HUMAN HEAT SHOCK GENE EXPRESSION AND THE MODULATION OF PLASMA MEMBRANE Na^+,K^+-ATPase ACTIVITY

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1. Introduction

The potential of hyperthermia in human cancer treatment has been recognised for some time [1,2], although a practical problem is the development of 'thermotolerance' [3]. Brief hyperthermic treatment of HeLa cells (45°C; 10 min) followed by an obligatory 'development' period of 2 h at 37°C results in the maximal induction of specific heat shock proteins (HSPs) at M_r 100 000, 72 000–74 000 and 37 000 [4]. The proteins at 100 000 M_r and 37 000 M_r are encoded by single mRNA species while the 72 000–74 000 M_r group are coded for by 7 mRNAs which show some sequence homologies but have widely differing sizes (1.9–6.3 kilobasepairs) [5,7]. In all cases the mRNAs are bimorphic, existing in poly(A^+) or poly(A^-) forms [5]. Northern blot analyses using cloned cDNA sequences encoding the 72 000–74 000 M_r group [7] confirm experiments with actinomycin D [4] suggesting control of gene expression at the transcriptional level. High M_r mRNA precursors at 15.8 kilobasepairs do not appear in nuclei until 1–2 h.

It has been speculated that HSPs may be required for the 'repair' or 'recovery' of cellular homeostasis. They may be involved in hexose transport and/or metabolism [9]. The conditions required for the induction of human HSP synthesis are quite similar to those that lead to the development of 'thermotolerance' [8]. In tumours a high rate of aerobic glycolysis is often encountered [10]. Racker et al. suggest that a rate limiting reaction in glycolysis is the hydrolysis of ATP to ADP and P_i. In certain tumour cells the responsible catalyst is the Na^+,K^+-ATPase of the plasma membrane [11,13].

Brief hyperthermia results in considerable loss of HeLa cell Na^+,K^+-ATPase activity. However subsequent incubation of these cells at 37°C for 2 h results in a partial recovery of activity. Evidence is presented to suggest HSP gene expression involvement in the modulation of Na^+,K^+-ATPase activity in HeLa cells.

2. Materials and methods

2.1. *HeLa cell culture: Heat and sodium arsenite treatment*

HeLa cells were grown in culture as monolayers in Eagle's minimal essential medium (Glasgow modification) supplemented with 10% (v/v) calf serum [4]. 2×10^7 cells were seeeded and grown for 2 days in rotating 80 oz. Winchester bottles. Hyperthermic treatment was carried out by immersion of the bottles in a water bath at 45°C for various times and in certain experiments the cultures were returned to normal growth temperature of 37°C for further incubation.

In other experiments the monolayer cultures were simply treated with sodium arsenite (5×10^{-5} M) at 37°C for 2 h.

2.2. *Estimation of Na^+,K^+-ATPase activity*

After harvesting the HeLa cells from each culture were washed with saline buffered with calcium acetate buffer (pH 7) suspended in 10 mM Tris–HCl (pH 7) at 4×10^6 cells/ml and disrupted by 20–25 passages in a glass, hand homogeniser.

Ouabain-sensitive Na^+,K^+-ATPase was determined following the method in [24]: 0.2 ml cell homogenate was incubated for 2 h at 37°C with 0.9 ml substrate solution containing 2 mM ATP, 40 mM histidine–imidazole buffer (pH 7.1), 80 mM NaCl, 33 mM KCl and 2 mM $MgCl_2$. The reaction was stopped by the addition of 1 ml ice-cold 10% (w/v) trichloroacetic acid and after removal of the precipitate, free phos-

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phate was determined in the supernatant using the procedure in [25].

Protein was determined as in [26] using bovine serum albumin as standard.

3. Results

In fig.1 it can be seen that treatment of HeLa cells at 45°C leads to a dramatic loss of Na^+,K^+-ATPase activity. If after 10 min at 45°C the cells were allowed to recover at 37°C there was a partial restoration of ATPase activity which reaches a peak after 2 h (fig.2). This recovery (or 'repair') however was impaired by

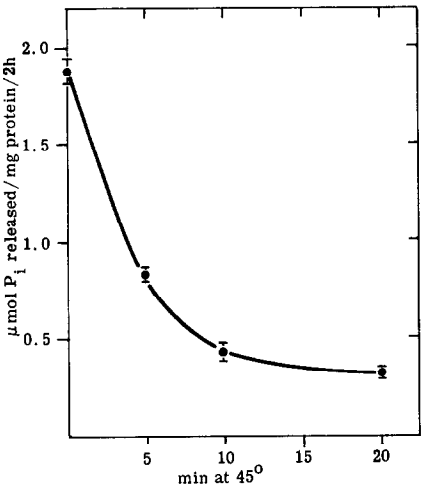

Fig.1. Loss of Na^+,K^+-ATPase activity in HeLa cells treated at 45°C. Monolayer cultures in 80 oz. bottles were treated at 45°C for various time periods by immersion in a water bath. The cells were then washed, collected and assayed for ATPase as in section 2. (Bars indicate extent of standard deviations.)

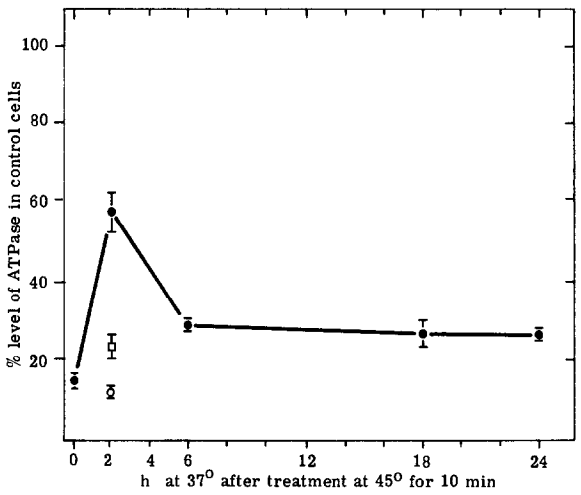

Fig.2. Recovery of Na^+,K^+-ATPase activity at 37°C after heat treatment of HeLa cells at 45°C. Monolayer cultures were subjected to heat treatment at 45°C for 10 min as in section 2. They were returned to the normal growth temperature of 37°C and cultures were removed, washed and assayed for ATPase at various times as in section 2. The resulting activities (●——●) are expressed as % of Na^+,K^+-ATPase activity in untreated control cells grown at 37°C; (□) relative enzyme activity in cells treated at 45°C for 10 min but then incubated for 2 h at 37°C in presence of cycloheximide (25 μg/ml); (○) activity in cells treated at 45°C for 10 min but then incubated for 2 h at 37°C in presence of actinomycin D (2 μg/ml). (Bars indicate extent of standard deviations.)

the addition of actinomycin D (2 μg/ml) or cycloheximide (25 μg/ml) to the culture medium immediately after the initial hyperthermic treatment (fig.2). Such data suggest the involvement of gene expression in this recovery process. Since neither actinomycin nor cycloheximide treatment has this marked effect on ATPase levels in untreated cells (table 1) the

Table 1
Effect of sodium arsenite treatment of HeLa cells on levels of Na^+,K^+-ATPase

Treatment of HeLa cells	Na^+,K^+-ATPase activity ($\mu mol \cdot mg\ protein^{-1} \cdot 2\ h^{-1} \pm SD$)
None	1.52 ± 0.01
Actinomycin D (2 μg/ml) 2 h 37°C	1.37 ± 0.06
Cycloheximide (25 μg/ml) 2 h 37°C	1.53 ± 0.07
Sodium arsenite (5×10^{-5} M) 2 h 37°C	2.31 ± 0.12
Sodium arsenite (5×10^{-5} M) + actinomycin D (2 μg/ml) 2 h 37°C	1.34 ± 0.13
Sodium arsenite (5×10^{-5} M) + cycloheximide (25 μg/ml) 2 h 37°C	1.73 ± 0.11

Treated monolayers were washed, collected and assayed for Na^+,K^+-ATPase as in section 2

effects of these inhibitors were not simply manifestations of enzyme protein turnover. Since the time of maximal recovery of activity at 37°C is similar to the time of maximal HSP synthesis during incubation at 37°C after hyperthermic treatment [4] it is possible that HSPs may be involved in this partial recovery, or 'repair', of enzyme activity. The recovery is also depressed by ~60% if the incubation after hyperthermia is carried out for 2 h at 4°C, rather than at 37°C. Such conditions also markedly depress HSP synthesis [8].

It is clear that agents other than heat, such as sodium arsenite, can elicit the induction of HSPs in HeLa cells [8], and in chick fibroblasts [14]. Treatment of HeLa cells with 5×10^{-5} M sodium arsenite for 3 h leads not only increased synthesis of HeLa cell HSPs but also elevated levels of Na^+,K^+-ATPase (table 1). This increase in ATPase activity can be prevented by inclusion of actinomycin D or cycloheximide in the culture medium along with the sodium arsenite, implying specific gene expression in the activation of ATPase rather than a direct effect of arsenite on the enzyme itself.

4. Discussion

The data obtained using heat or sodium arsenite suggest that HSPs may be involved in the in vivo modulation of Na^+,K^+-ATPase activity. Whether this might be achieved directly or indirectly cannot be established here. In addition the nature of the thermal damage to the ATPase after the hyperthermia is not clear. It need not simply be denaturation. For example heat treatment of reticulocyte lysates is believed to lead to the activation of a kinase which appears to inactivate the small subunit of the initiation factor eIF-2 by phosphorylation [15]. Thus phosphorylation of Na^+,K^+-ATPase could lead to its inactivation and its reversal might require the direct association of an HSP or the participation of an HSP in a dephosphorylation reaction. An alternative is to consider the structure of mammalian cell Na^+,K^+-ATPase [16, 22]. The plasma membrane enzyme comprises 2 α-subunits and 2 β-subunits and for example in mouse ascites tumour cells these are ~100 000 M_r and 53 000 M_r, respectively [17]. It is thus possible that one of the HSPs we observe at 100 000 M_r is in fact an α-subunit. In any event, the alterations to ATPase activity observed do not yield enzyme with increased thermal resistance in vivo (not shown). Thus whilst our observed effects may concern recovery or 'repair' of thermal damage they do not appear to be directly relevant to the generation of 'thermotolerance' [8]. In a comparative study we find that human, mouse and hamster cells are similar in that 3 major groups of HSPs are inducible (at ~37 000 M_r, 72 000–74 000 M_r and 100 000 M_r [8]. However avian cells (chick fibroblasts) may have 4 rather than 3 major groups of HSPs [18]. Nevertheless 2 of the avian groups have ~70 000 M_r and ~100 000 M_r and thus appear to correspond to the 2 higher M_r groups we describe in mammalian cells. Indeed the correspondence is considerable in the case of the 100 000 M_r proteins extending to similarities revealed by partial proteolytic mapping [18]. However, there is disagreement concerning the actual M_r of the 100 000 M_r HSPs common to avian and mammalian cells [4,18–20] (estimates range from 89 000–100 000 M_r). Nevertheless data are available to indicate a possible overlapping role for this particular group in oncogenesis and the heat shock response. A single viral protein ($pp60^{src}$) believed to influence plasma membrane components mediates the neoplasmic transformation of avian cells infected with Rous sarcoma virus. Immunoprecipitation of $pp60^{src}$ has revealed two cellular proteins to be associated with $pp60^{src}$ in a specific manner. One of these proteins corresponds to the above-mentioned large common HSP [20,21]. Although a similar situation may exist in human cells it is difficult to assess. However in addition to similar HSPs, normal and malignant human cells appear to contain proteins closely related antigenically to $pp60^{src}$ [23]. Thus it is possible that $pp60^{src}$-type proteins could influence the activity of Na^+,K^+-ATPase, either directly or indirectly, in a process involving HSPs. Whether the effects on Na^+,K^+-ATPase could in turn also influence hexose transport through electrogenic sodium symport systems [22] remain to be examined.

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