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Ornithine decarboxylase activity is affected in primary astrocytes but not in secondary cell lines exposed to 872 MHz RF radiation

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Abstract

Purpose: The effects of radiofrequency (RF) radiation on cellular ornithine decarboxylase (ODC) activity were studied in fibroblasts, two neural cell lines and primary astrocytes. Several exposure times and exposure levels were used, and the fields were either unmodulated or modulated according to the characteristics of the Global System for Mobile (GSM) communications.

Materials and methods: Murine L929 fibroblasts, rat C6 glioblastoma cells, human SH-SY5Y neuroblastoma cells, and rat primary astrocytes were exposed to RF radiation at 872 MHz in a waveguide exposure chamber equipped with water cooling. Cells were exposed for 2, 8, or 24 hours to continuous wave (CW) RF radiation or to a GSM type signal pulse modulated at 217 Hz, at specific absorption rates of 1.5, 2.5, or 6.0 W/kg. Cellular ODC activities of cell samples were assayed.

Results: ODC activity in rat primary astrocytes was decreased statistically significantly (p values from 0.003 to <0.001) and consistently in all experiments performed at two exposure levels (1.5 and 6.0 W/kg) and using GSM modulated or CW radiation. In the secondary cell lines, ODC activity was generally not affected.

Conclusions: ODC activity was affected by RF radiation in rat primary neural cells, but the secondary cells used in this study showed essentially no response to similar RF radiation. In contrast to some previous studies, no differences between the modulated and continuous wave signals were detected. Further studies with primary astrocytes are warranted to confirm the present findings and to explore the mechanisms of the effects.

Keywords: *Mobile phones, cell biology, ODC*

Introduction

Alterations in cellular ornithine decarboxylase (ODC) activity are related to cellular growth and differentiation. Increase in ODC activity has been reported to occur for example in cancerous cells. Overexpression of ODC by itself is not sufficient to induce tumours in normal cells, but increase in cellular ODC activity enhances tumour development in initiated premalignant cells (Clifford et al. 1995). Reports of increased ODC activity in cell cultures exposed to radiofrequency (RF) radiation (Byus et al. 1988, Litovitz et al. 1993, 1997, Penafiel et al. 1997) have raised concern about possible health effects, as use of mobile telephones has rapidly expanded. However, compared to the strong ODC activation caused by known chemical tumor promoters, enhancement in ODC activity in RF radiation exposed cells has generally been quite low, from 20%

(Penafiel et al. 1997) to 90% (Litovitz et al. 1993), and not all studies have been able to show the effect at all (Desta et al. 2003). In several studies, the reported increase in ODC activity was associated with amplitude-modulated RF radiation, and no effects were seen with non-modulated (continuous wave [CW]) radiation at similar exposure levels (Litovitz et al. 1993, 1997, Penafiel et al. 1997). Such modulation-specific effects would be particularly relevant to mobile phone exposure, as amplitude-modulated signals are used in digital mobile phone systems. The World Health Organization (WHO) has recommended further studies to explore effects of RF radiation on cellular ODC activity (Repacholi 1998).

We have previously reported that exposure to low-level (SAR levels of 0.2 or 0.4 W/kg) RF radiation using CW signals or modulation similar to that used in the global system mobile (GSM) mobile phone

system (217 Hz) did not affect cellular ODC activity in L929 cells (Höytö et al. 2006). The present study examined effects of CW or GSM modulated RF radiation on cellular ODC activity at higher exposure levels (SAR levels of 1.5, 2.5, and 6.0 W/kg). Exposures were carried out using murine L929 fibroblasts, which have been used in several other studies, as well as rat C6 glioblastoma, human SH-SY5Y neuroblastoma cell lines, and rat primary astrocytes to study effects on neural cells. Neural cells are of special interest, since mobile phones are usually held in the proximity of the brain tissues and the effects of RF radiation on ODC activity in neural cells have not been studied previously. Moreover, primary cells have been used seldom to study effects of RF radiation, although they are functionally closer to intact tissues than transformed cell lines.

Materials and methods

Secondary cell lines

Three secondary cell lines and primary cultured cells were used for RF radiation exposures. L929 murine fibroblasts (obtained from ECACC, European Collection of Cell Cultures, Salisbury, UK) were cultured as described previously (Höytö et al. 2006) and C6 rat glioblastoma cells (obtained from Dr Nikolaus Plesnila, University of Munich, Germany) were cultured in Dulbecco's Modified Eagle Medium (DMEM) and SH-SY5Y human neuroblastoma cells (obtained from Dr Sven Pålman, University of Uppsala, Sweden) in DMEM with Glutamax I, 4500 mg/l D-glucose, both supplemented with 10% heat-inactivated fetal bovine serum, penicillin (50 U/ml), and streptomycin (50 µg/ml) (all obtained from Gibco, Invitrogen, Carlsbad, CA, USA). Cells were harvested by trypsinisation (trypsin, Gibco, Invitrogen, Carlsbad, CA, USA). Approximately 20 h prior to exposure, cells were seeded into four 55 mm diameter glass Petri dishes (Schott, Mainz, Germany). To provide sub-confluent cultures for exposures, L929 cells were seeded at a density 2×10^6 cells/dish for shorter (2 and 8 h) exposures and at a density 1×10^6 cells/dish for 24 h exposures in 5 ml of culture medium. Cell densities for C6 cells were for 2 and 8 h exposures 1.5×10^6 cells/dish and for 24 h exposures 0.75×10^6 cells/dish. For SH-SY5Y cells the density was 4×10^6 cells/dish for all of the exposures.

Primary cells

Primary rat astrocyte cultures were derived from the brains of 2-days-old rat. Briefly, Wistar neonatal rats were sacrificed by decapitation and brains were isolated. The cerebral cortices were excised from

the medulla and meninges and blood vessels were removed carefully. The refined cortical tissue was minced into small pieces with a scalpel. Cells were separated by dispase treatment with 150 mg dispase (Gibco, Invitrogen, Carlsbad, CA, USA) in 50 ml phosphate buffered saline (PBS) w/o Ca^{2+} , Mg^{2+} (Oy Reagentia Ltd, Kuopio, Finland). During the dispase treatment, the tissue was stirred (300 rpm, 30°C) in 5–10 ml of dispase for 5 min and cleared solution was filtered through 100 µm nylon net filter (Millipore, Carrigtwohill, Ireland) and 5 ml of DMEM with 580 mg/l L-glutamine, containing 20% FBS, 50 µg/ml streptomycin, and 50 U/ml penicillin (all obtained from Gibco, Invitrogen, Carlsbad, CA, USA) was added. Dispace treatment was repeated until all of the dispase had been consumed. Then the solution containing dissolved tissues were centrifuged at 160 g for 5 min. The tissue sediments were triturated with DMEM. The cells were cultured in 75 cm² tissue culture flasks (Nunc A/S, Roskilde, Denmark) in 15 ml of medium. Flasks for pure astrocyte cultures were shaken vigorously before each medium (20 ml of DMEM with 10% FBS) change, which was done first after 2 days and then after every 4 days altogether 2–3 times. The purified astrocytes were detached by trypsinization and used for exposures at passage numbers 5–10. For exposures, 1.25×10^6 cells/dish (2 and 8 h) and 1.0×10^6 cells/dish (24 h) were plated into glass dishes 20 h prior to the exposure. The study plan for the preparation of rat primary astrocyte cultures was approved by the Research Animal Use Committee of the University of Kuopio.

Exposure

At the beginning of the exposures, cells were evenly distributed in the dishes and two randomly selected dishes were placed in the exposure chamber and the other two in the sham-exposure chamber. To avoid excess activation of ODC, the medium was never changed prior to the exposure. Exposures were carried out in a waveguide exposure system described earlier together with dosimetric details (Höytö et al. 2006). The exposure system involves a water circulation system for controlling the temperature of the cell culture dishes. In spite of the temperature control, some of the experiments reported in this paper were carried out with a slight temperature difference between the exposure and sham-exposure chambers (maximally 0.8°C lower in the latter), due to an improperly functioning temperature sensor. This error was detected and corrected after the initial experiments (all experiments with L929 cells and 1.5–2.5 W/kg experiments with C6 and SH-SY5Y cells), and

temperatures of the chambers were equal in the rest of the experiments. The effects of the small temperature difference are commented in the results and discussion sections. L929, C6, and SH-SY5Y cells were exposed to 872 MHz RF radiation for 2, 8, and 24 h at SAR levels of 1.5, 2.5, and 6.0 W/kg with or without modulation (pulse frequency 217 Hz; GSM-type modulation). Primary rat astrocytes were exposed for equal times and exposure levels, except for SAR level of 2.5 W/kg, which was not used.

ODC activity assay

The medium was removed from the dishes after exposure and the cells were washed twice with ice-cold (4°C) Ca²⁺- and Mg²⁺-free PBS. The cells were detached by scraping in 1 ml of ice-cold PBS. The cell suspension was centrifuged (500 g, 5 min, 4°C) and the supernatant was discarded. Dry cell pellets were frozen in -70°C for ODC activity analysis to be performed later.

ODC activity of coded samples was assayed in a blinded manner according to the method described by Jänne and Williams-Ashman (1971) with some modifications. Briefly, frozen cell samples were lysed in buffer containing 25 mM Tris pH 7.4 (Trizma Base, Sigma-Aldrich Chemie GmbH, Steinheim, Germany), 0.1 mM ethylenedinitrilotetraacetic acid, disodium salt dihydrate (EDTA, Titriplex III, Merck KGaA, Darmstadt, Germany), 0.1% Triton X-100 (Sigma-Aldrich Chemie GmbH, Steinheim, Germany), and 1 mM DL-dithiothreitol (DTT, Sigma-Aldrich Chemie GmbH, Steinheim, Germany) and vortexed. Homogenate was centrifuged (16,060 g, 20 min, 4°C) and an aliquot of supernatant was incubated using a shaking water bath (Memmert WB 22 & SV 1422, Schwabach, Germany) for 1 h at 37°C in reaction mixture containing 100 mM Tris pH 7.4, 4 mM EDTA, 4 mM DTT, 0.2 mM L-ornithinmonohydrochlorid (Merck KGaA, Darmstadt, Germany), 0.2 µCi/assay ¹⁴C-L-ornithine (specific activity 52.0 mCi/mmol, Amersham Biosciences, Buckinghamshire, UK), and 0.4 mM pyridoxal-5-phosphate (PLP, Sigma-Aldrich Chemie GmbH, Steinheim, Germany). ¹⁴CO₂ was liberated by addition of 2 M citric acid (Riedel-de Haën, Sigma-Aldrich, Seelze, Germany), and incubation was continued for 15 min. Liberated ¹⁴CO₂ was trapped in pleated filter paper (Chromatography paper 3MM Chr, Whatman International Ltd, Maidstone, UK) wetted with Solvable (Packard Instruments, Groningen, The Netherlands). Filter papers were transferred to scintillation vials (Perkin Elmer Life Sciences, Wallac Oy, Turku, Finland) containing 3 ml OptiPhase HiSafe 3 scintillant (PerkinElmer Life Sciences, Fisher Chemicals,

Loughborough, UK) and the activity was measured using a liquid scintillation counter (Wallac WinSpectral 1414, PerkinElmer Life Sciences, Wallac Oy, Turku, Finland). Protein content of the samples was measured using Bio-Rad protein assay kit (Bio-Rad Laboratories, Hercules, CA, USA).

Units of ODC activity were expressed as pmol ¹⁴CO₂ generated/h/mg protein at 37°C ± SEM ($n=5-12$). Due to day-to-day variation of ODC activity in control cultures results are expressed also as ODC activity ratio ± SEM ($n=3-6$), which is calculated by dividing the mean ODC activity of the two simultaneously exposed cultures by the mean of the two non-exposed control cultures.

Positive controls

Positive control experiments were carried out to prove the responsiveness of the cell lines used in the studies. Cells were plated in two dishes containing medium with serum and in two dishes containing medium without serum and incubated for 20 h. Fresh medium (with serum) was changed to one dish per group and all dishes were further incubated for 4 h. Cellular ODC activity of frozen cell samples was assayed.

Sham-sham experiments

Sham-sham experiments were carried out to check possible differences between the exposure chambers. In sham-sham experiments no RF radiation was applied and cells were placed in the chambers for 2, 8, and 24 h at equal cell densities as in real exposure experiments. This procedure was repeated with C6 and SH-SY5Y cells after calibration of the temperature sensors of the water circulation system, as some exposures were carried out after the calibration.

Statistics

Statistical analyses were carried out using SPSS for Windows release 11.5.1 (SPSS Inc., Chicago, Illinois, USA). ODC activity values were used for linear mixed model analysis to obtain an overall p value for all exposure times of a given exposure level. If the overall p value was significant ($p \leq 0.05$), additional analyses were performed as post-tests to identify significant p values at single exposure times. Treatment and exposure time (in the overall analysis) were used as a fixed factors and replicate as a random factor in the analysis. A replicate consisted of two dishes exposed and two dishes sham-exposed simultaneously. Random factor was included in the model since the ODC activities of the control cultures varied from day to day.

Results

Positive controls

Positive control experiments showed that all cell lines used in the present study were responsive to medium change and serum deprivation treatments. Compared to controls, ODC activity after changing of fresh medium was increased by 2.7% in C6 cells, by 54.2% in SH-SY5Y cells, and by 37.4% in rat primary astrocytes. Serum deprivation decreased ODC activity by 87.0% in C6 cells, by 87.6% in SH-SY5Y cells, and by 97.2% in rat primary astrocytes, when compared to corresponding controls. Changing of fresh medium to serum deprived cultures enhanced the ODC activity very strongly in all cells, by 213.7% in rat C6 glioblastoma cells, by 708.9% in rat primary astrocytes, and by 977.2% in human SH-SY5Y neuroblastoma cells. The results of positive control experiments for L929 cells were published earlier (Höytö et al. 2006).

Sham-sham experiments

A total of six sham-sham comparisons with no RF radiation applied were performed. In one such experiment with L929 cells, a statistically significant difference between the two exposure chambers was observed after 2 h of exposure (Table I). This difference is most likely related to the small temperature difference between the exposure chambers (see Materials and methods). No statistically significant differences were observed in the other sham-sham experiments (Tables II–IV).

Secondary cell lines

The results presented in Tables I–III show that cellular ODC activity in secondary cell lines was generally not affected by RF radiation at low SAR levels. The only significant difference below 6 W/kg was observed in murine L929 fibroblasts exposed to the GSM signal at 1.5 W/kg (Table I).

Table I. Cellular ODC activity in murine L929 fibroblasts exposed to continuous wave (CW) or GSM-modulated RF radiation at various exposure levels up to 6 W/kg.

Exposure time (h)	n C/E	Control (mean $\pm$ SEM)	Exposed (mean $\pm$ SEM)	P_1	P_2	ODC activity ratio
<i>Sham-sham</i>						
2	8/8	3686.7 $\pm$ 169.0	2685.2 $\pm$ 285.5	0.002	< 0.001	0.72 $\pm$ 0.06
8	6/6	1983.8 $\pm$ 480.0	1996.7 $\pm$ 472.5		0.960	1.00 $\pm$ 0.05
24	6/6	1480.1 $\pm$ 373.9	1224.9 $\pm$ 359.5		0.227	0.82 $\pm$ 0.10
1.5 W/kg GSM						
2	6/6	5564.2 $\pm$ 541.6	4257.8 $\pm$ 351.7	0.003	0.052	0.78 $\pm$ 0.07
8	6/6	2521.6 $\pm$ 160.5	2436.8 $\pm$ 281.4		0.549	0.95 $\pm$ 0.11
24	6/6	2217.4 $\pm$ 403.9	1507.6 $\pm$ 284.8		0.020	0.68 $\pm$ 0.05
1.5 W/kg CW						
2	6/6	3710.8 $\pm$ 235.1	3510.3 $\pm$ 252.5	0.405	—	0.95 $\pm$ 0.05
8	6/6	2086.3 $\pm$ 435.7	2168.1 $\pm$ 488.3		—	1.00 $\pm$ 0.15
24	6/6	1417.0 $\pm$ 230.8	1268.7 $\pm$ 219.3		—	0.89 $\pm$ 0.10
2.5 W/kg GSM						
2	5/6	3447.2 $\pm$ 130.9	3361.3 $\pm$ 425.0	0.814	—	0.98 $\pm$ 0.13
8	6/6	2772.7 $\pm$ 691.8	2840.1 $\pm$ 823.6		—	0.99 $\pm$ 0.05
24	6/6	1954.1 $\pm$ 156.3	2076.3 $\pm$ 349.8		—	1.09 $\pm$ 0.33
2.5 W/kg CW						
2	5/6	3514.4 $\pm$ 298.7	3227.1 $\pm$ 310.3	0.875	—	0.90 $\pm$ 0.09
8	6/5	2420.9 $\pm$ 291.5	2815.3 $\pm$ 233.9		—	1.21 $\pm$ 0.10
24	6/6	1193.7 $\pm$ 266.3	976.3 $\pm$ 105.9		—	0.89 $\pm$ 0.19
6.0 W/kg GSM						
2	6/6	4435.4 $\pm$ 311.3	3653.8 $\pm$ 387.8	0.934	—	0.83 $\pm$ 0.15
8	6/6	1736.0 $\pm$ 106.7	1916.3 $\pm$ 157.7		—	1.10 $\pm$ 0.01
24	6/6	795.8 $\pm$ 245.1	1344.6 $\pm$ 576.6		—	1.46 $\pm$ 0.34
6.0 W/kg CW						
2	6/6	5399.5 $\pm$ 251.0	4046.9 $\pm$ 225.1	0.002	0.004	0.78 $\pm$ 0.02
8	6/6	2202.5 $\pm$ 323.1	1924.7 $\pm$ 303.1		0.398	0.92 $\pm$ 0.13
24	6/6	747.4 $\pm$ 137.3	702.6 $\pm$ 103.5		0.724	1.01 $\pm$ 0.14

n (C/E) = number of control (C) and exposed (E) samples. The ODC activities are expressed as pmol $^{14}\text{CO}_2$ generated/60 min/mg protein at 37°C. P_1 = Overall p value. P_2 = p value for a single exposure time, calculated as a post-test only if the overall p value was significant. Significant p values (≤ 0.05) are indicated with bold font. ODC activity ratio (mean $\pm$ SEM) was calculated by dividing the mean ODC activity of the two simultaneously exposed cultures by the mean of the two non-exposed control cultures. Sham-sham = experiments in which both the 'exposed' and 'control' cultures were sham-exposed, i.e., kept in non-energized exposure chambers.

This significant overall p value (0.003) resulted mainly from the 32% reduction of ODC activity at the 24 h time point ($p = 0.020$). Like the difference seen in the sham-sham experiment with L929, this difference is most likely related to the small temperature difference between the exposure chambers. Also exposure to the CW signal at 6.0 W/kg resulted in a significant difference ($p = 0.002$) in L929 cells, mainly related to a 22% decrease of ODC activity after 2 h of exposure. In rat C6 glioblastoma cells, ODC activity was not altered statistically significantly at any time point after any kind of exposure, although there was a tendency towards decreased ODC activities at 6 W/kg (Table II). In the human SH-SY5Y neuroblastoma cell line, exposure to CW RF radiation at 6.0 W/kg resulted in a statistically significant p value of 0.002 (Table III), associated with a 15% decrease of ODC activity after 2 h of exposure.

Primary cells

In experiments with rat primary astrocytes, statistically significant differences were found at all

exposure levels and signals, with overall p values from <0.001 – 0.003 (Table IV). ODC activities were generally decreased (16 to 59%, statistically significant in 5 out of 8 cases) after 2 or 8 h of exposure, but the 24 h ODC activity was decreased (41%, $p = 0.042$) only in cells exposed to the GSM signal at 6 W/kg.

Discussion

Exposure to radiofrequency radiation similar to that emitted by mobile phones has been reported to enhance cellular ODC activity in cell cultures. In murine L929 fibroblasts, ODC activity increased by 40% after exposure to 840 MHz TDMA modulated RF radiation at 2.5 W/kg for 8 h (Penafiel et al. 1997). Byus and his co-workers (1988) exposed Reuber H35 hepatoma, Chinese hamster ovary, and human 294 T melanoma cells to 450 MHz RF fields sinusoidally amplitude modulated at 16 Hz, at a power density of 1.0 mW/cm^2 (peak envelope power) for 1 h and observed a 50% increase in ODC activity. However, in an attempt to replicate

Table II. Cellular ODC activity in rat C6 glioblastoma cells exposed to continuous wave (CW) or GSM-modulated RF radiation at various exposure levels up to 6 W/kg.

Exposure time (h)	<i>n</i> C/E	Control (mean ± SEM)	Exposed (mean ± SEM)	<i>P</i> ₁	ODC activity ratio
<i>Sham-sham</i>					
2	6/6	2467.5 ± 462.1	2413.0 ± 398.8	0.155	0.96 ± 0.07
8	6/6	1252.7 ± 101.1	728.3 ± 58.2		0.59 ± 0.08
24	6/6	1122.5 ± 402.5	1083.2 ± 343.0		0.98 ± 0.19
1.5 W/kg GSM					
2	10/10	5527.4 ± 688.0	4875.4 ± 568.7	0.075	0.90 ± 0.08
8	6/6	3239.3 ± 981.6	2863.1 ± 626.5		1.00 ± 0.17
24	6/6	1565.2 ± 260.8	1467.9 ± 257.6		1.00 ± 0.17
1.5 W/kg CW					
2	6/6	4759.6 ± 964.9	4114.4 ± 993.6	0.452	0.85 ± 0.10
8	8/8	4111.9 ± 271.2	4259.7 ± 474.5		1.02 ± 0.13
24	8/8	1420.4 ± 305.8	1540.8 ± 333.2		1.03 ± 0.06
2.5 W/kg GSM					
2	5/6	3750.0 ± 1244.7	3143.4 ± 1124.5	0.591	1.08 ± 0.13
8	6/6	2341.9 ± 495.8	2286.2 ± 481.0		0.98 ± 0.01
24	6/6	1102.8 ± 517.7	1075.8 ± 427.6		1.36 ± 0.30
2.5 W/kg CW					
2	6/6	6081.1 ± 611.0	5999.7 ± 574.9	0.599	0.99 ± 0.06
8	6/6	3811.7 ± 683.4	4180.7 ± 884.6		1.05 ± 0.20
24	6/6	1872.0 ± 656.7	1973.3 ± 565.8		1.33 ± 0.25
<i>Sham-sham</i>					
2	8/8	2928.8 ± 668.8	3279.1 ± 765.1	0.924	1.09 ± 0.08
8	6/6	2105.7 ± 586.3	1855.7 ± 533.5		0.90 ± 0.07
24	8/10	502.6 ± 194.8	523.2 ± 144.5		1.24 ± 0.29
6.0 W/kg GSM					
2	8/8	3019.6 ± 485.7	2634.4 ± 396.8	0.073	0.88 ± 0.06
8	8/8	1430.6 ± 276.8	1348.0 ± 459.1		0.84 ± 0.17
24	11/12	861.4 ± 230.1	570.4 ± 115.7		0.81 ± 0.25
6.0 W/kg CW					
2	6/6	3307.7 ± 408.8	2952.8 ± 436.6	0.096	0.88 ± 0.05
8	6/6	5106.6 ± 488.2	5055.4 ± 657.6		0.99 ± 0.02
24	10/10	1434.1 ± 450.0	928.6 ± 374.9		0.59 ± 0.10

Explanations as in Table I.

Table III. Cellular ODC activity in human SH-SY5Y neuroblastoma cells exposed to continuous wave (CW) or GSM-modulated RF radiation at various exposure levels up to 6 W/kg.

Exposure time (h)	<i>n</i> C/E	Control (mean ± SEM)	Exposed (mean ± SEM)	<i>P</i> ₁	<i>P</i> ₂	ODC activity ratio
<i>Sham-sham</i>						
2	5/6	1648.9 ± 255.1	1917.0 ± 247.8	0.951	—	1.07 ± 0.04
8	6/6	311.8 ± 44.8	206.7 ± 38.2		—	0.64 ± 0.07
24	6/6	204.0 ± 29.1	189.3 ± 56.4		—	0.86 ± 0.19
1.5 W/kg GSM						
2	6/6	1519.6 ± 237.4	1740.4 ± 327.4	0.165	—	1.11 ± 0.08
8	6/6	994.7 ± 180.0	1063.8 ± 132.0		—	1.14 ± 0.12
24	5/6	109.7 ± 35.6	126.5 ± 30.1		—	1.09 ± 0.15
1.5 W/kg CW						
2	5/6	1953.5 ± 275.5	2259.5 ± 288.4	0.137	—	1.22 ± 0.23
8	6/6	534.6 ± 94.5	556.5 ± 79.0		—	1.08 ± 0.09
24	6/6	328.3 ± 25.9	344.1 ± 25.8		—	1.06 ± 0.09
2.5 W/kg GSM						
2	6/6	1669.9 ± 184.2	1850.2 ± 111.2	0.410	—	1.12 ± 0.03
8	6/6	1173.0 ± 303.1	1134.8 ± 309.8		—	0.96 ± 0.08
24	7/7	147.2 ± 11.1	177.6 ± 36.1		—	1.40 ± 0.23
2.5 W/kg CW						
2	6/6	3989.0 ± 1141.5	3449.5 ± 1101.6	0.055	—	0.82 ± 0.06
8	5/6	688.8 ± 138.3	695.8 ± 191.7		—	1.06 ± 0.13
24	5/6	148.2 ± 10.1	109.9 ± 4.7		—	0.65 ± 0.14
<i>Sham-sham</i>						
2	6/6	2021.4 ± 372.6	1959.4 ± 436.3	0.797	—	0.93 ± 0.07
8	6/6	521.9 ± 75.2	543.5 ± 56.0		—	1.07 ± 0.07
24	6/6	113.8 ± 6.2	106.0 ± 17.4		—	0.95 ± 0.22
6.0 W/kg GSM						
2	5/6	1074.2 ± 87.5	1171.6 ± 75.7	0.210	—	1.06 ± 0.09
8	6/6	777.9 ± 75.0	828.0 ± 71.2		—	1.07 ± 0.03
24	8/7	97.8 ± 14.6	81.0 ± 15.8		—	1.18 ± 0.32
6.0 W/kg CW						
2	6/6	1746.2 ± 164.2	1494.3 ± 173.4	0.002	0.012	0.85 ± 0.04
8	6/6	325.6 ± 67.0	262.4 ± 58.5		0.264	0.82 ± 0.03
24	8/6	107.1 ± 16.6	96.3 ± 24.7		0.815	1.45 ± 0.42

Explanations as in Table I.

Table IV. Cellular ODC activity in rat primary astrocytes exposed to continuous wave (CW) or GSM-modulated RF radiation at various exposure levels up to 6 W/kg.

Exposure time (h)	<i>n</i> C/E	Control (mean ± SEM)	Exposed (mean ± SEM)	<i>P</i> ₁	<i>P</i> ₂	ODC activity ratio
<i>Sham-sham</i>						
2	6/6	1604.2 ± 441.3	1247.6 ± 370.3	0.787	—	0.84 ± 0.07
8	6/6	1704.0 ± 410.2	1746.1 ± 809.7		—	1.07 ± 0.44
24	6/6	132.2 ± 28.7	172.3 ± 59.6		—	1.15 ± 0.28
1.5 W/kg GSM						
2	6/6	2764.3 ± 453.3	2088.3 ± 482.9	<0.001	0.008	0.72 ± 0.07
8	6/6	1486.8 ± 202.8	685.1 ± 109.4		0.005	0.47 ± 0.06
24	6/6	49.0 ± 11.4	72.5 ± 35.2		0.440	1.33 ± 0.41
1.5 W/kg CW						
2	6/6	2253.3 ± 274.8	1815.9 ± 189.1	0.003	0.219	0.84 ± 0.14
8	6/6	1459.1 ± 163.3	599.9 ± 88.1		0.001	0.41 ± 0.00
24	6/6	155.0 ± 50.5	172.6 ± 105.3		0.849	0.96 ± 0.24
6.0 W/kg GSM						
2	6/6	1519.3 ± 371.2	1154.3 ± 401.3	0.002	0.158	0.65 ± 0.19
8	6/6	1175.1 ± 204.1	510.6 ± 126.5		0.017	0.50 ± 0.14
24	6/6	193.0 ± 79.2	70.5 ± 22.8		0.042	0.59 ± 0.24
6.0 W/kg CW						
2	6/6	1447.8 ± 150.4	766.2 ± 138.8	0.001	0.007	0.56 ± 0.18
8	6/6	174.4 ± 40.6	123.2 ± 33.5		0.170	0.74 ± 0.16
24	6/6	44.6 ± 16.4	26.4 ± 4.4		0.230	0.91 ± 0.32

Explanations as in Table I.

the study of Penafiel et al. (1997), Desta et al. (2003) failed to show any increase in ODC activity in L929 cells after 8 h of exposure to 835 MHz RF radiation with modulation typical to TDMA mobile phones at SAR levels from <1 W/kg to 15 W/kg. In our previous study, we also were unable to show enhancement of ODC activity in L929 cells exposed for 2–24 h to 872 MHz continuous or GSM modulated RF radiation at SAR levels of 0.2–0.4 W/kg (Höytö et al. 2006).

The results of the present study, consistently with our previous findings and those of Desta et al. (2003), did not show enhancement of ODC activity. Instead, a decrease in ODC activity was observed in some cases. Especially primary astrocytes were sensitive to RF radiation and ODC activity was suppressed as much as 59% in those cells. The results from experiments with primary astrocytes are clearly different from those with secondary cells, suggesting a cell type-dependent difference in sensitivity. Sensitivity of primary astrocytes to RF radiation is an interesting finding, as primary cells are not transformed and their function is closer to intact animal tissues than that of secondary cell lines. ODC activity of primary cells after RF radiation exposure has not been previously studied. Studies with whole animals have reported increased ODC activity in brain tissue after long-term (35 d) exposure to 16-Hz modulated 112 MHz fields at 0.75 W/kg (Paulraj et al. 1999) and to 2.45 GHz continuous-wave fields at 0.1 W/kg (Paulraj & Behari 2002), but no effects after short (2 h) exposure to 1.6 GHz pulse modulated RF field (Stagg et al. 2001).

Secondary cell lines were in general not affected by the RF exposure used in the present study. The results from the experiments with L929 cells were probably affected by the small initial temperature difference between the chambers (see Materials and methods), which is likely to explain the reduced ODC activity seen in the sham-sham experiment with this cell line. Our previous results have clearly shown that even small increase in temperature can result in decreased ODC activity (Höytö et al. 2006). Results from the other experiments with L929 cells do not show any differences clearly larger than those seen in the sham-sham experiment, so the two statistically significant p-values are also most likely explained by the small temperature difference. Apart from the differences seen in L929 cells, the only statistically significant difference in secondary cell lines was observed in SH-SY5Y cells exposed to the CW signal at 6 W/kg. This might be interpreted as an indication of an effect at high exposure level, but it might also be a chance finding, as many statistical comparisons were performed. The latter alternative is supported by the fact that no effect was observed at the same exposure level with the GSM signal.

All results from this study (reduced ODC activity in primary astrocytes and essentially no effects in secondary cell lines) were similar in experiments with the GSM and CW signals. Hence, the present study did not support the findings of the previous studies in which only modulated signal affected ODC activity.

The increased ODC activity reported in some earlier studies has been considered as an indication of potential harmful health effects. However, also decreased ODC activity (such as that seen in primary astrocytes in the present study) could lead to adverse effects on cells. Decrease in cellular ODC activity leads to impairment in polyamine synthesis and results in decreased proliferation. Inhibition of ODC may activate or prevent apoptosis, programmed cell death, depending on the cell type (Seiler & Raul 2005). It has also been suggested that polyamines act as free radical scavengers and therefore play a role in protecting cells from oxidative stress (Ha et al. 1998). Therefore, a decrease in ODC activity could impair cellular capability to protect DNA from free radical attack.

Conclusions

Decrease in cellular ODC activity was detected in rat primary astrocytes after exposure to 872 MHz RF radiation at SAR levels of 1.5 and 6 W/kg. Secondary cell lines, in contrast, showed very little evidence of any effects. This is a very interesting finding, since function of the primary cells is closer to normal tissues than that of secondary transformed cell lines. There are no previous studies on ODC activity in primary cells exposed to RF radiation. Further studies with primary astrocytes are warranted to confirm the present findings and to explore the mechanisms of the effects and their possible relevance to human health.

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