Differential effect of high pressure on NMDA receptor currents in X*enopus laevis* oocytes

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Key words
High pressure neurological syndrome (HPNS), neurophysiology, hyperbaric research

Abstract

(Mor A, Levy S, Hollmann M, Grossman Y. Differential effect of high pressure on NMDA receptor currents in X*enopus laevis* oocytes. *Diving and Hyperbaric Medicine*. 2008; 38: 194-196.)
Hyperbaric environments over 1.1 MPa induce in mammals and humans the high pressure neurological syndrome (HPNS). HPNS is characterized by cognitive and motor decrements associated with sleep disorders, EEG changes, tremor, and convulsions. Previously, it was proposed that augmented responses of the glutamatergic *N*-methyl-D-aspartate receptor (NMDAR) may be involved. Recently, we have reported that, in rat hippocampus brain slices, isolated NMDAR synaptic responses were augmented at high pressure. We now test how high pressure affects NMDAR ionic currents. Mammalian (rat) cRNA of the most common hippocampal NMDAR compositions NR1-1a / NR2A and NR1-1b / NR2A were injected into *Xenopus laevis* oocytes, and NMDAR ionic currents were recorded, applying the two-electrode voltage clamp technique, at normobaric and at hyperbaric pressure (10.1 MPa). Statistical analysis revealed that high pressure increased NR1-1a / NR2A current amplitudes. By contrast, high pressure decreased NR1-1b / NR2A current amplitudes. These preliminary results demonstrate a differential high pressure effect on two types of NMDAR subunits. Moreover, augmentation of the NR1-1a / NR2A composition further supports the recently reported increase of NMDAR synaptic response in rat hippocampus brain slices. These results support the notion that increased NMDAR response, in addition to other mechanisms, plays an important role in HPNS.

Introduction

Humans and mammals exposed to 1.1 MPa and above may develop the high pressure neurological syndrome (HPNS).[1] HPNS signs and symptoms include dizziness, nausea, stomach cramps, vomiting, muscle twitching and tremors, EEG changes, and a reduction in cognitive function.[2,3] At greater depths, myoclonia, convulsions, and seizures occur.[4,5] Neuropharmacological studies at high pressure suggested an increase in excitatory N-methyl-D-aspartate receptor (NMDAR) responses in CA1 pyramidal cells.[6,7] In the same brain region, our recent electrophysiological studies showed a significant increase in the synaptic NMDAR response followed by postsynaptic excitability changes.[8,9] These studies of field potentials suggest an increase in NMDAR ionic currents. However, no work has been done on direct measurement of identified NMDAR currents at high pressure. NMDAR is a hetero-tetrameric receptor-ion channel constituted of different combinations of 'NR1' with at least one 'NR2' subunit. NR1 subunit has eight alternative splicing isoforms: NR1-1a, b; NR1-2a, b; NR1-3a, b; NR1-4a, b (according to Hollmann et al terminology).[9] The NR2 subunit has four genes: NR2A, B, C, D.[10] NMDAR is activated by the co-agonists glutamate and glycine simultaneously with the removal of Mg^{2+} blockade by membrane depolarization. These, in turn, gate the cationic channel that is permeable to Na^{+}, K^{+} and Ca^{2+}. A considerable proportion (approximately 11 %) of the current is carried by Ca^{2+}.[11]

To date, there are incomplete data on the NMDAR subunits' spatial distribution and function(s) in the adult mammalian brain. For example:

- the NR1-1a isoform occurs extensively and approximately homogeneously throughout rat brain grey matter.
- the NR1-1b variant is found primarily in the sensorimotor cortex, thalamus, hippocampal CA3 field, and cerebellar granule cells.[12]
- the NR2A subunit is expressed widely throughout the whole adult rat brain.[13]
- the composition of the NMDAR subunits may change during development.[10]
- the deactivation rate is roughly four times faster for NR1-1b / NR2B receptors than for the NR1-1a / NR2B receptors and, therefore, may be involved in long-term synaptic modulation and learning.[14]

These examples reveal a large diversity of NMDAR roles in the mammalian brain.

The goal of the present study is to examine the NMDAR component alone without the contribution of the central nervous system (CNS) network. We aim at examining directly all available NMDAR combinations. We report here our preliminary results of high-pressure effects on the currents of the two abundant and important NMDAR combinations NR1-1a / NR2A and NR1-1b / NR2A.

Methods

OOCYTE PREPARATION

Animal experiments were carried out in accordance with the guidelines laid down by the Ben-Gurion University of the Negev ethics committee for the care and use of animals for

experimental work. *Xenopus laevis* oocytes were prepared and maintained in NDE96 solution (at 18^{O}C) containing (in mM): 96 NaCl, 2 KCl, 1 $MgCl_2$, 1 $CaCl_2$, 2.5 sodium pyruvate, 5 HEPES (pH 7.5) and 50 $\mu g \cdot ml^{-1}$ gentamycin. The oocytes were injected with cRNA for co-expressions of rat NR2A (5 ng) with either NR1-1a or NR1-1b, (5 ng) subunits (produced by Prof. M Hollmann's laboratory).[15] After 3–5 incubation days individual oocytes were placed in a recording chamber specifically modified for oocyte experiments and perfused (7–8 $ml \cdot min^{-1}$) with a frog physiological solution without Mg^{2+}. Solutions were introduced to the pressure chamber by means of a high-pressure pump ('mini-pump', LDC Analitical Inc, Fl, USA).

NMDAR CURRENT RECORDINGS

Oocytes were voltage-clamped at -70 mV employing the two-electrode voltage clamp technique using an Axoclamp-2B amplifier, (Molecular Devices, Axon Instruments Inc, CA, USA). The co-agonists glutamate (100 µM, Sigma, Israel) and glycine (10 µM, Sigma, Israel) were applied to the physiological solution; exposure duration was 20 s. NMDAR currents were acquired under control (0.1–0.3 MPa) and hyperbaric (10.1 MPa, compressed helium) conditions, and analyzed off-line. Recovery at 0.1 MPa was always attempted. Temperature was kept constant at 25 ± 1^{O}C. The pressure chamber, perfusion system, helium compression, and the experimental setup have been described in detail elsewhere.[8]

DATA AND STATISTICAL ANALYSIS

Under the experimental conditions noted above, NMDAR current (in nA) was composed of two peaks. The first, relatively fast peak probably reflects current flowing through the oocyte's native Ca^{2+} dependent Cl^- channels.[16,17] The second peak represents NMDAR cationic maximal inward current amplitude. Therefore, only the second peak was measured and analyzed. The results of maximal current amplitude are expressed as mean amplitude ± 1 standard error of mean (SEM); n denotes the number of successful experiments (number of oocytes) for each experimental protocol. In each experiment, control and hyperbaric conditions were tested on the same oocytes. We used paired samples *t*-tests for analysis, assuming electrophysiological recordings meet the conditions of a normal distribution. The degree of significance was denoted by the values of *P*; results were considered statistically different when $P < 0.05$. All statistical data were analyzed using SPSS 13.0 (SPSS Inc, Chicago, IL, USA). Graphical representations were made by using Microsoft Office Excel 2003 (Microsoft Inc, Redmond, WA, USA) and SPSS 13.0.

Results

NMDAR currents were blocked at normal pressure by 2 mM Mg^{2+} and by 20 µM DL-2-amino-5-phosphonopentanoic acid (AP-5, Tocris, Bristol, UK), confirming NMDAR responses (data not shown). High pressure increased NR1-1a / NR2A current amplitude by 42.7 ± 17.7 % (n = 11, P = 0.04). By contrast, high pressure decreased NR1-1b / NR2A current amplitudes by 11.6 ± 8.4% (n = 10, P = 0.003) (Table 1, Figure 1). The NMDAR currents exhibited only partial recovery (Figure 1). In the case of NR1-1a / NR2A, out of 11 attempts, eight showed at least partial recovery (decreased responses), and three did not recover. In the case of NR1-1b / NR2A, out of 10 attempts, six recovered (increased responses), one failed to recover, and data were not available for three.

Discussion

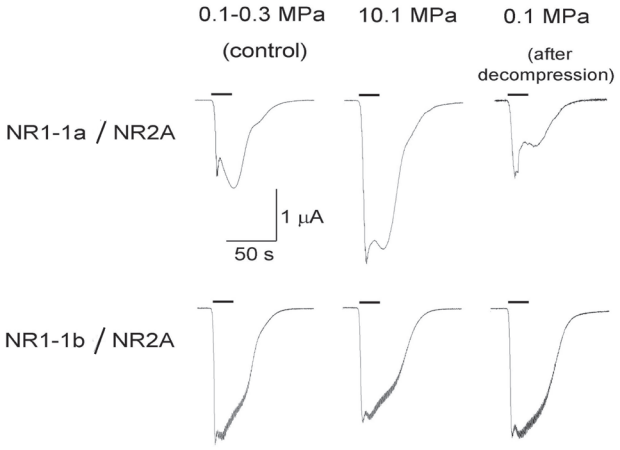
Our results reveal that high pressure selectively increases the NR1-1a / NR2A but reduces the NR1-1b / NR2A current amplitude. This pressure selectivity may point out important structural differences between the two NR1 subunits. However, it remains to be examined whether this difference is consistent for all other NR2 subunits and other NR1 1a/1b splice variant pairs. Another known difference is that NR1-1b / NR2B combinations produce currents with faster kinetics in comparison to the NR1-1a / NR2B combinations.[14] We assume that this is the reason for the greater proximity of the first peak (Ca^{2+} dependent Cl^- current) and the maximal NMDAR current (cationic current) peak recorded from the NR1-1b / NR2A combination in our experiment. The presence of the Cl^- current may distort to some extent the shape of the overall NMDAR current. At this stage we did not attempt to systematically separate the two components (e.g., by replacing Ca^{2+} with Ba^{2+}), in order to keep as close as possible to physiological conditions.

The NR1-1a is the most common NR1 subunit in the mammalian CNS.[18] Therefore, pressure effects on this subunit may determine the NMDAR pressure response in the CNS. These preliminary results are in accordance with our previous studies of pressure augmentation of NMDAR

Table 1
Statistical analysis of the NMDAR currents; n – number of experiments (oocytes); *P* – degree of statistical significance; SEM – one standard error of mean

Subunits composition	Amplitude (nA) Mean ± SEM 0.1 – 0.3 MPa	Amplitude (nA) Mean ± SEM 10.1 MPa	Amplitude (% change) Mean ± SEM 10.1 / 0.1 – 0.3 MPa	n	*P*
NR1-1a / NR2A	648 ± 141	925 ± 231	+42.7 ± 17.7	11	0.04
NR1-1b / NR2A	1858 ± 189	1561 ± 201	-11.6 ± 8.4	10	0.003

Figure 1
High pressure differentially modulates NMDAR currents in *Xenopus laevis* oocytes. NMDAR subtype NR1-1a / NR2A is increased and NR1-1b / NR2A decreased. The application of agonists (see text) is indicated by horizontal bars (20 s). The high-pressure effect is reversed after decompression.



synaptic responses in rat hippocampal (CA1) brain slices.[8,9] It is worth mentioning that an earlier study on NMDAR expressed in oocytes (isolated mRNA from rat cerebellum) exhibited pressure-increased responses.[19]

Conclusions

Our data support the postulated NMDAR involvement in HPNS hyperexcitability; however, they indicate a selective role for specific combination(s) of the receptor subunits. It is important to note that the NMDAR hyperactivity is only one factor in a multifactorial model for HPNS that may include reduced inhibition, synaptic frequency modulation, and dendritic boosting mechanisms.

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