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Effect of non-thermal radio frequency burst-type electromagnetic field irradiation on the microcirculation and vascular contractility.

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Abstract We investigated the effects of non-thermal radio frequency burst-type EMF irradiation on blood circulation of the rat liver in vivo. In order to investigate the cellular mechanisms, we also examined the direct effect of EMF irradiation on the contractility of organ-supplying artery and conduit artery and on endothelial function. EMF irradiation was achieved with an EMF generator coil used with 10-15 V peak-to-peak voltage. Changes in blood flow and blood mass in the rat liver were monitored with a laser Doppler flowmeter. Vascular contractility was measured in artery strips with a force transducer. Cytosolic Ca^{2+} concentration ($[Ca^{2+}]i$) in endothelial cells was monitored in situ on the bovine aortic valve strips by front-surface fluorometry of fura-2. EMF irradiation induced slight increase of hepatic blood flow without affecting blood mass. However, EMF irradiation had no direct effect on the vascular contractility and on the $[Ca^{2+}]i$ levels in endothelial cells in situ. Our results indicate that EMF irradiation may play a minor role in the regulation of microcirculation.

Introduction

The radio frequency electromagnetic field (EMF) has been shown to influence the regulation of physiological systems and effectiveness of medical therapies. Negative or positive effects have been reported, for example, on embryonic development, healing of bone fractures, corneal epithelial and other types of wounds, prevention of osteoporosis and DNA-damage repair mechanisms.[1-7] It is generally accepted that the most important mechanism involved the therapeutic effects of EMF may be the increase in blood flow mediated by vasodilatation, which may be induced by the elevation in tissue temperature associated with EMF irradiation. Non-thermal radio frequency burst-type EMF has also been shown to have biological effects. Laboratory and clinical applications of this type of EMF have shown its potential for accelerating hematoma reabsorption, promoting wound healing, reducing the severity of arthritis, and dilating the microvasculature.[8-11] However, there has been little basic research concerning the effects of non-thermal radio frequency burst-type EMF on internal organs and the blood vessels themselves.

Miura et al. demonstrated recently that

EMF radiation increased the formation of both nitric oxide (NO) and cyclic guanosine monophosphate (cGMP) in rat cerebellum supernatant.[12] NO induced by endothelial cells plays an important role in the regulation of vascular tone. In addition, vascular endothelial cells have numerous physiological functions, including prevention of coagulation, control of vascular permeability, maintenance of vascular tone and regulation of leukocyte extravasation.[13] Considering these findings together we hypothesized that non-thermal radio frequency burst-type EMF increases blood flow via vasodilatation mediated by the endothelium.

Thus, we carried out the present animal study to clarify in vivo whether hepatic blood flow and hepatic blood mass increase under non-thermal radio frequency burst-type EMF irradiation. We also examined in vitro whether vasodilatation occurs via direct action of non-thermal radio frequency burst-type EMF irradiation of the blood vessels. In addition, because elevation of cytosolic Ca^{2+} concentration ($[Ca^{2+}]i$) is essential for the activation of endothelial NO synthase (eNOS) and thus production of NO in endothelial cells, we investigated the effects of such irradiation on $[Ca^{2+}]i$ levels in endothelial cells in situ.

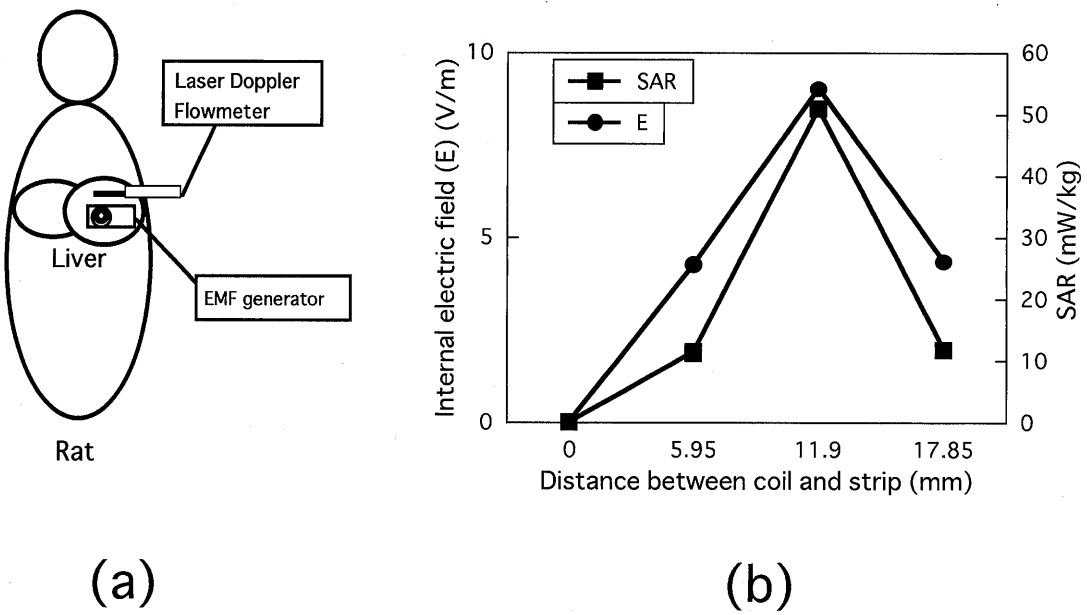
Materials and methods

Measurements of the hepatic blood flow and mass of the rats

Seven Male Wister rats weighing 250~300 g purchased from Chiyoda (Tokyo, Japan) were used to measure hepatic blood flow and blood mass during EMF irradiation. EMF irradiation was then delivered to the liver for 30 min. Figure 1(a) shows the position of the radio frequency burst-type EMF generator in relation to the rat liver. Hepatic blood flow and blood mass were measured without contact with a laser Doppler flowmeter (ALF21N, Advance Co., Ltd., Tokyo, Japan). Hepatic blood flow was taken as the hepatic blood mass multiplied by the blood velocity (blood flow = blood mass x blood velocity).

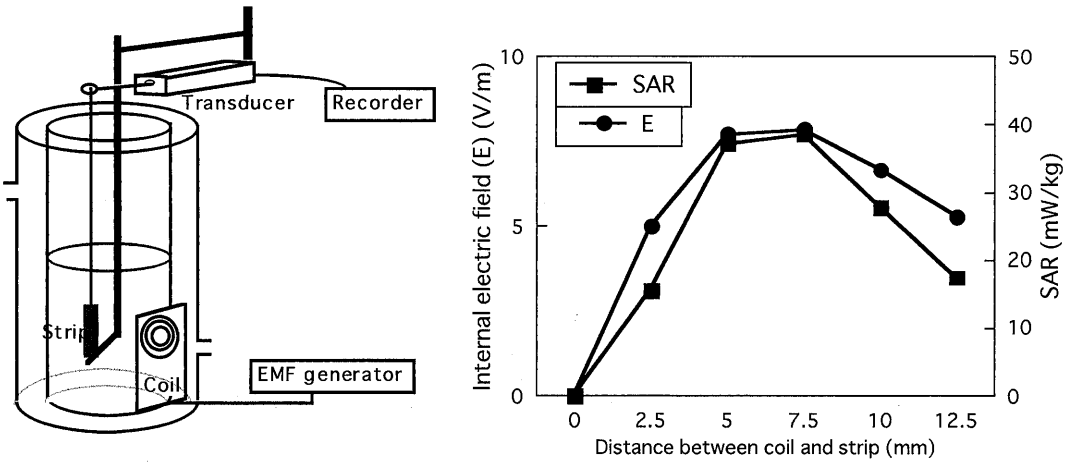
Measurements of tension development of vascular strips

Small strips (approximately 1 mm wide, 5



(a) (b)

Fig. 1 (a), Relation between the rat liver and the radio frequency burst-type EMF generator. The coil is located 2 mm over the rat liver. The laser Doppler flowmeter is located just outside the coil, with a distance of 11.9 mm between the coil and the laser Doppler flowmeter.
(b), Relation between the coil distance and the internal electric field (E) or specific absorption rate (SAR) of the rat liver.



(a) (b)

Fig. 2 (a), Diagram of the experimental instruments for tension measurements. The vascular strip mounted vertically in a bath is connected to a force transducer. The coil of the EMF generator is located on the surface of the chamber. (b), Relation between the coil distance and E or SAR of the strip.

mm long, and 0.1 mm thick) of pig coronary artery were obtained as previously described.[14] The adventitia was removed in some arteries. The inner surface of some strips was rubbed with a cotton swab to remove the endothelium. Thus, four types of strips (n = 8 each) were obtained: these with and without endothelium and those with and without adventitia.

Small strips (approximately 1 mm wide, 5 mm long, and 0.1 mm thick) of rat aorta were obtained as previously described.[15] Endothelial cells or adventitia or both were removed in some strips.

Pig coronary artery strips or rat aorta strips mounted vertically in a bath were connected to a force transducer (TB-612T, Nihon Koden, Japan), and isometric tension was measured at 37℃, as previously described.[16] The developed tension was expressed as a percentage, assuming the values obtained with normal PSS (5.9 mM K+) and in 118 mM K+ PSS to be 0% and 100%, respectively. Direct effects of the EMF on vascular tension was evaluated by application of the EMF (for 30 min) at rest (in normal PSS) and during the sustained and steady contraction induced by 100 nM U46619 (thromboxane analogue).

In situ measurement of [Ca^{2+}]i in endothelial cells

Bovine aortic valves leaflets (approximately 5 mm wide, 10 mm long, and 0.1 mm thick) were used to monitor [Ca^{2+}]i in the endothelial cells in situ at 25℃, as previously described.[16,17] The valve leaflets were cut into strips with care being taken not to touch the strip surface, and the strips were endothelium.

Fura-2 loading and measurements in endothelial cells in situ were carried out as previously described.[16-18]

A diagram of the instrumentation is shown in Fig. 3. The fluorescence intensities at alternating 340 nm (F340) and 380 nm (F380) excitation and the ratio (R = F 340/F380) were monitored at 510 nm emission, with a spectrofluorometer (Hitachi F 2000) that operates on our own program.[18] Double staining of fura-2 and fluorescent acetylated low-density lipoprotein revealed that fura-2 signals arose exclusively from monolayered endothelial cells on the surface of the aortic side of the aortic valves.[16,18-20] The fluorescence ratio values were normalized by assigning values of 0 % and 100 % to normal PSS and at the peak response to 10 mM ATP, respectively. Effects of

the radio frequency burst-type EMF was examined in the presence and absence of 10 μM ATP.

EMF irradiation

Hepatic blood flow and blood mass

The EMF was generated with a circular loop antenna (EW587, Matsushita Electric Works, Ltd., Osaka, Japan) carrying an alternating current and located 2 mm over the rat liver.

Figure 1(b) shows the relation between the coil and the internal electric field (E) and specific absorption rate (SAR) of the rat liver. E and SAR were measured with a the laser Doppler flowmeter. When the laser Doppler flowmeter was positioned at the center of the coil, parallel distance was 0 mm. When the laser Doppler flowmeter was gradually moved outside the coil parallel to the surface of the rat liver, E and SAR of the strip increased gradually and peaked at a distance of 11.9 mm, a point just outside the generator coil. To determine the dose rate (i.e., E and the corresponding SAR) of the electromagnetic field in biological tissue located close to the circular loop antenna, we calculated the dose rate according to the Maxwell equations in each region which was divided into three parts, as described previously[21] : AREA-1, a half free space above the antenna opposite the biological medium; AREA-2, the space between the antenna and the biological medium; and AREA-3, homogeneous biological medium.

$$\hat{E}_{\varphi} = -j\omega a\mu_m \hat{I}\int_0^{\infty} \frac{e^{-(\gamma h_1 + \kappa z)}}{\mu_\gamma \gamma + \chi} J_1(\lambda_a) J_1(\lambda_r)\lambda d\lambda$$

where *h1* is the distance between the antenna and the biological medium, *a* is the diameter of the antenna, *J1(χ)* is the first-order Bessel Function, μ is a permeability and *I* is the real and effective value of the loop current.

Accordingly, the SAR was given by

$$SAR = \frac{\sigma_m \left|\hat{E}_{\varphi}\right|^2}{\rho_m}$$

where ρm is the mass density of the biological specimen and σm is the mass conductivity of the biological specimen.[21] When the voltage of the generator coil was adjusted to 10 V peak-to-peak, the maximum EMF intensity was 9.024 V/m,

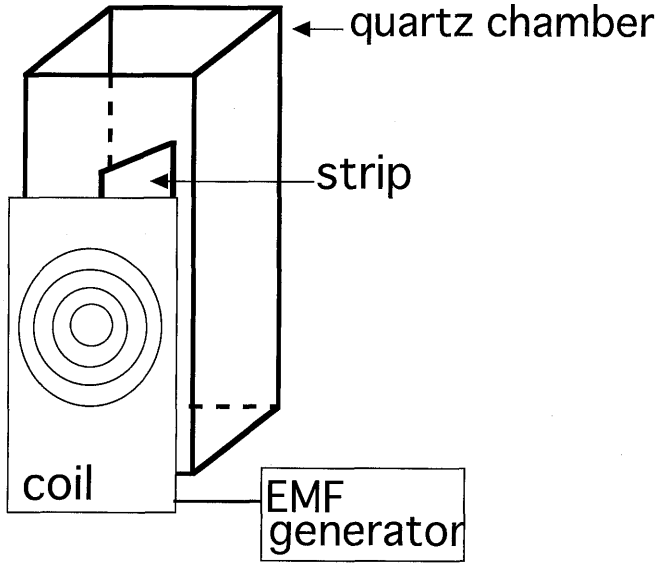


Fig. 3 Diagram of the experimental instruments for in situ measurement of the cytosolic Ca^{2+} concentration of endothelial cells in situ on the surface of bovine aortic valve. The circular loop antenna is located just outside the quartz chamber. The strip is located in the center of the quartz chamber.

and the maximum SAR was 50.900 mW/kg on the liver surface just outside the generator coil 21). The frequency of the basic sine waves during the burst was fixed at 10 MHz. The burst rate, i.e.,the frequency at which bursts were applied, was fixed at 10 kHz. The burst time, i.e.,the percentage of cycle time occupied by the burst, was fixed at 50%.

Tension development

A diagram of the instrumentation is shown in Fig. 2(a). To avoid interference to the EMF, no metals were used in the instrument within 5 cm of the strip. Figure 2(b) shows the relation of E or SAR to the distance between the coil and the strip. The EMF intensity was dependent on the distance between the coil and the strip; the peak value was at a distance of 7.5 mm. When the EMF voltage was 15.0 V peak-to-peak, E was 7.852 V/m, and SAR was 38.535 mW/kg at the distance of 7.5 mm.[21] Thus, the circular loop antenna was located at 7.5 mm to obtain the maximum EMF intensity, as shown in Fig. 2-(b).

The cytosolic Ca^{2+}-concentrations

The EMF generator, the circular loop antenna and the EMF irradiation conditions were as described for the measurement of tension development. The circular loop antenna was located just outside on the quartz chamber of the fluorometer. When the EMF voltage was 15.0 V peak-to-peak, the EMF intensity was 11.824 V/m, and SAR was 87.379 mW/kg.[21]

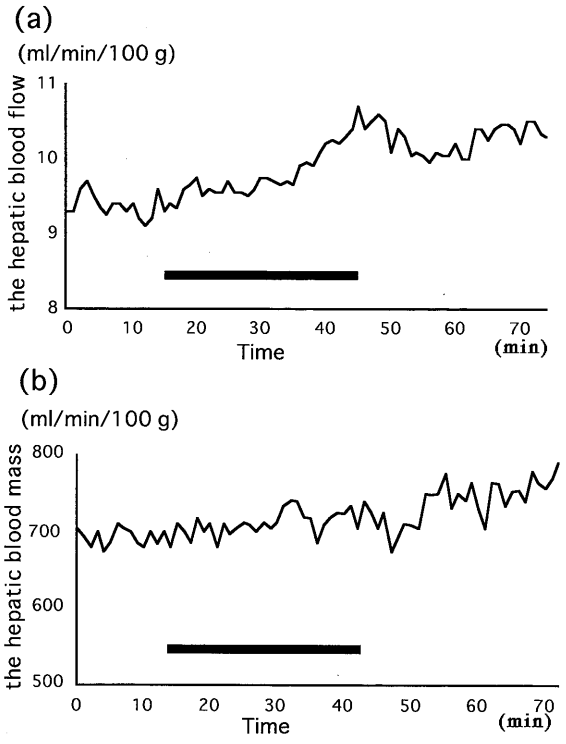


Fig. 4 Representative recordings of changes in hepatic blood flow (a) and hepatic blood mass (b) of the rat liver over time. The solid horizontal bar indicates the 30-min EMF irradiation that followed at least 15 min of stable blood flow or mass. Hepatic blood flow or mass was measured for at least 30 min.

Temperature elevation with the EMF irradiation

With irradiation, temperature elevation in the tissue was less than 0.001℃ even when the specimen was irradiated for 1 h. Thus, the thermal effect of EMF irradiation was neglectable in our experimens.

Drugs and solutions

Normal physiological salt solution (PSS) comprised the following (mM): NaCl 123, KCl 4.7, $NaHCO_3$ 15.5, KH_2PO_4 1.2, $MgCl_2$ 1.2,$CaCl_2$ 1.25 and D-glucose 11.5. High K^+ PSS was prepared by replacing NaCl with equimolar KCl. PSS was bubbled with a mixture of 95% O_2 and 5% CO_2, and the resulting pH was 7.4. U 46619 (9,11-dideoxy-9α, 11α-methanoepoxy prostaglandin F2α) was purchased from Funakoshi (Tokyo, Japan), probenecid, BK, 6-hydroxydopamine and tetrodotoxin were purchased from Sigma (St. Louis, MO, USA). Fura-2/AM was purchased from Dojindo (Kumamoto, Japan), ATP (adenisine 5'- triphosphate) was purchased from Kohjin Co. Ltd. (Tokyo, Japan) and cremophore was purchased from Nacalai Tesque (Kyoto, Japan).

Statistical analysis

Results are shown as mean ± standard error (SE). All satistical analyses were

performed by Student's t-test. A p value of less than 0.05 was considered significant.

Results

Effects of EMF irradiation on blood circulation of the rat liver in vivo

EMF irradiation induced a gradual increase in blood flow of the rat liver which peaked at 30 min, whereas EMF irradiation had no significant effect on blood mass (Fig. 4(a)and (b)). The increase in the blood flow was maintained for at least 40 min even after EMF radiation was stopped (data not shown). The hepatic blood flow levels during and after radiation (9.84 ± 4.53 ml/min/100 g and 10.10 ± 4.23 ml/min/100 g, respectively) increased significantly in comparison to levels before radiation (9.38 ±4.22 ml/min/100 g) ($p<0.05$). In contrast, hepatic blood mass during radiation (712.8 ± 159.6ml/min/100 g) did not increase in comparison to blood mass before radiation (693.6 ± 130.8 ml/min/100 g) ($p>0.05$). Hepatic blood mass was increased slightly after radiation (739.0 ± 184.6 ml/min/100 g) in comparison to that before irradiation, but the difference was not significant ($p>0.05$). According to the equation

hepatic blood flow = hepatic blood mass x blood velocity

the increase in hepatic blood flow induced by the EMF irradiation was due mainly to the increase in blood velocity not the increase in blood mass.

Effects of EMF irradiation on tension of pig coronary artery and rat aorta strip

No change in the blood mass during the EMF irradiation suggests that the EMF irradiation may not affect vascular contractility. Therefore, to evaluate directly the effect of the EMF irradiation on the vascular contraction, we investigated the effect of the EMF irradiation on the contraction of the vascular strips with and

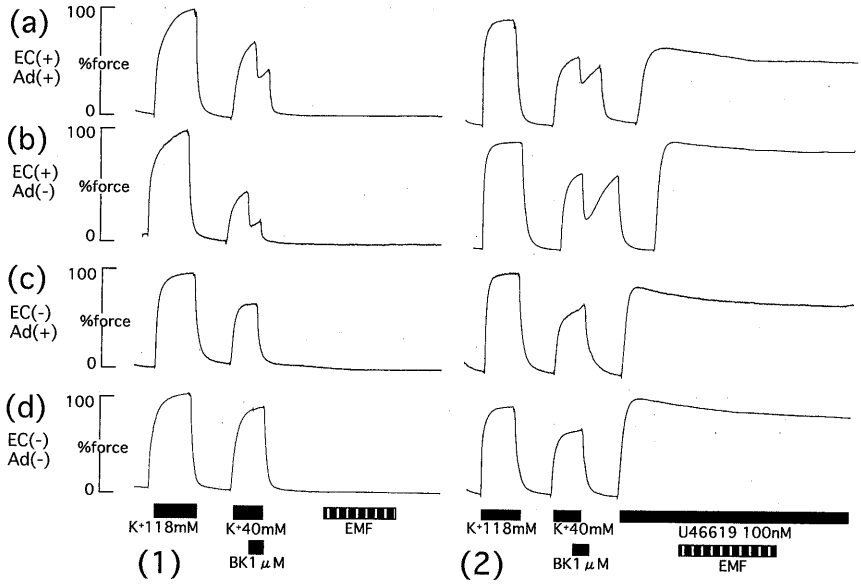


Fig. 5 Representative recordings showing the effects of EMF at rest or during contraction induced by 100 nM U46619 in pig coronary artery strips. EC(-) indicates removal of the endothelial cells and Ad(-) indicates removal of the adventitia. (a), strip of total vessel stratum. (b), strip with intact endothelium but without adventitia. (c), strip with adventitia but without endothelium. (d), strip of only smooth muscle. The presence and absence of endothelium were confirmed in relation to the relaxing response to 1 μ M bradykinin (BK).

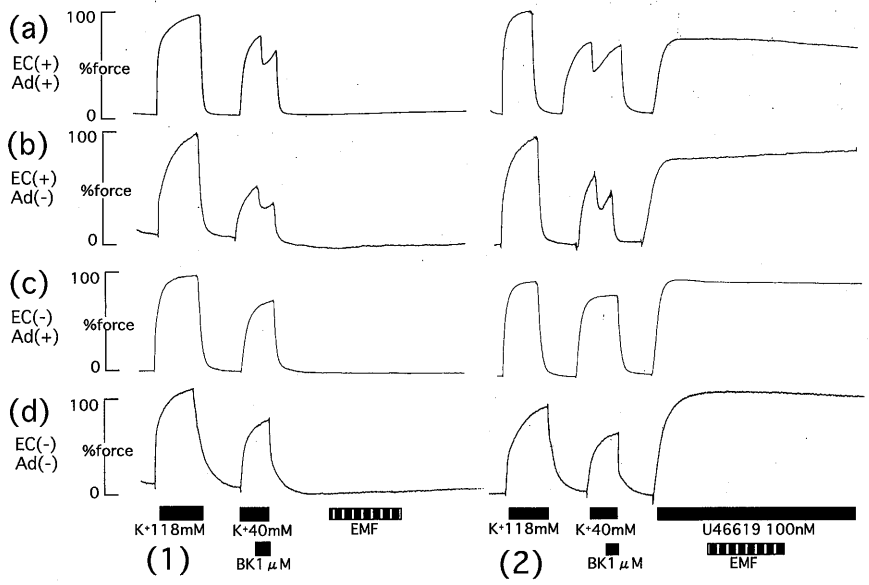


Fig. 6 .Representative recordings showing the effects of EMF at rest orduring contraction induced by 100 nM U46619 in rat aorta strips. EC(-) indicates removal of the endothelial cells and Ad(-) indicates removal of the adventitia. (a), strip of total vessel stratum. (b), strip with intact endothelium but without adventitia. (c), strip with adventitia but without endothelium. (d), strip of only smooth muscle.

without endothelium or adventitia (Figs. 5 and 6).

We selected the pig coronary artery as the artery supplying blood to the organ tissue (Fig. 5), because the roles of smooth muscle, endothelium, and vasoneuron have been extensively investigated in the coronary artery. EMF irradiation had no effect on the tension of the coronary artery strips either at rest or when stimulated by U46619, as shown in Fig. 5. Removal of the endothelium did not affect the contractile responsiveness to EMF irradiation, suggesting that the EMF irradiation has no effect on the smooth muscle contraction and on the endothelium-dependent vasorelaxation. Removal of the adventitia also did not affect the responsiveness to EMF irradiation, supporting an unimportant role of vasoneuron in the EMF irradiation. In addition to the mechanical removal of vasoneuron, we also blocked the vasoneuron pharmacologically by combined treatment of 1.2 μM 6-hydroxydopamine, 3 μM tetrodotoxin and 1 μM guanethidine. However, extensive pharmacological block of the vasoneuron did not influence contractile responsiveness to EMF irradiation (data not shown). These results strongly rule out involvement of vasoneuronal activity in the effects of EMF.

As shown in Fig. 6, we also investigated the effect of EMF irradiation on vascular contractility using rat aorta strips; rat aorta is used frequently as a conduit artery for the evaluation of vascular tone and functions of the endothelium and vasoneurons. We obtained the exact same result as that observed in the coronary artery. The results shown in Figs. 5 and 6 suggest that the EMF does not affect contraction of the vascular smooth muscle, endothelium-dependent relaxation, or the vasoneuronal function, whether the blood vessels are organ-supplying arteries or conduit arteries. This is consistent with our finding that EMF irradiation increases blood flow without affecting blood mass in the rat liver in vivo.

Effects of EMF irradiation on $[Ca^{2+}]i$ *of bovine aortic valvular strips*

Endothelium-dependent vasorelaxation is mainly mediated by NO and prostacycline

(PGI_2), both of which are produced by the elevation of $[Ca^{2+}]i$ in endothelial cells. Therefore, to investigate precisely the involvement of endothelium in EMF irradiation, we next investigated in situ the effect of the EMF irradiation on $[Ca^{2+}]i$ levels in endothelial cells on the surface of the aortic valves (Fig. 7), which produce vasorelaxing factors in response to the $[Ca^{2+}]i$ elevation induced by agonists.[17,22] EMF irradiation had no significant effect on $[Ca^{2+}]i$ levels of resting endothelial cells or on the extent of $[Ca^{2+}]i$ elevation induced by 10 mM ATP (Fig. 7). Therefore, it is highly likely that EMF irradiation is not able to produce NO or PGI_2 in endothelial cells. Indeed, we demonstrated that EMF irradiation did not influence endothelium-dependent relaxation (Figs. 5 and 6).

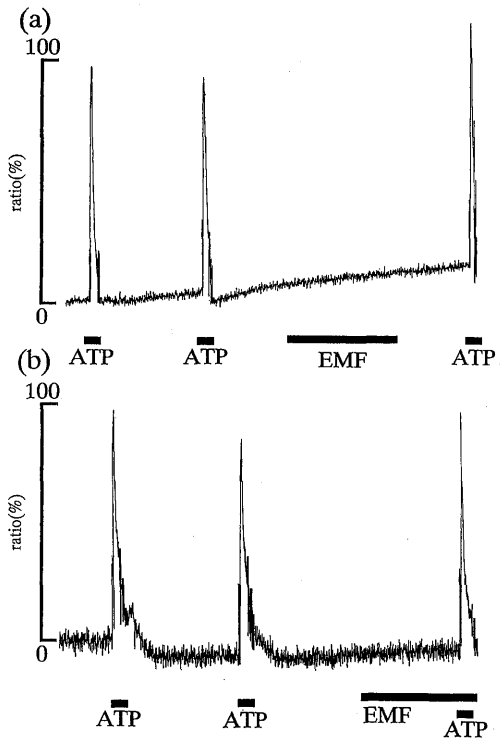


Fig. 7 The effects of EMF irradiation on $[Ca^{2+}]i$ levels of endothelial cells in situ. (a), EMF irradiation was applied in the interval of the repetitive application of 10 mM ATP, showing the effects of the resting level of $[Ca^{2+}]i$ in endothelial cells in situ. (b), EMF irradiation was applied before and during the application of 10 mM ATP.

Discussions

Whether EMFs have any influence on the life processes in living system is a question of importance. In spite of many investigations, the biological effects of EMFs are only partly understood, and the mechanism by which EMF irradiation-induced slight increase of blood flow remains poorly understood.

In the present study, we investigated the mechanism by which the EMF irradiation induced slihgt increase of blood flow without affecting blood mass. By direct evaluation of vascular contractility and endothelial and neuronal functions, we found EMF irradiation to have no direct effect on the smooth muscle contraction, endothelial function (endothelium-dependent relaxation and endothelial $[Ca^{2+}]i$ elevation), or vasoneuronal function (Figs. 5 - 7). These findings were also supported by the in vivo experiment demonstrating that the EMF irradiation induced slight increase of blood flow without affecting blood mass (Fig. 4), because blood mass corresponds to vascular contractility. Since blood flow is the product of blood mass times blood velocity, our results are compatible with the notion that EMF irradiation increases blood velocity without affecting vascular contractility.

The magnetic field has been reported to have several experimental effects on the nervous system. One is retarding motor activity of the ciliary apparatus [23] and the other is promoting nerve fiber myelinization.[24] Miura and Okada suggested that RF burst-type EMF radiation may increase in NO and cGMP of rat cerebellum supernatant[12] and that EMF irradiation of arterioles of the frog web may induce elevation of cGMP concentration and vasodilatation.[11] In addition, Ueno et al.[25] reported that the increase in the skin blood flow induced by EMF irradiation may be due to effects on the nervous system. Therefore, it is possible that EMF irradiation may

effect a vasoneuron release of neuronal NO and thereby induce vasorelaxation. However, in the present study we demonstrated that anatomical removal and pharmacological blockade of vasoneuron had no effect on EMF-regulation of contractility of organ-supplying or conduit arteries (with or without endothelium) either at rest or when contracted by a vasoconstrictor (Figs. 5 and 6). These results strongly suggest that vasoneuron does not contribute to the EMF-influenced regulation of blood flow. Our results do not rule out the posssibility that EMF irradiation affects vasoneuron; however, they support the notion that the effect of EMF irradiation on vasoneuron, if any, may not contribute to the regulation of vascular contraction.

In our rat hepatic blood flow and blood mass experiments, the EMF voltage was 10.0 V peak-to-peak, the maximum EMF intensity was 9.024 V/m, and the SAR was 50.900 mW/kg. In our vascular tension study, the EMF voltage was 15.0 V peak-to-peak, the maximum EMF intensity was 7.852 V/m, and the maximum SAR was 38.535 mW/kg. In the measurement of $[Ca^{2+}]i$ in endothelial cells in situ, the EMF voltage was 15.0 V peak-to-peak, the maximum EMF intensity was 11.824 V/m, and the SAR was 87.379 mW/kg. It was reported that arterioles of the frog web were actively dilated in response to radio frequency burst-type EMF irradiation without temperature elevation.[11] Miura et al. reported a maximum EMF intensity was 7.3 mG and 2.19 V/cm at an irradiation frequency of 10 MHz. However, when we recalculated their values according to more reliable equation reported by Terada et al.[21], which was used in the present study, the maximum EMF intensity was 2.076 V/m, and the SAR was 2.693 mW/kg. Because the EMF intensity and the SAR values used in the present study were much greater than those used (3.8-5.7 times for EMF intensity and 14-32 times for SAR) by Miura et al.[11], it is very unlikely that the negative results we obtained were due to insufficient EMF irradiation.

In the present study, we demonstrated that the EMF irradiation induced slight increase of blood flow without affecting blood mass and vascular contractility (Figs 4-6), indicating the increase in the blood velocity induced by the EMF irradiation. These results imply that EMF irradiation may not play a major role in the direct regulation of circulating systems. Therefore, it is possible that the EMF irradiation may affect the blood constituents, thereby increasing blood flow and velocity. Indeed, Likhachev reported that the EMF irradiation elevates the erythrocyte sedimentation rate (ESR) and the leucocyte count.[26] Further detailed studies must be done to clarify the mechanism of increase of the blood flow under EMF irradiation.

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