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A strong static magnetic field inhibits the poly-ADP-ribosylation of proteins in human kidney T1-cells

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Abstract

Exposure of monolayers of human kidney T1-cells to a strong static magnetic field (1.4 T, 30 min at 37°C) reduced the poly-ADP-ribosylation (PADPR) of the total cellular proteins to about 60% of its normal metabolic level. The inhibition was transient and showed dependence on the time of exposure and on the strength of the applied static magnetic field. PADPR relaxation kinetics followed a non-monotonous course reaching the metabolic level not earlier than 24 h after the termination of magnetic field interference. It is suggested that the magnetic field interacts with the cellular membrane components affecting the transfer of signals which controls the PADPR of proteins.

INTRODUCTION

Biological systems have been generally shown to respond to magnetic fields [1,2]. A static magnetic field (SMF) of 1.4 T affected thymidine kinase activity in bone marrow cells of whole body exposed mice [3,4]. Other studies implicated SMF effects on acetyl choline receptor–ligand binding in *T. californica* electric organ membranes [5], pineal cAMP content in the rat [6], and release of lysozyme and lactate dehydrogenase in human polymorphonuclear leukocytes [7]. However, the biophysical mechanisms underlaying the biological responses to SMF have remained ambiguous.

Poly-ADP-ribosylation (PADPR), a post-translational modification of cellular proteins, mainly nuclear proteins [8,9], has been shown to be involved in a variety

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of metabolic responses, such as DNA repair [10], cell cycle [9,11], cell differentiation and gene expression [9,12] and transfer of signals across the membrane via adenylate cyclase [12], G-protein [13] and ADP-ribosylation factor [14]. An increase and decrease in PADPR of nuclear proteins causes relaxation or condensation of the chromatin respectively [15]. These findings implicated the cellular PADPR reactions in intricate biological control processes [9,16–18]. We have observed that PADPR of cellular proteins is sensitive to external interventions [19] and could be the basis of biological responses.

Because of the obvious involvement of PADPR of proteins in the cellular metabolism in more than one way and because of the ability of the magnetic field to affect the cellular systems, we have monitored the PADPR of cellular proteins of human kidney T1-cells following exposure to a SMF at 37°C. We have addressed this investigation to see whether the PADPR of proteins is affected by SMF and whether this effect is transient or permanent. With this line of investigation we hope to contribute to the understanding of the effects of the static magnetic field at the metabolic level.

EXPERIMENTAL DETAILS

Chemicals

All chemicals were of highest purity grade and were used without further purification. ^{32}P-NAD$^+$ (high specific activity) was purchased from New England Nuclear, UK. Glass double-distilled water was used for preparing solutions.

Cell culture

Human kidney T1-cells were grown in Eagle's basal medium supplemented with 10% fetal calf serum, 0.1% antibiotics and 0.9% glutamine (full medium) in Leighton tubes (Nunc, Denmark) in a CO_2-incubator (5% CO_2; 95% humidity). 0.3×10^6 cells were seeded in 3 ml of full medium in each Leighton tube and were used for experiments on the fifth day when a confluent monolayer of 1.3 to 1.4×10^6 cells had fully grown in each Leighton tube.

Exposure to static magnetic field

An electromagnet, type B-E, 25 V (Bruker GmbH, Karlsruhe, Germany) was used to generate the SMF. To the T1-cell monolayer in 3 ml of full medium in Leighton tubes, 5 ml of fresh full medium was added and the tubes were placed transaxially to the poles of the SMF in a thermostated chamber at 37°C. The magnet was switched on to deliver the field strengths of 0.1, 0.425, 0.7, 1.095 and 1.4 T. The exposures were continued for 2, 5, 10 or 30 min at 37°C. Immediately after the termination of the exposure, T1-cells were assayed for PADPR of the cellular proteins. In another experiment, after the 30 min exposure to SMF (1.4 T

at 37°C), T1-cells were incubated at 37°C in full medium for various time intervals before the PADPR of proteins was monitored. The controls were maintained at 37°C for the same period of time without the SMF.

Assay of poly-ADP-ribosylation

The method, a modification of Surowy and Berger [11], has been described in detail in another communication to be published elsewhere [19]. In short, after discarding the medium from the Leighton tubes, the T1-cell monolayer was gently washed twice with 2 ml of PBS, pre-warmed to 37°C. The cells were then permeabilized by 6 ml of permeation (P) buffer (final concentrations: 10 mM Tris-HCl, pH 7.8/4°C; 1 mM EDTA; 4 mM $MgCl_2$; 30 mM 2-mercaptoethanol) for 15 min in ice. The P-buffer was aspirated out, and to the permeabilized T1-cell monolayer 0.6 ml of P-buffer and 0.3 ml of reaction buffer (final concentrations: 80 mM Tris-HCl, pH 7.8/37°C; 222 kBq ^{32}P-NAD^+ equivalent to approximately 1 μM NAD^+) were added and gently mixed. The Leighton tubes were placed horizontally in a water bath shaker at 37°C for 15 min. The reaction was terminated by the addition of 9 ml of cold 15% trichloroacetic acid (TCA). The tubes were allowed to stand for 30 min in ice. The 15% TCA insoluble precipitates were collected on a GF/C filter disc (2.1 cm; Whatman), by carefully scraping the entire content of the Leighton tubes, and washed with 25 ml cold 15% TCA. The discs were dried and the residual activity on the discs was counted in a Packard liquid scintillation counter using an instant scint. gel scintillation cocktail.

RESULTS

All results shown here are the mean $\pm$ SD of at least nine replicates. Each experiment had its own control, normalized to 100%. All controls were sham-exposed under strictly controlled conditions.

Poly-ADP-ribosylation as a function of duration of exposure to SMF

The T1-cell monolayer was exposed in full medium at 37°C to a constant field of SMF (1.4 T) for different time intervals. PADPR of total cellular proteins was monitored immediately after the exposure. The results are shown in Fig. 1. PADPR was inhibited to about 37% of the control after 2 min of exposure, to about 50% after 5 min exposure and reached a plateau of about 60% at 10 and 30 min exposures.

Poly-ADP-ribosylation as a function of field strength of SMF

The T1-cell monolayer was exposed in the full medium at 37°C to different field strengths of SMF for 10 min. PADPR of total cellular proteins was monitored immediately after the exposure. Figure 2 shows that the PADPR of proteins in

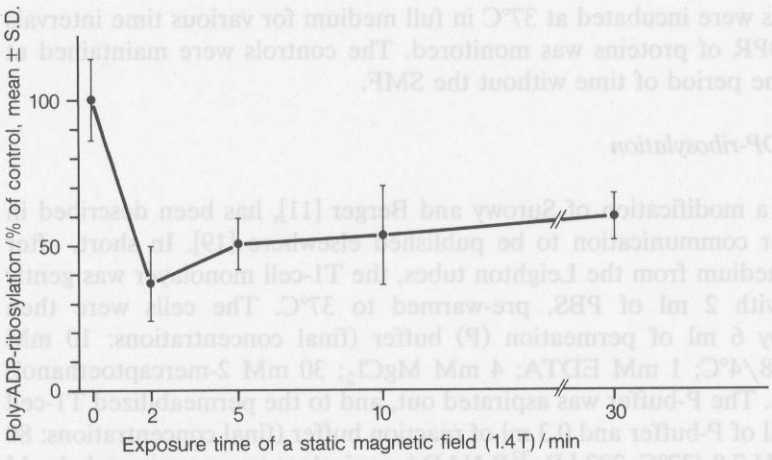

Fig. 1. Poly-ADP-ribosylation of cellular proteins of human kidney T1-cell monolayers as a function of duration of exposure at 37°C in full BME medium to a static magnetic field of 1.4 T. The results are mean ± SD of 9 to 27 replicates.

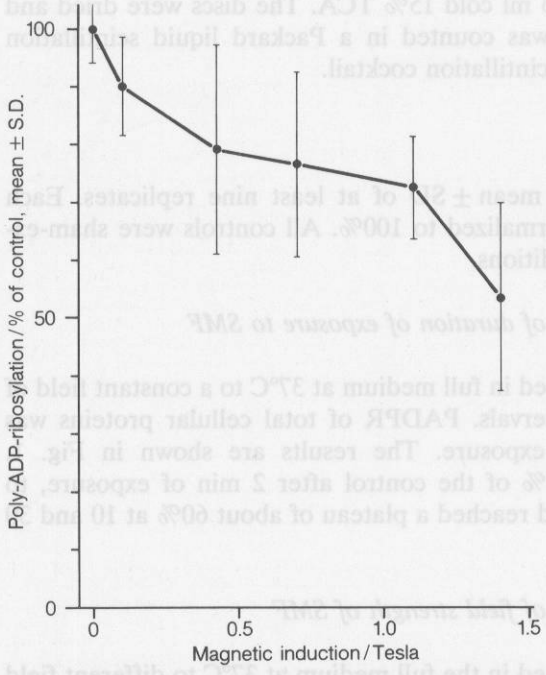

Fig. 2. Poly-ADP-ribosylation of cellular proteins of human kidney T1-cell monolayers as a function of strength of static magnetic field at 37°C in full BME medium for 10 min. The results are mean ± SD of 9 to 29 replicates.

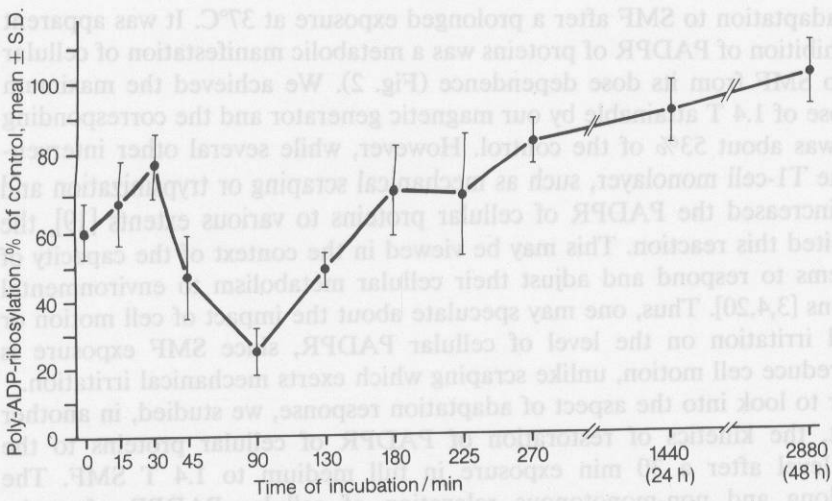

Fig. 3. Poly-ADP-ribosylation of cellular proteins of human kidney T1-cell monolayers as a function of time of incubation in full BME medium at 37°C following exposure to a static magnetic field of 1.4 T at 37°C in full BME medium for 30 min. The results are mean ± SD of 9 to 36 replicates.

T1-cells decreased with increasing field strength of SMF in a dose dependent manner reaching a value of about 53% of the control by 1.4 T.

Poly-ADP-ribosylation as a function of time after termination of exposure to SMF

The T1-cell monolayer was exposed in the full medium at 37°C to a SMF (1.4 T) for 30 min and was allowed to stand at the same temperature without the SMF for different periods of time before the total cellular PADPR was monitored (Fig. 3). The level of PADPR increased from 62% (measured immediately after the termination of SMF) to 77% of the control in the first 30 min after the SMF exposure. A second inhibition to about 25% of the control followed in the next 60 min. After this period of incubation the PADPR of cellular proteins showed a rise towards the control value. 24 h after the SMF exposure, the PADPR of total cellular proteins was about 93% of the control and reached a value of 103% after 48 h.

DISCUSSION

Various interventions, including SMF, have been found to affect the PADPR of cellular proteins of T1-cell monolayer [19]. We report here that the inhibition of PADPR of proteins of T1-cells by an exposure to 1.4 T of SMF at 37°C is already manifested at 2 min, the shortest time monitored. When the exposure continued for 30 min, the decrease in the level of PADPR of proteins was less compared with the 2 min value (Fig. 1). This indicates that T1-cells have a tendency to develop a

biological adaptation to SMF after a prolonged exposure at 37°C. It was apparent that the inhibition of PADPR of proteins was a metabolic manifestation of cellular response to SMF from its dose dependence (Fig. 2). We achieved the maximum possible dose of 1.4 T attainable by our magnetic generator and the corresponding inhibition was about 53% of the control. However, while several other interventions on the T1-cell monolayer, such as mechanical scraping or trypsinization and agitation, increased the PADPR of cellular proteins to various extents [19], the SMF inhibited this reaction. This may be viewed in the context of the capacity of living systems to respond and adjust their cellular metabolism to environmental interventions [3,4,20]. Thus, one may speculate about the impact of cell motion or mechanical irritation on the level of cellular PADPR, since SMF exposure is known to reduce cell motion, unlike scraping which exerts mechanical irritation.

In order to look into the aspect of adaptation response, we studied, in another experiment, the kinetics of restoration of PADPR of cellular proteins to the metabolic level after a 30 min exposure in full medium to 1.4 T SMF. The relatively long and non-monotonous relaxation of cellular PADPR after the withdrawal of SMF, as shown in Fig. 3, indicates that the cellular components involved in the expression of the SMF effect on the PADPR process are stable biomolecules of relatively long half life and not molecules with a high turn-over rate. Since, PADPR of proteins involves membrane bound components, such as adenylate cyclase [12], GTP-binding proteins [13], and ADP-ribosylation factor [14], it can be argued that SMF influences membrane components involved in regulating PADPR of cellular proteins. It is known that anisotropic, diamagnetic macromolecules of the membrane change their orientation under the influence of SMF [1,21,22]. Gravitational force, which should also cause change in the membrane organization due to condensation of membrane components, has been shown by us to cause inhibition of PADPR of T1-cell proteins [19]. In the light of this, it appears that exposure of T1-cells in full medium to 1.4 T SMF at 37°C for 30 min induces changes of the orientation of membrane components in a short time. These changes may have signalled inhibition of PADPR of cellular proteins. When SMF is withdrawn, the membrane components reorient themselves with time resulting in the restoration of the PADPR of proteins to the metabolic level.

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