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THE INVOLVEMENT OF CUTANEOUS RECEPTORS IN THE BIOLOGICAL EFFECTS OF ELECTROMAGNETIC MILLIMETER WAVES

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Abstract - The involvement of peripheral nerve terminations in the mechanisms of action of electromagnetic millimeter waves (mmW) was assessed. It is currently thought that mmW could be used in noninvasive complementary therapy because of their analgesic effect. However, the mechanisms of their antinociceptive effect and non-ionizing radiation are the subjects of controversy. The mechanisms of interaction of mmW and the cutaneous tissue have not been elucidated. We observed mast cell degranulation at the place of mmW action, a decrease of chronaxie and Turck reflex time, an increase in the number of afferent impulses after sciatic nerve at stimulation, as well as an increase electrocardiogram R-R interval of isolated frog heart after application of mmW. Based on these investigations, we propose that electromagnetic waves of millimeter length modify, through indirect mechanisms, the excitability and reactivity of peripheral nerve terminations.

Key words: cutaneous receptors; electromagnetic millimeter waves; peripheral nerves terminations.

INTRODUCTION

As we have previously reported (Anton et al., 2013), millimeter waves could be used in noninvasive complementary therapy because of their analgesic effect, however, the mechanisms of their effects are controversial (Usichenko et al., 2006). The many assumptions regarding electromagnetic wave interactions in the millimeter range (mmW) generated within biological tissues, and the presence of intracellular mmW generators, are due to the current absence of direct methods for the determination of these waves. The therapeutic effects of mmW radiation are based on these assumptions.

In the plasma membrane of living cells, mmW transform in electroacoustic oscillations that are maintained (on account of cellular metabolism) analogous to a super high frequency (SHF) generator (Devyadkov et al., 1994). On the other hand, another hypothesis highlights the resonance generated in biological structures by mmW; the model of resonant reception of the protein molecules in living organisms, in response to the external action of electromagnetic waves (Sit`ko et al., 1994).

Based on the probability of mmW autonomous generating in the cells and their possible relevance in the inter- and intracellular informing, external irradiation with mmW may be considered as an informative action, which synchronizes the informational-undulatory functions. Moreover, according to another concept, mmW interact with tissular water, causing critical hydration of membrane proteins, which ensures their passage from the passive into the active functional state. Regardless of the physicochemical and biophysical phenomena that initiate the interaction of mmW with biological structures, the physiological, and especially, therapeutic effects remain unclear.

Because the penetrative power of mmW into the skin is under 1 mm, the peripheral reflector element is of particular interest for research regarding the influence of these waves on the cutaneous nervous receptors. The mechanism of mmW interaction with cutaneous tissue is not understood and consequently the physiotherapeutic effects are not substantiated. The main purpose of our work was to assess the involvement of peripheral nerve terminations in the mechanisms of action for electromagnetic millimeter waves.

MATERIALS AND METHODS

The application of millimeter waves was performed with the 'КВЧ-универсал' (MTA-KB2) apparatus in a amplitude modulation regime with frequency 8 Hz for 15 min at 5.6 mm wave length, 10 mW cm^{-2} intensity. The point electrode was applied directly on the surface of irradiation.

In order to determine the excitability threshold (rheobase and chronaxie), after assessing the location of the biologically active point Gi4 with the electroanalgesimeter ДЭА-1, the 'Neuropulse electrostimulator' (Bulgaria) was used. The excitability parameters in healthy young volunteers were determined by double-checking under irradiation with mmW in the aforementioned regime.

Reflex time was determined according to the Turck spinal frog method, applying an increasing concentration of acetic acid (10, 15, 20%) to frog legs. After determining the initial value of the reflex time, the right leg reflex was radiated with mmW. The left leg was left unirradiated and served as control. Reflex time was determined immediately after irradiation and over 15 min after irradiation.

The electrocardiogram was recorded with the BIOPAC system of collecting data on isolated frog heart up to and after 15 min irradiation with mmW. In similar conditions, the frog tongue was subjected to irradiation in order to determine histologically the mast cells stained with toluidine blue.

RESULTS

The results of determining excitability of the nociceptors (Table 1) showed that the average values of the rheobase before and after irradiation do not essentially differ from the average values of the initial and final rheobase of the placebo effect. This phenomenon is probably caused by the fact that the mmW irradiation exposure time (15 min) is essentially shorter compared to that applied during treatment.

In addition, the Turck reflex time decreases in relation to the concentration of acetic acid and increases during the experiment. At the same time, the duration of the Turck reflex decreases obviously under the influence of mmW irradiation (Table 2).

Under similar conditions, the spontaneous and induced afferent impulses of the sciatic nerve up to and after the application of mmW were recorded. The recordings made with the BIOPAC MP 100 WSW system showed the increasing of the maximal amplitude of the recording data after applying mmW (unpublished data, Saulea et al.). In addition, histological studies of frog tongue have highlighted massive degranulation of mast cells in irradiated areas compared to regions where the mast cell granules were kept intact.

The decrease in the Turck reflex time indicates an increase in excitability of the nociceptive receptors without the involvement of the central nervous structures. This phenomenon is confirmed by in-

Table 1. The average values of the rheobase and chronaxie in the mmW irradiation.

Type of action	Averages for the rheobase and chronaxie			
	Ri nr = 30	Rf nr = 30	Cri nr = 30	Cr f nr = 30
Umm Pl Umm	32±10.48 32 ± 11.24	34±9.85 40 ± 14.77	0.203±0.08 0.184 ± 0.05	0.195±0.001 0.195 ± 0.09

(RI (mA) – rheobase up to irradiation, Rf (mA) – rheobase after irradiation, Cri (msec) – chronaxie up to irradiation, Crf (msec) – chronaxie after irradiation mmW – millimeter waves Pl mmW – placebo effect for mmW)

Table 2. The average values of the Turck reflex during mmW irradiation

	Immediate			after 15 min		
	10% nr = 8	15% nr = 8	20% nr = 8	10% nr = 8	15% nr = 8	20% nr = 8
LS LD	12.72±0.22 9.55±0.14*	9.57±0.27 6.65±0.27*	6.18±0.35 3.89±0.24*	17.0±0.19 12.55±0.18*	11.69±0.25 8.52±0.16*	7.84±0.21 5.01±0.26*

(LF – left foot of the frog. RF – right foot of the frog. *p <0.05 compared to LF and RF.)

creasing nervous impulses, particularly in response to the application of acetic acid on the foot skin.

DISCUSSION

Mast cell degranulation under mmW action is likely a consequence of the changes in the structured water from the cells or the intercellular space. Because mast cells are located at the periphery in the region of the peripheral nerve terminations, removal of the biologically active substances as a consequence of degranulation, mainly histamine, acts in a paracrine manner on the peripheral receptors by increasing their sensitivity.

Thus, mmW, through mast cell degranulation and elimination of biologically active substances through the paracrine pathway, change the excitability of peripheral nervous structures. This phenomenon is probably one of the mechanisms that ensure the therapeutic effect of mmW and can be explained by the inclusion of mast cell degranulation affecting the peripheral nerve structures.

The physicochemical mechanism regarding the apparent transformations in living tissue cells, including mast cell degranulation phenomenon under the action of mmW, is not currently understood. The irradiation with mmW of the skin of anesthetized frogs causes the heart rate to change its latency for almost 1 min, and the complete denervation of the heart reduces this phenomenon (Chernyakov et al., 1999). Also, the heart rate variability changes in anesthetized rats in response to upper thoracic vertebrae irradiation for 20 min with an intensity of 10 mW/cm^2 (Potekhina et al., 1992).

The spontaneous afferent impulses increase with mmW irradiation of the electroreceptors of the ampullae of Lorenzini in anaesthetized turbot, within a range of 1-4 mW/cm^2, which produces a local temperature rise of 0.1^{0}C. The intensity of 10 mW/cm^2 and higher causes inhibition of spontaneous afferentation. However, authors do not consider these responses to the mmW irradiation order to be specific (Akoev et al., 1995).

Spontaneous afferentation of the nerve of the frog bladder increases as a response to the mmW irradiation of the bladder wall with an intensity of 10 mW/cm^2 (42.19±0.15 GHz) for 20 min. Immediately after irradiation cut-off, the number of spontaneous afferent pulses drops, but not to the initial value. Infrared irradiation of an equivalent intensity to mmW radiation produces delayed effects after 1-1.5 h of irradiation, which do not disappear immediately after the cut-off (Sazonov et al., 1995).

In other investigations carried out on the extension receptors of the noble crayfish, it was found that infrared irradiation or mmW irradiation of equivalent values produces similar effects in increasing afferentation, implying frequency modulation only through caloric effect (Khramov et al., 1991). The possibility of alteration of the peripheral cutaneous receptors' function under the influence of low intensity mmW was exhibited in studies on the skin mechanoreceptors in anesthetized rats.

Also, the exposure to mmW (55, 61, 73 GHz and intensity of 2.90 and 7.81 mW/ cm^2) for 35 min caused a small increase in skin temperature (0.01°C), but did not directly excite the mechanoreceptors. The answer to the scaled mechanical excitation is through the increase in the excitation threshold, in the case of some receptors up to 180% compared to the initial value, while in others an initial decrease of 8-12% that disappears after 10 min, and increases up to 160% after 25 min from exposure, after which they become completely inactive for a long time and do not respond to mechanical stimulus. It is assumed that the sensations (vibration, heat, numbing, etc.), apparent in mmW irradiation, may be the consequence of functional disturbances and receptor blocking (Enin et al., 1992).

In the double experiences performed on volunteers it was found that mmW radiation is discerned by 30-80% of the subjects, which according to authors can be explained by the fact that the electromagnetic waves are perceived by different mechanical receptors and nociceptors (Lebedeva et al., 1995) or due to individual hypersensitivity.

Thus, the direct influence of mmW on the peripheral nerve structures, in particular the receptors, remains to be elucidated. First, the depth of penetration of the mmW equal to 0.6-0.8 mm in the human skin corresponds to the epidermal layer in which free nervous terminations are located, able to perceive thermal and aching stimuli, tactile and pressure sensations. In the deeper layers of the epidermis and dermis, at the level of dermal papillae, are located the Merkel discs and Meissner's corpuscles ensuring tactile perception. The Meissner corpuscles have a special structural feature, which is the presence inside the receptor capsule, beside the network of nervous fibers, of an additional nervous fiber originating in the initial network of the epidermis (Webster et al., 2005). The probability of direct involvement of the Ruffini or Pacini corpuscles in the reactions caused by mmW irradiation from a morphologically point of view is low because they are located deep in the dermis or even hypodermis.

Secondly, mmW radiation, as a rule, is applied to biologically active points, which are regarded as peripheral elements of the reflex zones. In terms of physics, these active biological points have low electrical resistance, which makes it possible to determine their exact location. The causes of the low impedance in these points are not clear, which gives rise to various controversial concepts. In some papers, it is stated that this phenomenon is determined by the presence of sebaceous and sweat glands, while in others it is thought that this phenomenon is determined by biophysical and structural peculiarities of the dermis (Webster et al., 2005).

Morphological studies of the biologically active points indicate the presence in their vicinity of nervous terminations, subcutaneous arterioles and veins. Obviously the blood vessels have an abundant vegetative innervation. Adjacent to the nerve terminations and blood vessels in the epidermis there are a large number of cells, among which mast cells predominate (Bauer et al., 2000). The mast cell's ability to synthesize and secrete a number of biologically active substances (histamine, proteases, serotonin, substance P, heparin, etc.) points not only to their involvement in local tissue regulation but also to neurohumoral influences related to the central nervous system. Additionally, mast cells are very sensitive to mechanical, thermal, chemical, radiant and electromagnetic waves actions. This sensitivity is due to the large number of membrane receptors through which mast cell degranulation is stimulated. Human skin mast cells respond to neuropeptide action by rapid release of histamine. In human skin, there is a diverse number of neuropeptides (substance P, somatostatin, VIP, angiotensins, etc) (Rossi et al., 1998. Ciobica et al., 2009, 2011).

Substance P stimulates mast cells to release histamine, and, on the other hand, being present in the afferent C nerve fibers, is a mediator of pain transmission. The somatostatin that is released from the dendritic cells also contributes to the activation of histamine degranulation from the mast cells. Histamine is also encountered in basophiles, in various skin cells, capillary endothelium, etc. Additionally, histamine release causes local inflammatory reactions, as well as sensations of itching or even pain (Arcan et al., 2013).

The presence of mast cells in the cutaneous structures and especially in areas with low electrical resistance (e.g. abundantly hydrated), allows the hypothesis of their inclusion in the response to mmW radiation primarily due to histamine release and other active substances, able to shape the sensitivity or to stimulate peripheral nerve structures. For example, the emergence of various symptoms in humans under the influence of electromagnetic waves generated by video terminals is explained by histamine releasing from dermal mast cells (Gangi et al., 2000).

The phenomenon of mast cell degranulation under mmW irradiation is attested in various works. Irradiation with mmW (10 mW/cm^2, λ – 5.6 mm) of the tongue of the impaired frog, *Rana esculenta* (medullary centers destroyed) highlighted the obvious degranulation of mast cells. In addition, experiments on animals (rodents) are difficult because of the occurring stressful situations, which cause the mast cells' degranulation (Theoharides et al., 1996).

REFERENCES

Akoev, G.N. Avelev, V.D. and *P.G. Semenjkov* (1995). Reception of low-intensity millimeter-wave electromagnetic radiation by the electroreceptors in skates. *Neuroscience* **66**. 15-17.

Arcan, O., Ciobica, A., Bild, W., Hritcu, L. and *D. Cojocaru* (2013). The effects of central angiotensin II and its specific blockers on nociception. Possible interactions with oxidative stress status. *J Med Biochem* **32**. 52-58.

Anton, E., Rotaru, A., Covatariu, D., Ciobica, A. and *CR Anton* (2013). A new interaction mechanism between extremely high frequency electromagnetic waves of athermic intensities and their biological manifestations. *Archives of Biological Sciences Belgr*. in press 2013.

Bauer, O. and *E. Razin* (2000). Mast Cell-Nerve Interactions. *News Physiol Sci* **15**. 213-218.

Chernyakov, G.M., Korochin, V.L., Babenko, A.P. and *E.V. Bigdai* (1989). Reactions of biological systems of various complexity to the action of low-level EHF radiation. *Millimeter Waves in Medicine and Biology* **1**. 141-167.

Ciobica, A., Bild, V., Hritcu, L., Padurariu, M. and *W. Bild* (2011). Effects of angiotensin II receptor antagonists on anxiety and some oxidative stress markers in rat. *Central European Journal of Medicine* **6**. 331-340.

Ciobica, A., Bild, W., Hritcu, L. and *I. Haulica* (2009). Brain renin-angiotensin system in cognitive function: pre-clinical findings and implications for prevention and treatment of dementia. *Acta Neurol Belg* **109**. 171-80.

Devyadkov, N.D. and *O.V. Betskii* (1994). Biological Aspects of Low Intensity Millimeter Waves. Seven Plus. Moscow.

Enin, L.D., Akoev, G.N., Potekhina, I.L. and *V.D. Oleiner* (1992). Effect of extremely high-frequency electromagnetic radiation on the function of skin sensory endings. *Patol Fiziol Eksp Ter* **5**. 23-25.

Gangi, S. and *O. Johansson* (2000). A theoretical model based upon mast cells and histamine to explain the recently proclaimed sensitivity to electric and/or magnetic fields in humans. *Med Hypothes* **54**. 663-671.

Khramov, R.N., Sosunov, E.A., Koltun, S.V., Ilyasova, E.N. and *V.V. Lednev* (1991). Millimeter-wave effects on electric activity of crayfish stretch receptors. *Bioelectromagnetics* **12**. 203-214.

Lebedeva, N.N. (1995). Neurophysiological mechanisms of biological effects of peripheral action of low-intensity nonionizing electromagnetic fields in humans. *Millimeter Waves in Medicine and Biology* **1**. 138-140.

Potekhina, I.L., Akoyev, G.N., Yenin, L.D. and *V.D. Oleyner* (1992). Effects of low-intensity electromagnetic radiation in the millimeter range on the cardio-vascular system of the white rat. *Fiziol Zn* **78**. 35-41.

Rossi, R. and *O. Johansson* (1998). Cutaneous innervation and the role of neuronal peptides in cutaneous inflammation: a minireview. *Eur J Dermatol* **8**. 299-306.

Sazonov, A., Zamuraev, I.N. and *V.G. Lukashin* (1995). Effect of the extremely high frequency electromagnetic radiation on bush-like receptors of the frog bladder. *Fiziol Zh* **81**. 46-49.

Sit`ko, S.P. and *L.N. Mkrtchian* (1994). Introduction to Quantum Medicine. Pattern. Kiev.

Theoharides, T.C. (1996). The mast cell: A neuroimmunoendocrine master player. *Int. J. Reac* **18**. 1-21.

Usichenko, T.I., Edinger, H., Gizhko, V.V., Lehmann, C., Wendt, M. and *F. Feyerherd* (2006). Low-intensity electromagnetic millimeter waves for pain therapy. *Evid Based Complement Alternat. Med.* **3**. 201-7.

Webster, R.A. (2005). Neurotransmitters. Drugs and Brain Function. John Wiley & Sons. London. 5-67.