Electromagnetic Modulation of Biological Processes: Bicarbonate Effect and Mechanistic Considerations in the Ca-Uptake by Embryonal Chick Tibia *in vitro*

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Z. Naturforsch. **38 c**, 465–467 (1983); received November 29, 1982/March 16, 1983

Pulsating Electromagnetic Fields, Ca-Uptake, Osteogenesis, Bicarbonate, Ca^{2+}-ATPase

Electromagnetically induced currents pulsating at very low frequency stimulated calcium uptake by chick embryo tibia rudiments only when bicarbonate was present in the culture medium. A bicarbonate-dependent Ca^{2+}-ATPase might be implicated in coupling of the electromagnetic signal with processes that promote Ca-transport and storage in bone tissue.

Introduction

It is well established that very low frequency electromagnetic fields stimulate bone growth and repair *in vivo* [1], Ca^{2+} efflux from brain tissue *in vitro* [2], and DNA synthesis by cartilage and bone cells in culture [3, 4]. Although the gross final effects are appreciated, little is known about the early molecular events that couple the electromagnetic field with the various biological processes. In our attempts at identifying such mechanisms, Ca-uptake by chick embryo tibia in short term culture was used as a model to obtain information about the effect of the fields on calcium mobilization by bone tissue.

Materials and Methods

Tibiae were isolated by standard procedures [5] from 8- to 10-day-old chick embryos (derived from white Leghorn eggs) and incubated in the given medium for 60 min at 37.5 ± 0.5 °C. Each tibia was placed in 600 µl Y medium, which consisted of 120 mM glucose, 90 mM NaCl, 6 mM KCl, 5 mM sodium phosphate, 1.2 mM $CaCl_2$, 1.2 mM $MgSO_4$, 10 mM $NaHCO_3$, initial pH 7.00, and contained 0.3 to 0.5 µCi $^{45}CaCl_2$. Because of the short term nature of the experiments, sterile conditions were not necessary, and no CO_2 gas was introduced into the atmosphere during incubation, a practice which is not uncommon [6]; but the culture was kept in the air flow of the incubator. Blanks consisted of culture medium, without tibia. The Ca-uptake was best determined by counting of ^{45}Ca in aliquots of the medium before and immediately after incubation [7, 8]; the % Ca-uptake was then calculated as 100× (initial-final)/initial.

The electromagnetic generator [9], provided by Electro-Biology, Inc., Fairfield, NJ, consisted of a power supply that delivered current in the form of asymmetric pulses into two Helmholtz air-gap coils, approximately square, 10 cm × 10 cm, 7 cm apart; the resulting signal had 15 mV main peak amplitude, 200 µs main and 20 µs opposite polarity duration. The pulses were delivered in trains of 5 ms duration with a repetition rate of 15 Hz. The energy content of these pulses is very small, since the induced extracellular fields are about 1 mV/cm or less and the current density is between 10 and 50 µ A/cm^2; the rate of change of the magnetic field is 0.1 G/µs for the main polarity pulse [10, 11].

The samples were placed in clear polystyrene, 12 mm × 75 mm, cylindrical Falcon culture tubes with round bottom and snap cap. The tubes were suspended in a plastic holder with the samples at a midline distance between the two coils; each holder contained up to 16 tubes.

Results

The data in Table I represent 3 sets of experiments with the same batch of eggs: one experiment in the absence of bicarbonate (Columns III, IV), one in he presence of bicarbonate but without phosphate (Columns I and II), and one with both bicar-

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0341-0382/83/0500-0465 $ 01.30/0

Table I. Effect of acetate, bicarbonate (BC) and phosphate (ph) mM concentrations on % Ca-uptake by embryonal chick tibia rudiments after 60 min culture in Y medium modified to contain the ingredients indicated in table. No. samples: 4; average deviation < ± 10%.

	Bicarbonate		Acetate	Phosphate	[Bicarbonate (BC) + Phosphate (ph)]			
	10.0	20.0	10.0	5.0	BC	10.0	20.0	50.0
	–	–	–	–	ph	5.0	5.0	5.0
	I	II	III	IV		V	VI	VII
Control	24.1	25.0	1.2	11.5		20.4	34.1	33.6
Field	28.9	29.1	1.3	11.7		28.1	46.8	38.2
Δ%	19	16	None	None		39	38	14
Δ/mM BC	0.48	0.21	None	None		0.77	0.64	0.09

bonate and phosphate (Columns V, VI, VII). The electromagnetic or field effect is expressed in % $\Delta = 100 \times$ [% Ca-uptake in the field − % Ca-uptake outside the field (control)]/control = 100 Δ/control, as well as in Δ/mM bicarbonate.

It became soon apparent that sufficient quantities of bicarbonate were required in order for the field to exert an effect on the Ca-uptake by the chick tibia rudiment. In the absence of bicarbonate (Columns III, IV), there was no effect of the field on the Ca-uptake, which was relatively meager, as indicated by the order of effectiveness acetate < phosphate < bicarbonate. In the presence of bicarbonate, even without phosphate (Columns I, II), the Ca-uptake was large and the effect of the field was marked and peaked at about 10 mM bicarbonate; the value of the field effect as Δ/mM bicarbonate became smaller as the bicarbonate concentration increased from 10 mM to 20 mM. Although also in the presence of phosphate (Columns V, VI, VII) the field effect peaked at about 10 mM bicarbonate, the greater values of %Δ and Δ/mM bicarbonate clearly indicate that phosphate potentiated the action of the bicarbonate inside the field (contrast with values in Columns I, II). The normal biological and experimental media contain both bicarbonate and phosphate. Notice the marked depressing effect of a large bicarbonate concentration Column VII.

The data in Table I are typical. The trends were reproduced consistently at least six times with six different batches of eggs. The values of the effect (%Δ) in normal medium (bicarbonate + phosphate, with moderate bicarbonate concentrations, Columns V, VI) were typical of tibiae of that age. However, such values peaked as high as 100% to 200% with tibiae somewhere between 8 and 8½ day, whereas the effect was more likely 20% or less on both ends, namely at 7½ and 9 to 11 days. This suggests that around the 8th day there may be a peak of cellular activity, which is typical of growing tissue and is affected most by the electromagnetic field.

Discussion

The discovery of the unique influence of bicarbonate on the Ca-uptake by embryonal chick tibia rudiments inside the electromagnetic field provides a novel lead toward identifying molecular mechanisms in coupling of the electromagnetic field with selected biological processes. A similarly unique role of bicarbonate in very low frequency pulsating fields was observed also in the efflux of Ca^{2+} from brain tissue *in vitro* [2]. Interestingly, at a concentration of 2.4 mM bicarbonate, the pulsating field stimulated Ca^{2+} efflux, whereas at 10× that bicarbonate concentration, the effect was reversed as the field depressed the Ca^{2+} efflux [12]. Peaks and reversals of effects were keenly observed by us with a number of agents (Mg^{2+}, Ca^{2+}, phosphate, bicarbonate, NaF, ethanol, and the signal's amplitude), which in optimal concentrations brought about the greatest stimulation of the Ca-uptake by chick tibia rudiments in the field [7, 8].

Mechanistic considerations cannot be but speculative at this time. The only though important conclusion is: In the absence of bicarbonate, there was no effect of the electromagnetic field on the Ca-uptake by chick tibia rudiment. Since, in spite of the bicarbonate's presence, the field had no effect on the Ca-uptake by either the blank, dead tibia, and nonliving artificial ion-sorbing systems [7], as

well as by isolated rat liver mitochondria and microsomes [8], a reasonable suggestion is that bicarbonate, a live system, and the plasma membrane are the specific requirements for coupling of the electromagnetic signal with the Ca-uptake by the chick tibia.

The possible participation of the cell membrane in signal coupling is consistent with several observations and hypotheses. One, the plasma membrane has been attributed a still undefined role in the spinning of suspended live cells under the action of high frequency electromagnetic fields [13]. Two, according to the electrical information transfer hypothesis, the electromagnetically induced currents must couple nonFaradaically with ion transport across the membrane [10]. Three, the same very low frequency fields that stimulated Ca-uptake by the chick tibia promoted also Na^+ flux and ATPase activity across red blood cell membranes [14, 15]. Four, since the same fields stimulate DNA synthesis and cell proliferation [3, 4, 16], which are regulated by the plasma membrane Na^+K^+ATPase [3, 17], the role of membrane ATPases in coupling of the signal with the biological processes becomes inevitable. Five, if a bicarbonate-dependent Ca^{2+} ATPase or Ca^{2+}-activated ATPase regulates Ca^{2+} transport across cell membranes [18], the involvement of this ATPase may explain the effect of bicarbonate on the stimulation of Ca mobilization that ensued when chick tibia (above), cartilage and bone tissues [1] and brain cells [2] were placed in the very low frequency fields. Six, these same fields markedly reduced production of cyclic AMP by cultured bone cells in response to parathyroid hormone [11], suggesting a direct stimulation of the membrane ATPase by the field in line with the hypothesis of a seesaw connection between ATPase and adenyl cyclase [17, 19].

Although every indication is that the electromagnetic field acts at least on the plasma membrane ATPases (and possibly also on other membrane systems), any attempt at formulating a mechanism would be premature at this time. We must first ascertain if the induced current of the field couples directly with the transmembrane ion fluxes, as the electrical information transfer hypothesis predicates [10], or the field acts on some vital part of the ATPase enzyme itself, which then drives the flux.

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