EFFECT OF HIGH PRESSURE ON LONG-TERM POTENTIATION IN RAT HIPPOCAMPAL CA1 REGION: AN ELECTOPHYSIOLOGICAL STUDY

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ABSTRACT

Pressures above 15 bar induce in mammals and humans the high pressure neurological syndrome (HPNS). This syndrome is characterized by various neurological disorders and motor decremented associated with memory disorders. However, a brief tetanic stimulation of an excitatory pathway in the hippocampus, synaptic transmission through the tetanized pathway is facilitated for a long period. This phenomenon is called long-term potentiation (LTP), and has been regarded as a neuronal correlate with learning and memory. Human and animal studies have shown that motor learning results in long-term potentiation (LTP)-like plasticity processes. Thus, it has been speculated that the occlusion of LTP-like plasticity after learning, indicative of how much LTP was used to learn, is essential for retention. In order to understand the pressure-induced alteration in memory processes, we aim to study the effect of high pressure on long term potentiation in rat hippocampal slide. The effect of high pressure on LTP was studied in vitro in CA1 region of hippocampal slices using electrophysiological recordings. Our results show that pressures above 50 bar inhibit the development of LTP. Pressure-induced disorders in glutamatergic transmission have been reported. Our findings suggest that the effect of pressure on LTP in CA1 could be related to the reduction of glutamatergic activity and/or a significant depolarization of CA1 that may involve voltage-dependent calcium channels.

Key words: *Long-term potentiation, Hippocampus, GABA, NMDA, CA1, calcium channels.*

1. INTRODUCTION

When human diver is exposed to high pressure, it leads to various neurological disorders called the high-pressure neurological syndrome (HPNS). HPNS is a condition encountered in deep-sea diving beyond a depth of 100 meters, a feat that is made possible by the breathing of special gas mixtures such as helium and oxygen. Confusion, drowsiness, dizziness, and impairment of cognitive skills are frequent expressions of this disorder (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., 1982) 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 (Bennett and Rostain, 2003).

Several studies showed that hydrostatic pressure, compression velocity and the nature of the gas breathing mixture are responsible for serious physiological disorders (Rostain et al., 1984; Rostain and Naquet, 1974) (Miller, 1972). Electrophysiological studies in animals during high pressure display EEG modification and perturbations of neurochemical processes. Studies performed *in vitro* on rat hippocampal slices exposed to high pressures revealed a decrease in the release of excitatory (Glutamate) and inhibitory neurotransmitters (γ-Aminobutyric acid GABA) as

well as a facilitation of mechanism involving N-methyl-D-aspartate (NMDA) receptors leading to neuronal hyperexcitability (Zinebi et al., 1988; Zinebi et al., 1990). In vivo studies demonstrated that the administration of NMDA receptor antagonists; 2-amino-7-phosphonoheptanoic acid (2-APH) and ketamine reduce tremor severity. It is probable that the anti-tremor effect and the electroencephalographic (EEG) changes resulting from 2-APH are due to decreased postsynaptic activity of an excitatory neurotransmitter (Wardley-Smith et al., 1986). Furthermore, Sodium valporate, an anticonvulsant used in the treatment of epilepsy, anorexia nervosa, panic attack, anxiety disorder, posttraumatic stress disorder, migraine and bipolar disorder, as well as other psychiatric conditions requiring the administration of a mood stabilizer, has been shown to improve behavioral disorders and EEG changes (Rostain et al., 1986). Disturbances of long-term memory and a decrease of psychomotor performance have been reported after HPNS episodes. 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 cortico-hippocampal 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). Initially observed in the CA1 region of the rat hippocampus, an area of the brain known to be fundamentally important in memory acquisition (Schwartzkroin and Wester, 1975), Long-term potentiation (LTP) is demonstrable in different structures of the brain. LTP is operationally defined as a long-lasting increase in synaptic efficacy following high-frequency stimulation of afferent fibers. Thus, following LTP induction, a fixed amount of presynaptic stimulation induces a "potentiated" postsynaptic response, for example, an increase in EPSPs (excitatory post-synaptic potentials). Since the first full description of the phenomenon in 1973, exploration of the mechanisms underlying LTP induction has been one of the most active areas of research in neuroscience. In several recent reviews, various authors have concluded not only that LTP is a viable mechanism for the induction and storage of memories but that it is the most promising candidate (Morris et al., 1991). Reyman and coll. (Reymann et al., 1982) showed that electrical stimulations with high frequencies could serve as stimuli for memorization. Thus, the PLT represents the classical model for studying learning and memory cellular mechanisms. The *in vitro* study under pressure should allow us to address a general analysis of memory disorders encountered in divers (Fowler et al., 1985). NMDA and GABA systems have been demonstrated to be involved in LTP. Several neuropharmacological studies showed that perfusion of rat hippocampal slices by aminophosphonovaleric acid (APV), an antagonist of NMDA receptors; block the LTP (Wigstrom et al., 1986). Moreover, blockade of GABAergic inhibition by GABA antagonists (Picrotoxin) induce a significant facilitation of the LTP (Wigstrom and Gustafsson, 1986). Lynch and coll. (Lynch et al., 1985) showed that LTP depends as well on the increased release of glutamate involved in synaptic transmission.

It appears that the GABAergic inhibition and the activation of NMDA receptors are important complementary factors that contribute to the control of LTP in CA1 pyramidal neurons. These findings suggest the induction of LTP by an increase of the synaptic efficacy provided by Schaffer-commissural afferents.

The aim of this present study was to evaluate the effect of high pressures on LTP to understand the cellular mechanisms involved in memory disorders recorded in deep diving. We are investigating whether the potentiation of neurons of the mammalian central nervous system is maintained under high pressures and to determine the threshold pressure for LTP modification. In other words, we are interested to know if the LTP and high pressures which involve the same NMDA and GABA mechanisms will add their effect and produce a better LTP.

This study was performed on rat hippocampal slices. Indeed, the hippocampus is a structure of the central nervous system that presents a simple anatomical organization which facilitates any electrophysiological study.

2. MATERIALS AND MATHODS

2.1 Experimental animals

All procedures for animal experiments were performed in accordance with European and National regulations.

2.2 Rats (Brain preparation)

Sprague-Dawley rats of both sexes (150–200g) were killed (pentobarbital, 60 mg/kg); their brain was extracted and submerged in artificial cerebrospinal fluid (4-6°C). Corticohippocampal slices (350µm) were prepared as previously

described (Dreier and Heinemann, 1991); (Talpalar and Grossman, 2003). Slices were cut in a horizontal microtome (Chopper) and conserved in an incubation chamber constantly oxygenated at 25°C for later utilization. Artificial cerebrospinal fluid contained (in mM) 124 NaCl, 4 KCl, 1.25 KH2PO4, 26 NaHCO3, 2.41 MgSO4, 2 CaCl2*2H2O, 10 D-glucose and 3 L-ascorbate. Solution was constantly bubbled with 95% O2-5% CO2 for a pH of 7.4.

2.3 Electrophysiological recordings

After positioning the various electrodes of stimulation and recording to obtain cellular responses (SP, EPSP) (Figure.1), the housing is closed and compression begins. The rate of compression is 1bar/min. This speed is similar to that used by (Fagni et al., 1985) in vivo. Controls values are taken at 1 bar helium before and after potentiation of pyramidal cells. Compression is done, according to the cuts, between 10 and 80 bar. Under these conditions of constant pressure, equilibrium is established between the ambient gas and the bath incubation. The partial pressures of O_2 and CO_2 remain constant. Partial pressure of helium does not exceed 15 bar in the incubation medium for a total pressure of 80 bar inside the box. In these experimental conditions, the liquid temperature incubation of hippocampal slices is maintained at 37°C throughout the experiment by monitoring a temperature sensor connected to a thermostat located outside of the box. The pH of the liquid nutrition is measured in the incubation bath. Thus, any changes that we observe at high pressures can related to pressure.

2.4 Statistical analysis

The results were analyzed by non-parametric tests adapted to small biological samples. Nonparametric testes do not assume a Gaussian distribution. We used the Mann Whitney U test for different batches and Wilcoxon W test for paired samples.

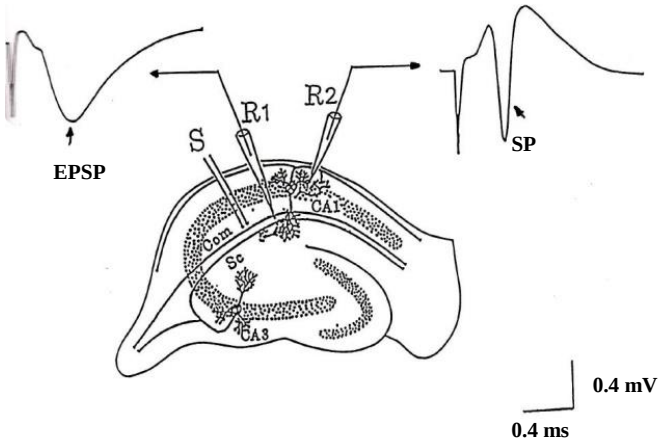


Figure 1 Positioning of stimulation and recording electrodes. S: Stimulation electrode activating CA1 pyramidal cells. R1: Reception electrode for EPSP. R2: Reception electrode for SP

3. RESULTS

3.1. CA1 pyramidal neurons response under atmospheric pressure

Shaffer collateral-commissural afferents that synapse onto CA1 pyramidal cells were electrically stimulated (80 µA). Two types of responses were recorded; excitatory postsynaptic potential (EPSP) and somatic potential (SP). These responses are recorded simultaneously on the same section of the hippocampus and represent an average of 4 responses that are stable to within ±15% variation for the gradient of EPSP and ±15% to ±20% for the amplitude of PS.

3.2. CA1 pyramidal neurons long-term potentiation under atmospheric pressure

Tetanization of Shaffer collateral-commissural afferents of CA1 cells by electrical impulses 2000ms/100 Hz induces a significant increase in the slope of EPSP and the PS amplitude (Figure.2a).

The effect of tetanization on the activity of pyramidal cells persists throughout the post-tetanization period (70 min).

3.3. High pressure alters long-term potentiation

The combined analyses of measurement parameters of cellular responses clearly show the depressant effect of pressure on LTP of CA1 pyramidal cells. In fact, the electrical stimulation of the Shaffer collateral-commissural afferents had allowed us to obtain both cellular responses; EPSP and SP. The application of tetanization under high pressure does not induce any change on the slope of field EPSP or PS Amplitude (Figure.2b). Indeed, these results are observed on Figure 4 showing that tetanization of pyramidal cells under atmospheric pressure (1 bar) induces a significant increase in PS amplitude up to 160%. However, the same tetanization under high pressure (80 bar) induces only an increase of 20 to 40% in PS amplitude (n=20, Mann-Withney U test $p<0.05$).

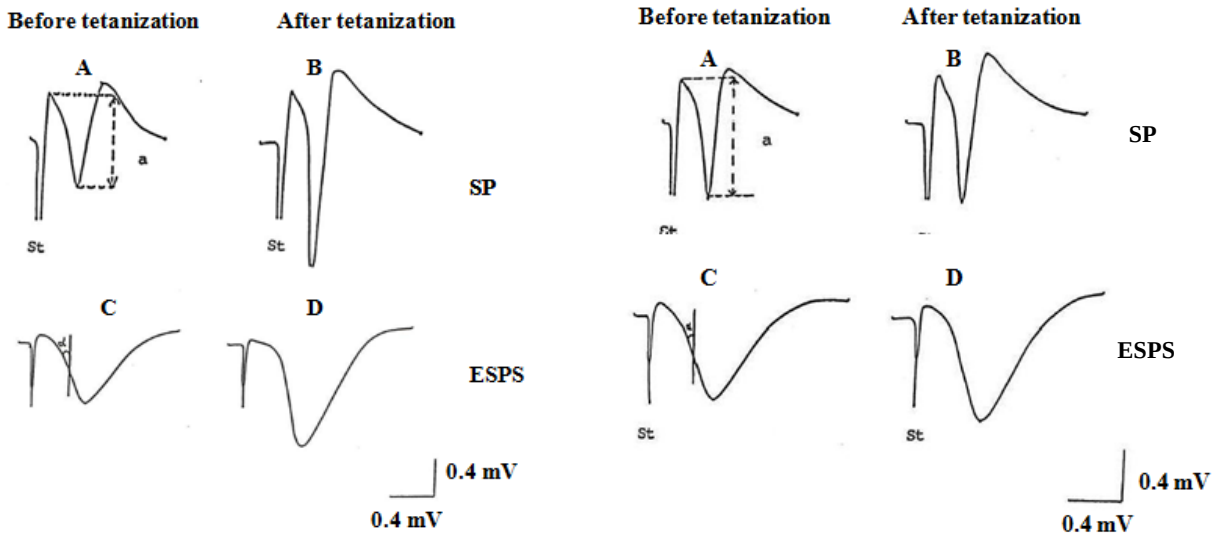


Figure 2 Effect of tetanization on orthodormic action potential evoked in the hippocampal CA1 region in vitro. A: Action potential of a population of pyramidal neurons (SP). C: Excitatory postsynaptic potential (ESPS) recorded simultaneously on the same section under 1 bar en before tetanization. B and D: the same cellular responses recorded at 1 bar and after tetanization. The initial slope of ESPS is represented by Alpha and the amplitude of SP is represented by "a" **2a,** Effect of tetanization on orthodormic action potential evoked in the hippocampal CA1 region *in vitro*. A: Action potential of a population of pyramidal neurons (SP). C: Excitatory postsynaptic potential (ESPS) recorded simultaneously on the same section under 80 bar en before tetanization. B and D: the same cellular responses recorded at 80 bar and after tetanization. The initial slope of ESPS is represented by Alpha and the amplitude of SP is represented by "a" **2b**

3.4. Pressure and tetanization separately induce an increase in CA1 cells excitability

Taking into account the previous results, we were interested in studying the effect of pressure and tetanization upon intrinsic excitability of CA1 pyramidal cells. The curve of variation of SP amplitude and the slope of field ESPS is an index of the dendro-somatic transmission meaning the flow of dendtritic current through the soma. As shown in Figure 3, high pressures (80 bar) as well as tetanization in atmospheric pressure move the curve to the left; this means that for all values of the slope of the ESPS, somatic recruitment of action potentials from the same population of cells increases. The tetanization of CA1 cells under 80 bars does not induce any significant increase in their excitability (Figure.4a). Indeed, we observe that a second tetanization of the CA1 cells under atmospheric pressure do not lead to significant change in terms of their excitability (Figure.4b).

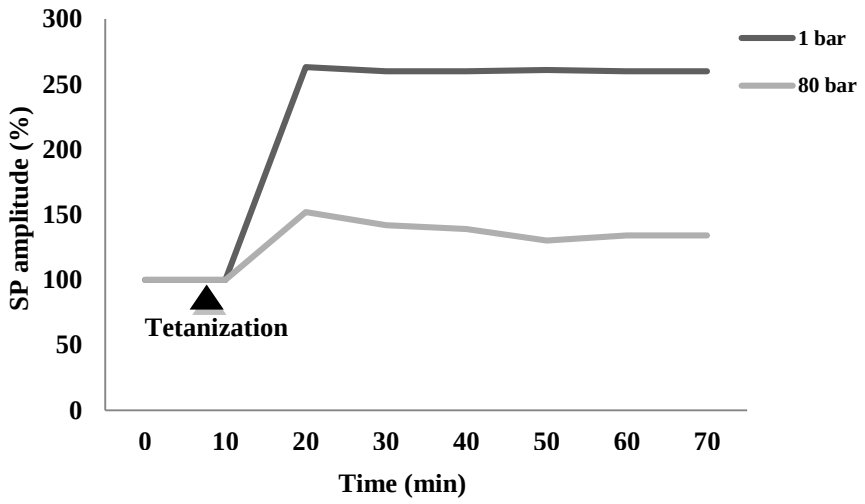
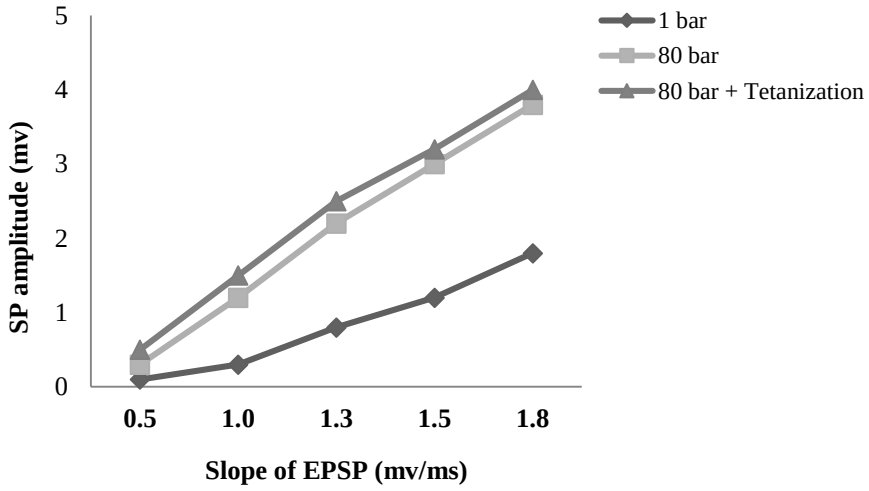


Figure 3 Significant depressant effect of high pressure (80 bar) on SP potentiation in CA1 region. ($p<0.05$, n=20, Mann Withney U Test)

3.5. Determination of the pressure value of LTP alteration

In order to determine the threshold of LTP alteration, we studied the variation in percentage of SP amplitude after tetanization. This was evaluated every 10 bar. Under atmospheric pressure (1 bar), tetanization of Scheffer collateral-commissural afferents of pyramidal cells induce a significant increase in SP amplitude (n=6 Wilconson test W $p<0.05$). Compared to the response obtained at atmospheric pressure after tetanization (Figure.5), we observe that the amplitude of the response is maintained up to 50 bar under pressure. From 60 bar, the response after tetanization is below that of the surface. Statistical studies show a significant decrease from 60 bar (Mann Whitney U test $p<0.01$). Changes in SP amplitude under different pressures compared to atmospheric response after tetanization lead to an evolution of the response of CA1 cells in function of pressure. There is a slight increase at 10 bar followed by a stabilization till 50 bar and finally a decrease from 60 bar (Figure 6).

a



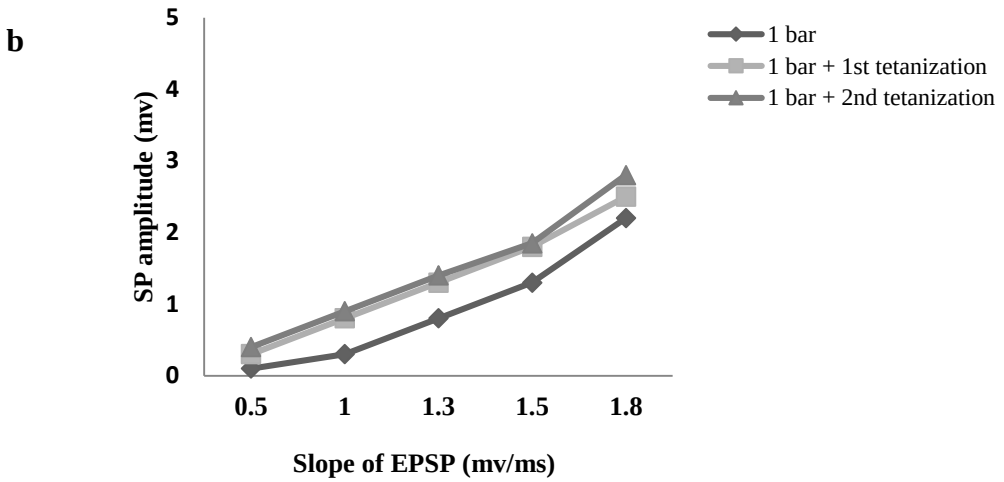


Figure 4 LTP is blocked under high pressures (Graph a) and during the first tetanization of CA1 pyramidal cells under normal pressure (Graph b). a: When the cells go form atmospheric pressure to high pressure, their excitability increases. Tetanization of cells under high pressure induces no change in their excitability. b: Under atmospheric pressure, the application of a first tetanization upon CA1 cells induces an increase in their excitability. However, a second tetanization has no effect on their excitability

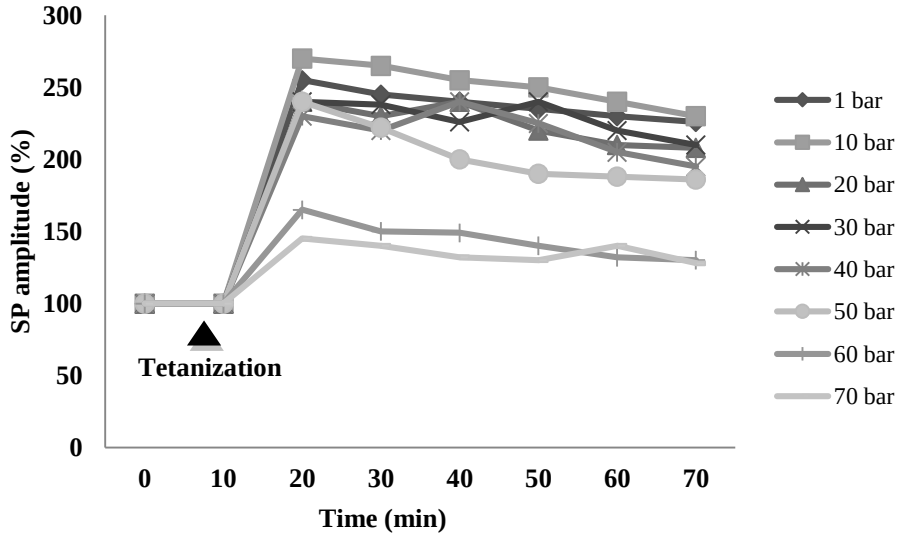


Figure 5 After tetanization, the increase of SP amplitude is at 60 to 70 bar is less important than 1 bar. The difference is significant ($p<0.01$ Mann Whitney U test)

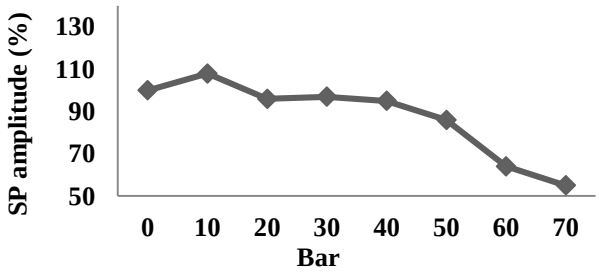


Figure 6 PS amplitude recorded at different pressures. After tetanization, PS amplitude decreases after 50 bar. The decrease at 70 bar is of 45% compared to 100% in normal response under atmospheric pressure

4. DISCUSSION

Our findings show that induced LTP by tetanic stimulation in CA1 pyramidal cells under normobar condition is altered by high pressures. This alteration appears at a pressure of 60 to 70 bar.

The depressant effect of the pressure on LTP suggests that the elementary neurochemical processes involved in the regulation of cellular excitability and leading to LTP generation were affected by pressure. Studies have shown that pressure induce a decrease in excitatory post-synaptic responses (EPSP, SP) due to changes in synaptic transmission relates to a decrease of glutamate release (Fagni et al., 1987). In parallel, pressure induces an increase of CA1 hippocampal pyramidal cells due to an overactivity of NMDA receptors and a decrease in GABA inhibition (Zinebi et al., 1988). Further studies performed by (Gustafsson et al., 1987) showed that LTP results from a hyperactivity of NMDA receptors. Moreover, the injection of NMDA receptors antagonists blocks the induction of LTP in pyramidal neurons during the application of impluses along the afferents. The blockade of GABA inhibition through the administration of antagonists such as Pirotoxin induces a large facilitation of LTP (Abraham et al., 1986). In the light of these data, we can see that LTP and high pressures play a complementary role in inducing an increase in NMDA receptors activity and a GABA disinhibition.

Although the mechanisms involved in both cases are similar, our results show a blockade of LTP rather than its facilitation. These findings lead us to believe that the high pressures, involving NMDA mechanisms and leading to loss of GABA inhibition, cause a depolarization of CA1 pyramidal cells highly enough so that cells could no longer respond to tetanic stimulation to induce LTP. In order to explain this blockade of LTP, we hypothesize that the decrease of GABA inhibition induced by the pressure may reduce the voltage-dependant blockade of potentiated NMDA receptor-coupled ion channels. The activity of these channels consist of increasing Ca^{2+} intracellular concentration at the postsynaptic cell leading to a decrease of Ca^{2+} extracellular concentration and liberation of neurotransmitters at the presynaptic terminal. (Lynch et al., 1985) reported that increased Glutamate level is mandatory in order induce LTP which means a decrease in the release of this excitatory neurotransmitter could block the development of LTP.

5. CONCLUSION

Pressures above 50 bar prevent the development of the long-term potentiation. This blockage may be related to an alteration of neurochemical processes involving GABAergic and NMDAergic systems leading to pressure-induced-hyperpolarization of hippocampal CA1 pyramidal cells. In our perspective, we would first perform further experiments in order to increase the precision of our statistical results and determine the mechanisms of LTP blockage at the levels of GABA-NMDA regulation, glutamate release and the level of Ca^{2+} channel activity.

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