

Original Article

Mechanical activity of isolated aorta strips after prolonged exposure to low frequency electromagnetic fields and its interaction with the cholinergic and adrenergic systems in male rat

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Abstract

Introduction: Public concern about the potential effects of electromagnetic field (EMF) on human health is increased by progressive usage of electrical devices in modern life. The aim of this study was to investigate the effect of prolonged exposure to low frequency EMF on the mechanical activity of isolated thoracic aorta strips in rats.

Methods: Fourteen male rats were randomly allocated into sham and experimental groups. Experimental group was continuously exposed to 1mT, 50Hz EMF, for 75 days in a magnetic coil box. Sham group was kept under conditions similar to experimental group, without being exposed to EMF. After 75 days, the rats were anaesthetized and the thoracic aorta was dissected and cut into 1cm strips. Aortic strips were suspended in organ bath chambers containing of Krebs' buffer and were bubbled with a gas mixture (5% CO₂, 95% O₂). Then the aortic isometric tension was measured during 20-min equilibration period and after cumulative administration of acetylcholine and phenylephrine in different concentrations.

Results: Increased vasocontraction responses to 10⁻⁶ M phenylephrine were observed in experimental group compared to sham group ($P<0.05$). Moreover, reduced vasorelaxation responses to 8×10⁻⁵ M acetylcholine were observed in the experimental group compared to sham group ($P<0.05$).

Conclusion: It can be suggested that prolonged exposure to EMF have an effect on the vascular sensitivity to cholinergic and adrenergic system, can lead to alteration of the vascular resistance.

Keywords:

Electromagnetic field;
Thoracic aorta;
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Introduction

Electromagnetic field (EMF) is the radiation that surrounds all electrical machines, tools, industrial instruments and power lines. Due to advances in

modern life style, public concern about the potential influences of EMF on human health has increased. Some researchers have indicated that EMF can affect growth and development of vessels. For instance, EMF raised growth rate and proliferation of endothelial cells and angiogenesis (Yen-Patton et al.,

1988). EMF increased angiogenesis in human umbilical vein endothelial cells by increasing fibroblast growth factor beta-2 (Tepper et al., 2004). Low-energy EMFs enhanced proliferation of bovine coronary and murine aortic smooth muscle cells (Kobbert et al., 2008). Endothelial cells in aorta and heart were transformed extremely by being exposed to EMF for a long time, therefore, it was assumed that it would affect cardiovascular system physically and functionally (Roshangar et al., 2012).

Most of the studies have reported vasodilatory effect by being exposed to EMF, as well as, increase in blood flow or blood pressure. For instance, EMF

stimulated arteriolar phosphate, GMP, in the rat cerebellum *in vitro* (Miura et al., 1993). It was also demonstrated that during and post EMF exposure periods, arteriole diameters increased meaningfully when compared with the pre-exposure period (Traikov et al., 2005).

On the contrary, some other studies have reported a decrease in blood perfusion or pressure and some other studies have reported no effect at all. Whereas some other studies found that EMF exposure could activate either vasodilation or vasoconstriction depending on the initial tone of the vessel. The results related to these studies are in Table 1.

Table 1: Summary of electromagnetic fields (EMFs) effects on blood perfusion and pressure

EMFs parameters	Time of exposure	Species	EMF effects	References
Burst-type EMF (10 MHz RF, 0.73 mT)	60 min	Frogs (web microcirculation)	Increased blood flow	(Miura and Okada, 1991)
EMF (3×10^8 to 3×10^{11} Hz RF)	12 min	Humans (cutaneous tissue of ear)	Increased blood flow	(Monfrecola et al., 2003)
Pulsed EMF	2 and 60 min	Rats (cremaster muscle)	Increased arteriolar diameter	(Smith et al., 2004)
Extremely low frequency - EMF (28 mT(rms), 10, 16 and 50 Hz)	33 min	Mice (microvasculature)	Increased arteriole diameter	(Traikov et al., 2005)
EMF (0.1 mT, 60 Hz)	5.5 h	Mice (liver)	increased NO	(Yoshikawa et al., 2000a)
Static magnetic field (70 mT)	15 min	Rats (skeletal muscle microvessels)	Modulatory effects *	(Morris and Skalak, 2005)
Static magnetic field (1, 5, and 10 mT)	10 min	Rabbits (cutaneous tissue of ear)	Modulatory effects	(Ohkubo and Xu, 1997)
Static magnetic field (1 mT)	10 min	Rabbits (cutaneous tissue of ear)	Modulatory effects	(Okano et al., 1999)
Static magnetic field (1 mT)	30 min	Rabbits (cutaneous tissue of ear)	Modulatory effects	(Okano and Ohkubo, 2001a; Okano and Ohkubo, 2001b)
Static magnetic field (0.4 T)	15 min (3 times)	Humans (cutaneous tissue)	Decreased blood flow	(Mayrovitz and Groseclose, 2005)
Static magnetic field (8 T)	20 min	Rats (cutaneous dorsal tissue)	Decreased blood flow	(Ichioka et al., 2000)
Static magnetic field (85 mT)	20 min	Humans (cutaneous tissue of middle finger)	No effect	(Mayrovitz et al., 2005)
Pulsed EMF (0.1 and 8.4 mT)	30 min	Humans (cutaneous tissue of foot)	No effect	(Schuhfried et al., 2005)
magnetic field (200- μ T, 60-Hz)	1 h	Microcirculation (skin)	No effect	(McNamee et al., 2011)

* Modulatory effects, when vascular tone was increased by a vasoconstrictor, vascular tone decreased by EMF and when vascular tone was decreased by a vasodilator, vascular tone increased by EMF.

Acetylcholine (ACh), a cholinergic agonist activate nitric oxide (NO) synthase in endothelial cells, stimulates the endothelial cells to produce NO, causing the vascular smooth muscle relaxation through increasing cGMP levels in smooth muscle (Rapoport and Murad, 1983; Wanstall et al., 2001). Phenylephrine (PE), a selective α_1 -adrenergic receptors agonist, increase vascular tone through activation of the phospholipase C which cleaves the membrane phospholipid phosphatidylinositol 4,5-bisphosphate into the inositol 1,4,5-trisphosphate (IP₃) and diacylglycerol (DAG), after that it leads to the opening of IP₃ receptor channels at the sarcoplasmic reticulum, and then induce Ca²⁺ release from intracellular stores and DAG activation of protein kinase C, leads to activation of the contractile machinery in the vascular smooth muscle cells (Wu et al., 1992; Graham et al., 1996; Zhong and Minneman, 1999; Gnus et al., 2013). Since, humans in modern society are exposed to low frequency of EMFs generated by power lines and household electric appliances during boarding, this study was designed to investigate the effect of exposure to low frequency EMF on mechanical activity of the isolated thoracic aorta and its interaction with the cholinergic and adrenergic systems.

Materials and methods

Chemicals

Materials were procured from the following sources: ACh bromide was obtained from British Drug House (BDH, England), PE hydrochloride from Sigma (St. Louis, MO, USA), NaCl and D-glucose monohydrate from Merck (Germany) and KH₂PO₄, MgSO₄, CaCl₂, NaHCO₃ and KCl were purchased from Applichem (Darmstadt, Germany).

Animals

The experimental protocol was approved by the Shiraz University, Ethical Committee, Shiraz, Iran. Fourteen male Wistar rats with the average weight of 200-250 g were used. The rats were maintained under the stable condition at room temperature (22-25°C, 12-hour light/dark, photo schedule); standard lab feed and water were provided to animal ad libitum. The rats were adapted to lab condition since 7-days prior to the experiment.

Animal treatment

Rats were randomly allocated into two groups, sham and experimental groups. Experimental group was continuously exposed to 1mT, 50Hz EMF, for 75 days in a solenoid. Sham group was kept under similar conditions with the experimental group, but without EMF.

EMFs inducing system

In order to produce 50Hz EMF, magnetic coils were used. A wooden box (100×100×35 cm) with open-end was built, then 600 turns of 1mm copper wire was wrapped around it and finally it was connected to an autotransformer, with a voltage percent scale, which was connected to 220V power. Calibration of the system was accomplished by a digital teslameter (Leybold Didactic GMBH 51662). Cages with animals were placed in the open-end wooden framework.

Preparation of aortic strips

At the end of the radiation exposure period, the subjects were anaesthetized by ether. Thoracic aorta was removed quickly and transferred to dish filled with ice-cold Krebs' buffer containing (in g/l): MgSO₄ 0.1324, KH₂PO₄ 0.1551, KCl 0.35, NaCl 6.896, NaHCO₃ 2.1, CaCl₂ 0.279 and D-glucose 1.98; pH 7.4. Fat and connective tissue were removed of aorta surface delicately to avoid touching the luminal surface and then they were cut into 4-5 mm long transverse segments.

Measurement of isometric tension

Organ chambers containing 40 ml of thermostated (37°C) Krebs' buffer, and were bubbled continuously with a gas mixture (5% CO₂, 95% O₂). Rat aortic strips were suspended by stainless steel hooks. The lower hook was fixed to a micrometer and the upper hook connected to a force-displacement transducer (Panlabs, Cornella, Spain) for measurement of isometric tension changes. The output of the force transducer was amplified by an ADInstruments Bridge Amplifier (ML110) and recorded via an ADInstruments PowerLab 2/25 (ML825) in computer using the chart software (version 5). The strips of aorta were gradually stretched to a resting tension in the range of 1-1.2 g, and allowed to equilibrate for 20 min with change of the medium every 20 min. After the 20-min equilibration period, different concentrations (1×10^{-6} , 1×10^{-5} , 4×10^{-5} , 8×10^{-5} and

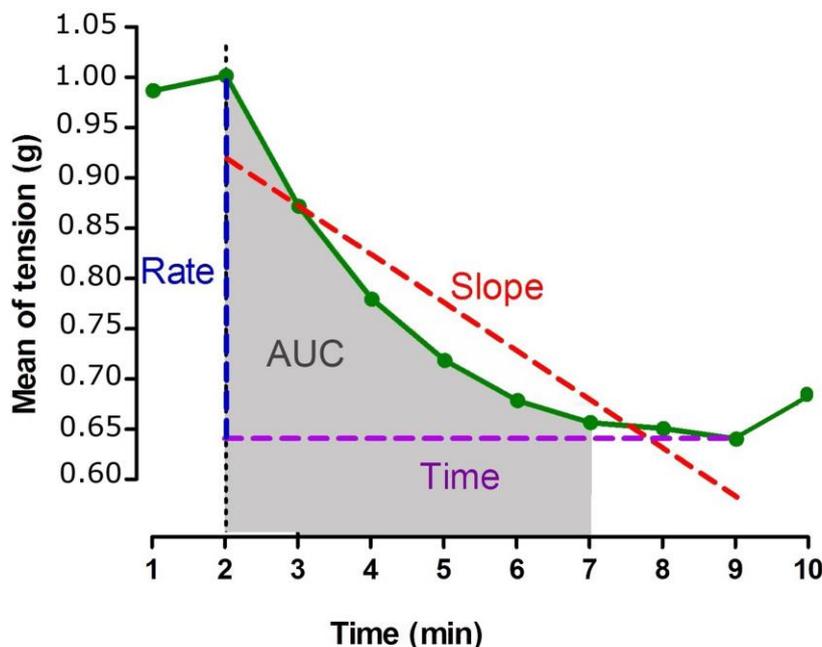


Fig.1. The corrected tension curves for evaluation of indices of area under the curve; AUC, time, rate and slope of the curve of relaxation.

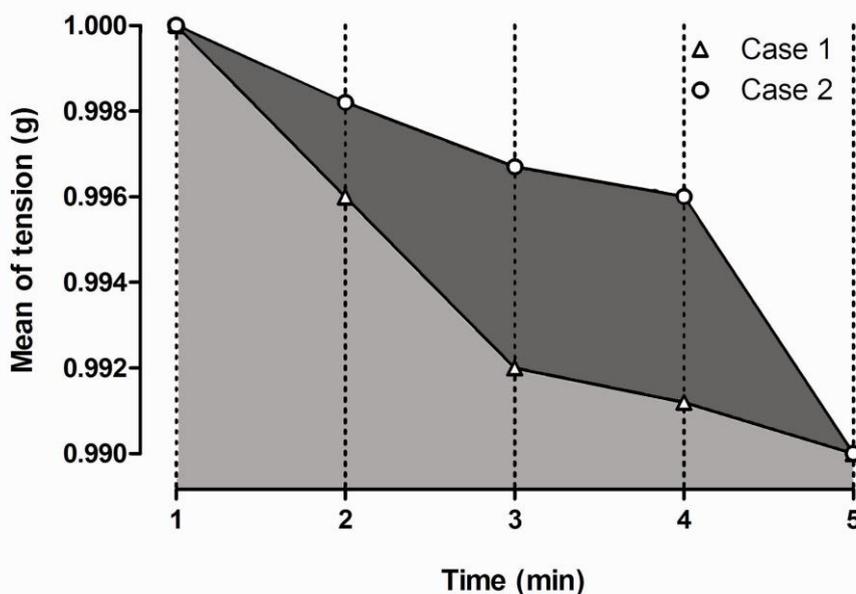


Fig.2. Samples of relaxation curve with the same rate, slope and time of relaxation, but different area under the curve (AUC). The AUC is calculated by adding the area of trapezoids together.

10^{-4} M) of Ach and different concentrations (1×10^{-7} , 1×10^{-6} , 4×10^{-6} , 8×10^{-6} and 10^{-5} M) of PE, were added cumulatively with an interval of 10 min into the organ bath chambers and cumulative dose-response curves were obtained.

Aortic tension curve uniformity

Averages of aortic tension in response to different doses of Ach and PE administration were calculated

every minute. The upward curved line of tension in response to PE and the downward curved line of tension in response to Ach were drawn using Excel software (office 2007, Microsoft co., USA). Since starting point of relaxation or contraction was obtained in the range of 0.69-1.05 g for relaxation and 0.61-1.08 g for contraction, to uniform the starting point of relaxation or contraction in all samples, average of aortic tension in the first minute

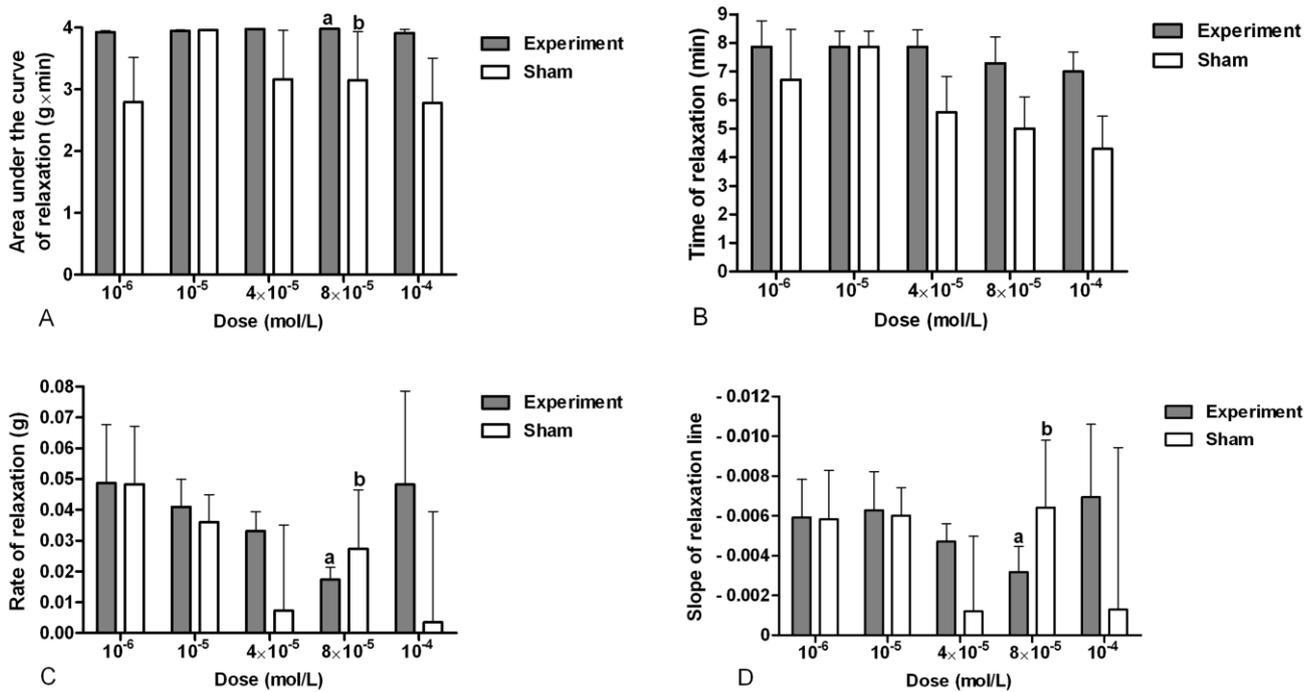


Fig.3. Comparison of mechanical activity of isolated thoracic aorta that were previously exposed to low frequency electromagnetic fields (EMF), one is the experimental group and the other is sham group using indices of corrected dose response curves of relaxation after administration of Ach. ^{a,b} different superscript letters show a significant different between groups in a single dose ($P < 0.05$).

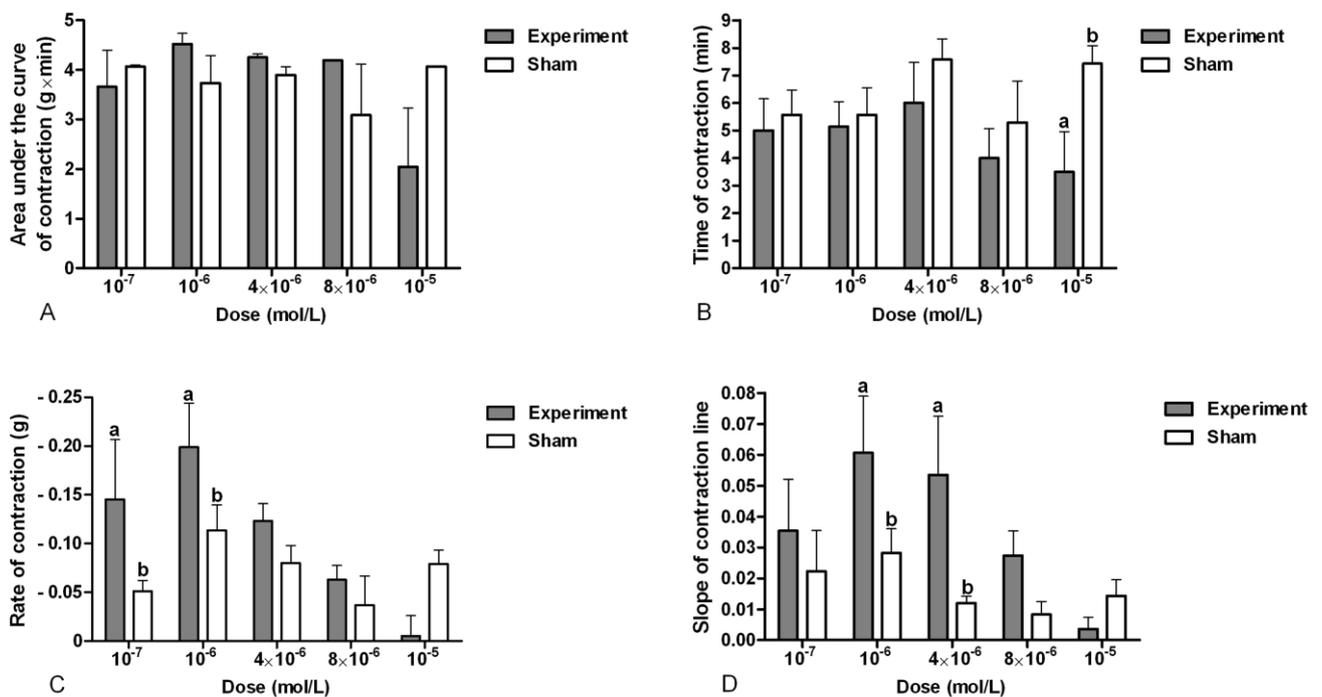
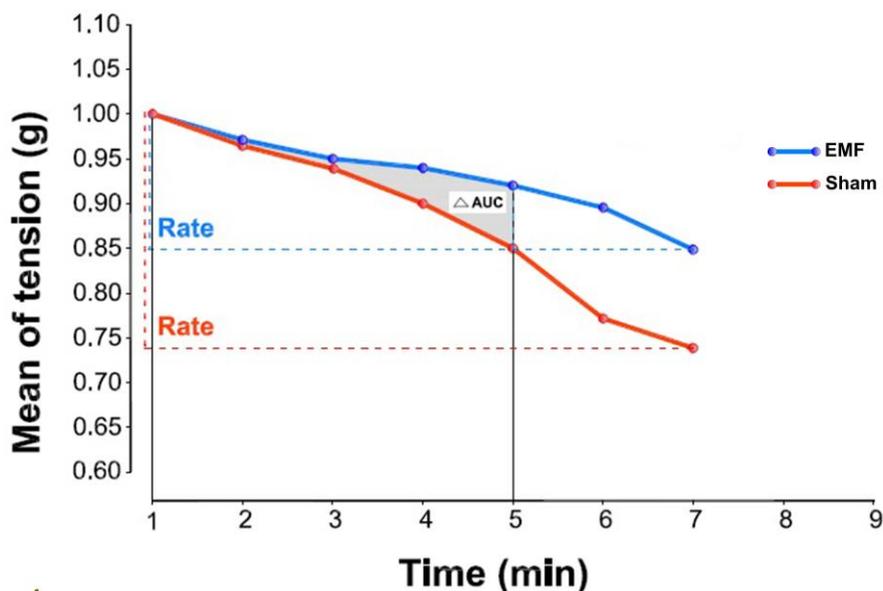
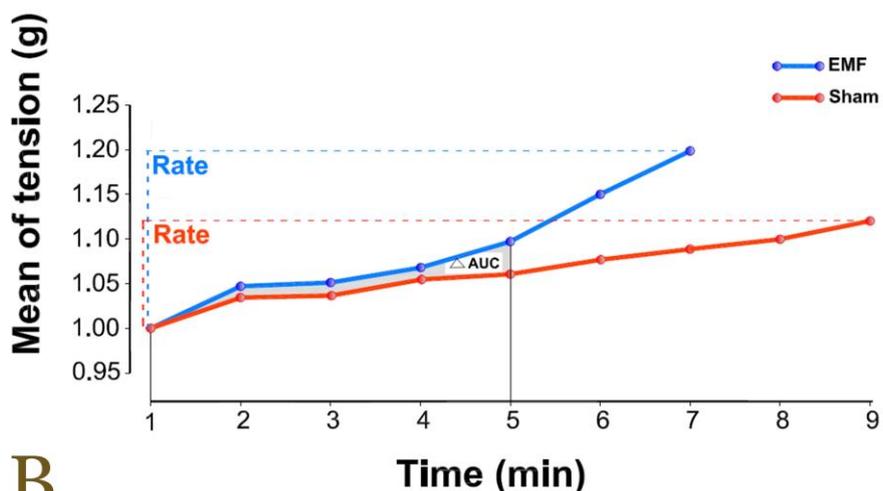


Fig.4. Comparison of mechanical activity of isolated thoracic aorta that were previously exposed to low frequency electromagnetic fields (EMF), one is the experimental group and the other is sham group by using indices of corrected dose response curves of contraction after administration of PE. ^{a,b} different superscript letters show significant different between groups in a single dose ($P < 0.05$).



A



B

Fig.5. Comparison of mechanical activity of isolated thoracic aorta that were previously exposed to low frequency electromagnetic fields (EMF), one is the experimental group and the other is sham group using indices of corrected dose response curves of A) relaxation after administration of effective dose of Ach ; and B) contraction after administration of effective dose of PE.

of relaxation or contraction was converted to “1” and then the average of aortic tensions in the next minutes were calculated using the following formula:

$$1-A=X$$

$$B+X=B'$$

where A, real average of aortic tension in the first minute of relaxation or contraction; X, calculated constant number; B, real average of aortic tension in the next minutes of relaxation or contraction; and B', calculated average of aortic tension in the next minutes of relaxation or contraction. Based on the calculated average tension numbers, new corrected curves were drawn.

Aortic tension curve indices

The corrected tension curves were evaluated using four indices (Tamadon et al., 2011) as follows (Fig. 1): a) Slope is the trend of the corrected descending or ascending curves of tension in response to administration of different doses of Ach and PE, respectively, during 10 min using trend line formula in Excel software. b) Rate is the subtraction of the onset in the aortic relaxation or contraction from the maximum relaxation and contraction average of the aortic corrected tension curves, respectively. c) Time is the number of minutes for relaxation or contraction after Ach and PE administration, respectively. d) Area

under the curve (AUC) was calculated from the onset of relaxation or contraction in the corrected curve for the next five min using the following formula:

$$\text{AUC} = [(a_0 + a_1)/2] + [(a_1 + a_2)/2] + [(a_2 + a_3)/2] + [(a_3 + a_4)/2]$$

Where a_i is calculated for the average of aortic tension in the corrected curve from the onset until the 5th min.

Based on the observation of corrected curves between two groups in the indices for rate, slope and time were equal, but since the pattern for contraction and relaxation were different between these groups (different in AUC). Therefore, measuring the area under the contraction and relaxation curve seems to represent the differentiation in depth and severity between groups (Fig. 2).

Statistical analysis

Data for corrected aortic tension curve indices (AUC, rate, time and slope) in response to different doses of Ach and PE administration was calculated by Kolmogorov-Smirnov test using SPSS (SPSS for Windows, version 11.5, SPSS Inc, Chicago, Illinois). In dose-response curves, log transformed data for rate and slope between dose groups were compared using one way ANOVA and LSD post hoc test. In addition, in dose-response curves, abnormal AUC data and time were compared using Kruskal-Wallis test and Tukey post hoc test. Values of $P \leq 0.05$ were considered significant. Group means and their standard errors are reported in the figures (GraphPad Prism version 5.01 for Windows, GraphPad software Inc., San Diego, CA, USA).

Results

There was no difference in weight gain (g) during experimental period between rat groups ($P > 0.05$). In dose-response curves, AUC of relaxation (g \times min) in response to administration of 8×10^{-5} M of Ach in experimental group was more than sham group ($P < 0.05$, Fig. 3A). However, there were no difference between AUCs of relaxation in sham and experimental groups in the other doses ($P > 0.05$). However the mean of time of relaxation (min) in response to administration of 8×10^{-5} M of Ach in experimental group was more than sham group but they were not significantly different in all doses ($P > 0.05$, Fig. 3B). Rate of relaxation (g) after 8×10^{-5}

M Ach administration in experimental group was less than sham group ($P < 0.05$, Fig. 3C). However, there were no difference between rates of relaxation in sham and experimental groups in the other doses ($P > 0.05$). Slope of relaxation line after 8×10^{-5} M Ach administration in experimental group was less than sham group ($P < 0.05$, Fig. 3D). However, there were no difference between slope of relaxation line in sham and experimental groups in the other doses ($P > 0.05$). In dose-response curves, there were no difference between AUC of contraction in sham and experimental groups in all doses ($P > 0.05$, Fig. 4A). However, mean of AUC of contraction after 10^{-6} M PE administration in experimental group was non-significantly more than sham group. Time of contraction (min) in response to administration of 10^{-5} M of PE in experimental group was less than sham group ($P < 0.05$, Fig. 4B) and in other doses, time of contraction (min) in response to administration of PE in experimental group were non-significantly less than sham group. Rate of contraction (g) after 10^{-7} and 10^{-6} M PE administration in experimental group was more than sham group ($P < 0.05$, Fig. 4C). Slope of contraction line after 10^{-6} and 4×10^{-6} M PE administration in experimental group was more than sham group ($P < 0.05$, Fig. 4D).

Discussion

Prolonged exposure to low frequency EMFs decreased the vascular sensitivity to cholinergic system by decreasing extent and severity of relaxation responses to administration of effective dose of Ach in isolated rat aorta (Fig. 5A). Also, it increased the vascular sensitivity to adrenergic system by obtaining greater level of contraction with more severity and time of contraction in response to effective dose of PE administration in exposed group compared sham group (Fig. 5B).

Prolonged exposure to low frequency EMFs decreased the extent and severity of vascular relaxation after Ach administration and increased the severity and time of contraction after PE administration. Our findings are in disagreement with the study of Öcal and Günay (2004) that indicated "decreased contraction and increased relaxation responses of the isolated thoracic aorta strips to administration of PE and Ach in the healthy and diabetic rats that were exposed to 50Hz magnetic

field, compared to control rats". They had concluded the positive effect of magnetic field on relaxations is the indicator for the role of endothelium-derived hyperpolarizing factor as well as endothelium-derived relaxing factor. There are a number of potential reasons for the variation in the reports of EMF effects on blood vessels and blood pressure such as: various duration of exposure, parameters of EMF exposure, the method of anesthetics and organs that were used in the experiments.

Most of the studies have indicated a vasodilatory effect of being exposed to EMFs that increases blood flow or blood pressure (Miura and Okada, 1991; Yoshikawa et al., 2000a; Monfrecola et al., 2003; Smith et al., 2004; Traikov et al., 2005). Other studies have also shown that EMF exert their vasodilatory effect by activating NO production via activation of NO synthase, causing relaxation of smooth muscle cells through cGMP pathways (Miura and Okada, 1991; Yoshikawa et al., 2000b). In addition, it was reported that EMFs promotes the growth rate of endothelial cells, angiogenesis and also endothelial cells in the presence of EMFs were observed to be lengthened morphologically (Yen-Patton et al., 1988). Moreover, a study showed that EMFs stimulated angiogenesis in human umbilical cord (Tepper et al., 2004). Also, in a ultrastructural study of aorta showed that in the EMFs exposed rats, there was significant decrease in the height of the endothelial cells and the mean thickness of aortic wall (Roshangar et al., 2012). Some studies reported the modulatory effect of EMF on vascular tone and blood pressure. When blood pressure was increased using a vasoconstrictor, exposure to EMF caused a significant vasodilation. This led to a significant increase in blood flow. On the contrary, when blood pressure was decreased using vasodilator, the EMF caused a significant increase in blood pressure during and post-exposure and led to vasoconstriction (Ohkubo and Xu, 1997; Okano et al., 1999; Okano and Ohkubo, 2001a; Morris and Skalak, 2005). In line with our study, other studies supported the negative effect of EMF exposure on blood flow or blood pressure (Ichioka et al., 2000; Mayrovitz and Groseclose, 2005). The remaining studies reported no effect (Schuhfried et al., 2005; McNamee et al., 2011). Through *in vitro* and *in vivo* studies reported the effects of EMF exposure were various according to the frequency, period of exposure and strength of

EMFs at cellular and organism levels. Further research is thus needed to achieve more definite answers regarding the potential adverse effects of EMF.

Conclusion

In conclusion, prolonged exposure to low frequency EMFs affected the vascular sensitivity to Ach and PE due to alteration the vascular resistance. It can be assumed that exposure to low frequency EMFs (1mT, 50Hz for 75 days 24 hr/day), may increase vascular resistance via decreasing sensitivity to cholinergic system and increasing sensitivity to adrenergic system; however further investigation is required.

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Conflict of interest

The authors have declared no conflict of interest.

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