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Effects of pulsed electromagnetic fields on rat skin metabolism

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In an attempt to approach the mechanism of action of pulsed electromagnetic fields (PEMF) on biological systems, the effects on protein synthesizing activity and on membrane transport have been examined in rat skin PEMF characterized by specific physical parameters stimulate the incorporation of L-[U-^{14}C]isoleucine into the proteins of rat skin as well as the α-amino[1-^{14}C]isobutyric acid uptake during incubation in buffer medium with extracellular electrolyte composition. Analogous incubation experiments carried out in an intracellular medium results in an inhibitory effect of PEMF on both biological functions. Addition of 10^{-3} M ouabain to the incubation medium, partially blocking the Na^+/K^+-ATPase pump mechanism, apart from reducing amino acid transport, results in an overall disappearance of any stimulatory effects by PEMF. PEMF applied to the skin in the presence of 10^{-3} M 2,4-dinitrophenol uncoupling the oxidative phosphorylation in the mitochondria and seriously restricting protein synthesis, still provides a limited stimulatory effect on protein synthesizing activity and on membrane transport. The effects of PEMF may well be understood by an increased availability of precursor elements controlled at the cell membrane level. Indeed the observed effects may even be simulated outside electromagnetic fields by modifications in the electrolyte composition of the incubation medium

Introduction

Electric and electromagnetic fields have been shown to affect metabolism when clinically applied in the treatment of non-union of bone [1–3] Furthermore epiphyseal growth and nerve generation have been reported to be stimulated by electro and electromagnetic application [4–6] In experimental studies pulsed electromagnetic fields (PEMF) appear to interact with biological systems at different levels, by reducing cyclic AMP, by affecting Ca^{2+} flow patterns and by stimulating the protein synthesizing activity in skin tissue [7–12] Even plant growth regulator systems have been reported to be affected by PEMF [13]

In order to approach the mechanism of action of PEMF, protein synthesis and membrane transport activity have been examined in rat skin

Materials and Methods

The skin from the back of locally inbred male Wistar R rats was used during the rest phase after the first haircycle at 21 days of age The hair was plucked skin flaps measuring 5 by 6 cm and 0 5 mm thickness were isolated After the manual removal of excessive subcutaneous fat and connective tissue with a scalpel, the skin was longitudinally halved One half was to be used as non-treated control, while the other half was treated with a PEMF (Fig 1) Each skin segment was attached with wooden plugs on a perspex frame, placed in a plastic container, submerged in either a buffer medium at pH 7 4 characterized by an extracellular high Na^+, low K^+, or an intracellular low Na^+, high K^+ electrolyte composition (Table I) 100 ml of incubation medium always contained 100000 units of penicillin-K (Sigma, St Louis, MO) 100 mg of streptomycin sulfate (Sigma) and 20 mg of gentamycin sulfate (Sigma) as well as 1 ml of a L-amino acid mixture (Vamin, Vitrum, Stockholm 12, Sweden) This resulted in a final concentration of 0 55 mmol of total free amino acids and 0 03 mmol of L-isoleucine in 100 ml of incubation medium 40 μCi of L-[U-^{14}C]isoleucine (spec radioact 150 mCi/mmol, Amersham International, Amersham, Bucks, U K) were added to 100 ml of buffer to follow the incorporation into the proteins To evaluate the amino acid transport activity through the cell membrane, 40 μCi of α-amino[1-^{14}C]isobutyric acid (AIB) (spec radioact 60 mCi/mmol, Amersham International) were added to 100 ml of buffer

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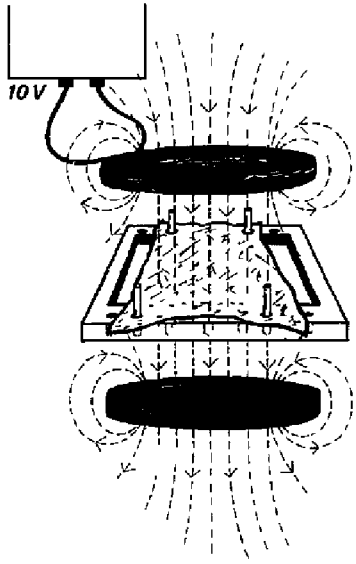

Fig 1 Schematic representation of skin tissue fixed on a perspex frame and treated by inductively coupled PEMF

During the incubation periods from 15 min up to 360 min in a thermostatically controlled waterbath at 37 °C a PEMF was applied to one skin segment while the collateral half was identically incubated outside the reach of the electromagnetic field

Ouabain (Sigma) to block the Na^+/K^+-ATPase and 2,4-dinitrophenol (DNP, Sigma) to uncouple the oxidative phosphorylation were supplementary added to specific experiments in final concentrations of 10^{-3} molar The electromagnetic field source (E S A T., Faculty of Engineering, University of Leuven) contained five variable parameters driving voltage from 5 to 20 V, pulse frequency from 0 5 to 10 kHz, pulse widths from 10 to 150 μs, pulse-burst frequency from 1 to 70 Hz and number of pulses per pulse burst from 1 to 100. The wave-form applied to the coils was rectangular and the field was applied perpendicularly on the surface of the skin [9]

TABLE I

Electrolyte composition of the incubation buffers

Composition	Extracellular characteristics	Intracellular characteristics
Na^+ (mequiv)	115	10
K^+ (mequiv)	10	115
Cl^- (mequiv)	15	15
HCO_3^- (mequiv)	10	10
HPO_4^{2-} (mequiv)	85	85
$H_2PO_4^-$ (mequiv)	15	15
Glucose (mM)	194	194
Osmolality (mosmol/kg)	330	320

After incubation with isoleucine, 100 mg portions of skin tissue were weighed and placed in 5 ml of 20% trichloroacetic acid (TCA, Merck, Darmstadt, F R G) to precipitate the proteins After centrifugation, the tissue was furthermore washed three times with 10 ml of 5% TCA and finally placed in 3 ml of Lumasolve (Lumac Systems AG, Basel, Switzerland) to be dissolved overnight in an oven at 60 °C After the addition of 10 ml of Lipoluma (Lumac Systems AG) the radioactivity in the samples was counted in a liquid scintillation spectrometer (Rack Beta, LKB, Wallac, OY, Turku, Finland) After incubation with AIB, the tissues were washed four times with 10-ml fractions of ice-cold physiological saline containing 30 mmolar non-radioactive AIB (Sigma), finally to be dissolved in Lumasolve and processed as described for the amino acid incorporation

The radioactivity incorporated into the proteins or taken up by the cells was expressed as disintegrations per min (dpm) ± S E Every experiment was carried out on skin of at least six different rats Statistical evaluations of the significance was carried out according to Student's *t*-test

Results

Effects of PEMF on protein synthesizing and membrane transport activities

Specific inductively coupled magnetic fields applied perpendicularly to the skin tissue were able to affect metabolism With driving voltages between 5 and 20 V and with 20 pulses per pulse burst, stimulatory effects of identical magnitude were obtained with pulse frequencies between 1 and 5 kHz, pulse-widths of 10 to 20 μs and pulse-bursts frequencies between 10 and 30 Hz. Instead of pulse bursts, continuously running pulses resulted in analogous observations Outside these limits modifications of pulse frequencies, pulse-widths or pulse-bursts frequencies considerable reduced the stimulatory effects These electromagnetic fields applied during incubation of the skin at 37 °C for 15, 30, 60, 120, 180 and 360 min in a buffer medium characterized by an extracellular electrolyte composition resulted in an increased incorporation of L-[U-^{14}C]isoleucine into the proteins amounting to 27%, 19%, 20%, 21% ($P < 0.05$), 26% ($P < 0.01$) and 52% ($P < 0.01$), respectively (Fig 2)

Analogous experiments carried out in the presence of α-amino[1-^{14}C]isobutyric acid led to an increased amino acid analogue uptake ($P < 0.01$) by the skin amounting to respectively 41%, 48%, 40%, 23%, 25% and 12 5% in function of incubation time (Fig 3)

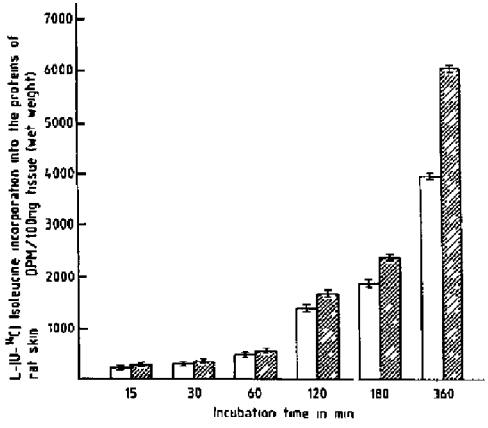

Fig. 2 The effects of PEMF on protein synthesizing activity The denuded skin from the back of 21 days old Wistar R rats was longitudinally halved and incubated for 15, 30, 60, 120, 180 and 360 min in 100 ml of buffer characterized by extracellular characteristics (Table I) at 37°C containing 40 μCi of L-[U-^{14}C]isoleucine (spec radioact 150 mCi/mmol) One half of the skin served as control while the other half was electromagnetically stimulated with continuously running pulses characterized by a driving voltage of 10 V, a pulse frequency of 5 kHz and pulse widths of 20 μs After incubation 100 mg skin tissue portions were taken and prepared for liquid scintillation counting. The amino acid incorporation into the proteins of control skin is represented by open blocks and the PEMF-treated tissue by shaded blocks Each value represents the mean of eight experiments ± S E

Interaction of buffer composition on PEMF effects

Although PEMF application on skin tissue for a period of 120 min in a buffer medium characterized by extracellular ionic composition, stimulated the incorporation of L-[U-^{14}C]isoleucine into the proteins by 18% ($P < 0.01$), analogous experiments carried out in an intracellular medium resulted in an inhibitory effect of PEMF by 14% ($P < 0.01$) The control incorporation values into an extracellular medium were slightly higher than those observed in an intracellular medium were slightly higher than those observed in an intracellular medium The α-amino[1-^{14}C]isobutyric acid uptake during PEMF stimulation in an extracellular medium was increased by 19% ($P < 0.01$) while PEMF stimulation applied during incubation in an intracellular medium decreased label uptake by 9% ($P < 0.05$) Amino acid transport was already considerably reduced in the intracellular medium compared to the extracellular buffer (Table IIA)

Without applying any PEMF, the transfer of the skin tissue from an extracellular medium, where it had been preincubated for 30 min, into an intracellular medium containing the amino acid tracer, reduced the incorporation by 13% ($P < 0.01$) over a further 60 min of incubation, compared to tissue continued to be incubated in the extracellular buffer However when after preincubation in an intracellular medium, incubation with labeled amino acids present took place in an extracellular medium, the incorporation was increased by 29% ($P < 0.01$) Analogous experiments without any PEMF stimulation carried out in the presence of α-amino[1-^{14}C]isobutyric acid resulted in an inhibition of 31% ($P < 0.01$) when the tissue after preincubation in extracellular medium was transferred to intracellular buffer containing the labelled amino acid analogue Transfer after a preincubation in an intracellular medium to an extracellular buffer containing the tracer resulted into a stimulatory effect of 53% ($P < 0.01$) (Table IIB)

Interaction of ouabain on PEMF effects

In the presence of ouabain added to a buffer medium characterized by extracellular electrolyte composition, the stimulatory effects of PEMF on amino acid incorporation into the proteins disappeared completely Equally the amino acid transport already being reduced

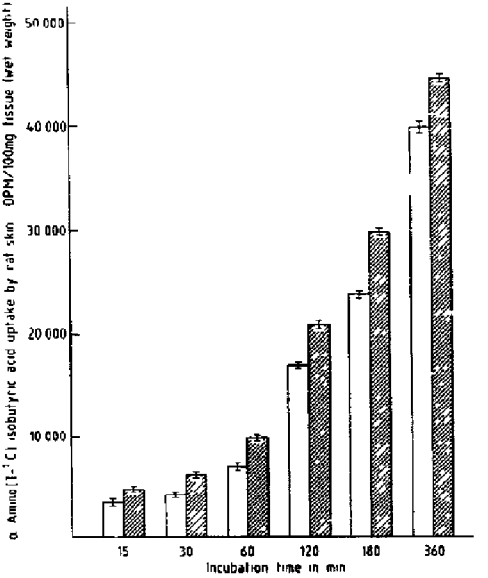

Fig 3 The effects of PEMF on membrane transport Skin tissue flaps were prepared and incubated with or without PEMF treatment identically as in Fig 1 but in the presence of 40 μCi of α-amino[1-^{14}C]isobutyric acid (spec radioact 60 mCi/mmol) After incubation the tissue was prepared for liquid scintillation counting. The control amino acid analogue uptake is expressed by open and the PEMF-treated one by shaded blocks Each value represents the mean of eight experiments ± S E.

TABLE II

Effects of electrolyte composition on PEMF interaction and on protein synthesis and membrane transport

A Skin tissue flaps prepared as in Fig. 2 were incubated for 120 min either in buffer medium characterized by an extracellular (E) and intracellular (I) electrolyte composition in the presence of L-[U-^{14}C]isoleucine or α amino[1-^{14}C]isobutyric acid, with and without PEMF stimulation

B After a preincubation period of 30 min either in (E) or (I) medium, each tissue was transferred, respectively, into (I) or into (E) medium and further incubated for 60 min in the presence of L-[U-^{14}C]isoleucine or α amino[1-^{14}C]isobutyric acid without PEMF treatment. Each value represents the mean of eight experiments ± S E

Incubation characteristics	L-[U-^{14}C]-Isoleucine incorporation (dpm/100 mg tissue (wet wt))	α-Amino[1-^{14}C]-isobutyric acid uptake (dpm/100 mg tissue (wet wt.))
A		
E. Controls	952 ± 39	13946 ± 251
E. PEMF-stimulated	1175 ± 21	16507 ± 439
I Controls	904 ± 42	10934 ± 237
I PEMF-stimulated	789 ± 24	10149 ± 303
B		
Preincubation and incubation in E	947 ± 41	14507 ± 406
Preincubation in E followed by incubation in I	810 ± 30	10016 ± 356
Preincubation and incubation in I	882 ± 22	11102 ± 7 9
Preincubation in I followed by incubation in E	1095 ± 29	16908 ± 995

by ouabain could not further be stimulated by the application of PEMF (Table III)

Comparing the sole effect of the presence of 10^{-3} M ouabain without considering PEMF effects, over incubation periods of 120, 240 and 360 min, isoleucine incorporation into the proteins became reduced by, respectively, 20%, 10% and 7% ($P < 0.05$) while α-aminoisobutyric acid uptake by this tissue over periods of 120, 240 and 360 min became reduced in the presence of ouabain by 26%, 16% and 22%, respectively ($P < 0.01$)

Interaction of 2,4-dinitrophenol (DNP) on PEMF effects

Although drastically reducing protein synthesis, in the presence of DNP, a stimulatory effect by PEMF was still observed amounting to 19% ($P < 0.05$), 10% ($P < 0.05$), 17% ($P < 0.05$), 23% ($P < 0.01$) and 22% ($P < 0.01$) over incubation periods of, respectively, 60, 120, 180, 240 and 360 min The α-aminoisobutyric acid uptake remained stimulated by PEMF in the presence of DNP by 4%, 3%, 7% ($P < 0.05$), 7 5% ($p < 0.05$) and 7% ($P < 0.05$) over the same incubation periods The

TABLE III

Effect of PEMF on protein synthesizing activity and on membrane transport in the presence of ouabain

Ouabain in a concentration of 10^{-3} M was added to the extracellular incubation buffer in which rat skin tissue was incubated for 60, 120 180, 240 and 360 min in the presence of L-[U-^{14}C]isoleucine to evaluate the incorporation into the proteins or in the presence of α-amino[1-^{14}C]isobutyric acid to follow the transport of the amino acid analogue into the cells Preparation of the samples and treatment with PEMF took place as in Fig. 2 Each value represents the mean of eight experiments ± S E

Incubation time (min)	L-[U-^{14}C]Isoleucine incorporation (dpm/100 mg tissue (wet wt.))		α-Amino[1-^{14}C]isobutyric acid uptake (dpm/100 mg tissue (wet wt))	
	− PEMF	+ PEMF	− PEMF	+ PEMF
60	450 ± 35	595 ± 47	7455 ± 544	7462 ± 246
120	845 ± 39	820 ± 52	12723 ± 488	12712 ± 421
180	1770 ± 62	1752 ± 58	17531 ± 532	17988 ± 386
240	2688 ± 44	2754 ± 64	18262 ± 568	18862 ± 531
360	3445 ± 42	3421 ± 61	20222 ± 498	20669 ± 359

significance of this stimulatory effect however became greatly reduced (Table IV)

Compared to control incubations, carried out in extracellular buffer medium, DNP in a concentration of 10^{-3} M reduced the amino acid incorporation by 90% ($P < 0.01$) while the α-aminoisobutyric acid transport was only reduced by approx 10% ($P < 0.05$) over periods of 120, 240 and 360 min

Discussion

It has already been observed that the stimulatory effects of PEMF on protein synthesizing activity and on membrane transport are essentially conditioned by

TABLE IV

Effect of PEMF on protein synthesizing activity and on membrane transport in the presence of 2,4 dinitrophenol (DNP)

Skin tissues prepared as in Table II were incubated in buffer characterized by extracellular electrolyte composition containing 10^{-3} M of 2,4-dinitrophenol in the presence of L-[U-^{14}C]isoleucine or of α-amino[1-^{14}C]isobutyric acid PEMF treatment was carried out as described in Fig 2 Each value represents the means of eight experiments ± S E

Incubation time (min)	L-[U-^{14}C]Isoleucine incorporation (dpm/100 mg tissue (wet wt))		α-Amino[1-^{14}C]isobutyric acid uptake (dpm/100 mg tissue (wet wt))	
	− PEMF	+ PEMF	− PEMF	+ PEMF
60	187 ± 14	242 ± 11	9547 ± 366	10120 ± 412
120	259 ± 17	314 ± 14	12354 ± 569	13028 ± 406
180	293 ± 20	375 ± 21	17133 ± 347	18586 ± 387
240	382 ± 23	492 ± 18	20082 ± 334	21674 ± 321
360	501 ± 24	622 ± 17	28120 ± 581	30064 ± 536

specific electromagnetic parameters [9] Several experimental approaches have illustrated that electric fields induce ATP synthesis in different energy-transducing membranes [14–16] Kinetic studies on the ouabain inhibitor effect indicate that Na^+/K^+-ATPase is capable of responding to a transmembrane electric field [17] Furthermore several biological activities stimulated by pulsed electric fields appear to be mediated by Na^+/K^+-ATPase [16] Thus stimulated pumping of ions against concentration gradients can be achieved by energy derived from external electric fields [15,16] Our present results seem to provide illustrative and confirmatory evidence of the 'electroconformational coupling' concept proposed by Tsong and Astumian [18] A protein such as Na^+/K^+-ATPase provided with two accessible conformation states of different electric moments may absorb free energy from an external oscillating electric field to be transduced into chemical or transport work Theoretically as calculated on the basis of a four-state model, one cycle of sinusoidal electric field could effectively induce the enzyme to turnover once when the system is in resonance with the alternating field [19,20] Enzyme recycling by modulation of the stationary potential to an oscillating wave form would essentially occur in a changing field [18] As pulsed electromagnetic fields in proximity of the tissue induce alternating currents and as pulse frequencies exceeding 5 kHz reduce or abolish the stimulatory effects on metabolism, it would appear that an accelerated change in polarity could destroy the electroconformational coupling, blocking precursor substances and ions to transgress the cell membrane

It has already been suggested that stimulatory effects of PEMF on protein metabolism may be due to an increased availability of precursor elements across the cell membrane [7,21]

Furthermore as membrane transport stimulation becomes proportionally reduced over the incubation period, stimulation of the amino acid incorporation continues to increase with time, indicating protein synthesis consecutive to the availability of precursors Moreover, the kind of PEMF effect greatly depends on the electrolyte composition of the incubation medium For the amino acid incorporation into the proteins as for the AIB transport across the cell membrane, PEMF treatment results in an inhibitory effect on both parameters when PEMF is applied during incubation of the skin in a buffer characterized by an intracellular electrolyte composition The crucial importance of the electrolyte composition is clearly demonstrated by the evaluation of metabolism in intra- and extracellular electrolyte buffer after an initial preincubation in extra- or intracellular buffer, illustrating the overall reduction in metabolism during incubation in an intracellular medium

In order to block artificially or considerably inhibit either membrane transport or protein synthesis the effects of ouabain and of DNP are analysed

It has previously been observed that addition of ouabain in vitro blocking the Na^+/K^+-ATPase or removal of Na^+ from the incubation medium inhibit incorporation of leucine into proteins of rat skeletal muscle [22]

Considering the various more or less specific transport systems serving different groups of amino acids and analogues we essentially analyze the AIB uptake mainly by the A system which is inhibited by ouabain Cellular uptake of L-isoleucine is equally performed by the A system although outward transport occurs more likely by the L system [23,24]

In the presence of ouabain AIB transport as well as the isoleucine incorporation into the proteins is reduced while PEMF treatment remains without effect Ouabain added to the skin tissue in an extracellular incubation medium considerably inhibits the amino acid transport while the simultaneous reduction in protein synthesizing activity seems secondary to the reduced availability of precursors The described digitalis insensitivity of the rat appears essentially due to an excessively fast hepatic clearance for ouabain, demonstrated by in vivo experiments The binding of glycosides to Na^+/K^+-ATPase of intact cells is less species dependent and is promoted by intracellular Na^+ and inhibited by extracellular K^+ concentrations [25–27] Our observations are in agreement with the initial assumption that a common gradient could be responsible for K^+ and amino acid transport, with the energy required for the amino acid transport, provided by the potential energy of the potassium gradient Thus amino acid uptake will be reduced when extracellular potassium is increased competing with amino acid uptake or when extracellular Na^+ levels decrease at constant K^+ concentrations, thus mainly implicating the Na^+ electrochemical potential gradient in amino acid transport mechanism [24,28,29]

With DNP however, uncoupling the oxidative phosphorylation in the mitochondria, PEMF treatment continues to stimulate the AIB uptake across the membrane and the precursor incorporation into the proteins although at a much reduced rate Although ATP levels may remain unaffected during the uncoupling, ATP synthesis does not occur and the requirements for protein synthesis of high-energy intermediates of the oxidative phosphorylation appear confirmed in this experimental system Inhibitory effects on DNA synthesis in the presence of DNP has been attributed to the failure to synthesize proteins essential for nuclear DNA formation [30] DNP added to a buffer medium characterized by extracellular electrolyte composition essentially reduces protein synthesis while the concommittant slight reduction in amino acid transport, without excluding an additional membrane effect, may even be due to the generally reduced metabolic activity requiring only small

quantities of amino acids to be available for incorporation

The present experimental approach to the mechanism of action of PEMF indicates that electromagnetic fields affect protein synthesizing activity through modifications in transport activity at cellular membrane level which adequately relate to the 'electroconformational coupling' concept [18] Changes in electrolyte and amino acid transport induced by PEMF explain the observed metabolic effects which can be simulated outside electromagnetic fields by modifications in the electrolyte composition of the incubation medium

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