Electromagnetic Modulation of Biological Processes: ATPase Function and DNA Production by Raji Cancer Cells *in vitro*

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Addition of either ATP or ouabain to the culture medium markedly depressed thymidine incorporation into DNA in Raji cells. The electromagnetic field, pulsating at very low frequency, did not affect DNA synthesis in normal culture media nor did it alter its ouabain-inhibition, but it partially reversed the ATP-inhibition. In spite of the presence of ATP, ouabain prevented stimulation of ATPase and DNA synthesis by the field. Although no mechanism is known for the action of either ATP or the field, the results may be interpreted in light of existing speculations. In the absence of the field, external ATP may go into an ATP pool that either blocks ATPase or feeds adenyl cyclase, which hinders DNA synthesis. In contrast, the electromagnetic field may either turn off adenyl cyclase or simply stimulate the ATP-depressed ATPase.

Introduction

Very low frequency pulsating electromagnetic fields are known to stimulate Na^+ flux and ATPase activity across red blood cell membrane [1, 2], DNA synthesis and cell proliferation in cartilage and bone cells *in vitro* [3, 4], limb regeneration in amphibians [5], and certain other cell, tissue, and organ processes *in vitro* [6–8] and *in vivo* [9]. Although the mechanism of coupling of the field with the biochemical functions is still unknown, all the data dealing with Na^+ flux [1, 2], DNA synthesis [3, 4, 10] and Ca^{2+} mobilization [6–8] tend to suggest that the field stimulates plasma membrane ATPases.

Since promotion of Na^+K^+ATPase leads to increased DNA synthesis and cell proliferation [3, 4, 11], the ATPase-DNA binomial may be used in our search for mechanisms of coupling of the electromagnetic signal with the given cell processes. Raji cancer cells were chosen because of their availability.

Materials and Methods

Chemicals

Radioactive [^{3}H]thymidine was purchased from New England Nuclear, Boston, MA. Salt and buffer solutions, and culture media were prepared with reagent grade and best grade materials in deionized water.

Cell cultures

The human lymphoma Raji line cells were kindly provided by Drs. H. Azar and U. Desai in the Department of Pathology at the University of South Florida. The cells were grown in suspension at 37 °C in plastic Corning flasks on Ames aliquot mixer (Miles) inclining at 56° 30′ angle inside a humidified incubator provided with flow of the usual 5% CO_2 and 95% air mixture.

The culture medium RPMI 1640 (GIBCO Laboratories, Grand Island, NY) consisted basically of Eagle's essential medium with the additions suggested in previous reports [12, 13]. The medium composition was: 105 mM NaCl, 24 mM $NaHCO_3$, 15 mM Hepes, 11 mM glucose, 5.6 mM Na-phosphate, 5.3 mM KCl, 0.42 mM $CaCl_2$, 0.41 mM $MgSO_4$, 1% L-glutamine, 2% Pen-Strep (2 ml in 100 ml medium of GIBCO Cat. # 600-5070, penicillin 5000 units/ml and streptomycin 5000 µg/ml), and 10% fetal calf serum, pH 7.3. The cells were split in fresh medium twice a week at 3- to 4-day intervals to an initial concentration of 10^6 cells/ml. Viability was determined by the Trypan blue dye exclusion test. The cells for each experiment were harvested after 22 h, when the concentration was $3 \pm 1 \times 10^6$ cells/ml and viability was 97 ± 1%.

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Thymidine incorporation

After 22 h the culture flasks, containing 20 ml suspension each, were removed from the incubator; the contents of 3 flasks were combined, and radioactive thymidine was added to a final count of about 600000 cpm/ml. Aliquots of 2 ml culture were immediately transferred into (12 mm × 75 mm) cylindrical polystyrene Falcon tubes with round bottom and snap cap; the additives cited under each experiment were supplied in solution in small volumes, and the equivalent water volumes were added to the reference samples. The cultures were then incubated for 16 h at 37 °C, in the warm room, inside the electromagnetic field, with an equal number of samples (control) outside the field.

Electromagnetic generator

This was provided by Electro-Biology, Inc., Fairfield, New Jersey [14]. The functional characteristics of the apparatus were already described [8, 15, 16].

Analyses

Thymidine incorporation was determined by scintillation counting in the DNA fraction, which was isolated according to described methods [17]. The counts reported were obtained from duplicate aliquots of 250 µl out of a total volume of 3 ml of the hydrolyzed DNA in 0.5 N $HClO_4$. Aquasol (10 ml) was used as scintillation fluid.

Results

The field barely affected DNA synthesis by Raji cells in the ordinary medium. Addition of either Ca^{2+} or Mg^{2+} 2.5 mM to the medium had also no effect. As expected, ouabain 1 mM produced a marked decrease in DNA-thymidine, which the field was unable to reverse (Table I).

Addition of sodium ATP to the medium caused a large decrease in thymidine incorporation, decrease which was greater with increasing ATP concentration. Unlike with ouabain, in the presence of ATP the field stimulated the otherwise depressed thymidine incorporation, but to values that were appreciably below the control level. Both the effect of ATP and that of the field in the presence of ATP were potentiated by Mg^{2+}. Addition of 1 mM ouabain with 2.5 mM Mg-ATP eliminated the effect of the field and gave results similar to those with ouabain alone (see Table I), indicating that in all cases the field was ineffective in the presence of ouabain.

Discussion

The experiment reported in Table I is typical. The trends were reproduced several times with different cell batches. The field had little if any effect on thymidine incorporation and DNA synthesis by Raji cells in normal culture medium. Only in one out of six different cell batches was there a small effect of the field ($\Delta = 6\%$), which otherwise was zero. It is not known if there could be a greater effect at other growth stages or after incubation for times longer than 16 h in the thymidine uptake experiment.

The fact that ouabain blocked thymidine incorporation is consistent with the knowledge that plasma membrane Na^+K^+ATPase regulates DNA synthesis [3, 11]. The inability of the field to reverse the ouabain effect in Raji cells bears a similarity to the results obtained with red blood cells, where the field had no effect on the inhibition of the Na^+ flux and Na^+K^+ATPase by ouabain [1, 2]. It is not known if ATP, products of its breakdown, or some other undefined messenger entered the Raji cell; nor is it known if ouabain blocked thymidine incorporation into DNA *per se* or thymidine transport into

Table I. [^{3}H]Thymidine incorporation in cpm in the DNA fraction of 500 000 cells after 16 h at 37 °C, outside (control) and inside electromagnetic field. Number of samples 3, average deviation ± 500 cpm.

	–	+ 1 mM ouabain	+ 2.5 mM Na-ATP	+ 2.5 mM Mg-ATP
Control	33 000	16 000	22 000	16 000
Field[a]	33,000 (100)	16 000 (100)	26 000 (118)	29 000 (181)

[a] The values in parentheses are % of control values.

the cell and the nucleus. That notwithstanding, for the sake of simplicity in this discussion, we shall assume that the terms thymidine incorporation, ATPase activity, and DNA synthesis are interchangeable. We also assume as a model the seesaw balance that has been postulated by others between ATPase and adenyl cyclase in the plasma membrane [11].

Consequently, the depressing effect of exogenous ATP on DNA synthesis by Raji cells and its reversal by the field become consistent with (a) the possibility that this ATP stimulates adenyl cyclase, which in turn inhibits Na^+K^+ATPase and DNA synthesis, and (b) the observation that the same fields markedly reduced the production of cyclic AMP which had been stimulated by the parathyroid hormone in cultured bone cells [16]. Both ATPase inhibition by exogenous ATP and reactivation by the field in Raji cells were potentiated by Mg^{2+}, a feature which is consistent with the known role of Mg^{2+} cofactor in the activation of the two enzymes [18]. Irrespective of whether the field acts on ATPase, adenyl cyclase, or both, the mechanism of coupling is still unknown.

In any case, the foregoing data confirm two important concepts. One is that the coupling is probably at the level of the plasma membrane [7, 8, 10, 16]. The other is that the effect of the field is conspicuous in the presence of activators or inhibitors, such as ATP in Raji cells (above) and the parathyroid hormone in bone cells [16], or bicarbonate in the cases of Ca^{2+}-mobilization in brain tissue [6] and chick tibia [7, 8]. Clearly, the very low frequency low energy pulsating electromagnetic fields [14–16] are a new compelling tool to investigate (a) the structural and functional relationships among the biochemical entities regulating cell division and differentiation, and (b) the therapeutic implications of the fields' interaction with the receptor-messenger apparatus of the plasma membrane.

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