Cell Bathing Medium as a Target for Non-thermal Effect of MMW on Heart Muscle Contractility

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Abstract— The comparative study of the effects of weak intensity specific absorption rate (SAR = 1.8 mW/g) of 4Hz modulated 160 GHz millimeter wave (MMW) and near Infrared (IR) irradiation on thermodynamic properties, specific electrical conductivity (SEC) of physiological solution (PS) and hydrogen peroxide (H_2O_2) formation in it as well as the effect of MMW-treated PS on heart muscle contractility, ^{45}Ca uptake was performed. The heat fusion capacity of MMW-pretreated PS after freezing by liquid nitrogen (N_2) is significantly less than the heat fusion capacity of sham and IR-treated PS. MMW unlike IR, has time-dependent elevation effect on water SEC and SAR, which is accompanied by the increase of H_2O_2 formation in it. The direct MMW radiation, MMW-pretreated PS and H_2O_2-containing PS have increasing effect on heart muscle contractility. The MMW-pretreated PS and the H_2O_2–containing PS have activation effect on ^{45}Ca uptake and dehydration effect on heart muscle contractility. Thus, the obtained data allow us to consider water dissociation as a main target through which the non-thermal effect of MMW on physicochemical properties of water is realized, while the MMW-induced formation of H_2O_2 in cell bathing medium serves as a messenger through which the modulation of intracellular metabolism takes place.

1. INTRODUCTION

The phenomenon of non-thermal biological effect of low intensity Millimeter Waves (MMW) has been known for several decades (Devyatkov 1973; Adey 1981; Lin 2004; Belyaev 2005). Although, it is more and more widely used in alternative treatments of a variety of diseases (Ziskin 2006; Markov 2007), the physicochemical mechanisms underlying the non-thermal biological effect of MMW still remain unclear.

As MMW penetration in body depths is only a few tenths of a millimeter, it is suggested that the therapeutic effect on organisms is initiated by water within the skin components ($\sim 70\%$), for which the absorption coefficient is the largest (Ziskin, 2006). However, the nature of low intensity MMW-induced changes of physicochemical properties of extracellular water, which could modulate cell metabolic activity, is not clear yet.

Although the higher sensitivity of hydrogen bonding makes water dissociation as one of the most variable properties of water, adequate attention has not been paid by investigators to water ionization as a universal and extra sensitive "primary" target for the biological effects of weak intensity environmental factors, including electromagnetic fields (EMF) (Szent-Gyorgyi 1968; Klassen 1982). It is known that even partial alignment of water molecules with the electric field may bend or break the hydrogen bonding (Chaplin 2008). Therefore, it is predicted that the MMW-induced water dipole vibration could increase water dissociation and in presence of oxygen (O_2) form reactive oxygen species (ROS), which are strong modulators for cell metabolism. The formation of hydrogen peroxide (H_2O_2) upon the high intensity MMW has been demonstrated (Gudkova et al. 2005). However, the possibility of ROS generation in water and water solutions upon the influence of extremely low intensity MMW is not clear yet.

For checking the above mentioned hypothesis whether the weak intensity MMW could modulate the water dissociation and generate H_2O_2, through which the effect of MMW on heart contractility is realized, the following two series of experiments were performed in present work:

1. A comparative study of the effects of MMW and near IR (1–100 THz) radiation on physicochemical properties of PS.
2. A comparative study of the effects of MMW-pretreated and H_2O_2-containing PS on heart muscle contractility, ^{45}Ca-uptake.

2. METHODS

As an experimental model serves PS (composition (in mM) NaCl-80, KCl-4, $CaCl_2$-7, Tris-HCl-5, pH-7.5) and isolated-intracordial perfused heart muscle of snail *Helix pomatia*. The MMW generator

"Artsakh-04M" (Russian production), designated for clinical applications, which generates 90–160 GHz MMW modulated by 4 Hz EMF was used. As IR source serves the "NOVAFLEX" Fibber Optic Illuminator (World Precision Instruments, USA) with near IR light filter. The determination of SAR ($SAR = C_{w(PS)}\Delta T/\Delta t$) of PS and heart for MW and IR radiation was used a high-precision differential calorimetric device "Biophys MWD-001" (Simonyan et al. 2006). For the determination of H_2O_2 content, the enhanced chemiluminescence method in a peroxidase-luminol-*p*-iodophenol system was used. The chemiluminescence and ^{45}Ca uptake by muscle were quantified by "Walac" liquid scintillation counter (Finland production).

2.1. The Comparative Study of MMW and IR Radiation on Physicochemical Properties of PS

As the SEC of water solutions is determined by ions, in order to find out whether MMW radiation leads to water ionization, the comparative study of the effects of MMW and IR radiation on SEC of PS was performed.

The data presented on Figure 1 show that at 18°C the SEC of 10-min-MMW-pretreated PS is significantly higher (4.1%) than in case of equivalent intensity IR-preheated and sham-treated PS. It is worth to note that the second 10 min of MMW exposure (a') has more pronounced elevation effect (50.2 %) on PS SEC than the first 10 min of exposure (a), while in case of IR irradiation both, the first (b) and the second (b') 10 min of exposures have the same effect on PS SEC (Figure 1(a)).

It is known that the SAR of water is related to electrical conductivity (σ) and density (ρ) expressed as $SAR = \sigma E^2/\rho$, where E is electric field intensity. Therefore, it is predicted that the MMW-induced possible changes of water dissociation could cause time-dependent changes of water SAR during MMW radiation. To check this hypothesis the thermal effects of double exposure of MMW and IR radiation on PS for 10 min (with 10 min intervals) were studied. During 10 min inter-exposure period the temperature of PS samples was returned to the initial temperature (18°C).

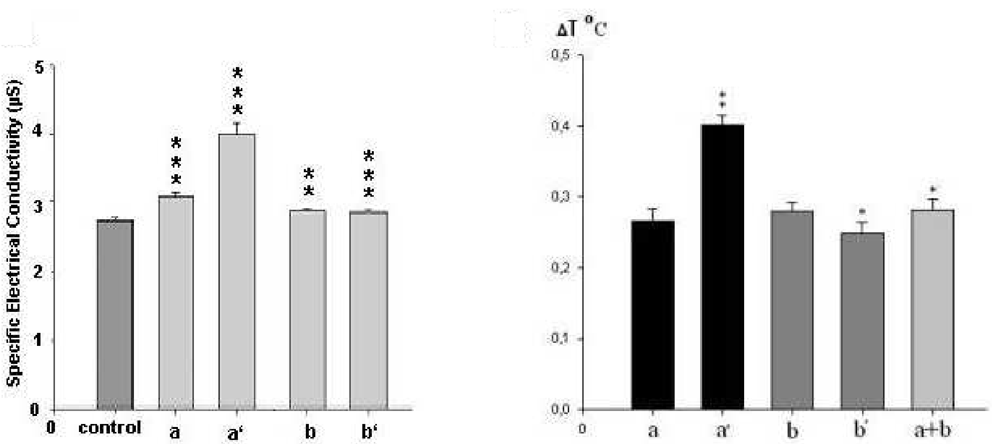
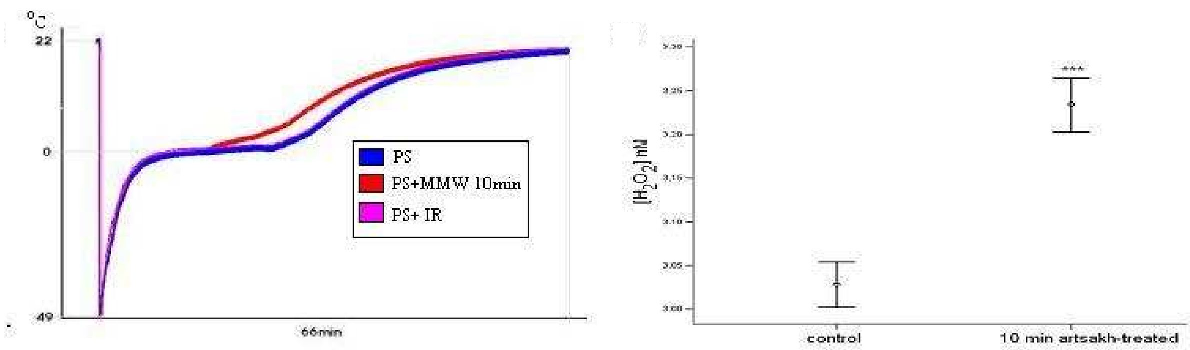


Figure 1: The effect of MMW and IR irradiation on specific electrical conductivity (a) and thermal capacity (b) of PS: (a) and (b) the first (a, b) and the second (a', b') 10 min of MMW and IR exposures, correspondingly and (b) ($a+b$) — 10 min MMW exposure followed by 10 min IR irradiation. ** $P < 0.0001$; *** $P < 0.000001$.

The data presented on Figure 1(b) show that 5.8 mW/sm^2 MMW-induced thermal effect on PS after the second 10 min of exposure (a') was higher than after the first 10 min of exposure (a) (0.22°C). While in case of IR radiation, its thermal effect was the same after the first (b) and the second (b') 10 min of exposures. The increase of MMW-induced heating after the second 10 min of exposure could be the result of either the decrease of the thermal capacity or the increase of SAR of PS. The absence of the elevation of the thermal effect after IR exposure (10 min) on MMW-pretreated (10 min) PS ($a + b$) could be explained by MMW-induced increase of PS SAR.

As water structure changes predict the adequate changes of its ice structure, in the next series of experiments the kinetics of melting process of sham-, MMW- and IR-treated PS frozen in liquid N_2 (−50°C) were studied.

As it can be seen from these data, the melting curve of MMW-pretreated PS (a) is significantly different from sham- and IR-pretreated PS. The heat fusion time of MMW-pretreated PS is twice shorter than in case of sham- and IR- pretreated PS. These data could serve as an evidence of non-thermal structural (poliarity) changes of PS upon MMW irradiation.



(a) (b)

Figure 2: (a) The time-dependent melting curves of sham (PS), MMW- (PS+MMW) and IR- (PS+IR) pretreated PS frozen in liquid N_2. MMW and IR radiation was performed 3 times with 10min intervals. Typical curves of one of 20 experiments. (b) The amount of H_2O_2 in control and 10 min MMW-treated PS. ($n = 10$).

In order to find out whether MMW-induced elevation of water dissociation leads to the formation of ROS in DW and PS, in the next series of experiments the concentration of hydrogen peroxide (H_2O_2) in non-treated and MMW-treated PS was determined.

As it can be seen on Figure 2(b) the level of H_2O_2 in PS was increased by 10 min MMW irradiation (SAR = 1.8 mW/g) from 3.03 to 3.24 nM.

2.2. The Comparative Study of MMW-pretreated and H_2O_2 Containing PS on Heart Muscle Contractility

As it is presented on Figure 3 both, 10-min-direct-exposure of MMW (a) and MMW-treated PS (b) have contraction effect on heart muscle contractility brining to fully stop of heart beating. Previously it was shown that H_2O_2-containing PS has also inhibitory effect on heart muscle beating (Ayrapertyan et al. 2007). Such similar depressing effects of MMW-pretreated and H_2O_2-containing PS on heart muscle contractility could be considered as coincide data for the hypothesis that the MMW-induced formation of H_2O_2 in PS could serve as a pathway through which the biological effects of MMW are realized.

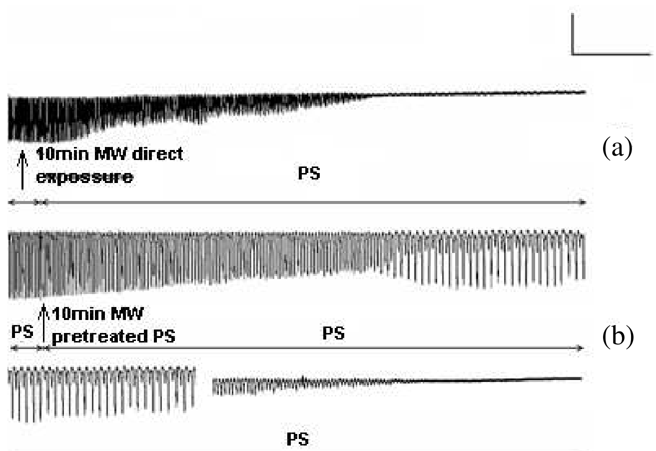


Figure 3: The effect of direct MMW 10 min-exposure (a) and MMW-pretreated PS (b) on heart muscle contractility. The interruption period of the recording was 15 min. Calibration: contraction amplitude-250 mg, time-5 min.

However, to finalize the above mentioned data we need to clarify if MMW-pretreated and H_2O_2-containing PS have a common metabolic pathway through which they modulate heart muscle contractility. As the intracellular Ca ion has a crucial role in the regulation of muscle contractility in the next series of experiments the study of the effects of MMW-treated and H_2O_2–containing PS on ^{45}Ca uptake was performed. The heart muscle 10min-exposure by H_2O_2 (5×10^{-9} M) containing PS and 5 min exposure by MMW has elevation effect on ^{45}Ca uptake in heart muscle by $20 \pm 1.9\%$ ($n =$ 10), while H_2O_2 (10^{-7} M) and 1 min MMW direct exposure leads to the decrease of ^{45}Ca uptake by 30%. This data indicate that MMW exposure could increase the local H_2O_2 concentration until 10^{-7} M. Thus, the obtained data allow us to conclude on common ^{45}Ca-dependent mechanisms through which their effects on muscle contractility are realized.

This conclusion is in close agreement with an early suggestion of Arber and Lin (Lin 2004) showing that the MMW effect on snail neuron was triggered by the increase of intracellular free Ca^{2+}, which was completely eliminated by intracellular injection of Ca^{2+} chelating agent-EDTA.

3. CONCLUSIONS

The obtained data show that the non-thermal biological effects of MMW could be explained by dissociation increase of cell bathing aqua solution bringing to generation of ROS through which the modulation of Ca-dependent metabolic mechanisms responsible for heart muscle contractility is realized.

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