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The effect of long-term exposure to low frequency electromagnetic field on cholinergic system and nitric oxide level of isolated distal colon of male rats

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Abstract
Objective: To evaluate the effects of long-term exposure to low frequency electromagnetic field **(**EMF) on the cholinergic system and nitric oxide level of isolated distal colon.
Methods: Fourteen adult male Sprague-Dawley rats were randomly divided into sham and experimental groups. Experimental group was exposed to 1mT and 50Hz electromagnetic field, continuously for 140 days 24 hrs per day in a solenoid. Sham group was housed under conditions same as experimental group, without EMF. At the end of the exposure period, the animals were anaesthetized with ether and the distal colon was removed and the tension changes of distal colon tissue were recorded in response to acetylcholine and L-N^G-nitroarginine methyl ester (L-NAME).
Results: The tension changes of distal colon tissue to acetylcholine and L-NAME was higher in the experimental group in compared to the sham group. Significant difference was detected between groups (p<0.05).
Conclusion: Long-term exposure to low frequency electromagnetic field increased sensitivity of acetylcholine receptors and decreased produce of nitric oxide in distal colon.

Keywords: Electromagnetic Field, Cholinergic System, Nitric Oxide, Colon, Rat

Introduction

Many field and laboratory investigators have showed that EMFs originating from residentially proximate power line, household electrical wiring, medical devices, cellular phone, and wireless communication produce a variety of behavioral and physiological changes in animals [1, 2]. Furthermore, fundamental research for EMFs suggested that changes of neurotransmitter concentration, activity stimulation of numerous enzymes and hormones, stimulation of oxidation-reduction processes and cellular synthesis by EMFs are resulted in the biological effects of EMFs [3-6]. Modern societies are pervaded by Non-ionizing 50-60 Hz low frequency electromagnetic field exposure. The possible health effects of different electromagnetic spectrum emitted by electrical device have resulted in performance of researchers in this field [7].

Acetylcholine (ACh) and Nitric oxide (NO) are two important neurotransmitter in entric system. ACh is well known as an important exhibitory neurotransmitter and the primary neural regulator of gastrointestinal motility, and NO is as a neuromuscular neurotransmitter of noradrenergic no cholinergic (NANC) inhibitory nerves in the parasympathetic [8] and the enteric nervous systems [9] that is produced endogenously during arginine metabolism by isoforms of nitric oxide synthase (NOS), enzymes possessing a wide range of physiological and pathophysiological activities [10, 11].

Modulation of cholinergic nerve activity that directly affects smooth muscle contractility is mediated by the muscarinic receptors expressed on smooth muscle [8]. Increased density of muscarinic receptors and decreased acetylcholine esterase (ChAT) activity might be a cause of formation of intestinal adhesion in postoperative [18]. Several studies showed increased expression and activity of inducible nitric oxide synthase (iNOs) in human colon adenomas [13]. In addition, iNOs expression in inflamed mucosa of patients with ulcerative colitis and gastritis demonstrate the production of NO and its potential involvement in the pathogenesis of these diseases [12]. Furthermore, after abdominal surgery, increase of iNOs damages intestinal pacemaker cells [14].

The data on effects of EMF on human gastrointestinal tract and neurotransmitter are scarce and most of the findings concentrate on the influence of EMF on secretion of neurotransmitter in brain. Therefore, the aim of the current study was to assess the effects of exposure to low frequency EMF on the cholinergic system and nitric oxide level in large intestine.

Materials and methods

Fourteen adult male Sprague-Dawley rats at an average weight of 180-200 g obtained from Razi Vaccine and Serum Research Institute, Shiraz, Iran. The rats were kept in the animal house under the standard condition at temperature (22-25 °C, 12-hour light/dark, photo schedule); fed pellets and water ad libitum. These animals were adapted to laboratory condition since 7-days before beginning of the study. Rats were randomly divided into two groups, sham and experimental groups. Experimental

group was exposed to 1mT, 50 Hz low frequency EMF, for 140 days 24 h/day in a solenoid. Sham group was housed under conditions similar to experimental group, without EMF. At the end of the exposure period, the animals were anaesthetized with ether and the distal colon was removed and placed in a Petri dish containing physiological solution consisting of NaCl 118, NaHCO3 25, MgSO4 1.2, KH2PO4 1.2, KCl 4.7, CaCl2 2.5, and glucose 11 in mM. The distal colon 1.5 cm in length were prepared and suspended in an organ bath containing 25 ml of physiological solution warmed at 37°C and bubbled with 95% O_2 : 5% CO_2.The strips were mounted vertically. One end of the segment was connected to an electrode placed at the bottom of the bath and the other end was mounted on a transducer that connected to an bridge amplifier and power lab A to D system that muscle activities were converted to electrical signals was visible by computer monitor. Before the start of the experiments, the resting tension was adjusted to 0.5 g and the tissue was allowed to equilibrate for 40 min during which time the solution was renewed every 10 min. Contractions of the longitudinal muscle were recorded in response to concentrations of acetylcholine 10^{-6},10^{-5} and 2×10^{-5} M and L-NAME 10^{-4} and 10^{-3} M. The experimental protocol was performed based on the Animal Care and Use Protocol, Shiraz University, Shiraz, Iran.

Electromagnetic fields inducing system

Continuous 50 Hz EMF were produced by magnetic coils. The solenoid was attached with 600 turns of 1 mm copper wire on a 70×120×30 cm wooden framework. The electrical source of solenoid was an autotransformer with the input of 50 Hz and 220 V. The system was calibrated by a digital electromagnetic field tester (EMF 827, Lutron). Cages with animals were placed symmetrically on both sides of the coils.

Drugs

Drugs used were acetylcholine, a muscarinic agonist, and L-N^G-nitroarginine methyl ester (L-NAME) (Sigma), a non-specific NOs inhibitor. All drugs were prepared as stock solutions (100 mM) in distilled water. Then dilutions were made for each drug and added in the bathing medium. Previous experiments have shown that distilled water has no effect on spontaneous contractions of the tissues.

Statistical analysis

Data were extracted of recorded graphs at intervals of 2 minutes (1,3,5,7,9 min) and 5 minutes (5,10,15,20,25,30,35,40) for acetylcholine and L-NAME, respectively. Data were analyzed by independent t-test, using the statistical package of SPSS version 16. In all tests $P<0.05$ was considered as statistically significant level. All data were presented as mean±standard deviation (SD).

Results

In the current study, the tension changes of distal colon tissue were recorded in response to acetylcholine for 10 minutes and doses were cumulatively added. The tension changes of distal colon tissue to acetylcholine was higher in the experimental group as compared to the sham group. There was significant difference in the experimental group than the sham group in some minutes (table 1, 2 and 3).

Table 1: Effect of acetylcholine 10^{-6} M on distal colon contraction (mean±SD) of the rat

Time (min)	Experimental group(n=7)	Sham group(n=7)	P value
1	6.02±0.77	6.24±1.02	0.87
3	4.56±0.49	3.55±2.22	0.36
5	2.83±0.44	1.99±0.19	0.12
7	2.53±0.23	2.26±0.89	0.56
9	2.61±0.47	1.92±0.39	0.35

Table 2: Effect of acetylcholine 10^{-5} M on distal colon contraction (mean±SD) of the rat

Time (min)	Experimental group (n=7)	Sham group (n=7)	P value
1	2.72±0.73	3.52±1.1	0.55
3	2.69±0.29	1.59±0.61	0.1
5	2.61±0.34	1.24±0.48	0.03*
7	1.93±0.25	0.94±0.42	0.05
9	2.31±0.48	1.25±0.91	0.15

*$P<0.05$ as statistically significant level

Table 3: Effect of acetylcholine 2×10^{-5} M on distal colon contraction (mean±SD) of the rat

Time (min)	Experimental group (n=7)	Sham group (n=7)	P value
1	2.46±0.84	2.08±1.01	0.72
3	2.26±0.31	1.52±1.45	0.28
5	2.39±0.36	0.94±0.62	0.57
7	2.13±0.42	1.56±0.82	0.51
9	1.92±0.21	0.81±0.5	0.04*

Two doses of L-NAME 10^{-4} and 10^{-3}M were added to the medium of organ bath with an interval of 40 minutes and cumulatively. The contraction of distal colon tissue in response to L-NAME 10^{-4} M was higher in the experimental group as compared to the sham group but no significant difference was detected between groups ($p>0.05$) (tables 4 and 5).

Table 4: Effect of L-NAME10^{-4} M on distal colon contraction (mean±SD) of the rat

Time (min)	Experimental group (n=7)	Sham group (n=7)	P value
1	0.634±0.308	0.581±0.229	0.679
5	0.690±0.153	0.534±0.551	0.364
10	0.528±0.395	0.598±0.525	0.554
15	0.446±0.165	0.490±0.246	0.927
20	0.443±0.138	0.598±0.492	0.718
25	0.430±0.149	0.531±0.397	0.816
30	0.441±0.137	0.524±0.375	0.860
35	0.640±0.208	0.407±0.262	0.610
40	0.691±0.210	0.420±0.221	0.540

Table 5: Effect of L-NAME 10^{-3} M on distal colon contraction (mean±SD) of the rat

Time (min)	Experimental group (n=7)	Sham group (n=7)	P value
1	0.770±0.170	0.314±0.143	0.008*
5	1.006±0.286	0.593±0.345	0.115
10	0.851±0.336	0.529±0.218	0.195
15	1.306±0.644	0.684±0.646	0.235
20	1.507±0.87	0400±0.232	0.081
25	1.359±0.805	0.539±0.08	0.139
30	1.424±0.50	0.475±0.325	0.028*
35	1.336±0.548	0.304±0.159	0.021*
40	0.728±0.151	0.438±0.239	0.077

Exposure of distal colon tissue with L-NAME10^{-3} M significantly increased tissue tension in the experimental group (p<0.05).

Discussion

In the present study, the effect of long-term exposure to low frequency electromagnetic field on cholinergic system and nitric oxide level of isolated distal colon of rats has been investigated. Our results showed that low frequency electromagnetic field increased response to acetylcholine in the experimental group. Increased response to acetylcholine in the experimental group as compared to the sham might be due to increased sensitivity or expression of acetylcholine receptors. This result is in similar to the result of study of Katiaand colleagues. They concluded that PEMF treatment evokes an up-regulation of the A2A adenosiner eceptors and alters the response of this receptor subtype in human neutrophils. Other cause of increased response to acetylcholine might be increased intracellular calcium concentration. Since muscarinic receptors act through intracellular calcium, therefore, increased intracellular calcium is another reason for increased contraction. Similarly, Casteelset *et al*. concluded that the smooth muscle cells of the guinea-pig taenia coli have an intracellular store of Ca^{2+} which participates in excitation-contraction coupling. The store is sensitive to muscarinic agonists, P-agonists and monovalent ions, but is not affected by depolarization of the outer membrane [15]. Also, Afnan *et al*. studied effects of short-term exposure to (1mT, 50Hz) electromagnetic fields on calcium concentration in different brain regions of Mice and resulted that ELF EMFs exposure increased Ca^{2+} concentrations in the hippocampus and the brainstem [19]. On the other hand, the decrease of acetylcholine esterase (AchE) activity can be another cause for this increased contraction. Hong wang *et al*. studied effect of very low frequency electromagnetic radiation on acetylcholine esterase activity and concluded that very low frequency electromagnetic field radiation decreased AchE activity when the intensity ranged from 120v/m to 140 v/m and exposure time ranged from 20 time to 120 time (17). In the current study, long-term exposure to low frequency EMF increased the amount of distal colon tissue tone in response to L-NAME in the experimental group. L-NAME is a non-specific NOs inhibitor. Nitric oxide is produced by a family of NO synthases (NOSs) which causes the relaxation of smooth muscle. Increased tension of distal colon tissue showed in this study might be a cause to decrease of NOS enzyme activity in the experimental group. This result is in agreement with the result of study of Antonia and colleagues. They observed that exposure to electromagnetic field decreases the activity of the nitric oxide synthase [16].

In conclusion, long-term exposure to low frequency electromagnetic field increased sensitivity of acetylcholine receptors and decreased producion of nitric oxide in distal colon.

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