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# Excitatory and inhibitory amino-acidergic determinants of the pressure-induced neuronal hyperexcitability in rat hippocampal slices

**F. ZINEBI, L. FAGNI, and M. HUGON**

*Biologie des Hautes Pressions, CNRS-URA 1330, Faculté de Médecine Nord, Bd. Pierre Dramard, 13326 Marseille, Cedex 15, France*

Zinebi F, Fagni L, Hugon M. Excitatory and inhibitory amino-acidergic determinants of the pressure-induced neuronal hyperexcitability in rat hippocampal slices. Undersea Biomed Res 1990; 17(6):487–493—In a previous study we found that the intrinsic excitability of the hippocampal CA1 pyramidal cells increased under helium pressure (80 bar). We presently show that drugs inhibiting γ-aminobutyric acid (GABA) uptake or facilitating GABA binding partially reversed the pressure-induced hyperexcitability of the CA1 pyramidal cells. When these drugs were simultaneously applied with 2-D,L-aminophosphonovaleric acid, a specific antagonist of *N*-methyl-D-aspartate (NMDA) receptors, the effect of pressure on the neuronal excitability was nearly abolished. These results suggested that the observed pressure-induced hyperexcitability of pyramidal cells resulted from reduced efficiency of GABA transmission and facilitated excitation mediated by NMDA receptors.

γ-aminobutyric acid
*N*-methyl-D-aspartate
pressure
hippocampus

Man and animals exposed to elevated pressures of helium develop neurologic disturbances such as tremors and myoclonies, accompanied by EEG changes (1). Pressure effects were also observed in rat hippocampal slices (2). Thus, we found that for a given size of the CA1 excitatory postsynaptic field potential (fEPSP), the concomitant CA1 population spike (PS) was enhanced under pressure. This change indicated a facilitated electronic transfer of excitatory currents from dendrites to soma of CA1 pyramidal cells, and thus an increased intrinsic excitability of these cells.

Under normal pressure, the excitability of CA1 pyramidal cells is controlled by both dendritic and somatic γ-aminobutyric acid (GABA) inhibitions (3). On the other hand, these inhibitions are balanced by excitatory mechanisms mediated by *N*-methyl-D-aspartate (NMDA) receptors (4). These data suggest that the pressure-induced hyperexcitability of the CA1 pyramidal cells might result from reduced GABA inhibition or facilitated NMDA-mediated excitation or both.

The aim of the present study was to test this hypothesis. Thus, the effects of drugs that potentiate GABA inhibition or reduce NMDA-mediated processes of CA1 hippocampal pyramidal cells were examined, in vitro, under normal and high helium pressures. These drugs were: a) nipecotic acid (NA) and *p*-chloromercuriphenylsulphonic acid (CMPSA), which are known to block GABA uptake systems (5, 6); or b) diazepam (DZ), which enhances GABA binding on neuronal membranes (7), or 2-D,L-aminophosphonovaleric (2-APV), which blocks NMDA receptors (8).

## METHODS

Hippocampal slices were prepared from male Sprague-Dawley rats weighing 150–200 g, according to the procedure described by Dunwiddie and Lynch (9). They were maintained in a recording chamber at a temperature of 34°–35°C. The composition of the perfusion medium was (in m*M*): NaCl (124), KCl (3), $Mg \cdot SO_4$ (2.5), CaCl2 (2), $KH2 \cdot PO_4$ (1.25), $NaH \cdot CO_3$ (26), D-glucose (10), and L-ascorbic acid (3). The solution was continuously bubbled with 95% $O_2$: 5% $CO_2$.

Bipolar stimulation electrodes were positioned in the stratum radiatum to activate the Schaffer-commissural fibers. Single, constant-current pulse stimulations (0–200 μA amplitude, 0.1 ms duration) were delivered at a rate of 0.04 Hz. The fEPSPs and PSs evoked by these stimulations were simultaneously recorded in stratum radiatum and stratum pyramidal, respectively, by means of glass micropipettes filled with 2 *M* NaCl (impedance = 5–15 MΩ). The initial slope of the fEPSP and the amplitude of the negative component of the PS were continuously measured on a digital storage oscilloscope. The amplitude of the PS was plotted against the initial slope of the fEPSP and the curves obtained in the same preparation under normal and high pressures of helium, in the presence and in the absence of a drug, were compared.

Helium pressures up to 80 bar applied at a rate of 2 bar $\cdot$ $min^{-1}$ in a high-pressure vessel designed for microphysiologic studies (10). Additional tests were performed in the same preparations at 1 bar, 10 min after decompression (2 bar $\cdot$ $min^{-1}$). Drug applications consisted of shifting from normal perfusion medium to perfusion medium containing NA (0.5 m*M*) or CMPSA (0.005 m*M*) or DZ (0.1 m*M*) and/or 2-APV (0.03 m*M*). All drugs were purchased from Sigma, St. Louis, MO.

## RESULTS

Stimulations evoking single PSs of maximal amplitude, under normal pressure, evoked multiple PSs under 80 bar. An example of such a pressure effect is shown in Fig. 1 (*Aa–Ad*). Fig. 1 *B–E* shows that 2-APV reduced the number and amplitude of the pressure-induced multiple discharges.

Under normal pressure, perfusion of either NA, CMPSA, DZ, or 2-APV (data not shown) or combinations of these drugs (Fig. 2) at concentrations indicated above, did not significantly affect the fEPSP nor PS nor the transfer curve relating the fEPSP to the PS (Mann-Whitney test, $n = 6$, $P \leq 0.05$). Concentrations of these drugs, twofold higher than those indicated above, induced a marked decrease in the amplitude of the studied evoked field potentials, and therefore were not studied under high pressure. Indeed, the effects of such concentrations of GABA mimetics would have been merely superimposed to the pressure effects and thus eventual variations in the

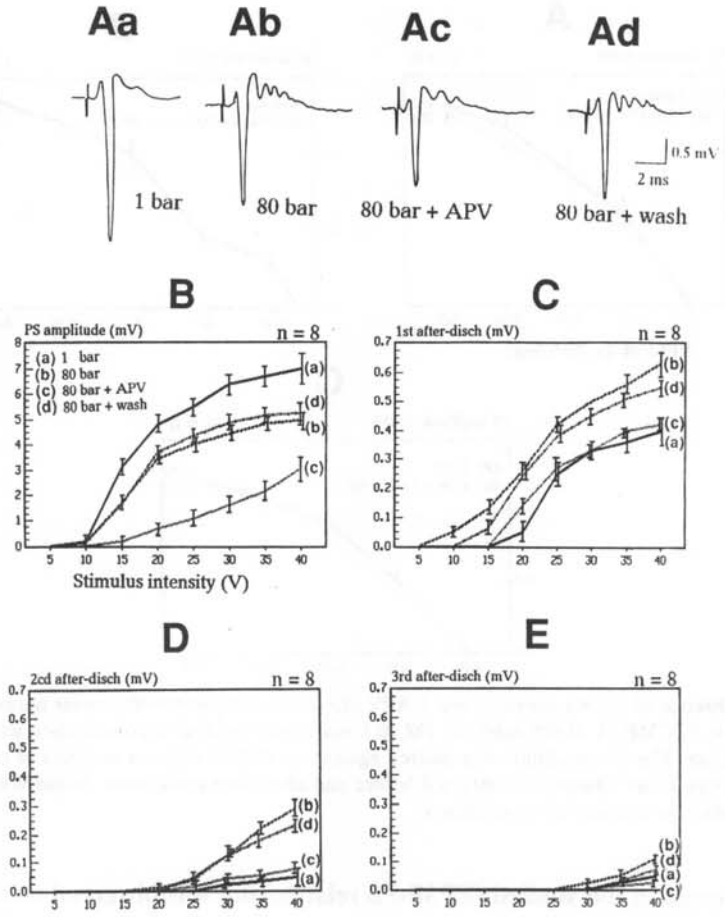


Fig. 1. Effect of 2-APV on the pressure-induced after-discharges. *A*, Maximal PS was recorded under normal (*Aa*) and high pressure, before (*Ab*) and after (*Ac*) injection of 2-APV (0.03 m*M*) and after washout (*Ad*). *Note* the appearance of after-discharges under pressure. *B*, the amplitudes of the PS (*B*) and after-discharges (first: *C*; second: *D*; and third: *E*) were plotted against stimulus voltage, under normal and high pressures, before and during injection of 2-APV and after washout. *Note* the decrease in amplitude of the PS and all afterdischarges during addition of 2-APV (10-min perfusion period of the drug) under high pressure.

size of the field potentials under pressure, in the presence of these drugs, would have been difficult to interpret.

Under 80 bar, NA, CMPSA, DZ, and 2-APV depressed the PS amplitude without affecting the fEPSP. As shown in Fig. 3 *A–C*, for a given value of the initial slope of the fEPSP, the amplitude of the PS increased under pressure, in the absence of a drug. The same figure shows that NA, CMPSA, DZ, or 2-APV, tested separately, partially antagonized this pressure effect. Effect of the drugs appeared 6 min after the beginning of the application and became stable 5–10 min later. All drug effects returned gradually to baseline after 15-min washout. When either NA, CMPSA, or DZ was applied simultaneously with 2-APV, a complete reversion of the pressure-

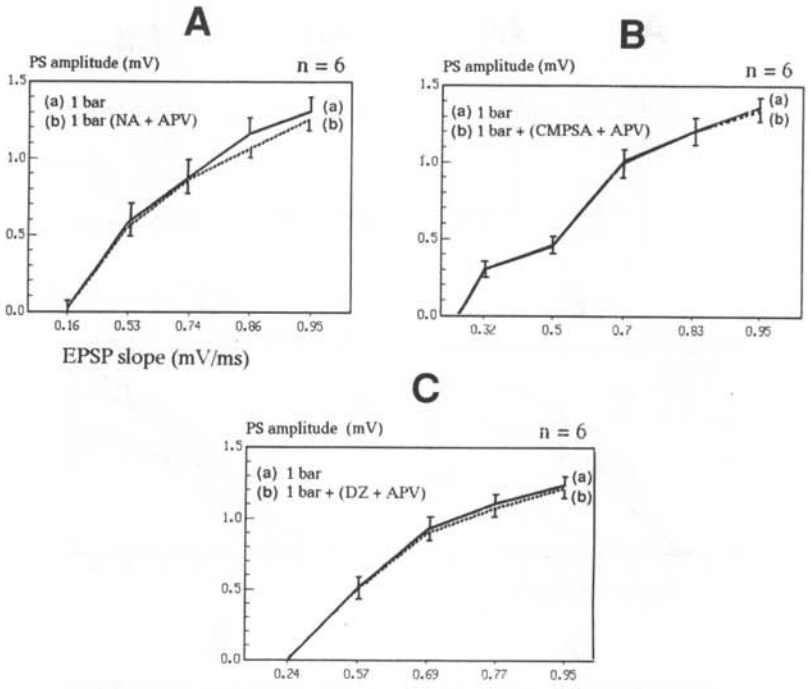


Fig. 2. Absence of GABA mimetic and 2-APV effects on cell excitability under normal pressure. *A*, NA (0.5 m*M*); *B*, CMPSA (0.005 m*M*); *C*, DZ (0.1 m*M*) were applied in combination with 2-APV (0.03 m*M*) under 1 bar. The PS amplitude was plotted against the fEPSP slope as an index of cell excitability. *Note* that no significant change was obtained before and after drug application. Mann-Whitney test, $P \leq 0.05$, $n$ indicates the number of experiments.

induced change of the studied fEPSP-PS relationship was observed (Mann-Whitney test, $n = 6$, $P \leq 0.05$, Fig. 3).

All the pressure-induced changes were partially reversible after decompression.

## DISCUSSION

The present results show that pressure facilitated the transfer curve relating fEPSPs to PSs, indicating that pressure increased the intrinsic excitability of CA1 pyramidal cells. This result corroborates our previous findings (2). The pressure-induced afterdischarges of the CA1 pyramidal cells were partially reversed by 2-APV. This indicates that the NMDA receptors are involved in pressure-induced hyperexcitability. GABA synergistic agents also antagonized pressure-induced hyperexcitability. This suggests that GABAergic transmission was also affected under high pressure. As could be expected from these results, additive effects of the studied GABA synergists and the NMDA antagonist, 2-APV, completely reversed the pressure effects. Since these drugs were used at concentrations that did not affect cell excitability under normal pressure, we can assume that their protective effect against pressure-induced hyperexcitability did not merely result from summation of opposite effects of these drugs and pressure on the recorded field potentials. Further experiments are needed to understand the mechanism of this apparent specific action of these drugs.

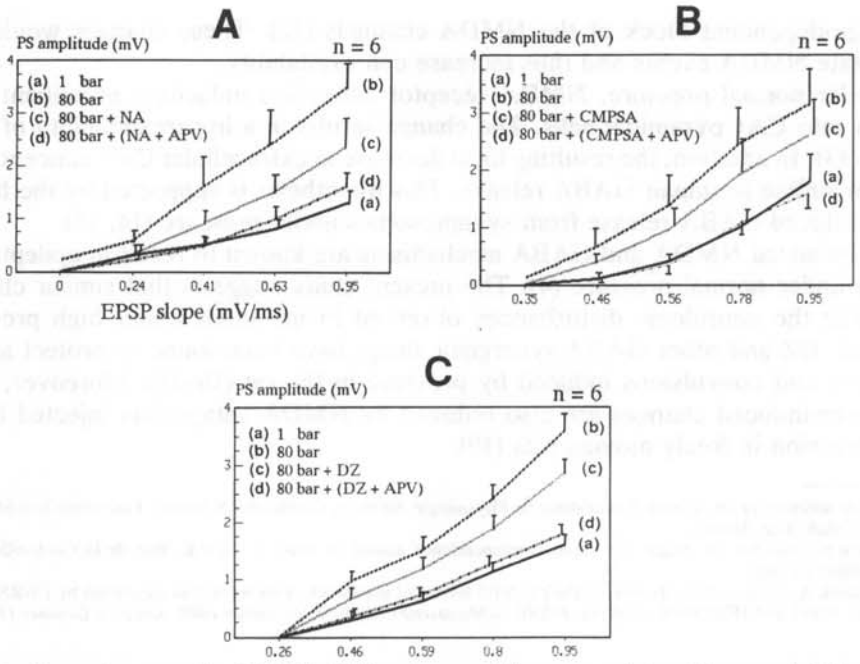


Fig. 3. Drugs that potentiate GABA inhibition decreased the pressure-induced hyperexcitability. *A–C*, the PS amplitude was plotted against the fEPSP initial slope under normal and high pressures. *Note* that for a given value of the fEPSP slope, the amplitude of the PS was facilitated under pressure. This change indicates a hyperexcitability of CA1 pyramidal cells. *A*, application of NA (0.5 m*M*) decreased the pressure-induced hyperexcitability. *Note* that combined application of NA (0.5 m*M*) and 2-APV (0.03 m*M*) induced a complete reversion of the pressure effect. Mann-Whitney test, $P \leq 0.01$. CMPSA (0.005 m*M*, *B*) and DZ (0.1 m*M*, *C*) had similar effects than NA.

The present findings suggest that alteration of GABAergic and NMDA processes would be sufficient to explain the development of pressure-induced hyperexcitability of CA1 pyramidal cells. Two GABAergic systems have been identified in the CA1 region of the hippocampus: a recurrent and a feed-forward system (3). Our experiments did not distinguish between the effects of pressure on these two types of inhibition. Previous work has shown that the depressing effects of exogenously applied GABA on the fEPSP and PS are not significantly affected under pressure (11). This result suggests that the observed pressure-induced hyperexcitability of hippocampal pyramidal cells resulted neither from GABA receptor alteration, nor from alteration of their coupled ionic channels, nor from facilitated GABA uptake. A likely hypothesis would be that pressure impaired GABA release and thus reduced tonic inhibition of CA1 pyramidal cells. An additional mechanism favoring our hypothesis is suggested hereafter.

According to Dingledine (4), reduced inhibition of pyramidal cells would unmask NMDA excitatory events and thus increase cell excitability. Thus, the NMDA component of the pressure-induced hyperexcitability of pyramidal cells might result from the reduction of the inhibitory process suggested above. Other nonexclusive possibilities would be that NMDA receptor sensitivity increased under pressure or that a slight pressure-induced depolarization of pyramidal cells would reduce the

voltage-dependent block of the NMDA channels (12). These changes would also facilitate NMDA events and thus increase cell excitability.

Under normal pressure, NMDA receptor activation induces a prominent $Ca^{2+}$ influx into CA1 pyramidal cells. The change results in a hyperexcitability of these cells (13). In addition, the resulting local decrease in extracellular $Ca^{2+}$ concentration would suffice to impair GABA release. This hypothesis is supported by the finding of a reduced GABA release from synaptosomes under pressure (14, 15).

Unbalanced NMDA and GABA mechanisms are known to result in epileptic seizures under normal pressure (4). The present study suggests that similar changes underlie the neurologic disturbances observed in mammals under high pressure. Indeed, DZ and other GABA synergistic drugs have been found to protect against tremors and convulsions induced by pressure in the rat (16–18). Moreover, these pressure-induced changes are also reduced by NMDA antagonists injected before compression in freely moving rats (19).

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Present address for Dr. Zinebi: Laboratoire de Physiologie Animale, Faculté des Sciences, Université Sidi-Mohamed Ben-Abdellah, Fez, Maroc.

Present address for Dr. Fagni, to whom correspondence should be sent: C.C.I.P.E. Rue de la Cardonille, 34000 Montpellier, France.

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