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THE HYDROGEN PEROXIDE AS A POSSIBLE MESSENGER FOR 4HZ MECHANICAL VIBRATION TREATMENT OF HEART MUSCLE CONTRACTILITY

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It was shown that MV increased the melting duration (polarization) of PS by 21.33±4 %, and decreased the H_2O_2 concentration in PS by 5±0.9%. MV-treated PS increased amplitudes of heart muscle. On the basis of the obtained data it is suggested that MV-induced increase of heart muscle contraction's amplitudes is due to decrease of H_2O_2 concentration in cell bathing medium.

Mechanical vibration – heart muscle contractility – H_2O_2

Ցույց է տրվել, որ 4 Հց մեխանիկական տատանումների ազդեցությամբ տեղի է ունենում ֆիզիոլոգիական լուծույթի հալման տևողության մեծացում 21.33±4 %-ով և H_2O_2-ի կոնցենտրացիայի նվազում 5±0.9%-ով: Մեխանիկական տատանումներով մշակված ֆիզիոլոգիական լուծույթը խթանել է սրտամկանի կծկողունակությունը: Ստացված տվյալներից ենթադրվում է, որ սրտամկանի կծկողունակության խթանումը մեխանիկական տատանումների ազդեցությամբ կատարվում է H_2O_2-ի կոնցենտրացիայի նվազման շնորհիվ:

Մեխանիկական տատանումներ – սրտամկանի կծկողունակություն – H_2O_2

Было показано, что механическая вибрация продливает период плавления физиологического раствора на 21.33±4 %, уменьшает концентрацию H_2O_2 на 5±0.9%. После воздействия механической вибрации увеличивается амплитуда сократимости сердца. Предполагается, что увеличение амплитуды сократимости сердца под влиянием механической вибрации происходит благодаря уменьшению концентрации H_2O_2 в жидко-клеточной среде.

Механическая вибрация – сократимость мышцы сердца – H_2O_2

In our previous works it was shown that Mechanical Vibration (MV) has a frequency dependent effect on physicochemical properties of water and water solutions, which is accompanied by gas composition changes (decreasing CO_2 and increasing O_2) [1, 2, 11]. These changes were more pronounced at the 4Hz (30dB) frequency. The 4 Hz MV-treated water and water solutions have modulation effects on plant seed germination [4], microbes' growth and development [11, 12] and heart muscle contractility [5, 6]. However, the nature of the messenger(s) able to transfer the MV-induced changes of physicochemical properties of cell bathing medium to the intracellular metabolic cascade, as well as the metabolic pathway, through which the biological effect of MV is being achieved are not clear yet. It is known that the water dissociation products collision with soluble oxygen could generate the reactive oxygen spices (ROS) [7, 8, 9, 13]. Therefore it is suggested that MV-induced changes of water dissociation could bring to the changes of hydrogen peroxide (H_2O_2) (ROS with the longest life time) content in cell bathing medium, which would modulate the cell metabolic activity. To check this hypothesis the effect of 4Hz 30dB MV on melting process of Physiological Solution (PS) after freezing in nitrogen (N_2) liquid, and H_2O_2 concentration in PS was studied.

Materials and methods. A special device was assembled, which allowed treating the PS by MV in range of 1–20 Hz frequency. A glass test tube with 10 mm diameter and 10 ml volume was fixed in the holder of the vibrator. The vibrator was driven by the sine–wave generator (PASCO, CA, USA) through the power amplifier.

To obtain the stable amplitude for vertical vibration, a feedback coil was used. The vibrator was constructed in the department of engineering at UNESCO Chair–Life Sciences International Postgraduate Educational Center. For matching the output power of the sine–wave generator with the driving power vibration, a special power amplifier (Institute of Radiophysics and Electronics Armenian NAS, Yerevan, Armenia) was used.

The snail (Helix Pomatia) isolated hearts were cannulated and suspended in bath with PS (pH 7.5). The PS contained: NaCl (80 mM), KCl (4 mM), $CaCl_2$ (7 mM), Tris–HCl (5 mM). Continuously the intra- and extra-heart perfusion with PS was applied. The intra-cordial pressure was controlled by keeping the perfusion solution in reservoirs vessel at a constant level. To record the heart contraction, a special setup was constructed (fig. 1). The transducer was fixed on the bottom of the heart by a silk suture. Up and down movements of the records baseline showed the heart contraction and relaxation, correspondingly.

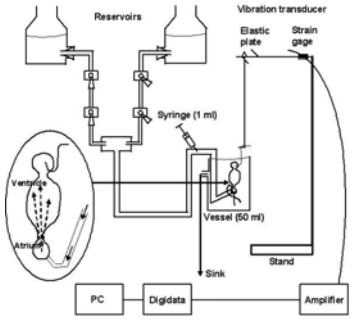

Fig.1. The setup for the registration of heart muscle contractility. Heart contractions were recorded isotonically and displayed on PC through Digidata 1322A.

The melting duration of frozen PS samples was estimated by the following method: the 15-min MV-pretreated samples (per 0.5 ml) were placed in a hermetic plastic tube (volume 1 ml) that had a thermo-sensor at the bottom. Then, the tube was inserted into the Dewar vessel containing liquid N_2 for deep freezing (up to -75^{o}C). After withdrawing from the liquid N_2, the tube was left to melt in the thermostat at 22 ± 0.5^{o}C condition. The temperature of the samples was recorded during the melting process by extra-sensitive thermometer Biophys-TT (elaborated by the Institute of Radiophysics and Electronics of Armenian NAS, Yerevan, Armenia) connected to PC through Digidata 1322A data acquisition system (Molecular Devices, Sunnyvale, CA). The data were analyzed by dataTrax 2–computer software.

The quantity of H_2O_2 in PS samples was determined by the method of sensitive assay based on enhanced chemiluminescence in peroxidase–luminol–p-iodophenol system [11], which allowed detecting the H_2O_2 content at a range of nanomolar concentrations. The chemiluminescence of samples was quantified with 1450 MicroBeta liquid scintillation and luminescence counter Wallac–1450. For luminescence counting, the 24-well sample plates (1450-402) set on 1450-102 cassettes was used. The ratio of sample's and "counting solution"s volumes in each well of plate was 1:1 (v/v). The "counting solution" contained 10 mM Tris–HCl buffer (pH = 8.5), 50 μM p-iodophenol, 50 μM luminol, and 1 nM horseradish peroxidase (Sigma-Aldrich Chemie Gmbh, Steinheim, Germany). The "counting solution" was prepared immediately prior to the measurement. The H_2O_2 concentration in nM was quantified according to the calibration curve.

The Sigma-Plot (Version 8.02A) was used for the data analysis.

The mean value and standard error of H_2O_2 concentration, were calculated and the statistical probability was determined by Student's paired t-test with the help of the computer program Sigma-Plot (Version 8.02A). The statistical probability was expressed on figures with the help of asterisks (*).

Results and Discussion. Previously it was shown that 4Hz MV has more pronounced modulation effect on physicochemical properties of water [1, 2]. In present work the effect of 4 Hz MV on melting duration of after freezing in liquid N_2 and H_2O_2 content as well as 4 Hz MV-treated PS effect on snails heart muscle contractions were studied.

It is known that the melting duration of water solution depends on its polarity, which determines by the quantity of hydrogen bounds between water molecules [10]. As can be seen on (fig. 2), 4 Hz MV has more pronounced effect (20±2%) on melting duration of PS than 5 Hz and 3 Hz MV have.

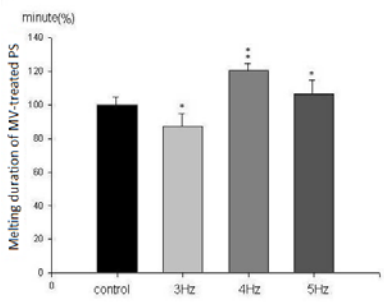

Fig.2. Melting duration of 15 min. MV-treated PS at 3Hz, 4Hz, 5Hz frequencies (expressed in %, compared to their control value). *P<0.05, **P<0.001.

Four Hz MV-induced increase of melting duration of PS could be explained by depression of water and electrolytes' molecules dissociation. In consequence the decrease of dissociation products of water molecules would decrease the possibility of the oxygen molecules collusion with them and would depress the H_2O_2 formation.

To check this suggestion, in the next series of experiments the effect 4 Hz of MV on the H_2O_2 level in PS was studied.

Data presented on fig. 3, show that 4 Hz MV treatments has a decreasing effect on H_2O_2 content in PS (fig. 3A). As the H_2O_2 contents in cell bathing PS depends on both, its formation and degradation, it was interesting to perform the same experiments in isolated snail heart containing PS (fig. 3B).

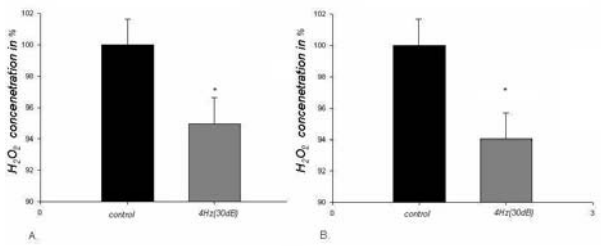

Fig.3. (A and B). The measurement of H_2O_2 in 15 min 4Hz (30 dB) MV-treated PS (A) and in heart containing MV-treated PS (B). (expressed in %, compared to their control value). *P<0.05.

Thus, the obtained data indicate on 4 Hz MV-induced depressing effect on H_2O_2 formation in PS. To find out weather such MV-induced decrease of the H_2O_2 in PS could serve as a potential messenger, through which MV could modulate the heart muscle contractility or not, in the next series of experiments the effect of MV -treated PS on heart muscle contractility was studied. Obtained data show that 4Hz MV exposure causes to the decrease of H_2O_2 level in heart containing PS more (6±1.2%) (fig. 3B) as

compared with heart-free PS (5±0.9%) (fig. 3A), which probably could be explained by the increase of H_2O_2-destroing enzymes in muscle or by heart muscle-induced structural changes of PS. For the final conclusion we need more detailed investigations.

One of the typical recordings of 10 experiments shows that 4 Hz MV-treated PS has clearly pronounced increasing effect on the amplitude of heart contractions (fig. 4). It is worth to note that in all our experiments, the MV-induced activation of heart muscle contractility has trace effect, the elucidation of which needs more detailed study. For elucidating if the observed MV-induced stimulating effect of muscle activity is the direct result of modulation of the metabolic activity of muscle or not, the similar protocol of experiments was performed in cold (+10^{o}C) PS (fig. 4B). As can be seen on (fig. 4B), the MV-treated PS-induced effect on heart muscle contractility was temperature insensitive. Therefore the comparative insensitivity of MV-treated PS effect on muscle contractility allows us to suggest on non metabolic nature of the mechanism(s) responsible for observed effect of MV-treated PS on muscle.

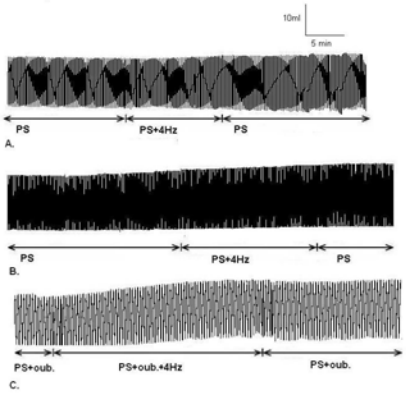

Fig.4. The effect of 15 min. 4Hz (30dB) MV-treated PS on the heart muscle contractility at room temperature (A), in cold medium (10°C) (B), and in ouabain containing PS (10^{-4}M) (C).

For clarifying the role of Na+/K+ ATP-ase in realization of MV effect on heart muscle contractility, in the next series of experiments, the effect of MV-treated ouabain (inhibitor of Na+/K+ ATP-ase) containing PS (10^{-4} M) and MV-treated K-free PS effects on heart beating was studied. As can be seen on one of typical records of 10 experiments, the MV-treated PS effect on heart contractility was insensitive to pump inhibitor-ouabain. Same data was observed in case of K-free induced pump inhibition (data are not presented). The data of present experiments performed in MV-treated ouabain (inhibitor of Na+/K+ ATP-ase) containing PS (10^{-4} M) (fig. 4C), and in MV-treated K-free PS (data are not presented) indicate to the non sensitivity of 4Hz MV-treated PS–induced effect on Na^+/K^+ pump. In all experiments MV-induced activation of heart muscle contractility had trace effect and it was necessary to perfuse heart by non treated PS longer than 30 min to remove this effect.

The obtained data allow us to make the following conclusions:

1. MV leads to decrease of H_2O_2 concentration in cell bathing medium as a result of depression of water molecules dissociation.
2. MV-treated PS increases the amplitude of heart muscle contractility.

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