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EFFECTS OF MODULATED MICROWAVE AND X-RAY IRRADIATION ON THE ACTIVITY AND DISTRIBUTION OF CA^{2+}-ATPase IN SMALL INTESTINE EPITHELIAL CELLS

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Abstract

The distribution and activity of Ca^{2+}-ATPase were investigated by histochemical methods in small intestine epithelial cells of mice following total body 2450 MHz low frequency (16 Hz) microwave and X-ray irradiation. In the control animals, enzyme activities were found in the brush border and on lateral membranes, including junctional areas of the cells. The enzyme activity of lateral membranes was inhibited by quercetin, a specific inhibitor of Ca^{2+}-ATPase.

Immediately after square modulated (16 Hz) 2450 MHz microwave irradiation at 1 mW/cm^2 power densities, we observed a decreased activity of Ca^{2+}-ATPase on the lateral membrane regions.

The X-ray irradiation (1 Gy) induced a similar decrease of Ca^{2+}-ATPase activity which was reversible within 24 hours. "5 Gy" doses resulted in a decrease of enzyme activities on both apical and lateral membrane areas persisting up to 24 hours following irradiation.

Key Words: X-radiation, modulated microwave irradiation, Ca^{2+}-ATPase activity, transmission electron microscopy.

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Introduction

Calcium ions play an important role in regulating some cell functions by acting as an intracellular second messenger. A wide variety of external stimuli induce elevation of intracellular calcium levels (Nicholls, 1986; Carafoli 1987; Bement 1992; Putney and Bird, 1993; Scharff and Foder, 1993), which exerts an influence on the activity of calcium dependent enzymes. Enzymatic components of different signal transduction systems [i.e., protein kinase C (Bement, 1992; Wright *et al.*, 1993), phospholipase C (Morgan *et al.*, 1993), phospholipase A_2 (Churcher *et al.*, 1990) calmodulin (Cheung, 1982), endonuclease (Zhivotovsky *et al.*, 1993)] are included in this system.

The cellular calcium stores, signal transduction systems and cellular transporters of calcium build up a complicated network within the cell (reviewed recently by Scharff and Foder, 1993; Putney and Bird, 1993). The relatively stable intracellular calcium concentration is maintained by calcium pump mechanism(s), which transport the calcium out of the cells or to calcium stores in the cytoplasm. The chief device to pump out calcium from the cells is the plasma membrane calcium transporting ATPase system (Carafoli and Guerini, 1993). According to recent data the calcium pump ATPase is a membrane spanning enzyme which protrudes into the cytosol and is in contact with the extracellular space (Carafoli and Guerini, 1993).

The activity of ATPase enzymes can be revealed by electron microscopic cytochemical methods (El-sherif and Bácsy, 1989; Peute *et al.*, 1990; Stahl and Baskin, 1990; Eisenmann *et al.*, 1992; Baller *et al.*, 1993) which, however, do not distinguish the Ca^{2+}-transporting ATPase form from other ATPase(s) (Stahl and Baskin, 1990). The two types of enzyme activities can be separated by the use of specific inhibitors quercetin or vanadate for blocking the activity of the calcium pump and other transport ATPases, respectively (Peute *et al.*, 1990; Yoshioka *et al.*, 1990; Zivkovic *et al.*, 1990).

It is well known that ionizing and electromagnetic

irradiation deeply influence the calcium homeostasis of the cells. Modulated weak electromagnetic fields have been reported to induce calcium uptake in leukemic and normal lymphocytes (Emilia *et al.*, 1985; Wallaczek and Liburdy, 1990; Lyle *et al.*, 1991), in mitogen activated lymphocytes (Conti *et al.*, 1985; Liburdy *et al.*, 1993), and in chick and mouse small intestine (Klavins *et al.*, 1991; Somosy *et al.*, 1993b). There are observations about the effects of ionizing radiation on calcium transport in rat thymocytes (Zhivotovsky *et al.*, 1993) and in mouse small intestine (Somosy *et al.*, 1993a). The observed changes in local cellular calcium concentration may play a role in radiation-induced functional changes of cells and tissues, e.g., in apoptotic cell death and in modification of paracellular transport of the small intestine and blood vessel wall (Blackman *et al.*, 1979; Blackman, 1990; Walleczek and Liburdy, 1990; Kandasamy and Harris, 1992; Liburdy, 1992; Story *et al.*, 1992; Walleczek, 1992; Somosy *et al.*, 1993a,b; Zhivotovsky *et al.*, 1993). However, data about radiation-induced changes of calcium pump ATPase activity are incomplete. This prompted us to investigate whether the changes in calcium distribution following modulated and continuous microwave and X-ray irradiation in mouse small intestine described by us earlier (Somosy *et al.*, 1993 a,b) are connected to changes in the activity of plasma membrane Ca^{2+}-ATPases.

Materials and Methods

Animals

Male CFLP mice of 25-30 g maintained in standard laboratory conditions were used in our experiments. Experimental groups consisting of 3 animals each were irradiated as indicated below. Three parallel experiments were carried out.

Microwave exposure and dosimetry

The mice were total body irradiated with amplitude modulated microwave for 3 hours at either 0.1, 0.5 or 1 mW/cm^2 energies. The irradiation conditions and dosimetry were detailed in our earlier paper (Somosy *et al.*, 1993b). Tissue samples for enzyme cytochemistry were fixed immediately, and 1, 3 and 24 hours after irradiation.

X-ray irradiation

The mice were total body X-ray irradiated with 1 and 5 Gy doses and pieces of small intestine were fixed immediately, as well as 1, 3 and 24 hours after irradiation. X-ray irradiation was performed with a THX-250 machine. Conditions: 200 kV, half value layer 1.0 mm Cu, source-surface distance 90 cm, dose rate 0.317 Gy $water.min^{-1}$.

Figures 1-6 on facing page 615.

Figure 1. Ca^{2+}-ATPase reaction of control small intestine. The enzyme reaction product appears on the apicolateral region of the epithelial cells. MV = microvillar surface, lateral membranes. Bar = 0.5 μm.

Figure 2. Ca^{2+}-ATPase reaction of control small intestine in the presence of quercetin. The electron dense deposits are located only on the surface of microvilli (MV). Reaction products are absent in the lateral membrane regions (→). Bar = 0.2 μm.

Figure 3. Ca^{2+}-ATPase reaction of control small intestine in the presence ouabain. The enzyme reaction products is present on the apicolateral region of the epithelial cells. MV = microvillar surface, (→) lateral membrane region. Bar = 0.25 μm.

Figure 4. Ca^{2+}-ATPase reaction of control small intestine in the absence of ATP (**A**) or calcium (**B**). The intensity of reaction is weak. MV = microvilli. Bar = 0.2 μm.

Figure 5. Ca^{2+}-ATPase reaction in the presence of EGTA. Reaction products were absent. Bar = 0.5 μm.

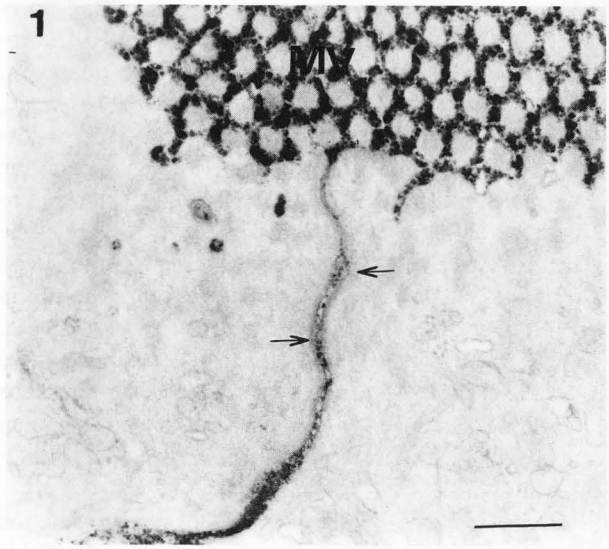
Figure 6. Calcium-ATPase reaction of modulated (16 Hz) microwave irradiated (1 mW/cm^2) small intestine after 1 hour. Reaction products are primarily localized on the microvillar region (MV) of the cells. (→) lateral region of the cells. Bar = 0.2 μm.

Electron microscopy, Ca-ATPase cytochemistry

The irradiated and control animals were anaesthetized with Nembutal and then perfused through the ascending aorta with a mixture of ice-cold 3% paraformaldehyde and 1% glutaraldehyde, 0.25 M sucrose in 0.05 M cacodylate buffer (pH 7.4) for 30 minutes. A piece of small intestine from the duodenal region (about 0.5 cm) was quickly cut from each, and 1-2 mm sections were washed in the same buffer with 0.25 M sucrose (pH 7.4) for 45 minutes.

The ATPase activity was demonstrated by the modified method of Salama *et al.* (1987). The sections were incubated in a medium containing Tris-maleate buffer (70 mM, pH 7.2), ATP (1 mM), $CaCl_2$ (3 mM), $CeCl_3$ (2 mM), $MnCl_2$ (5 mM) and levamisole (inhibitor of alkaline phosphatase, 5 mM) for 1 hour at room temperature. To see whether the reaction products represent the activity of Na/K-ATPase or Ca^{2+}-ATPase, parallel sections were incubated in the above mixture along with either ouabain (1 mM, inhibitor of Na/K-ATPase) or quercetin (1 mM, inhibitor of Ca^{2+}-ATPase).

Control incubations were done by omitting ATP or with $CaCl_2$ or 10 mM ethylenebis(oxyethylenenitrilo)-tetraacetic acid (EGTA) added to the reaction mixture. Incubation was followed by rinses in Tris-maleate buffer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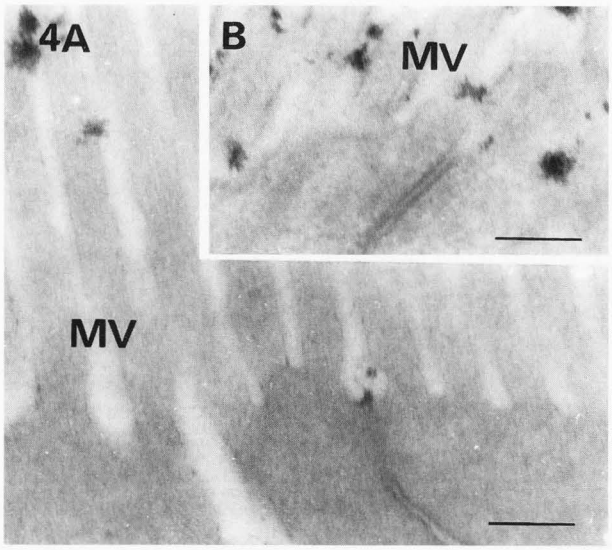
4A
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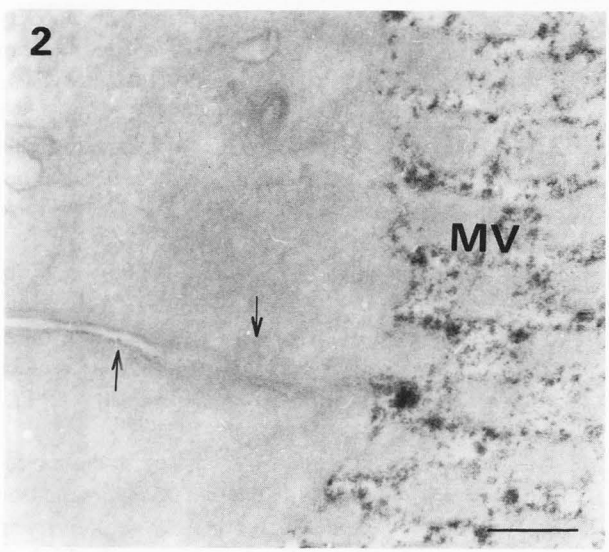
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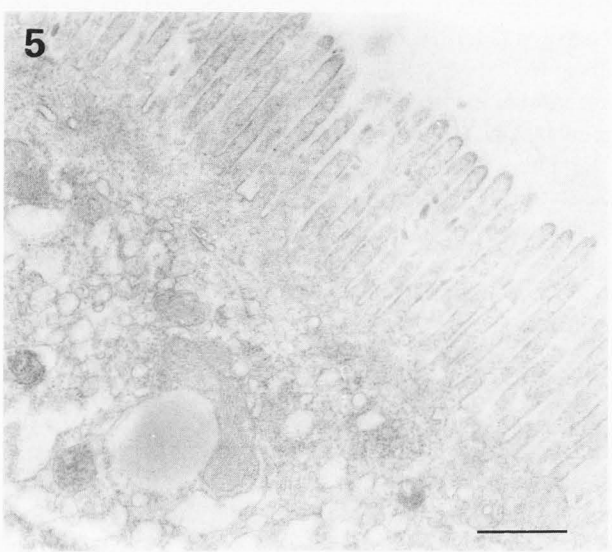
MV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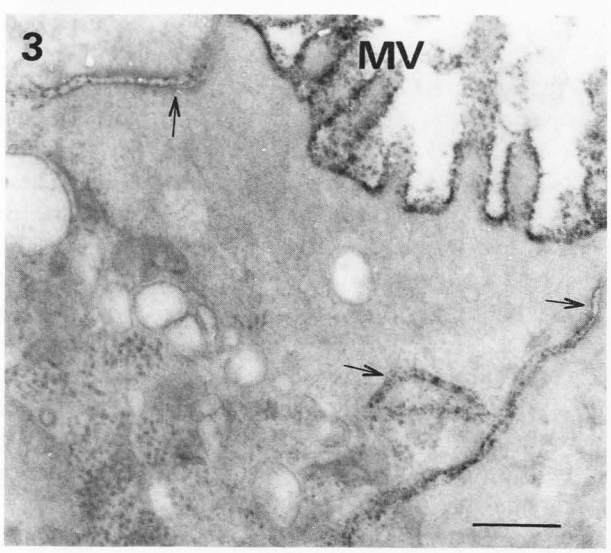
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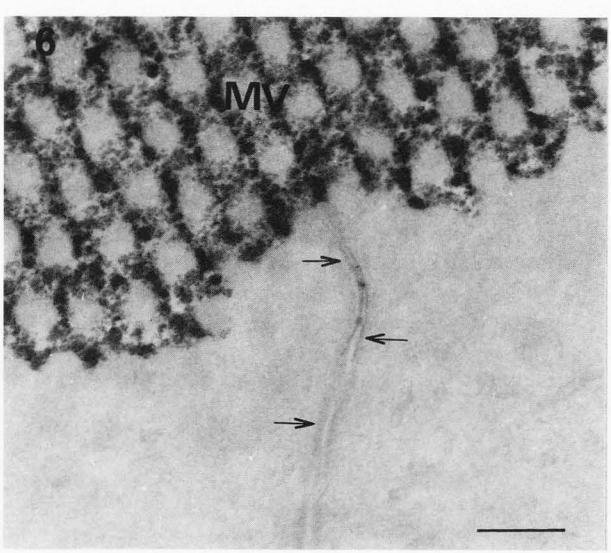
MV


6
MV

and the samples were then postfixed in 1% OsO_4.

After fixation the pieces of tissue (five from each sample) were dehydrated through graded ethanol series to propylene oxide and embedded in Durcupan AC (Fluka, Basel, Switzerland). Pre-embedding counter staining with 2% uranyl acetate was carried out in 70% ethanol for 1 hour. The samples were cut with a diamond knife on an LKB ultratome and the sections were examined in a JEOL 100CX transmission electron microscope (TEM).

Results

In the control animals, globular, electron-dense reaction products of ATPase activity were located in the apicolateral regions of the epithelial cells (Fig. 1).

When the incubation of experimental material was carried out in the presence of quercetin, the electron dense deposits of enzyme reaction appeared on the surface of microvilli, but were absent on the lateral membrane regions (Fig. 2). Ouabain, on the other hand, did not influence the distribution of electron dense reaction product which was similar to that of the controls (Fig. 3). The cytochemical reaction was weak when either $CaCl_2$ or ATP was omitted (Fig. 4). In the presence of EGTA no reaction products appeared on the apicolateral surfaces of the cells (Fig. 5). Taken together Ca^{2+}-ATPase activity is present on the lateral membranes of the cells. The apically located cerium deposits are probably products of ecto ATPases without calcium pump activity.

Immediately, 1 and 3 hours after the 1 mW/cm^2 power density modulated microwave irradiation, a decrease in enzyme activity was detected on the lateral cell membranes (Fig. 6). However, the intensity of histochemical reaction on the apical regions of cells did not change (Fig. 6). One day later, the amount and distribution of reaction products had returned to control levels (Fig. 7). Continuous microwave irradiation with the same energy does not alter the enzyme activity of epithelial cells.

X-ray irradiation (1 and 5 Gy, for 1 hour) also resulted in a decrease of lateral membrane enzyme activities (Fig. 8a and 8b). This effect was transient when 1 Gy was applied (Fig. 9); however, a markedly reduced staining pattern persisted up to 24 hours following irradiation with 5 Gy doses (Fig. 10).

Discussion

Histochemical methods for demonstration of Ca^{2+}-ATPase activity have been widely used to study the localization of this enzyme in a variety of cells (El-sherif and Bácsy, 1989; Watson *et al.*, 1989; Peute *et al*, 1990; Stahl and Baskin, 1990; Wild and Schraner, 1990; Zivkovic *et al.*, 1990; Eisenmann *et al.*, 1992; Sato *et al.*, 1993). The specificity and localization of the reaction can be greatly enhanced by the use of quercetin, a specific inhibitor of Ca^{2+}-ATPase (Yoshioka *et al.*, 1990). Our results show that the electron dense deposits, which appear on the apical and lateral surface of the small intestine epithelial cells following incubation, are products of at least two different types of ATPases; one of which is quercetin sensitive and located on the lateral membranes, the other(s) are not influenced by quercetin and probably represent apically located ecto-ATPases. The observed lateral distribution of quercetin sensitive (calcium pump) ATPase is in agreement with previous reports, which suggested the lateral localization of pump ATPase in other cell types (Eisenmann *et al.*, 1992; Doneen, 1993).

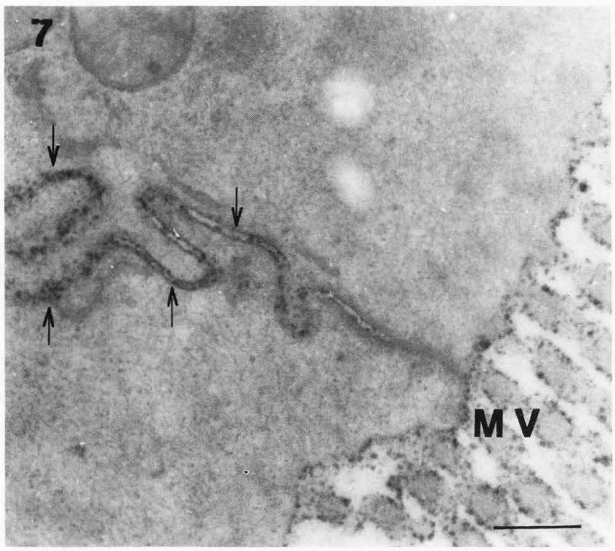
Cellular membranes are targets of ionizing radiation and of weak, low frequency electromagnetic fields (Somosy *et al.*, 1993a,b), which can disturb the activity of membrane bound enzymes. X-ray irradiation can modify the activity of adenylate and guanylate cyclase and protein kinase C (Somosy *et al.*, 1988; Hallahan *et al.*, 1991). Weak, low frequency electromagnetic fields also altered the activity of 5'nucleotidase, acetylcholinesterase, alkaline phosphatase and Na^+, K^+-ATPase (Blank and Soo, 1992; Moses and Martin, 1992).

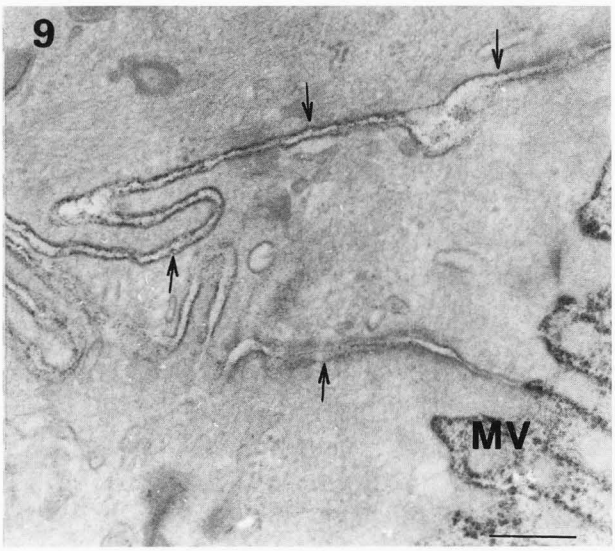
Data regarding changes in Ca^{2+}-ATPase activity upon exposure with electromagnetic fields are lacking. However, a decrease of its enzyme activity upon ionizing radiation has been reported in several studies (Riskulova and Ivashcenko, 1976; Karmalkov *et al.*, 1991; Dreval, 1992; Baller *et al.*, 1993).

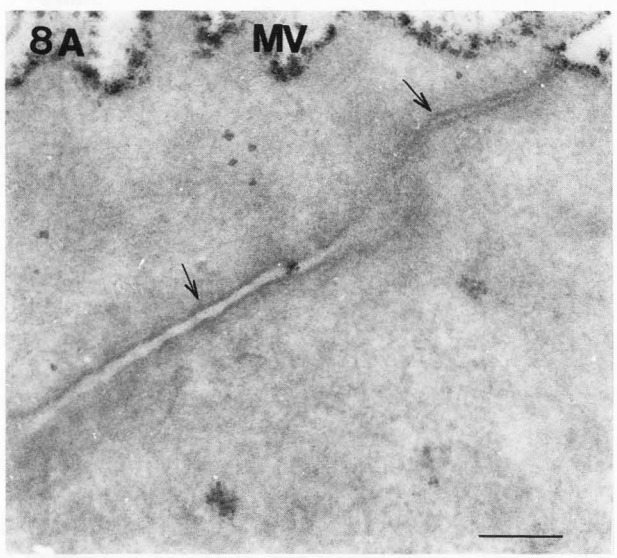
Our data presented here further show that the modulated microwave and moderate dose of X-ray irradiation (1 Gy) decrease the calcium pump ATPase activity in small intestine epithelial cells. In addition, the high (5 Gy) dose of X-ray irradiation markedly decreases both calcium pump and other ATPase activities.

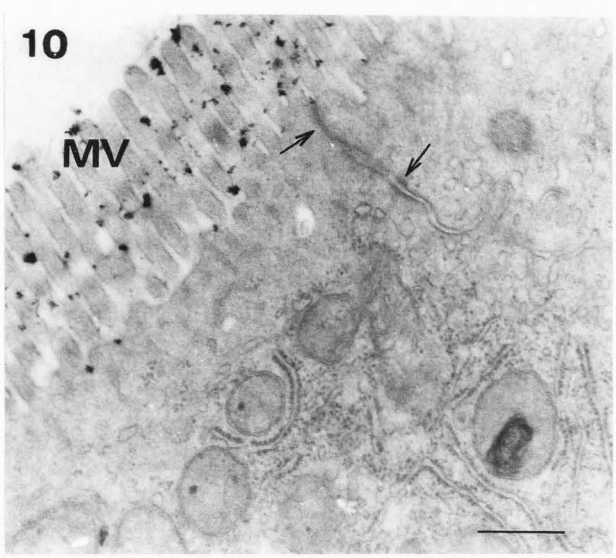
In earlier studies, we showed that X-rays and modulated microwave irradiation resulted in a decrease and redistribution of calcium in the lateral areas of the small intestine epithelial cells (Somosy *et al.*, 1993a,b). These phenomena are probably connected to the decrease of calcium-ATPase activity observed in the present study. Based on these data, it is conceivable that the lateral membrane associates pump ATPase has any role in the regulation of calcium content bound at same membrane area. Similar findings were published by Eisenman *et al.* (1992), who observed a concomitant reduction of membrane-associated calcium and Ca^{2+}, Mg^{2+}-ATPase activity after vinblastine treatment in rat incisor maturation ameloblasts.

Modulation of Ca^{2+}-ATPase activity and/or calcium pump gene expression are regulated by multiple second









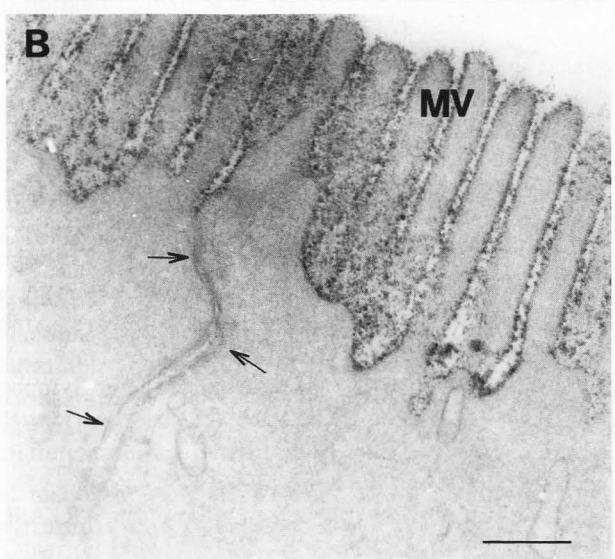


Figure 7. Calcium-ATPase reaction of modulated (16 Hz) microwave irradiated (1 mW/cm^2) small intestine after 24 hours. The distribution of deposits of enzyme reaction products is similar to that of the control. MV = microvilli, (→) lateral membrane. Bar = 0.2 μm.

Figure 8. Calcium-ATPase reaction upon X-ray irradiation. (**A**) 1 Gy, 1 hour; (**B**) 5 Gy, 1 hour. Enzyme reaction products appear only on the microvillar surfaces (MV). (→) lateral membrane region. Bars = 0.2 μm.

Figure 9. Calcium-ATPase reaction in X-ray irradiated small intestine with 1 Gy dose after 24 hours. Cytochemical reaction products appears on apicolateral regions of cells. MV = microvillar region, (→) lateral membrane. Bar = 0.25 μm.

Figure 10. Calcium-ATPase reaction in X-ray irradiated small intestine with 5 Gy dose after 24 hours. Markedly reduced staining pattern persists. MV = microvillar region, (→) lateral membrane. Bar= 0.5 μm.

messenger pathways that include protein kinase C-, cAMP- and Ca^{2+} dependent mechanisms (Carafoli and Guerini 1993; Kuo *et al.*, 1993; Wright *et al.*, 1993), are influenced by membrane fluidity (Su *et al.*, 1993). The ion transport ATPases are also targets for free radical damage (Rohn *et al.*, 1993). Since both modulated microwave and X-ray irradiation can act on elements of the membrane signal transduction processes (Somosy *et al.*, 1988, 1993a,b; Hallahan *et al.*, 1991), presumably they play a role in radiation induced changes of calcium pump ATPase activity.

Acknowledgments

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References

Baller JFW, Poelstra K, Hardonk MJ, Bakker WW (1993) A modified cerium-based histochemical method for detection of experimentally-induced ATPase impairment in glomeruli of the rat kidney. J. Histochem. Cytochem. **41**, 1105-1109.

Bement WM (1992) Signal transduction by calcium and protein kinase C during egg activation. J. Exp. Zool. **263**, 382-397.

Blackman CF (1990) ELF Effects on calcium homeostasis. In: Extremely Low Frequency Electromagnetic Fields: The Question of Cancer. Wilson BW, Stevens RG, Anderson LE (eds.). Battelle Press, Columbus, OH. pp 188-208.

Blackman CF, Elder JA, Weil CM, Benane SG, Eichinger DC, House DE (1979) Induction of calcium-ion efflux from brain tissue by radio-frequency radiation: Effect of modulation frequency and field strength. Radio Sci. **14**(6S), 93-98.

Blank M, Soo L (1992) Threshold for inhibition of Na,K-ATPase by ELF alternating currents. Bioelectromagnetics **13**, 329-333.

Carafoli E (1987) Intracellular calcium homeostasis. Ann. Rev. Biochem. **56**, 395-433.

Carafoli E, Guerini D (1993) Molecular and cellular biology of plasma membrane calcium-ATPase. Trends Cardiovasc. Med. **3**, 177-184.

Cheung WY (1982) Calmodulin: an overview. Fed. Proc. **41**, 2253-2257.

Churcher Y, Allan D, Gomperts BD (1990) Relationship between arachionate generation and exocytosis in permeabilized mast cells. Biochem. J. **275**, 157-163.

Conti P, Gigante GE, Alesse E, Cifone MG, Fieschi C, Renale M, Angeletti PU (1985) A role of Ca^{2+} in the very low frequency electromagnetic field on the blastogenesis of human lymphocytes. FEBS Lett. **181**, 28-32.

Doneen BA (1993) High-affinity Ca^{2+}-Mg-ATPase in kidney of euryhaline *Gillichthys mirabilis*: Kinetics, subcellular distribution and effects of salinity. Comp. Biochem. Physiol. **106B**, 719-728.

Dreval VI (1992) The effect of ionizing radiation on the Ca^{2+}, Mg^{2+} ATPase activity of plasma membranes. Radiobiologiia **32**, 222-224.

Eisenmann DR, Salama AH, Zaki AME (1992) Effects of vinblastine on calcium distribution pattern and Ca^{2+},Mg^{2+}-adenosine triphosphatase in rat incisor maturation ameloblast. J. Histochem. Cytochem. **40**, 143-151.

El-sherif G, Bácsy E (1989) Ultrastructural study of the Ca^{2+}-dependent ATPase activity in rat adenohypophyseal cells. Histochemistry **91**, 501-505.

Emilia G, Torelli G, Ceccherelli G, Donelli A, Ferrari S, Zuccini P, Cardossi R (1985) Effect of low-frequency low-energy pulsing electromagnetic fields on the response to lectin stimulation of normal human and chronic lymphocytic leukemia lymphocytes. J. Bioelectricity **4**, 145-161.

Hallahan DE, Virudachalam S, Sherman ML, Huberman E, Kufe DW, Weichselbaum RR (1991) Tumor necrosis factor gene expression is mediated by protein kinase C following activation by ionizing radiation. Cancer Res. **51**: 4565-4569.

Kandasamy SB, Harris AH (1992) Effect of prostaglandins, inositol 1,4,5-triphosphate, and phorbol esters on radiation induced decreases in calcium influx in rat brain synaptosomes. Radiat. Res. **131**, 43-46.

Karmalkov SA, Miliutin AA, Konoplia EF (1991) The immediate and late effects of ionizing radiation on the Ca^{2+} accumulating capacity of the plasma membranes of rat aorta smooth muscle cells. Radiobiologiia **31**, 48-51.

Klavins IE, Galvanovsky YY, Dreimanis AP (1991) Low-frequency electromagnetic pulses enhance Ca uptake by chick small intestine in vitro. Bioelectrochem. Bioenergetics **25**, 437-445.

Kuo TH, Liu B-F, Diglio C, Tsang W (1993) Regulation of the plasma membrane calcium pump gene expression by two signal transduction pathways. Arch. Biochem. Biophys. **305**, 428-433.

Liburdy RP (1992) Calcium signaling in lymphocytes and ELF fields. Evidence for an electric field metric and a site of interaction involving the calcium ion channel. FEBS Lett. **301**, 53-59.

Lyle DB, Wang X, Ayotte RD, Sheppard AR, Adey WR (1991) Calcium uptake by leukemic and normal T-lymphocytes exposed to low frequency magnetic fields.

Bioelectromagnetics **12**, 145-156.

Morgan A, Wilkinson M, Burgoyana RD (1993) Identification of exo2 as the catalytic subunit of protein kinase-A reveals a role for cyclic AMP in Ca^{2+} dependent exocytosis in chromatin cells. EMBO J. **12**, 3747-3752.

Moses GC, Martin AH (1992) Effects of extremely low-frequency electromagnetic fields on three plasma membrane-associated enzymes in early chicken embryos. Biochem. Intern. **28**, 659-664.

Nicholls DG (1986) Intracellular calcium homeostasis. Br. Med. Bull. **42**, 353-358.

Peute J, van Linder ATM, Zandbergen MA, de Bruijn WC (1990) Ultrastructural localization of calcium and Ca^{2+}-ATPase activity in gonadotrops and stellate cells of the catfish pituitary. Histochemistry **94**, 601-607.

Putney JW, Bird GSJ (1993) The signal for capacitive calcium entry. Cell **75**, 199-201.

Riskulova ST, Ivashchenko AT (1976) Action of X-radiation on the activity of ATPases of plasma membranes and other subcellular liver fractions. Radiobiologiia **16**, 652-658.

Rohn TT, Hinds TR, Vincenzi F (1993) Ion transport ATPases as targets for free radical damage. Protection by an amino steroid of the Ca^{2+} pump ATPase and Na/K pump ATPase of human red blood cell membranes. Biochem. Pharmacol. **46**, 525-534.

Salama AH, Zaki AE, Eisenmann DR (1987) Cytochemical localization of Ca^{2+}-Mg^{2+} adenosine triphosphatase in rat incisor ameloblasts during enamel secretion and maturation. J. Histochem. Cytochem. **35**, 471-482.

Sato I, Shimada K, Ezure H, Sato T, Lance VA (1993) Distribution of calcium-ATPase in developing teeth of embryonic American alligators (*Alligator mississippiensis*). J. Morphol. **218**, 249-256.

Scharff O, Foder B (1993) Regulation of cytosolic calcium in blood cells. Physiological Rev. **73**, 547-581.

Somosy Z, Takáts A, Kubasova T, Sudár F, Köteles GJ (1988) Effects of X-irradiation on adenylate cyclase activity and cyclic AMP content of primary human fibroblasts. Scanning Microsc. **2**, 2165-2172.

Somosy Z, Kovács J, Siklós L, Köteles GJ (1993a) Morphological and histochemical changes in intercellular junctional complexes in epithelial cells of mouse small intestine upon X-irradiation: Changes of ruthenium red permeability and calcium content. Scanning Microsc. **7**, 961-971.

Somosy Z, Thuróczy G, Kovács J (1993b) Effects of modulated and continuous microwave irradiation on pyroantimonate calcium content in junctional complex of small intestine. Scanning Microsc. **7**, 1255-1261.

Stahl WL, Baskin DG (1990) Histochemistry of ATPases. J. Histochem. Cytochem. **38**, 1099-1122.

Story MD, Stephens LC, Tomasovics SP, Meyn RE (1992) A role of calcium regulating apoptosis in rat thymocytes irradiated in vitro. Int. J. Radiat. Biol. **61**, 243-251.

Su Z, Yang X-D, Li Y-J, Chen X (1993) Effects of hydrogen peroxide on membrane fluidity and $Ca^{(2+)}$-transporting ATPase activity of rabbit myocardial sarcoplasmic reticulum. Acta Pharmacol. Sinica **14**, 393-396.

Walleczek J, Liburdy RP (1990) Non-thermal 60 Hz sinusoidal magnetic-field exposure enhances $^{45}Ca^{2+}$ uptake in rat thymocytes: dependence on mitogen activation. FEBS Lett. **271**, 157-160.

Walleczek J (1992) Electromagnetic field effects on cells of the immune system: the role of calcium signaling. FASEB J. **6**, 3177-3185.

Watson LP, Kang Y-H, Falk MC (1989) Cytochemical properties of osteoblast cell membrane domains. J. Histochem. Cytochem. **37**, 1235-1246.

Wild P, Schraner EM (1990) Parathyroid cell polarity as revealed by cytochemical localization of ATPases, alkaline phosphatase and 5-nucleotidase. Histochemistry **94**, 409-414.

Wright LC, Chen S, Roufogalis BD (1993) Regulation of the activity and phosphorylation of the plasma membrane Ca^{2+}-ATPase by protein kinase C in intact human erythrocytes. Arch. Biochem. Biophys. **306**, 277-284.

Yoshioka T, Fujimoto K, Tanaka O (1990) Ca-ATPase cytochemistry in the spinal cord microvasculature of prenatal rats. Histochem. J. **22**, 134-142.

Zhivotovsky B, Nicotera P, Bellomo G, Hanson K, Orrenius S (1993) Ca^{2+} and endonuclease activation in radiation induced lymphoid cell death. Exp. Cell Res. **207**, 163-170.

Zivkovic D, Créton R, Zwaan G, de Bruijn WC, Dohmen MR (1990) Polar localization of plasma membrane Ca^{2+}/Mg^{2+} ATPase correlates with the pattern of steady ionic currents in eggs of *Lymnae stagnalis* and *Bithynia tentaculata* (Mollusca). Roux's Arch. Dev. Biol. **199**, 134-145.

Discussion with Reviewers

W.C de Bruijn: How consistent is the distribution of the precipitates among the specimens, in other words, how reliable are the images shown, and do they represent a true sample from the total population?

Authors: The distribution of calcium-ATPase reaction was consistent in this cell population.

W.C de Bruijn: The amount of reaction product is not measured, so the steps to interfere with the activity like

quercetin have to rely on "absence" or "presence" (0 or 100%), but unfortunately there is some remaining, though differently located product left. Are all the sites (cells?) in the specimens investigated reacting, in the same way or are local differences in the enzyme activity noted? Do you have any information whether the remaining activity is abolished by this vanadate treatment?

Authors: Our results show that the reaction products localized on adjacent lateral cell membranes do not disappear totally after quercetin treatment. This fact may be explained by remaining activity of pump ATPase and/r other ecto-ATPase(s) activity. Work is in progress in our laboratory to solve this question by applying vanadate treatment.

L-G. Friberg: In combined irradiation and cytotoxic agent treatment of experimental solid tumors it should be of great interest to find out the concentration of labeled cytotoxic agent as a result of induced changes of calcium pump-ATPase activity?

Authors: Yes, this problem is interesting, however we are not working on this topic and therefore, we do not have any information about it.

L-G. Friberg: Could, based on your results, cisplatinum side effects on the small intestine be a problem given together with irradiation of the pelvic tumor?

Authors: It is known that the toxic effects of cisplatinum are related to the changes of calcium homeostasis of cells (Walker *et al.*, 1990). However, the possible relationship between the cisplatinum induced changes of calcium homeostasis and irradiation induced changes of calcium pump ATPase activity in the small intestine remains to be elucidated.

Additional Reference

Walker EMJ, Fazekas May MA, Bowen WR (1990) Nephrotoxic and ototoxic agents. Clin. Lab. Med. **10**, 323-354.