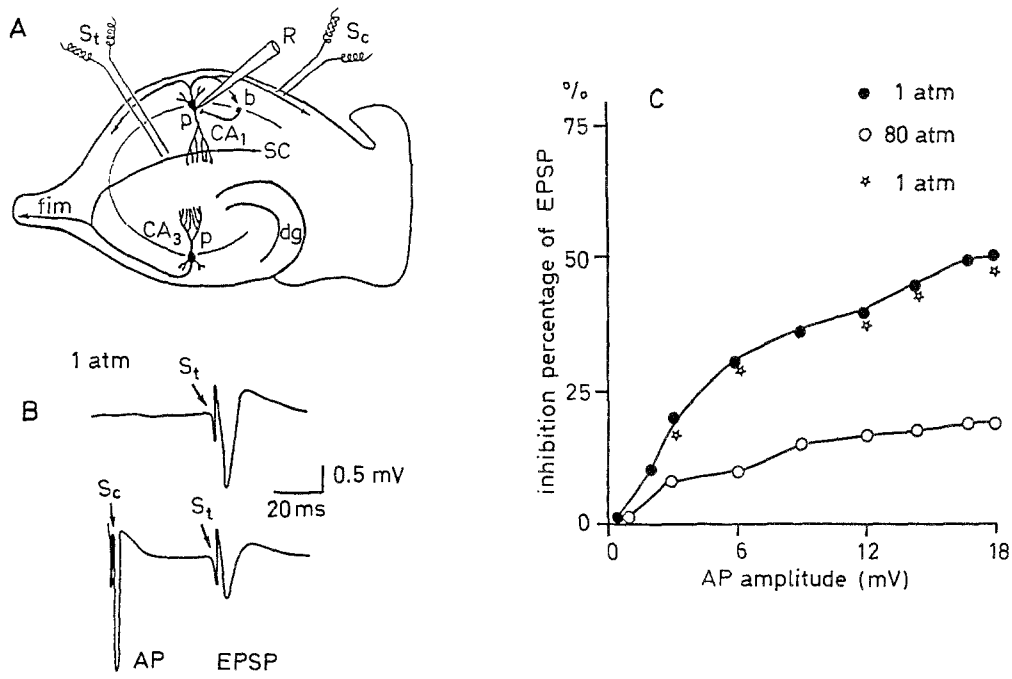

Fig 3. Effect of high pressure on the antidromic-orthodromic paired-pulse inhibition of CA1 pyramidal cells. **A** The GABAergic interneuron (*b* basket cell) was activated by antidromic conditioning stimulation (*SC*) of CA1 pyramidal cell (*p*) axon. Conditioning stimulation was followed by a test stimulation (*St*) of Schaffer/commissural afferents (*SC*) to orthodromically activate *p* cell. **B** population EPSP evoked singly (upper trace) and subsequently to a conditioning field antidromic potential (*AP*; lower trace) under normal pressure. Arrows indicate stimulus artefacts. **C** Percentage inhibition of EPSP expressed as a function of AP amplitude, obtained before compression (filled circles), at high pressure (open circles) and after decompression (asteriks), in the same slice. Note the decreased inhibition obtained under pressure

EPSP was preceded by a conditioning EPSP. These paired-pulse depressions were taken as index of CA1 pyramidal cell inhibition. High pressure induced roughly 50% fall in the efficiency of both tested paired-pulse inhibitions (Mann-Whitney U-test, $p < 0.05$, $n = 14$). These changes were reversible after decompression.

GABA and muscimol depressed field potentials of CA1 pyramidal cells under normal pressure. The inhibitory effect of these drugs was quantified, like for the depolarizing effect of the excitatory amino acids, by measuring the percentage decreases with time induced by the drugs on the field AP and EPS. We found no significant change in the inhibitory effect of GABA and muscimol under high pressure (result not shown).

Effects of drugs which block NMDA receptors or potentiate GABA inhibition under high pressure

Under normal pressure, perfusion of either nipecotic acid (NA, 0.05 mM), a blocker of GABA uptake, or 2-amino-phosphonovaleric acid (2-APV, 0.03 mM),

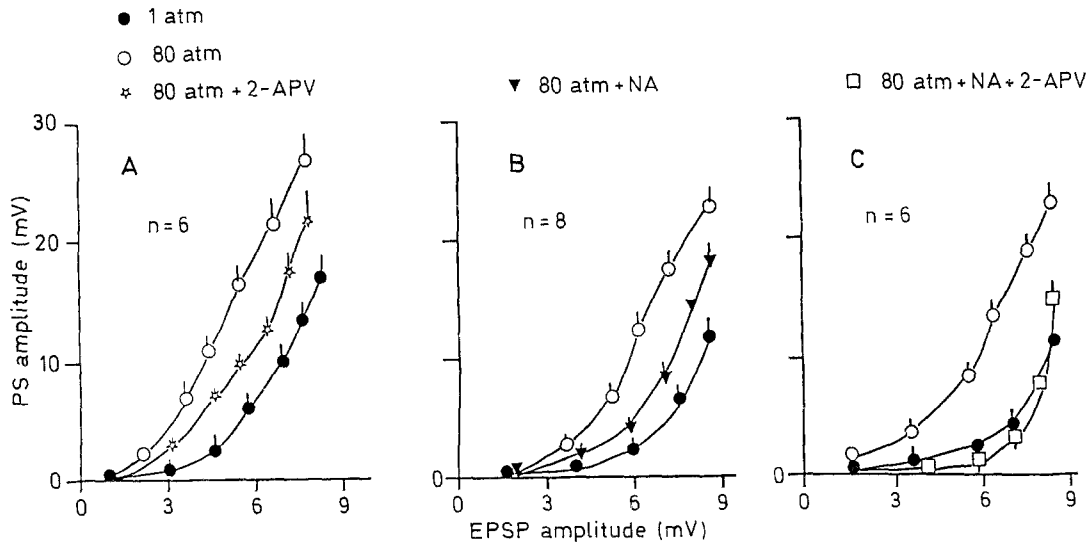

Fig. 4. Effects of 2-amino-phosphonovaleric acid (*2-APV*) and nipecotic acid (*NA*) on the pressure-induced hyperexcitability of CA1 pyramidal cells. The amplitude of the population spike (*PS*) was plotted against the amplitude of the dendritic EPSP. Each graph (**A**, **B**, and **C**) was obtained from a same set of slices (n = number of experiments). For all graphs, the values (mean S.D.) obtained under normal pressure were significantly different from those obtained under elevated pressure ($p < 0.05$; Mann-Whitney U-test). Note that in the absence of drug, for a given value of the amplitude of the EPSP, the amplitude of the PS increase under pressure. Bath application of either 2-APV (0.03 mM; **A**) or NA (0.05 mM; **B**) partially reversed the pressure-induced change. Note in graph **C** that this pressure effect (pyramidal hyperexcitability) was completely reversed by simultaneous application of 2-APV and NA (same concentrations). Similar drug applications did not modify the control curves (not represented)

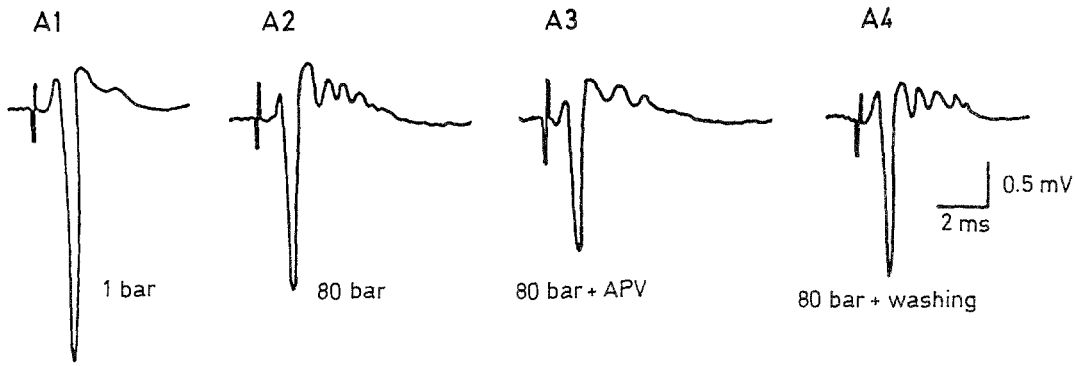

Fig. 5. Effect of 2-APV on pressure-induced afterdischarges. Maximal PS was recorded under normal (**A1**) and high pressures, before (**A2**) and after (**A3**) application of 2-APV (0.03 mM) and after washing of the drug (**A4**). Figure **A2** shows the appearance of afterdischarges (multiple PS) under pressure. Note the decrease in amplitude of all these afterdischarges in presence of 2-APV (10 min perfusion period of the drug)

a NMDA antagonist, did not significantly affect the EPSP and PS nor the transfer curve relating the EPSP to the PS (Mann-Whitney test, $p < 0.5$, $n = 6$). Under high pressure, these drugs (at the same concentrations) depressed the PS amplitude without affecting the EPSP. Thus, when CA1 pyramidal cells were perfused with 2-APV or NA under pressure, the pressure-induced facilitation of the transfer curve relating EPSP to PS partially decreased (Fig. 4A and B). When 2-APV was applied simultaneously with NA, a complete reversion of the pressure-induced hyperexcitability indicated by the facilitation of the studied transfer curve was observed (Fig. 4C, Mann-Whitney test, $p < 0.05$ $n = 8$). Moreover, stimulations evoking single PS of maximal amplitude under normal pressure, evoked multiple PS under high pressure (80 atm; $n = 5$). An example of this pressure effect is shown in Figure 5 (A1 and A2). 2-APV reduced the number and amplitude of the pressure-induced multiple discharges.

Discussion

High pressure of helium, in absence of drug, decreased field potentials (EPSP, PS and AP) recorded in the CA1 region of the hippocampus. Bath application of excitatory amino acids depressed EPSP and AP like previously reported [5, 6, 10]. This effect is an index of CA1 pyramidal cell depolarization. Helium pressure enhanced the actions of NMDA and *L*-homocysteate without significantly affecting the action of the other tested amino acids. The transfer curve relating EPSP to PS is an index of cell intrinsic excitability. High pressure shifted this curve to the left. This result indicates that pressure facilitated the transfer of currents from dendrites to soma of CA1 pyramidal cells. In addition to these changes, pressure decreased recurrent and feed-forward GABA inhibitions of these cells. No change was observed in the effect of GABA when the transmitter was bath applied. Drugs which block NMDA receptors and/or potentiate

GABA inhibition antagonized the pressure-induced epileptic-like activity (multiple PS) of CA1 cells. The NMDA antagonist 2-APV decreased the amplitude and number of the pressure-induced afterdischarges.

The synaptic receptors involved in the studied excitatory transmission are non-NMDA receptors [7, 10]. Their activation by quisqualate, *L*-glutamate, kainate or *L*-aspartate was not significantly affected under pressure. This result suggests that the sensitivity of aminoacidergic excitatory synaptic receptors of CA1 pyramidal cells did not significantly change under pressure. Therefore, the depression in the synaptic responses observed under pressure might have resulted from lesser release of neurotransmitter, which itself might have resulted from pressure-induced depolarization of nerve endings.

The facilitation observed under pressure in the transfer of currents from the dendrites to the soma of CA1 pyramidal cells could have partially resulted from an increase (250%) in the sensitivity of the NMDA processes of pyramidal cells. Thus, the blockade of NMDA receptors by 2-APV partially antagonized this pressure-induced hyperexcitability. Enhancement of NMDA mechanisms under pressure could have resulted from receptor hypersensitivity or any change in pressure-sensitive membrane properties (e.g. membrane fluidity: see Wann and Macdonald, 1980 for review). An alternative hypothesis could be that the voltage-dependent block of NMDA channels by Mg^{2+} ions [19, 22] was reduced under pressure. This could not result from a reduction of extracellular concentration of Mg^{2+} because the rapid perfusion rate (1 chamber volume/min.) of the slices should have maintained normal ionic concentrations in the extracellular fluid. Moreover, we showed that the facilitation in the NMDA response of CA1 pyramidal cells obtained by switching from a Mg^{2+}-containing medium to a Mg^{2+}-free solution was not significantly affected under pressure [27]. A more likely hypothesis would be that the increased effect of NMDA observed under pressure resulted from a moderate but sustained depolarization of CA1 pyramidal cells. This depolarization could be sufficient to reduce the voltage-dependent block of NMDA channels but weak enough to not significantly affect the actions of the other amino acids tested.

On the other hand, the disinhibitory effect of pressure observed in the hippocampus is consistent with the findings of Grossman and Kendig [16] showing reduced GABAergic inhibition under pressure in the isolated crustacean neuromuscular junction. Decrease in GABA inhibition under pressure could not result from lesser sensitivity of GABA receptors, nor from alteration of GABA uptake, since effects of exogenously applied GABA or muscimol were not significantly affected under pressure. Pressure-induced disinhibition could result from a depression in GABA release, which itself could be a consequence of several possible changes: decrease in GABA synthesis or reduction in GABA interneuron activation by excitatory afferent or alteration of pre-synaptic Ca^{2+} transport as suggested by Gilman et al. [15] or a combination of them.

Pressure-induced hyperexcitability of CA1 pyramidal cells was partially reduced by NA which is known to potentiate GABA effect by blocking GABA

uptake [17]. Moreover, blockade of NMDA mechanisms with 2-APV reduced the facilitatory transfer from dendrites to soma. 2-APV also reduced the number and amplitude of pressure-induced afterdischarges (epilepticlike activity of pyramidal cells).

These results suggest that NMDA mechanisms are involved in pressure-induced epileptic activity. These findings suggest that both GABA and NMDA mechanisms are involved in the development of the pressure-induced alterations of CA1 cells. According to Dingledine [8], reduced inhibition of pyramidal cells would unmask NMDA excitatory events and thus increase cell excitation. Also, reduction in GABA inhibition should result in a depolarization of CA1 cells which could be the main cause of the potentiation in NMDA mechanisms observed under pressure. Another hypothesis could be a direct pressure-induced depolarization of CA1 cells. Unbalanced NMDA and GABA controls of CA1 pyramidal cells are known to result in epileptic seizures under normal pressure [8]. Our results suggest that similar changes would underlie the neurological alterations observed in mammals under pressure. Indeed, these pressure-induced changes are also reduced by NMDA antagonists injected before compression in freely moving rats [20]. Thus, NMDA antagonists should have a high protective effect against several symptoms of the high pressure nervous syndrome.

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