ORIGINALNI NAUČNI RADOVI
ORIGINAL STUDIES

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Originalni naučni rad
Original study
UDK 616.441:537.8:599.323.4

MORFOFIZIOLOŠKI STATUS TIREOIDNE ŽLEZDE PACOVA NAKON SUBHRONIČNOG IZLAGANJA NISKOFREKVENTNOM ELEKTROMAGNETNOM POLJU

MORPHOPHYSIOLOGICAL STATUS OF RAT THYROID GLAND AFTER SUBCHRONIC EXPOSURE TO LOW FREQUENCY ELECTROMAGNETIC FIELD

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Sažetak - Ispitivan je uticaj niskofrekventnog elektromagnetnog polja na tireoidnu žlezdu mužjaka pacova soja Mill Hill. Životinje su izlagane polju frekvencije 50 Hz, intenziteta 500 μT do 50 μT i 10 V/m, počevši od 24 časa nakon koćenja, 7 sati na dan, 5 dana u nedelji u trajanju od tri meseca. Rezultati histološke i stereološke analize pokazali su povećanje volumena tireoidnih folikula, smanjenje visine folikularnog epitela, nakupljanje koloida u folikularnom lumenu, smanjenje indeksa aktivacije tireoidne žlezde, povećanje volumena parafolikularnih ćelija, smanjenje volumena interfolikularnog vezivnog tkiva i povećanje broja degranulisanih mastocita u životinja izlaganih polju u odnosu na kontrolne životinje. Određivanje koncentracije tireoidnih hormona u krvnom serumu radioimunološkom analizom pokazalo je smanjenje ukupnog T4 i smanjenje ukupnog T3 u životinja izlaganih polju u odnosu na kontrole. Dobijeni rezultati ukazuju da je tromesečno izlaganje životinja niskofrekventnom elektromagnetnom polju dovelo do morfofunkcionalnih promena tireoidne žlezde koje ukazuju na njenu smanjenu aktivnost.
Ključne reči: Tiroidna žlezda; Elektromagnetna polja; Pacovi

Summary *- The objective of this study was to examine the impact of low-frequency electromagnetic field on male rat thyroid gland of Mill Hill strain. Animals were exposed to 50 Hz frequency, of decaying intensity from 500 μT to 50 μT and 10 V/m field, beginning 24 hours after birth, 7 hours a day, 5 days a week during three months. Results of histological and stereological analysis showed increased volume density of thyroid follicles, decreased thickness of the follicular epithelium, intrafollicular colloid content in lumen, decreased thyroid activation index, increased volume density of parafollicular cells, decreased volume of interfollicular connective tissue and increased number of degranulated mast cells in exposed animals in regard to control animals. Radioimmunologic assays were used to examine thyroid hormone concentrations in the blood serum revealing decrease of the total T4 as well as of total T3 in animals exposed to electromagnetic field in regard to controls. The obtained results show that a three month-exposition of animals to low frequency electromagnetic field led to morphofunctional alterations of the thyroid gland that can be referred to as reduced activity of the gland.*
Key words: *Thyroid Gland; Electromagnetic Fields; Rats*

Uvod

Elektromagnetna polja (EMP) pripadaju grupi nejonizovanih zračenja koja, pored prirodnih izvora, emituju i brojni veštački izvori, prvenstveno električne centrale i elektrodistributivne mreže (trafostanice, dalekovodi), ali i čitav niz potrošača električne energije kao što su telekomunikacioni sistemi, elektrificirani gradski saobraćaj i železnica, industrijska oprema, i brojni kućni aparati. Elektromagnetna polja koja nastaju iz ovih izvora jesu niskofrekventna (NF-EMP) čija je veličina učestalosti do 300 Hz. U

Introduction

Electromagnetic fields (EMF) pertain to the group of non-ionizing radiations and they originate from both natural and a number of artificial sources. A large group of artificial EMF sources comprises power-stations and electrodistributive networks (power-lines) as initial sites of EMF generation and users of electric current, such as telecommunication systems, electrified traffic and railway, industrial equipment and a number of home appliances. Electromagnetic fields that originate from these sources

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Skraćenice

cAMP	- ciklični adenozinmonofosfat
DNK	- dezoksiribonukleinska kiselina
EMP	- elektromagnetno polje
NF-EMP	- niskofrekventno elektromagnetno polje
PAS	- *periodic acid Schiff*
T3	- trijodtironin
T4	- tetrajodtironin, tiroksin
TSH	- tireostimulirajući hormon

okviru ovog frekvencijskog opsega, u čovekovoj životnoj sredini najprisutnija su EMP koja stvara elektrodistributivna mreža sa frekvencijom od 50 Hz (u Evropi) i 60 Hz (u SAD), čijem je delovanju čovek stalno izložen i na radnom mestu i u svom domu.

Ova činjenica nameće pitanje da li NF-EMP u interakcijama sa ljudskim organizmom mogu ostvariti štetan efekat i samim tim uticati na njegovo zdravlje? Ovaj je problem u poslednjih desetak godina privukao pažnju velikog broja istraživača u svetu, tako da danas sa sigurnošću možemo reći, prema rezultatima epidemioloških i eksperimentalnih istraživanja, da NF-EMP izazivaju različite efekte u organizmu, i to: stupaju u interakciju sa molekulom DNK [1], dovode do promena fluksa kalcijumovih jona kroz membranu ćelije [2], utiču na metabolizam neurotransmitera [3], na aktivnost enzima [4], na kardiovaskularni sistem [5], na imuni sistem [6], na endokrini sistem [7] i na procese učenja i pamćenja [8].

O uticaju NF-EMP na tireoidnu žlezdu postoji malo podataka koji su pri tome i kontradiktorni. Naime, izvestan broj autora među kojima i Ossenkopp i saradnici [9], Udinsteva i saradnici [10], Zagorskaia i Rodina [11] i Matavulj i saradnici [12] ukazuje na osetljivost tireoideje na NF-EMP. Nasuprot tome, radovi Lafreniere i Persingera [13] i Svedensta i Johansona [14] pokazuju da ne postoji odgovor tireoidne žlezde na uticaj ovih polja. To ukazuje na potrebu za daljim istraživanjem interakcije NF-EMP i tireoideje, posebno kada se ima u vidu izuzetan značaj uticaja njenih hormona na aktivnost gotovo svih ćelija u organizmu, što znači da bi se efekti eventualnih poremećaja tireoidne funkcije pod uticajem NF-EMP višestruko uvećali.

Materijal i metode

Ispitivanje uticaja promenljivog elektromagnetnog polja na tireoidnu žlezdu izvršeno je na mužjacima laboratorijskh pacova soja *Mill Hill.* Tokom eksperimenta životinje su bile smeštene u prostorijama Zavoda za fiziologiju Medicinskog fakulteta u Novom Sadu i podvrgnute prirodnom dnevno-noćnom ritmu svetlosti sa relativno konstantnom temperaturom vazduha od oko 20±2°C. Hranjene su standardnom peletiranom hranom za laboratorijske životinje

Abbreviations

cAMP	- *cyclic adenosine monophosphate*
DNK	- *deoxyribonucleic acid*
EMP	- *electromagnetic field*
NF-EMP	- *low frequency electromagnetic field*
PAS	- *periodic acid Schiff*
T3	- *triiodothyronine*
T4	- *tetraiodothyronine, thyroxine*
TSH	- *thyroid stimulating hormone*

are low frequency fields (LF-EMF) with a frequency value up to 300 Hz, but the most common one to which humans are exposed at work and at home is 50 Hz (in Europe) or 60 Hz (in USA).

Due to abundant presence of 50/60 EMFs in living environment, a question arises whether these fields affect human health during interaction with organisms. This dilemma became the issue of interest for many researchers in the past few decades. Results of epidemiological and experimental studies have demonstrated that LF-EMF exert diverse effects on living organisms such as: interaction with DNA molecule [1], alteration of Ca^{2+} flux through cell membrane [2], impact on: metabolism of neurotrasmitters [3], enzyme activity [4], cardiovascular system [5], immune system [6], endocrine system [7] and learning and memory processes [8].

Up to now, data on LF-EMF effects on thyroid gland are insufficient and, moreover, contradictory. Some authors like Ossenkopp and associates [9], Udinsteva and associates [10], Zagorskaia and Rodina [11] and Matavulj and associates [12] have pointed to the thyroid as an organ sensitive to LF-EMF. On the contrary, investigations performed by Lafreniere and Persinger [13] and Svedenst and Johanson [14] failed to prove any alterations of the thyroid structure and function upon LF-EMF effects. These opposite results are pointing to the necessity for further investigations of the LF-EMF-thyroid interaction, primarily if broad range of effects that thyroid hormones exert in the organism are taken into consideration. Therefore, possible alterations of thyroid gland function caused by LF-EMF would multiply in relation to target tissues.

Material and methods

The experiment was performed on 23 male rats of Mill Hill strain at the laboratory of the Department of Physiology of the Faculty of Medicine Novi Sad. The animals were housed in laboratory conditions at 20±2°C temperature and subjected to natural photoperiod. Access to water and standard food for laboratory animals (Department of Veterinary Medicine, Subotica) was unlimited. The system producing LF-EMF was made of a single coil of 2,5-mm thick wire placed on a wooden frame with 1320 turns. Cages

(Veterinarski zavod, Subotica), a vodu su uzimale po potrebi. Aparaturu pomoću koje je dobijeno EMP sačinjavao je kalem, priključen na strujnu mrežu, od žice debljine 2,5 mm namotane u 1320 namotaja na drvene ramove. Izlaganje životinja (n=12) EMP, frekvencije 50 Hz, intenziteta od 500 μT (sa strane kaveza bliže kalemu) do 50 μT (sa suprotne strane kaveza) i 10 V/m, počelo je 24 h nakon koćenja, sa dinamikom izlaganja od po 7 sati na dan, 5 dana u nedelji u trajanju od tri meseca. Kontrolne životinje (n=11) držane su u odvojenoj prostoriji gde nije bilo mogućnosti da budu izložene uticaju EMP.

Morfofunkcionalni status tireoidne žlezde određen je:

1. *Histološkom analizom* sprovedenom na parafinskim i polutankim presecima. Nakon žrtvovanja životinja dekapitacijom, izvađene tireoidne žlezde namenjene za ispitivanja na parafinskim presecima fiksirane su u Bouinovom fiksativu 24 h, kalupljene u parafinu i sečene na Reichertovom rotacionom mikrotomu u serijske preseke debljine 5 μm. Komadići žlezde namenjeni za ispitivanja na polutankim presecima isečeni su pod kontrolnom lupom u puferisanom glutaraldehidu, kalupljeni u EPON smoli i sečeni na Bromma ultramikrotomu u rezove debljine 1μm. Za prikazivanje folikularnih ćelija, koloida i interfolikularnog veziva parafinski preseci su bojeni metodom po Florentinu, hematoksilin-eozinom i PAS alcian plavim. Prisustvo parafolikularnih ćelija prikazano je Fernandez-Pascualovom metodom impregnacije sa $AgNO_3$, a mastocita toluidin plavim i Dominicijevom metodom sa eozinom, oranž G i toluidin plavim. Polutanki preseci su bojeni toluidinsko plavo-krezil violetom;

2. *Stereološkom analizom* koja je izvršena na svakom četvrtom parafinskom serijskom preseku počevši od sredine žlezde ka periferiji, pri čemu je korišćen Reichertov mikroskop pri uvećanju objektiva 63 puta i imerzionog objektiva i Wildovog okulara koji uvećava 10 puta sa ugrađenim Weibelovim mnogonamenskim testnim sistemom sa 42 tačke. Folikuli, interfolikularno vezivo i krvni sudovi analizirani su na 60 vidnih polja po životinji, a parafolikularne ćelije i mastociti na 100 vidnih polja. Pored stereološke analize numeričke i volumenske gustine mastocita, analizirana je i numerička i volumenska gustina odgovarajućih morfoloških podtipova ovih ćelija koje je opisao Banni-Sacchi [15] u cilju određivanja zastupljenosti degranulisanih mastocita;

3. *Merenjem koncentracije* T3 i T4 u krvnom serumu životinja radioimunološkom analizom uz korišćenje dijagnostičkih kompleta za kvantitativno određivanje tireoidnih hormona (INEP, Zemun).

Signifikantnost razlika dobijenih ispitivanjem pojedinih stereoloških parametara i ispitivanjem koncentracije tireoidnih hormona u kontrolnih i životinja izlaganih EMP proverena je Studentovim t-testom.

with animals were placed symmetrically on both sides of the coil. The coil produced 50 Hz LF-EMF of decaying intensity of magnetic field along the cages from 500 μT on the side of the cage nearby the coil to 50 μT on the opposite side, while the intensity of electric field was 10 V/m. Twelve animals were exposed to LF-EMF 24 h after birth, 7 hours a day, 5 days a week during a period of three months. Eleven animals were controls and were under similar conditions without exposure to LF-EMF.

Morphofunctional status of the thyroid gland was examined by:

1. *Histological analysis* performed on paraffin and semithin sections. After the animals were sacrificed (by decapitation) the thyroid glands were fixed in Bouin's solution, embedded in paraffin and cut on the Reichert rotation microtome in 5 μm thick sections. Thyroids designated for semithin sections were fixed in glutaraldehyde, prefixed in osmium-tetroxide, embedded in Epon resin and cut on Bromma ultramicrotome in 1 μm thick sections. Paraffin slices were stained with haematoxylin-eosin, after using the method of Florentin and PAS alcian blue for general preview of thyroid structure. The $AgNO_3$ impregnation method after Fernandez-Pascual was used in order to determine presence of parafollicular cells and toluidine blue as well as the method after Dominici with eosin, orange G and toluidine blue for mast cells. The semithin sections were stained with toluidine cresyl violet;

2. *Stereologic analysis* was performed on every fourth serial paraffin section beginning from the middle of the gland to the periphery using grid M42 placed in the ocular of Reichert light microscope at magnification of 10 and with objective magnification at 63 and 100. Thyroid follicles, interfollicular tissue and capillary network were analyzed on 60 test fields per animal, while parafollicular and mast cells were analyzed on 100 test fields. Apart from stereologic analysis of numerical and volume density of mast cells, numerical and volume density of three morphologic types of these cells (according to classification of Banni-Sacchi [15]) were also examined in order to estimate participation of the degranulated mast cells;

3. *Measurement of T3 and T4 concentrations* in blood serum by radioimmunologic analysis (RIA) using kits for thyroid hormone quantitative analysis (INEP, Zemun).

Statistical significance of differences between control animals and animals exposed to LF-EMF were examined using the Student's t-test.

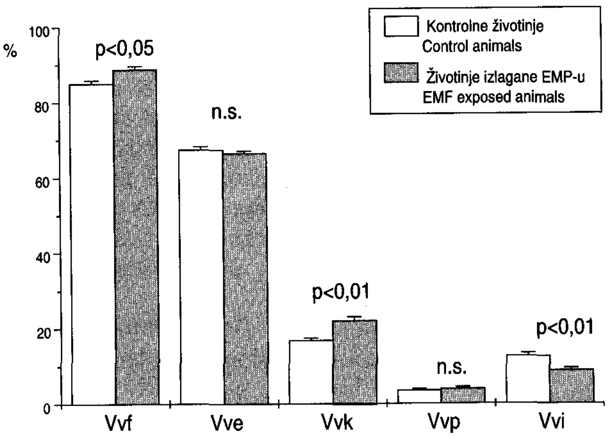
Rezultati

U tireoidnim žlezdama životinja izlaganih NF-EMP tri meseca zapaža se česta pojava makrofolikula sa niskoprizmatičnim epitelom u središnjim delovima lobusa žlezde koji su raspoređeni u grupama ili pojedinačno, a okruženi su mikrofolikulima. Lumen mikro- i makrofolikula u ovih životinja je veći u odnosu na žlezde kontrolnih životinja, što sve doprinosi povećanju ukupnog volumena folikula u životinja izlaganih EMP. Histološka analiza takođe pokazuje smanjenje visine folikularnog epitela u odnosu na kontrolnu grupu životinja, dok se pojedini regioni žlezde karakterišu folikulima sa izrazito niskim, skoro endotelomorfnim epitelom. U folikulima životinja izlaganih EMP zapažaju se izrazito ravne apikalne membrane tireocita, a u lumenu folikula došlo je do nakupljanja izrazito PAS pozitivnog koloida.

Ispitivanje parafolikularnih ćelija pokazalo je da su u životinja izlaganih NF-EMP pojedine ćelije hipertrofisane, ali se isto tako uočavaju i degranulisane ćelije. Takođe se uočava i čest kontakt parafolikularnih ćelija sa mastocitima.

Interfolikularni prostor tireoidne žlezde pokazao se senzitivnim na delovanje NF-EMP. Najznačanije promene interfolikularnog vezivnog tkiva ogledaju se u smanjenju ukupnog volumena veziva u životinja izlaganih NF-EMP. Ispitivanjem mastocita uočen je manji broj ovih ćelija u životinja izlaganih NF-EMP, ali i češća pojava degranulisanih mastocita u odnosu na kontrole. U životinja nakon tri meseca izlaganja NF-EMP zapaža se i dilatacija krvnih kapilara tireoidne žlezde.

Histološke nalaze potvrdila je i stereološka analiza tireoidne žlezde, koja je pokazala značajno pove-



Grafikon 1. Prosečna vrednost (x(SG) volumenske gustine folikula (Vvf), epitela (Vve), koloida (Vvk), parafolikularnih ćelija (Vvp) i interfolikularnog veziva (Vvi) tireoidne žlezde kontrolnih životinja i životinja izlaganih EMP

Graph. 1. *Volume density of thyroidal follicles (Vvf), epithelium (Vve), colloid (Vvk), parafollicular cells (Vvp) and interfollicular tissue (Vvi) in control animals and EMF exposed animals*

Results

The thyroid glands of exposed animals revealed presence of macrofollicles (solitary or in group) with low columnar epithelium in the middle portion of the thyroid lobe surrounded by microfollicles. The lumen of micro- and macrofollicles is enlarged comparing with controls and the total volume of thyroidal follicles is increased in LF-EMF exposed animals as well. Histological analysis also demonstrated a decreased follicular epithelial thickness which is extremely low in some areas of the gland, while apical membranes of follicular cells show absence of apical protrusions. Colloid demonstrated an explicitly PAS positive reaction and was accumulated in the follicular lumen.

Animals exposed to LF-EMF, presented hypertrophy of some parafollicular cells and degranulated cells as well. These cells were often in contact with mast cells.

The thyroidal interfollicular space is sensitive to effects of LF-EMF demonstrating a decreased total volume of connective tissue, decreased number of mast cells, but often presence of degranulated cells, and dilatation of blood capillaries comparing to controls.

The observed histological alterations of thyroid gland were substantiated by stereologic analysis. The mean value of the volume density of follicles (Vvf) was significantly increased ($p<0.05$) as well as the volume density of colloid (Vvk)($p<0.01$), while the volume density of follicular epithelium (Vve) decreased, but insignificantly (Graph 1). Decreased Vve and increased Vvk provided a significantly decreased ($p<0.01$) activation index of thyroid gland (Ia) (Graph 2), which represents the ratio of Vve to Vvk. Stereologic analysis of parafollicular cells showed insignificant decrease of its numerical density (Nvp) (Graph 3) and insignificant increase of the volume density (Vvp) of these cells (Graph 1) in comparison with controls.

Stereologic analysis demonstrated that the volume density of intrafollicular tissue (Vvi) significantly decreased ($p<0.01$) in exposed animals (Graph 1), while decrease of the numerical density of mast cells (Nvm) in this tissue and increase of the volume density (Vvm) of these cells were insignificant. Apart from stereologic analysis of total Nvm and Vvm, three types of mast cells (A, B and C) were also analyzed. This was performed after Banni-Sacchi classification, according to the degree of mast cell granulation as an important sign of their activity. Mast cells completely filled with granulations are designated as type A, cells with a number of cytoplasmic processes as type B and type C are degranulated mast cells surrounded with extruded granules dispersed in intrafollicular space. Stereologic analysis revealed insignificant increase of the mean value of numerical density of mast cells (Nvm) type A, insignificant reduction of volume ćanje ($p<0,05$) prosečne vrednosti volumenske gustine folikula (Vvf), smanjenje volumenske gustine epitela (Vve) koje se nije pokazalo statistički značajnim i značajno povećanje ($p<0,01$) volumenske gustine koloida (Vvk) u životinja izlaganih NF-EMP u odnosu na kontrolnu grupu (grafikon 1). Iz smanjenja Vve i povećanja Vvk proizašlo je značajno smanjenje ($p<0,01$) indeksa aktivacije (Ia) tireoidne žlezde (grafikon 2), koji predstavlja količnik ovih vrednosti (Vve/Vvk). Stereološka analiza numeričke gustine parafolikularnih ćelija (Nvp) pokazala je njeno smanjenje u životinja izlaganih NF-EMP u odnosu na kontrolne životinje, koje se nije pokazalo statistički značajnim (grafikon 3), kao ni povećanje volumenske gustine parafolikularnih ćelija (Vvp)(grafikon 1).

Stereološka analiza volumenske gustine interfolikularnog veziva (Vvi) pokazala je njeno značajno smanjenje ($p<0,01$) u životinja nakon tromesečnog izlaganja NF-EMP u odnosu na kontrole (grafikon 1), zatim, smanjenje numeričke gustine interfolikularnih mastocita (Nvm), a povećanje njihove volumenske gustine (Vvm) koje se nisu pokazale statistički značajnim. Pored stereološke analize Nvm i Vvm ukupnog broja mastocita stereološkom analizom su obuhvaćena i tri tipa mastocita (A, B i C) klasifikovanih po stepenu granulisanosti, budući da je to važan pokazatelj njihove aktivnosti. Tip A predstavljaju mastociti koji su potpuno ispunjeni granulama, tip B poseduje brojne citoplazmatične produžetke, dok tip C predstavljaju degranulisani mastociti oko kojih se u interfolikularnom prostoru nalaze izlučene granule. Stereološka analiza Nvm tipa A pokazala je nesignifikantno povećanje prosečne vrednosti ovog parametra u životinja nakon tromesečnog izlaganja NF-EMP, nesignifikantno smanjenje Vvm tipa A, nesignifikantno smanjenje Nvm tipa B, nesignifikantno povećanje Vvm tipa B, nesignifikantno povećanje Nvm tipa C (grafikon 3), i signifikantno povećanje ($p<0,05$) Vvm tipa C u odnosu na kontrolnu grupu životinja (grafikon 4). Stereološkom analizom volumenske gustine kapilarne mreže (Vvs) pokazano je povećanje prosečne vrednosti ovog parametra u životinja izlaganih NF-EMP u odnosu na kontrolu, koje se nije pokazalo statistički značajnim (grafikon 4).

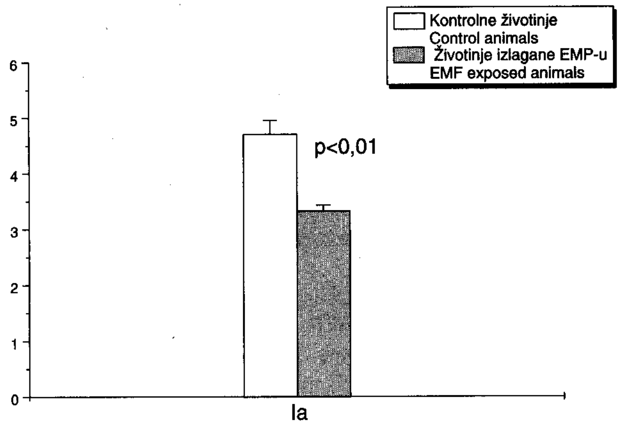
Rezultati radioimunološke analize pokazali su smanjenje koncentracije ukupnog T3 i povećanje ukupnog T4 u krvnom serumu životinja izlaganih NF-

Tabela 1. Koncentracija T3 i T4 (nmol/L) u krvnom serumu kontrolnih životinja i životinja izlaganih EMP

***Table 1.** T3 and T4 blood serum concentrations (nmol/L) in control animals and EMF exposed animals*

	T3		T4	
	Kontrola	EMP	Kontrola	EMP
x	0,76	0,66n.s.	84,6	87,8 n.s.

n.s. - nije signifikantno/*n.s. - not significant*

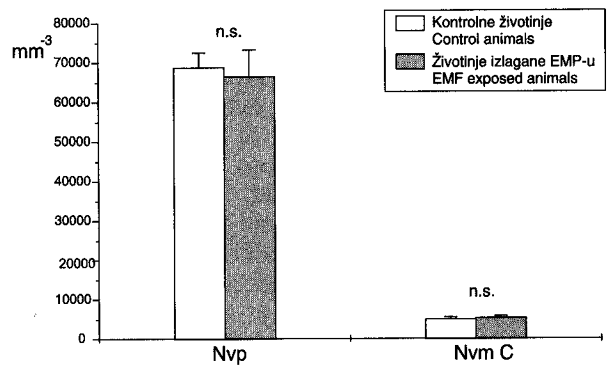


Grafikon 2. Prosečna vrednost (x±SG) indeksa aktivacije (Ia) tireoidne žlezde kontrolnih životinja i životinja izlaganih EMP

***Graph. 2.** Activation index (x±SG) of thyroid gland (Ia) in control animals and EMF exposed animals*

density of mast cells (Vvm) type A and Nvm type B, insignificant increase of Vvm type B and Nvm type C (Graph 3), while Vvm type C significantly increased ($p<0.05$) (Graph 4) in comparison with control animals. Stereologic analysis of capillary network showed that the mean value of volume density of capillaries (Vvs) increased in exposed animals, but insignificantly (Graph 4).

Results of radioimmunologic analysis demonstrated insignificant reduction of total T3 concentration and insignificant increase of total T4 concentration in the serum of exposed animals (Table 1).



Grafikon 3. Prosečna vrednost (x±SG) numeričke gustine parafolikularnih ćelija (Nvp) i mastocita tipa C (Nvm C) tireoidne žlezde kontrolnih životinja i životinja izlaganih EMP

***Graph. 3.** Numerical density (x±SG) of parafollicular cells (Nvp) and C type mast cells (Nvm C) in control animals and EMF exposed animals*

Discussion

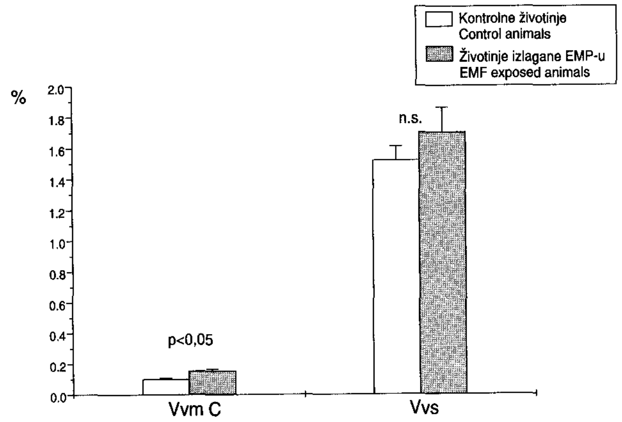
In regard to the degree and type of alterations of investigated qualitative and quantitative histological parameters, exposure to LF-EMF induced diminished thyroid function.

EMP. U odnosu na kontrolnu grupu životinja utvrđene razlike nisu bile statistički značajne (tabela 1).

Diskusija

Sudeći po stepenu i vrsti promene ispitivanih kvalitativnih i kvantitativnih histoloških parametara, tireoidna žlezda životinja neposredno nakon tri meseca izlaganja NF-EMP pokazuje znake smanjene aktivnosti.

Rezultati našeg istraživanja pokazuju da je u životinja izlaganih NF-EMP došlo do smanjenja indeksa aktivacije tireoidne žlezde (Ia=Vve/Vvk) za koji je pokazano da stoji u pozitivnoj korelaciji sa nivoom TSH u plazmi [16]. Konstatovane strukturne promene tireoidne žlezde bi, prema tome, mogle biti rezultat smanjenog nivoa TSH, pri čemu bi izostao stimulativni efekat koji ovaj hormon ostvaruje na folikularni epitel. To bi moglo objasniti pojavu ravnih apikalnih membrana tireocita i smanjenu visinu folikularnog epitela koje smo konstatovali u našim eksperimentalnim uslovima. Međutim, budući da je u životinja izlaganih NF-EMP indeks aktivacije tireoidne žlezde izrazito snižen, što upućuje na smanjenu koncentraciju TSH u krvi ovih životinja, postavlja se pitanje na koji bi način moglo doći do povećanja koncentracije T4 nakon perioda izlaganja. Od mnogih pretpostavljenih mehanizama koji bi mogli delovati, mišljenja smo da bi nam najviše od pomoći mogao biti jedan od najznačajnijih pretpostavljenih mehanizama delovanja EMP u kome se kao medijator između delovanja EMP i promena u biološkim sistemima pojavljuje jon kalcijuma [17,18]. Naime, *in vitro* istraživanja pokazala su da pod uticajem EMP dolazi do značajnog povećanja intracelularne koncentracije jona kalcijuma i da je ovo povećanje u potpunosti zavisno od influksa ovih jona iz ekstracelularnog medijuma [19]. Poznato je da u sekreciji tireoidnih hormona, pored TSH, ulogu imaju i peptidi poreklom iz parafolikularnih ćelija koji se oslobađaju u ekstracelularnu tečnost i parakrinim putem deluju na susedne folikularne ćelije menjajući bazalnu i TSH stimulisanu sekreciju tireoidnih hormona [20]. Intracitoplazmatične granule parafolikularnih ćelija sadrže serotonin za koji je pokazano da deluje stimulativno na folikularne ćelije [21]. Sekreciju serotonina stimuliše TSH, ali isto tako i povećane koncentracije ekstracelularnih kalcijumovih jona [22]. Ovaj efekat Ca^{2+} joni ostvaruju povećanim influksom iz ekstracelularnog medijuma kroz odgovarajuće membranske kanale ili delujući kao primarni glasnici kada se vezuju za membranske receptore na parafolikularnim ćelijama (tzv. Ca^{2+} sensing receptor) aktivirajući sistem drugog glasnika (fosfolipazu C i A2 ili cAMP) [23]. Kako u našem eksperimentu snižene vrednosti indeksa aktivacije tireoidne žlezde upućuju na sniženi nivo cirkulirajućeg TSH, izostao bi njegov



Grafikon 4. Prosečna vrednost (x±SG) volumenske gustine mastocita tipa C (Vvm C) i volumenske gustine kapilara (Vvs) tireoidne žlezde kontrolnih životinja i životinja izlaganih EMP
Graph. 4. *Volume density (x±SG) of C type mast cells (Vvm C) and volume density of capillary network in control animals and EMF exposed animals*

Our results showed, that thyroid activaty index (Ia=Vve/Vvk) is decreased in animals exposed to LF-EMF and according to Kališnik [16] this stereolgic parameter is in positive correlation with the plasma TSH level. Therefore, these alterations of the thyroid gland could be the consequence of decreased TSH level, since the stimulative effect of this hormone on follicular epithelium would be reduced. This state could also provide an explanation for absence of apical protrusions on thyrocytes and reduced thickness of follicular epithelium that was noted in our experimental procedure. However, reduction of thyroid activaty index was significant, pointing at reduced level of TSH in the circulation of exposed animals, an intriguing question is how could the level of T4 rise after the period of exposition? Since multiple pathways could be involved to provide this effect, the authors of this paper considered the possible role of Ca^{2+} in this process, whose function is well investigated in bioelectromagnetic interactions. Namely, one of the most important mechanisms of EMF effects involves calcium ion as the mediator of EMF interaction with the living systems [17,18]. *In vitro* experiments demonstrated a significant increase of intracellular calcium concentration under EMF effects and this elevation completely depends on calcium ion influx from extracellular medium [19]. It is known that, besides TSH, biologically active peptides originating from parafollicular cells also take part in thyroid hormone ecretion. They are released extracellularly and by paracrine action affect the nearby-situated follicular cells altering the basal and TSH-stimulated thyroid hormone secretion [20]. Intracytoplasmic granules of parafollicular cells contain serotonin which stimulates follicular cells [21]. Serotonin secretion is stimulated by TSH, but also by increased extracellular concentration of calcium ions [22]. This effect of stimulatorni uticaj na sekreciju serotonina. Moguće je da bi u takvim uslovima još uvek bio dovoljan efekat jona kalcijuma na održavanje sekrecije serotonina iz parafolikularnih ćelija i održavanja njegovog uticaja na tireocite. To znači da bi tireoidna žlezda i dalje mogla održavati relativno normalnu sekreciju T4, što bi objasnilo blago povećanje koncentracije ovog hormona u serumu životinja nakon tromesečnog izlaganja EMP.

Smanjenje indeksa aktivacije tireoidne žlezde konstatovano je i u našim ranijim istraživanjima pri istovetnoj dinamici izlaganja životinja kao i u ovom radu, ali nakon dužeg vremenskog perioda od pet [24] i šest [25] meseci. Međutim, podaci koji se sreću u literaturi o uticaju NF-EMP na koncentraciju TSH i tireoidnih hormona kontradiktorni su s obzirom na dužinu trajanja izlaganja i jačinu polja. Ispitujući efekat NF-EMP (50 Hz; 10 μT) na ljudski organizam, kojim su obuhvaćena 32 ispitanika muškog pola starih između 20 i 30 godina, Selmaoui i saradnici [26] konstatovali su da devetočasovno i 24-časovno izlaganje polju ne dovodi do promena nivoa TSH niti T4 i T3. S druge strane, Udinsteva i saradnici [10] izlagali su pacove NF-EMP (50 Hz; 20 mT) tokom 18 sati i pokazali da ovi eksperimentalni uslovi dovode do povećanja nivoa TSH i tiroksina u krvi ovih životinja. Međutim, jednokratno izlaganje pacova NF-EMP intenziteta 20 mT dovelo je do promene aktivnosti tireoidne žlezde i pada koncentracije tireoidnih hormona u cirkulaciji ovih životinja [11].

Kako su naši rezultati pokazali, u životinja nakon tromesečnog izlaganja NF-EMP došlo je do smanjenja volumena interfolikularnog vezivnog tkiva. Budući da je u ovih životinja došlo do povećanja volumena tireoidnih folikula, verovatno se taj proces odvijao na račun vezivnog tkiva. Izlaganje NF-EMP dovelo je i do smanjenja ukupnog broja mastocita, što je u saglasnosti sa smanjenjem indeksa aktivacije tireoidne žlezde, odnosno koncentracije TSH u krvi na koje ono upućuje, budući da je ustanovljeno da je broj mastocita u korelaciji sa nivoom ovog hormona [27]. Uočeno je i povećanje volumena mastocita tipa C, odnosno degranulisanih mastocita, što su zapazili i Iurina i saradnici [28] ispitujući *in vivo* efekat EMP (50 Hz, 32 kA/m) na intestinalne mastocite miševa, pacova i zečeva izlaganih polju u trajanju od 1,5 do 2 meseca. Dilatacija kapilara koju smo konstatovali u našim eksperimentalnim uslovima verovatno je nastala delovanjem vazodilatatornih medijatora iz mastocita.

Lin i saradnici [29] i Vernhes i saradnici [30] smatraju da se delovanje EMP može smatrati specifičnom stresnom situacijom. Obe grupe autora su uočile da je odgovor ćelija na uticaj EMP gotovo istovetan odgovoru na delovanje drugih fizioloških stresora (npr. hipertermija, oksidativni stres). Prema

Ca^{2+} is exerted by increased influx from extracellular medium either through membrane channels or by Ca^{2+} action as primary messenger when it bounds to membrane receptors of parafollicular cells (Ca^{2+} sensing receptor) and activating the secondary messenger system (phopholipase C and A2 or cAMP) [23]. As decreased thyroid activaty index found in our expeperiment refers to lowered TSH level, the stimulative action of TSH on serotonin secretion would be reduced. Under these conditions, it could be presumed that the effect of calcium ions on parafollicular cells would still be sufficient to maintain serotonin secretion and its effect on thyrocytes. This would further mean that the thyroid could preserve relatively normal T4 secretion and therefore cause small increase of this hormone in exposed animals.

Decreased activaty index of thyroid gland was obtained in our previous investigations as well, in the same experimental procedure as in this paper, but with five [24] and six [25] months of exposure. Differences in results regarding the LF-EMF effect on thyroid hormone concentration and TSH obtained by other authors are contradictory to ours, which is based mainly on differences in experimental design, primarily duration of exposure period and intensity of applied EMF. Selmaoui and associates [26] investigated effects of LF-EMF (50 Hz; 10 mT) on 32 men, aged between 20 and 30, and found that after 9 and 24 hour exposition the concentration of TSH, T4 and T3 remained unaltered. On the contrary, Udinsteva and associates [10] attained the increased level of TSH and thyroxine in circulation of rats exposed to LF-EMF (50 Hz; 20 mT) for 18 hours. However, a single exposure to 20 mT LF-EMF altered the thyroid gland activity in rats and decreased the concentration of circulating thyroid hormones [11].

As our results demonstrated a three-month exposition of rats to LF-EMF caused reduction of the intrafollicular connective tissue volume. Since the thyroid follicles are enlarged in exposed animals, it probably occurred at the account of connective tissue reduction. Reduction of total number of mast cells due to LF-EMF effects which is in accordance with the decreased activaty index of the thyroid gland reflects a lowered TSH concentration, since the number of mast cells is in positive correlation with the level of TSH [27]. The increased volume of type C or degranulated mast cells is in agreement with data reported by Iurina and associates [28] who gained similar results investigating the *in vivo* effect of EMF (50 Hz; 32 kA/m) on intestinal mast cells in mice, rats and rabbits exposed during 1.5 to 2 months. Dilatation of intrathyroidal capillaries found in our experiment is probably the consequence of vasodilatative action of some mast cell mediators.

According to Lin and associates [29] Vernhes and associates [30] effects of the EMF believed to be a specific stress situation. These authors found that cells respond to EMF affect similarly as to other physiologic stressors (such as hyperthermia, oxidatome bi, pri posmatranju uticaja EMP na tireoidnu žlezdu, trebalo imati u vidu i delovanje EMP kao stresogenog faktora.

Zaključak

Tromesečno izlaganje pacova NF-EMP dovelo je do morfofunkcionalnih promena tireoidne žlezde koje ukazuju na njenu smanjenu aktivnost. Međutim, potrebna su dalja istraživanja kako bi se ustanovilo da li su konstatovane promene rezultat direktnog delovanja NF-EMP na tireoidnu žlezdu ili su posledica uticaja polja na neki od viših nivoa regulacije tireoidne funkcije.

tive stress). In regard to these findings, the EMF effects on thyroid gland should also be considered as a stressor for the thyroid.

Conclusion

A three-month exposition of rats to LF-EMF led to morphofunctional alterations of thyroid gland designated as decreased gland function. However, further investigations are necessary in order to establish whether the obtained changes are the result of LF-EMF direct effects on the thyroid, or maybe the result of some indirect effects on thyroid function.

Literatura

1. Singh N, Lai H. 60 Hz magnetic field exposure induces DNA crosslinks in rat brain cells. Mutat Res 1998;400(1-2): 313-20.
2. Menendez RG. Three molecular mechanisms to explain some biological effects of electromagnetic fields and hypogravity. Med Hypotheses 1999;52(3):239-45.
3. Burchard JF, Nguyen DH, Richard L, Young SN, Heyes MP, Block E. Effects of electromagnetic fields on the levels of biogenic amine metabolites, quinolinic acid, and beta-endorphin in the cerebrospinal fluid of dairy cows. Neurochem Res 1998;23(12):1527-31.
4. Kumlin T, Alhonen L, Janne J, Lang S, Kosma VM, Juutilainen J. Epidermal ornithine decarboxylase and polyamines in mice exposed to 50 Hz magnetic fields and UV radiation. Bioelectromagnetics 1998;19(6):388-91.
5. Bortkiewicz A, Zmyslony M, Gadzicka E. Exposure to electromagnetic fields with frequencies of 50 Hz and changes in the circulatory system in workers at electrical power stations. Med Pr 1998;49(3):261-74.
6. Mehta S, Johnson K, Wanebo H, Cherlin D, Polk C. Human IL-2 production and binding to its receptor proteins on activated T-cells is affectected by 60 Hz, 1 G sinusoidal magnetic field exposure. Second World Congress for Electricity and Magnetism in Biology and Medicine, Bologna, 1997. Abstract Book p 61.
7. Wilson BW, Matt KS, Morris JE, Sasser LB, Miller DL, Anderson LE. Effects of 60 Hz magnetic field exposure on the pineal and hypothalamic-pituitary-gonadal axis in the Siberian hamster (Phodopus sungorus). Bioelectromagnetics 1999;20 (4):224-32.
8. Sienkiewicz ZJ, Haylock RG, Saunders RD. Deficits in spatial learning after exposure of mice to a 50 Hz magnetic field. Bioelectromagnetics 1998;19(2):79-84.
9. Ossenkopp KP, Koltek WT, Persinger MA. Prenatal exposure to an extremely low frequency-low intensity rotating magnetic field and increases in thyroid and testicle weight in rats. Dev Psychobiol 1972;5(3):275-85.
10. Udinsteva NA, Serebrov VY, Tsyrov GI. Vliyanie peremennogo magnetnogo polya promyshlenoj chastoty na funktsionalnoe sostoyanie shchitovidnoj zhelezi i pogloshehenie tiroksina organamikrys. Bull Eksper Biologii i Meditsini 1978;86:544-6.
11. Zagorskaia EA, Rodina GP. Reaction of the endocrine system and peripheral blood of rats to a single and chronic exposure to pulsed low-frequency electromagnetic field. Kosm Biol Aviokosm Med 1990;24(2):56-60.
12. Matavulj M, Rajković V, Ušćebrka G, Žikić D, Matavulj A, Lukač T, Lažetić B. Interactions of thyroid gland with non-ionizing electromagnetic fields In: Lažetić B, Sudakov KV, eds, Basic and clinical aspects of the theory of functional systems. Novi Sad: University of Novi Sad, Medical Faculty and P.K. Anokhin Institute of normal Physiology RAM Moscow, 1998:99-206.
13. Lafreniere GF, Persinger M.A. Thyroid morphology and activity does not respond to ELF electromagnetic field exposures. Experientia 1979;35(4):561-2.
14. Svedenst BM, Johanson KJ 5-Iododeoxyuridine-125I incorporation in vivo after exposure to a 50 Hz magnetic field. In Vivo 1998;12(5):531-4.
15. Bani-Sacchi T. Sul numero e la distribuzione delle mastzellen nell'omento del rato albino in rapporto all'eta ed al sesso. Arch It Anat Embriol 1966;71:91-8.
16. Kališnik M. Histometric thyroid gland activation index (preliminary report). J Micr (Oxford) 1971;95:345-8.
17. Balcavage WX, Alvager T, Swez J, Goff CW, Fox MT, Abdullyava S, King MW. A mechanism for action of extremely low frequency electromagnetic fields on biological systems. Biochemical and Biophysical Research Communications 1996; 222(2):374-8.
18. Morgado-Valle C, Verdugo-Diaz L, Garcia DE, Morales-Orozco C, Drucker-Colin R. The role of voltage-gated Ca^{2+} channels in neurite growth of cultured chromaffin cells induced by extremely low frequency (ELF) magnetic field stimulation. Cell Tissue Res 1998;291(2):217-30.
19. Cho MR, Thatte HS, Silvia MT, Golan DE. Transmembrane calcium influx induced by ac electric fields. FASEB J 1999;13(6):677-83.
20. Ahren B. Regulatory peptides in the thyroid gland-a review on their localization and function. Acta Endocrinologica (Copenh) 1991;124:225-32.

21. Russo AF, Clark MS, Durham PL. Thyroid parafollicular cells. An accessible model for the study of serotonergic neurons. Mol Neurobiol 1996;13(3):257-76.

22. Tamir H, Hsiung SC, Yu PY, Liu KP, Adlersberg M, Nunez EA, Gershon MD. Serotonergic signalling between thyroid cells: protein kinase C and 5-HT2 receptors in the secretion and action of serotonin. Synapse 1992;12(2):155-68.

23. Hebert SC, Brown EM, Harris HW. Role of the Ca(2+)-sensing receptor in divalent mineral ion homeostasis. J Exp Biol 1997;200:295-302.

24. Matavulj M, Rajković V, Ušćebrka G, Gudović R, Stevanović D, Lažetić B. Structural and stereological analysis of rat thyroid gland after exposure to an electromagnetic field. Acta Veterinaria 1996;46(5-6):285-92.

25. Matavulj M, Rajković V, Ušćebrka G, Lukač T, Matavulj A, Žikić D, Stevanović D, Lažetić B. Magnetic field effects on the thyroid gland. A morphological study. Proceedings of the Second International Conference "Electromagnetic Fields and Human Health", Moscow 1999:278-9.

26. Selmaoui B, Lambrozo J, Touitou Y. Endocrine functions in young men exposed for one night to a 50-Hz magnetic field. A circadian study of pituitaty, thyroid and adrenocortical hormones. Life Sci 1997;61(5):473-86.

27. Ericson LE, Hakanson R, Melander A, Owman C, Sundler F. TSH-induced release of 5-hydroxytryptamine and histamine from rat thyroid mast cells. Endocrinology 1972;90: 795-801.

28. Iurina NA, Radostina AI, Savin BM, Remizova VA, Kosova IP. Mast cells as a test of body state during electromagnetic exposure of varying intensity. Aviokosm Ekolog Med 1997;31(2):43-7.

29. Lin H, Opler M, Head M, Blank M, Goodman R. Electromagnetc field exposure induces rapid, transitory heat shock factor activation in human cells. J Ceel Biochem 1997;66(4): 482-8.

30. Vernhes MC, Cabanes PA, Teissie J. Chinese hamster ovary cells sensitivity to localized electrical stresses. Bioelectrochem Bioenerg 1999;48(1):17-25.

Rad je primljen 30. X 2000.
Prihvaćen za štampu 25. XI 2000.
BIBLID.0025-8105:(2001):LIV:3-4:119-127.