BIOCHEMICAL EFFECTS OF ELECTROMAGNETIC WAVES ON RATS

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ABSTRACT

Because of the great growing in mobile phone communications and their services, it was so important to study their electromagnetic waves (EMW) effects on melatonin and biogenic amines (epinephrine, norepinephrine, dopamine, serotonin, and histamine) of the neonatal rat brains during the prenatal period until different postnatal days (P1, P7, P14, P21 and P28).

Female pregnant rats were exposed to EMW from mobile phone antenna (3G SAR 1.25 W/kg body) and the irradiation continued until the 28th day of parturition time. After parturition, neonatal rats were decapitated under red light and their brains were removed. Melatonin and biogenic amines concentrations were estimated by HPLC (UV detector). Results showed that in the group of neonatal rats that exposed to EMW, there was a significant (P<0.05) increase in melatonin levels accompanied with a significant decrease in dopamine, histamine, norepinephrine and epinephrine in comparison to normal group. Exposure to EMW may cause many disturbances in the nervous system of the neonatal rats.

Key words: Electromagnetic waves, Neonatal rats, Histamine, Dopamine, Epinephrine, Norepinephrine, Serotonin and Melatonin.
INTRODUCTION

After replacing the land lines, and in addition to internet services, mobile phone becomes the main way of telecommunications. According to Grupe Spécial Mobile Association (GSMA) the global mobile subscription reached 6.9 billion by the end of 2013 (GSMA, 2014).

Electromagnetic fields (EMF) are a kind of radiation consists from waves of electric and magnetic energy and emitted together each perpendicular to another in one direction in the space. Science these waves consists of tops and bottoms, the distance between two consecutive tops or two bottoms is called the wavelength. Frequency is a term that used to describe the EMF especially radio and microwaves and it measured by Hertz (Hz). Hertz is the number of waves that passes a certain point in one second. EMF is classified in the magnetic spectrum by their wavelengths and frequencies (ICNIRP, 1998).

There are two kinds of radiation in the magnetic spectrum, ionizing radiation and nonionizing radiation. Ionizing radiation with high frequency waves has energy with ability to make molecules lose an electron and that can make a great damage to cellular structures and affect the health. Ionizing radiation includes radiation from radioactive elements like uranium and radon. Nonionizing radiation includes the radio frequencies (RF) with low frequency and it does not have enough energy to make molecule loses an electron (Johnson and Guy, 1972; Foster, 2000 and Challis, 2005)

Radio frequency (RF) is a term used to describe radio and microwaves together and it ranged from 3 kilohertz (kHz) to 300 gigahertz (GHz) in the magnetic spectrum. It is most used in telecommunication technologies like, radio, televisions, GPS technology, satellites and mobile phones and there other technologies like microwave ovens and radar (OTA, 1992).

RF in telecommunications has specific amplitude distribution that modulated either to continuous wave (CW) like in the analog mobile phone systems or to pulse amplitude like in GSM mobile phone systems. Cell phones
transmit or receive data calls from and to the base station through the radio frequencies that contain two bands. Uplink bands used to transfer data from the cell phone to the base station, and downlink bands to transfer the data from the base station to the cell phone (Gupta, 2014).

Global System for Mobile Communications (GSM) and the Universal Mobile Telecommunications System (UMTS) are the two main systems that used today in the mobile communications. In 1980s, analog phones technology has been used first generation (1G) with two bands, 450 and 900 MHz. In 1990s, GSM introduced the second generation (2G) of digital phones that used 900 and 1900 MHz bands. After that in 2000, UMTS introduced 3G that uses 1900–2200 MHz and then in 2011, it introduced the 4G that uses 2000–8000 MHz (Andersson, 2001; De Vriendt et al., 2002 and Ruippo et al., 2010).

With the massive wide spread of mobile phone and its services, Federal communication commission (FCC) had set standard limits to make the exposure to radio frequency from mobile phone more safe. These limits are determined by the absorbed energy by the head and body during using of mobile phone and it known by specific absorption rate (SAR). FCC admitted that the safe SAR for every mobile phone must not exceed 1.6 watts per kilogram (1.6 W/kg) and mobile phone manufactures have to commit these limits. SAR for every mobile phone device can be found in the manufacture website by using the device ID (FCC, 2014).

However with the long term use of mobile phones or exposure to electromagnetic fields (EMF) from base stations antennas could induce many harmful effects. Many studies reported that RF can affect health like causing brain tumors in adults after using mobile phone for several years (ICNIRP, 2009 and 2011). Also acute exposure to EMF from mobile phone was found to affect the auditory system and hearing threshold level (Alsanosi et al., 2013 and Gupta et al., 2015) besides it changed the blood pressure levels and the variability parameters of the heart rate in humans and affected the muscle
contraction of the amphibians (Barker et al., 2007; Andrzejak et al., 2008 and Mortazavi et al., 2015).

Many theories were suggested to explain the actual mechanisms of non-ionizing EMF on biological systems, like inducing thermal effects or heating that caused by low levels, while the high levels were suggested to induce electrical effects at both sides of the cell membrane and that lead to consequence changes in membrane ions and current flow, or it may oxidize some molecular structure and induce free radical and oxidative stress that causes the major damage at the end (Marino et al., 2009 and Manikonda et al., 2014). It also may cause rearrangements in amino acids sequences of protein especially of the membrane channels and tight junctions in the brain like the leakage that happened in the blood brain barriers (BBB) and caused the passage of harmful molecules (Belyaev et al., 2006 and Qiu et al., 2011).

Oxidative stress is the major effect that EMF was found to be induced in the many body organs like liver, spleen, kidneys, testes, blood cells, skin, brain and bone marrow. (Gorlitz et al., 2005; Ceyhan et al., 2012; Atlı-Şekeroğlu et al., 2013 and Cetin et al., 2014).

Also, it was found that EMF changed the secretion system of many glands, such as decreasing protein secretion and salivary rate in parotid gland, changing melatonin concentrations in pineal gland, decreasing the catecholamine produced by the adrenal gland (Belousova and Kargina-Teren'eva, 1999; Sukhotina et al., 2006 and Goldwein and Aframian, 2010). In addition, it changes cortisol, T₃ and T₄ levels in the blood (Shahryar et al., 2009) and inducing tumor in the mammary glands (Hruby et al., 2008).

Brain is the most affected organ by EMF and many researches manipulated the effects on the brain by many ways. EMF was found to change the distribution of the cerebral blood flow especially in the region close to antenna (Huber et al., 2002 and Aalto et al., 2006) and induced some behavioral changes, memory impairment and spatial learning difficulties
(Fragopoulou et al., 2010b; Ntzouni et al., 2011 and Li et al., 2012). Increasing the permeability of the blood brain barriers (BBB) after exposure to RF was found (Nittby et al., 2009), and alteration in brain biogenic amines levels were recorded (Inaba et al., 1992; Burchard et al., 1998 and Maaroufi et al., 2014). For such previous reasons EMF could be a coordinating agent with other pathogens in many neurodegenerative disorders like headache, dementia, Alzheimer's disease, Parkinson's disease, autism spectrum disorder (ASD) and sleep disorders.

The small skull volume and the sensitive body systems in children make them more vulnerable to EMF irradiation than the adult. Wiart et al. (2008) found that the amount of energy of EMF that can be absorbed by the brain (SAR) in children is two times higher than adults also Christ et al. (2010) found that the absorption by bone marrow is ten times greater than adults. Such reasons, many studies concern to direct great attentions to the effects of EMF on embryos and infants that exposed to the irradiation during the pregnancy period or during the early life period. Some of these studies suggested that EMF could induce developmental changes during the fetal period, (Magras, and Xenos, 1997; Ferreira et al., 2006; Fragopoulou et al., 2010a and Mortazavi et al., 2013) or it could be related to increasing the risk of tumor induction in children (Aydin et al., 2011).

In addition, prenatal exposure to 900 MHz EMF affected on the development of the dentate gyrus granule cells in the rat hippocampus, the subject that could lead to behavioral defects, learning difficulties and memory impairments in children (odaci et al., 2008; Abramson et al., 2009 and Aldad et al., 2012 ).
AIM OF THE STUDY

This study aimed to investigate the biological effects of EMF sourced from ringing mobile phone at many times of the day on neonatal rats that were previously exposed to EMF during the gestation phase. Biogenic amines (dopamine, norepinephrine, epinephrine, serotonin and histamine) and melatonin concentrations were estimated in neonatal rat brain immediately after post-partum at the postnatal 1st day (P1), P7, P14, P21 and P28.
SUMMARY

Perchloric acid (Sigma, Germany) was used for homogenization. Melatonin, serotonin hydrochloride, histamine dihydrochloride (Sigma, Germany), epinephrine, (Misr Co. For Pharmaceuticals and Chemical Industries, Egypt), norepinephrine (Mylan, Egypt) and dopamine hydrochloride (EUP for EMIC) were used for HPLC standard solutions. 3,4-Dihydroxybenzylamine hydrobromide (Sigma, Germany) was used as the internal standard solution.

Ten adult females and ten adult males of albino rats (Sprague Dawely strain) five-weeks old were obtained from Research Institute of Ophthalmology, Giza, Egypt. Rats were housed in plastic cages (35 L × 24 W × 20 H cm) and raised in the animal house of Center of Virology, Faculty of Agriculture, Cairo University.

Females were separated from males and two of the same gender were housed together in one cage before beginning the experiment. Animals were kept under normal healthy laboratory constant conditions, temperature (25±2°C), humidity (70%) and 12-hour light-dark cycle, adjusted by 24 h timer. The cages of rats were kept in a small room that was inside other large room, the two rooms were without any windows and any holes to prevent the sneak light into rooms during the dark period, and the inner room was supported with air condition. Light was switched on at 9.00 am and switched off at 9.00 pm. Rats were adapted on free access of tap water and fed basal diet regularly for two months before beginning of the experiment. The animal experiments were performed with the approval of Ethics Committee for experimental animals of the Faculty of Agriculture, Cairo University, Giza, Egypt, which followed the recommendation of the National Research Council guide for Care and use of laboratory animals (NRC, 1985).
Nokia mobile phone model (model: C6-01.3, SAR: 1.00 W/kg for head and 1.25 W/kg for body, SAR information obtained from Nokia and FCC network site) was used as a source of electromagnetic waves. Mobile phone was put at 15 cm distances from cages that were arranged in a circular way around the mobile phone. To make sure that the antenna of the mobile phone send its waves in all directions with the same velocity, mobile phone was fixed in the middle by a piece of foam to be in a standee state. Also, it was connected to the electricity by a charger to ensure that it is working all the time. The mobile phone was adjusted at the silent mode and etisalat 3G (2100 MHz).

Animals were fed with basal diets contain 77% starch, 10% casein, 8.0% corn oil, 1.0% vitamin mixture and 4.0% mineral mixture according to Ulloa et al. (1988). Vitamins mixture and salts mixture were prepared as described in AOAC (2000).

At the beginning of the experiment, on 6th of May 2013, each male was allowed to mate with one female in one cage, irradiation of females was started at the first day of mating and continued through the pregnancy period until the 35th day of postpartum (postnatal day 35 or P35).

After one week from mating all males were isolated in separated cages. Pregnant rats in the experiment were divided to two groups. The first group (five female rats) was exposed to irradiation from mobile phone that was ringed for 15 times, each of 40 seconds, at five intervals through the day, (at 10:00 am, 1:00 pm, 5:00 pm, 9:00 pm and 12:00 am, 3 times for each time). The other group of pregnant female rats (five female rats) was used as normal group that was housed in another place that was so far from the exposed group. Female rats in both groups were weighed in the day before mating and then they were weighed again at the 1st, 7th, 14th, 21st, 28th and 35th day of postpartum days. In the normal group and exposed group each pregnant female rat delivered a different number of pups, 7, 7, 9, 11 and 13 pups in the normal group and 7, 8, 9, 9 and 10 pups in the exposed group. Neonatal rats were left
with their mothers without weaning in the same cages until the end of the experiment. The neonatal rats were irradiated with their mothers until the end of the date of decapitation, from postnatal days 1 (P1) to 28 (P28).

Three neonatal rats from the normal group and five from the exposed group (one neonatal rat from each mother) at postnatal days one (P1), P7, P14, P21 and P28, were weighed and then they were decapitated under red light between 8:00 am and 9:00 am, according to Kato et al. (1993). The whole brain was isolated from each neonatal rat. The decapitation process was carried out as follow, every rat was quickly decapitated close to the skull by a sharp scalpel, then the head was fixed by the left hand in a flat plate filled with ice and covered with aluminum sheet. With the right hand, skin was cut along the length of the head by a small scissors and then removed. Skull was cut along the length of the brain, along the longitudinal fissure that separates the two hemispheres, and then at the end of the cut line a circular cut around each hemisphere was done. The two flaps of the skull then were pulled back. At the end point of the previous long skull cut line, another cut line was continued to cut the skull above the cerebellum until reached to the end. Then, the other two flaps above the cerebellum were totally cut from the side of the skull. After that, the brain was totally exposed in the head without the surface of the skull. Then, straight needle was carefully immersed at the side of the cerebrum to push it up from the cranium floor. Before the cerebrum was totally separated, the olfactory bulbs were cut off, and then the two hemispheres were pushed out. After that, the whole brain was pushed out with the two hemispheres. The whole brain isolation included cerebrum, cerebellum, pons and medulla oblongata. The brain isolation process was performed within 3 min of decapitation. The whole brain was weighed and then stored in deep freezer at -25°C.

Samples were prepared for HPLC injection according to Cao and Hoshino (1996) after some modification. A portion of of perchloric acid (0.2 M) was freshly prepared by dilution 1.7 ml of perchloric acid 70% with 50 ml
of distilled water in a glass beaker, and then it was completed to 100 ml by distilled water and kept in a refrigerator overnight. Brain tissues were thawed at room temperature. Each brain was homogenized with certain volume of 0.2 M perchloric acid, in a tube that was immersed in larger falcon tube filled with ice. Diluted perchloric acid that used in homogenization was kept in a container filled with ice during the work. Homogenization was carried out for 2 min with Heidolph homogenizer. Then, homogenized solution was centrifuged at 18745 xg at 4ºC for 20 minutes by laboratory centrifuge (Sigma 2K15). After that, the whole supernatant of each sample was transferred to a new eppendorf and then 10 µl (1mg/5ml) of internal standard (3, 4 dihydroxybenzylamine hydrobromide) were added to the supernatant and filtered through centrifugal microfilter (Hamilton syring; 13 mm of diameter and 0.2 µm pore size). Then, 25 µl of supernatant were injected into the HPLC with the standards at the same time and under the same condition.

Exactly, 5 µl of each melatonin, serotonin hydrochloride and histamine dihydrochloride (5 mg/5 ml of 0.2 M perchloric acid) were injected into HPLC. A portion of 10 µl of each 3.4-dihydroxybenzylamine hydrobromide as internal standard (5 mg/5 ml of 0.2 M perchloric acid) (Wallwork et al., 1982), epinephrine (1mg/1ml), norepinephrine (2mg/1ml), and dopamine hydrochloride (200 mg/5 ml) were injected into HPLC (Agilent Technologies, 1200 series), using C18 reversed phase column (Zorbax ODS, 250 mm × 4.6 mm). The HPLC was equipped with autosampling injector, solvent degasser, ultraviolet (UV) detector set at 205 nm and quaternary HP pump (series 1100), and the column temperature was proceeded at 35ºC. HPLC procedure that used in the present study was done according to Kerddonfak et al. (2010). Mobile phase was a mixture of methanol and 10 mM sodium heptane sulfonate in water with pH 3.5 containing 0.0002 M EDTA (23:77) and flow rate 1.0 ml/minute at 35ºC. HPLC chromatogram of the standards mixture of biogenic amines, melatonin and internal standard.
The results obtained could be summarized as follow

1. Effect of EMF on weights of mothers and neonatal rats

Weights of normal and EMF-exposed females rats before pregnancy and after parturition showed no significant changes were observed after postpartum. But, the fluctuation in the weight of EMF-exposed female rats was more obvious from 256.0±6.5 to 233.4±12.2 g than that found in normal female rats from 266.5±16.6 to 258.5±9.5 g. At the end (P35), weights of EMF-exposed female rats were insignificantly decreased from 256.0±6.5 to 242±7.7 g comparing to the slight decrease in weights of normal female rats from 266.5±16.6 to 260.5±9.6.

Exposure to EMF may decrease the weight of female rats. It may be confirmed by the present results which show that the weights of EMF-unexposed female rats after parturition were significantly increased than the rats weights before mating. While, the weights of EMF-exposed female rats after postpartum were not almost significantly changed.

2. Effects of EMF on biogenic amines and melatonin concentrations in neonatal rats

At the beginning, the present study was to investigate the changes of biogenic amines concentrations in normal brains of neonatal rats during the postnatal period extended from the first day of delivery (P1) to 28th day, and comparing the obtained results with those obtained from the brains of EMF-exposed rats. Results of biogenic amines and melatonin concentrations in normal neonatal rats were tested by the L.S.D. Data show significant differences ($P<0.01$ and 0.05) between the different postnatal days representing specific patterns (P1, P7, P14, P21 and P28). These significant changes ($P<0.01$ and 0.05) were disrupted in the EMF-exposed neonatal rats, representing different new patterns. Therefore, the present results concerned about the effects of EMF exposure on biogenic amines and melatonin
concentrations were compared with unexposed rats, each at the same age and the obtained results are presented in Table (6).

Melatonin concentrations in exposed group showed significant \((P<0.05)\) increases at P7 and P28 (1.80±0.14 and 1.56±0.16 µg/g tissue, respectively) as compared to the unexposed rats at the same age (0.67±0.21 and 0.48±0.09 µg/g tissue, respectively). Therefore, the significant differences between P1-P7, P1-P28 and P14-P28 in brain melatonin concentrations of normal rats (Table 7) were changed by EMF exposure to be significant differences between P7-P21, P14-P21 and P21-P28.

Moreover, dopamine concentrations in exposed group showed significant \((P<0.05)\) decreases at P7 and P14 (3.40±0.18 and 6.37±0.64 µg/g tissue, respectively) as compared to normal rats, at the same age (5.30±0.46 and 16.7±0.24 µg/g tissue, respectively). Significant variations of brain dopamine in postnatal period of normal neonatal rats were localized between the P14 and other postnatal days. While in rats exposed to EMF, the significant differences involved P14, P21 and P28.

In addition, norepinephrine concentrations in the exposed group showed a significant \((P<0.05)\) decrease at P7 as compared to the normal group at the same age (5.42±0.19 vs 13.56±2.80 µg/g tissue, respectively). The same trend was observed in brain histamine where the histamine concentrations in exposed group showed a significant \((P<0.05)\) decrease at P7 as compared to unexposed rats at the same age (4.99±0.34 vs 9.39±1.18 µg/g tissue, respectively).

Almost, there are significant differences between the concentrations of both norepinephrine and histamine in the brain of normal neonatal rats at the seventh day of parturition and the other days (P1, P14, and P28) that is completely disappeared in rats exposed to EMF.

Epinephrine concentrations in exposed group showed a significant \((P<0.05)\) decrease at P21 as compared to unexposed rats at the same age (2.89±0.40 vs 4.45±0.78 µg/g tissue, respectively). Results of serotonin
concentrations did not show any significant changes. At the P7, P21 and P28, the concentrations of brain serotonin and norepinephrine in normal rats were significantly differed than those at the other days. After exposure to EMF, the significant differences of serotonin between the neonatal days were shrinked to be between P1-P21 and P1-P28 only, while in brain epinephrine the significant differences were extended almost allover the comparison pairs.

The biogenic amines in the brain act as both neurotransmitters and neurohormones and their concentrations greatly differ depending upon (1) specific rhythm as melatonin (light-dark cycle), (2) different circumstances as epinephrine and norepinephrine (during anger, fear and terror), dopamine (during movement, fight or flight response and mental disorders) and serotonin (during the regular cycles in adrenocortical secretion, pineal secretion and emotional stress) as reported by Cools et al. (2011) and Colin-Gonzalez et al. (2015).

There are structural relations between some of biogenic amines, for example dopamine is a precursor of both epinephrine and norepinephrine. In addition, serotonin is a precursor of melatonin. Therefor, the change in the concentration of some biogenic amines may directly affect the biosynthesis and concentration of another biogenic amine. Moreover, there are functional relations between biogenic amines through the regulation of endocrine functions or through all parts of central nervous system. For example, norepinephrine released at the postganglionic neurons where pass to the pineal gland increase the biosynthesis of N-acetyltransferase enzyme and thus increases melatonin secretion (Missale et al., 1998 and Peliciari-Garcia et al., 2013).

According to the previous reasons, the present study has to do a statistical correlation between the normal concentrations of each biogenic amine during the postnatal beginning from the first day of parturition to the 28th day of delivery. The present data indicate significant correlations between each of brain melatonin, serotonin, histamine, dopamine, norepinephrine and
epinephrine throughout the postnatal period. It is concluded that there are neurohormonal patterns for the postnatal newborn rats.

The preservation of these neurohormone patterns is very important for the normal neuronic growth of the nervous system during the postnatal period of rats. The disturbances in these patterns due to EMF or any another factor may lead to great risk in nervous system development and whole body metabolism.

Pearson correlation of biogenic amines and melatonin in normal rat brain indicate the presence of significantly positive relations between (1) melatonin and serotonin, (2) norepinephrine and epinephrine and (3) histamine and norepinephrine. These patterns are completely absent in brains of EMF-exposed rats but rather a new significantly negative correlation between dopamine and serotonin was observed.
CONCLUSION

All of these changes that caused by EMF in melatonin and biogenic amines that play important roles as neurohormons and neurotransmitters could lead to many disturbances in many physiological processes in the nervous system, such the disturbances in newborn brain might cause many developmental problems and mental difficulties that affect their whole life.