Heat shock activation of NFkB in rat liver is mediated by interleukin-1

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Abstract Exposure to high temperature (heat shock) activates the transcription factor NFkB in the liver of the living rat, but is not effective in hepatoblastoma cells in culture: on the contrary, activation of the heat shock transcription factor (HSF) occurs under both conditions. Pre-treatment of the rat with IL-1 receptor antagonist suppresses the activation of NFkB, which seems to be mediated by the release of this cytokine, but does not hamper the activation of HSF and the concurrent induction of hsp 70 mRNA. IL-1 activity actually shows a strong, albeit transient, increase in the blood of heat shocked rats.

Key words: Heat shock; Liver; NFkB; Interleukin-1; Heat shock factor

1. Introduction

Exposure of cells to high temperature or to a variety of other environmental stresses can alter gene expression: in particular, a definite set of genes is activated, that were termed initially heat shock genes and are now more generally referred to as stress genes [1]. The best known of these genes are those coding for the hsp 70 protein family [2]. The transcriptional induction of heat shock genes in eukaryotic cells is mediated by the binding to the *cis*-acting heat shock element (HSE) of an heat shock transcription factor (HSF) which is activated under heat shock and repressed under non-shock conditions, most probably by small amounts of hsp 70 constitutively present in the cytoplasm [3]. This example of negative regulation is in many ways similar to the negative control exerted by the inhibitor IkB on the transcription factor NFkB, a pleiotropic DNA-binding protein complex which regulates the expression of a wide variety of genes, particularly those involved in immune and inflammatory responses [4,5]. Agents or conditions that activate NFkB stimulate phosphorylation and proteolytic degradation of the inhibitor, which dissociates from NFkB: only after this process has been completed NFkB can translocate to the nucleus and bind to DNA target sequences in the enhancers of a variety of genes [6]. In tissue cultures NFkB can be activated by H_2O_2 and other reactive oxygen species, but exposure of cells to heat shock, which is likely to increase oxidative events in the cells, has never been shown to activate NFkB [4,5]. A study on activation of NFkB in organs of a living animal would seem unjustified but in vivo cooperativity between different types of cells, and association of different activating pathways, could result in effects not obtained with a single cell type. In the present paper we have studied the effects of heat shock on the activation of NFkB and HSF in the liver of rats and HepG2 hepatoblastoma cells: after showing that only in the living rat there is activation of both transcription factors, we have been able to suppress activation of NFkB by pretreatment of the rat with the interleukin-1 receptor antagonist (IL-1 RA), which does not hamper the activation of HSF and the expression of hsp 70 mRNA. In confirmation of data obtained in the mouse [7], and as additional evidence of the role of IL-1 in the heat shock-induced activation of NFkB, we have been able to detect consistent, increasing activities of IL-1 in the blood of heat shocked rats.

2. Materials and methods

2.1. Animals

Male Wistar rats (220–250 g) housed and handled as prescribed by EC regulations, were used throughout. For the induction of heat shock they were anesthesized with sodium thiopental (4.5 mg/100 g body weight, intraperitoneally) and made hyperthermic by wrapping in a heating pad: the rectal temperature of 41°C, monitored by a thermocouple, was reached in 10–15 min and was maintained between 41 and 42°C for a further 50 min [8]. The rats were either killed immedialy by decapitation or left to recover at room temperature for 30 or 60 min.

2.2. Treatment with IL-1 RA

Human recombinant IL-1 RA, expressed in *B. subtilis*, of 98% purity, 1×10^6 IU/mg, was a kind gift of Dr. Diana Boraschi, Centro Ricerche Dompè, L'Aquila (Italy). After dilution with sterile apyrogen saline it was injected intraperitoneally at total doses ranging from 100 μg to 400 μg per 100 g b.wt., given in two equal portions, the first at the beginning of hyperthermic treatment, the second after about 25 min of hyperthermia.

2.3. Serum IL-1 biological activity

Blood was taken by cardiac puncture. IL-1 was measured by the growth promoting activity of sera on the IL-1-dependent T cell clone D10.G4 [9]. The test was performed as described [10] and final data reported in the text represent cpm/10^4 D10 cells/well.

2.4. Molecular biology techniques

Extraction of total RNA or nuclear factors, electrophoretic mobility shift analysis of DNA–protein complexes (EMSA) and determinations of steady-state levels of hsp 70 mRNA were performed as recently described [11]. Cellular nuclear extracts were prepared as described [12]. Supershift experiments were performed by incubating 1 μl of primary antibody with nuclear extracts in binding buffer for 1 h at 4°C prior to the addition of labeled oligonucleotide.

3. Results and discussion

3.1. DNA binding of HSF and NFkB is activated by exposure to heat shock in vivo

The first step in the induction of gene expression is the binding of a definite transcription factor to the corresponding consensus sequence in the promoter of the gene. To assess a possible role of NFkB in the heat shock response we performed EMSA experiments with nuclear extracts of livers from heat shocked animals reacted with a kB-motif or with an HSE-like oligonucleotide (Fig. 1), the latter as a positive control of gene

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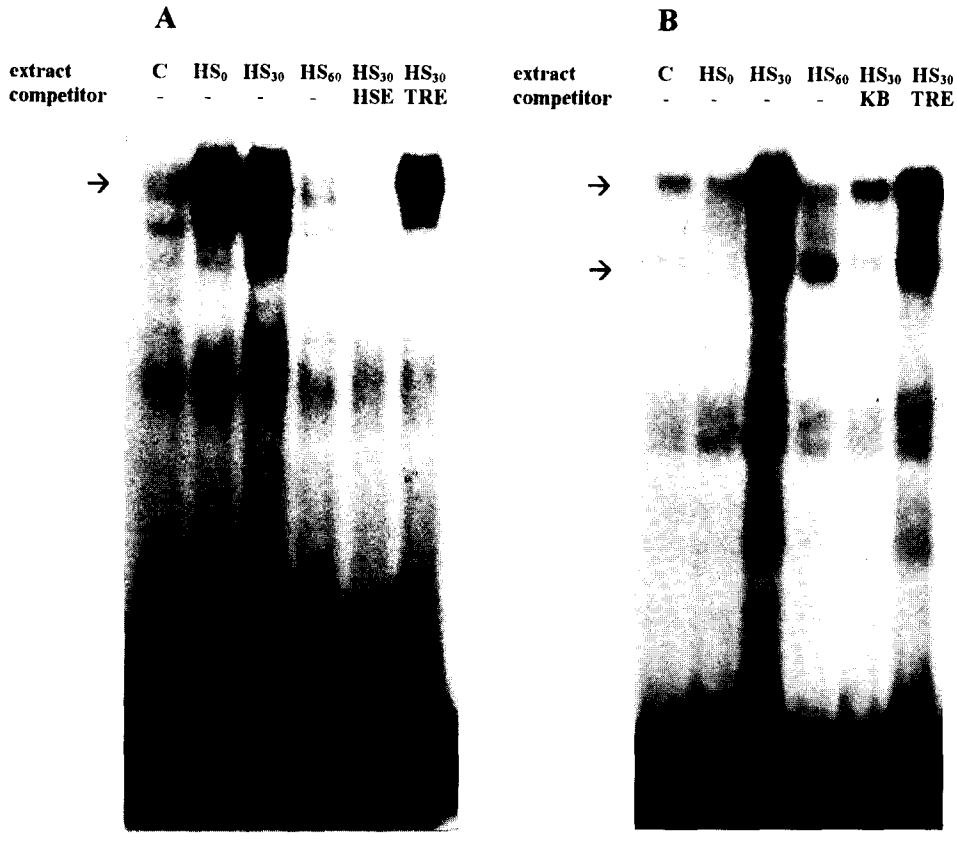

Fig. 1. Electrophoretic mobility shift analysis of nuclear extracts from rat livers before heat shock (control; C) at the end of heat shock (HS_0) or 30 and 60 min later (HS_{30} and HS_{60}), reacted with HSE-like (A) and kB-motif (B) labelled oligonucleotides. Specific competitors = unlabelled HSE and kB; unspecific competitor = unlabelled TPA-responsive element (TRE) oligonucleotide.

reprogramming caused by hyperthermia [13]. Among several bands observed in control livers tested with HSE-like oligonucleotide one is specifically increased by heat shock (arrow), persists for 30 min after exposure and disappears 60 min after the end of hyperthermia. The specificity of this complex is demonstrated by complete competition by a 10-fold excess of the unlabelled oligonucleotide (specific competition) and is not competed by a TRE-like oligonucleotide (lack of unspecific competition). In the same nuclear extracts of normal rats the kB-motif gives two faint bands, indicating a low but detectable level of constitutive binding. Activation, demonstrated by increased intensity of these two bands (arrows), does not appear at the end of exposure to high temperature, but only after a lag of 30 min after the end of heat shock, and declines, but does not entirely disappear, 60 min after heat shock: there is specific competition (kB) and lack of unspecific competition (TRE). We conclude that both HSF and NFkB are activated in heat-exposed animals, but the response of NFkB is less prompt than HSF. The delay in activation of NFkB may depend on some intrinsic characteristics of this transcription factor, but might also indicate the existence of an intermediate step along the pathway leading to its activation, that could possibily depend on cooperation between different cell types present in the liver. This hypothesis is supported by the observation that activation of NFkB does not occur in HepG2 hepatoblastoma cells which, under heat shock conditions, induce the activation of HSF (Fig. 2A). HepG2 cells express a constitutive active form of NFkB revealed by three bands (Fig. 2B), specifically competed by the unlabelled kB-motif and uncompeted by TRE, plus an extra band competed by both kB and TRE, which do not increase appreciably under conditions of heat shock. Use of antibodies raised against p50 and p65 subunits of NFkB allowed the identification of two of these bands.

3.2. Pretreatment with IL-1 RA abolishes only NFkB activation

The most likely cooperation that can be envisaged under these circumstances is that between Kupffer cells and hepatocytes [14]. Among the possible mediators of the effect, first of all we choose to consider IL-1, which is produced by Kupffer cells [15] and possibly, only under definite circumstances, by primary cultured liver cells [16] and is a well-known activator of NFkB in many different cell types [4,5,17–19]. In the attempt to suppress a response tentatively ascribable to IL-1 we used the IL-1 RA, a member of the IL-1 family which can prevent the binding to the cells of both IL-1α and IL-1β [20]. Pretreatment of the animals with IL-1 RA prevents the binding of the kB-motif oligonucleotide to the nuclear extracts of heat shocked rats in a way that depends on the dose. A total dose of 100 μg IL-1/100 g b.wt. prevents the induction due to heat shock, but does not affect the constitutive expression of NFkB; at doses of 200, 300 and 400 μg both constitutive and induced expression of NFkB are suppressed (Fig. 3A). The specificity of activation is validated as usual by the use of unlabelled kB-motif and TRE oligonucleotides. In the same nuclear extracts of IL-1 RA-treated rats the band corresponding to the HSE-HSF complex, competed by the unlabelled HSE-like and

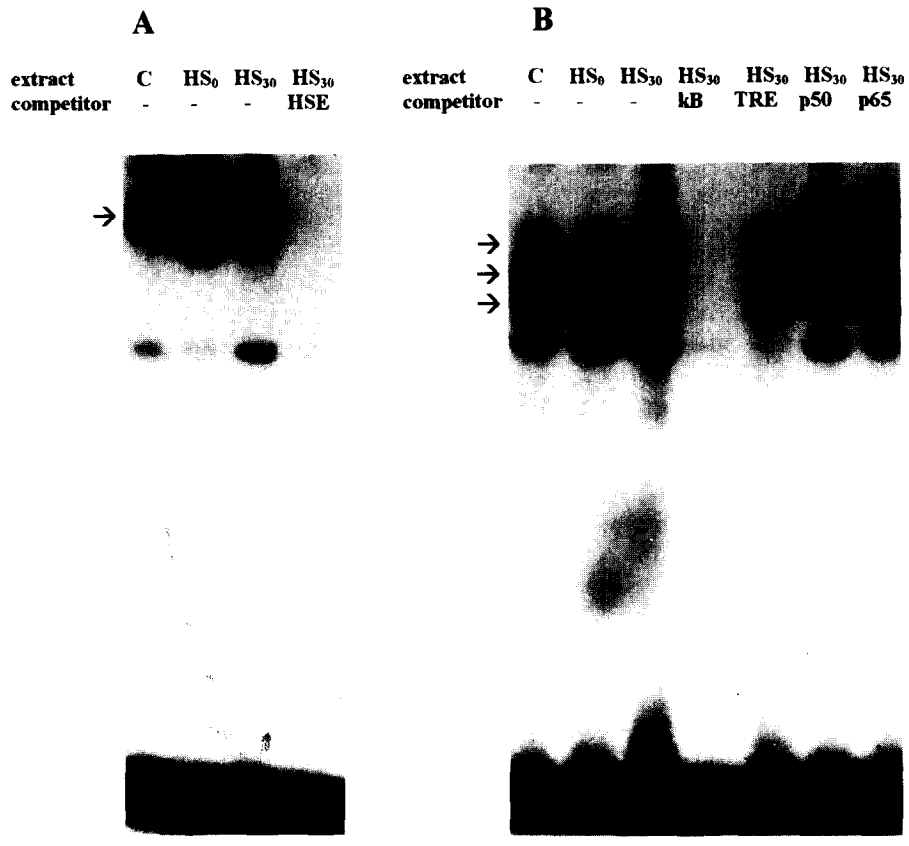

Fig. 2. EMSA of nuclear extracts from HepG2 hepatoblastoma cells, reacted with HSE (A) and kB-motif (B). Symbols as in Fig. 1; p50 and p65 = samples reacted with antibodies raised against p50 and p65 subunits of NFkB.

incompeted by the TRE-like oligonucleotide, is essentially maintained (Fig. 3B). Under conditions of repressed NFkB activation the livers of heat shocked rats accumulated mRNA for hsp 70 (Fig. 3C), which is obviously the outcome of the process started by activation of HSF: but we thought it wise to ascertain the induction of this mRNA in view of some reported cases of lack of correlation between DNA binding activity and transcription of hsp 70 [21–25].

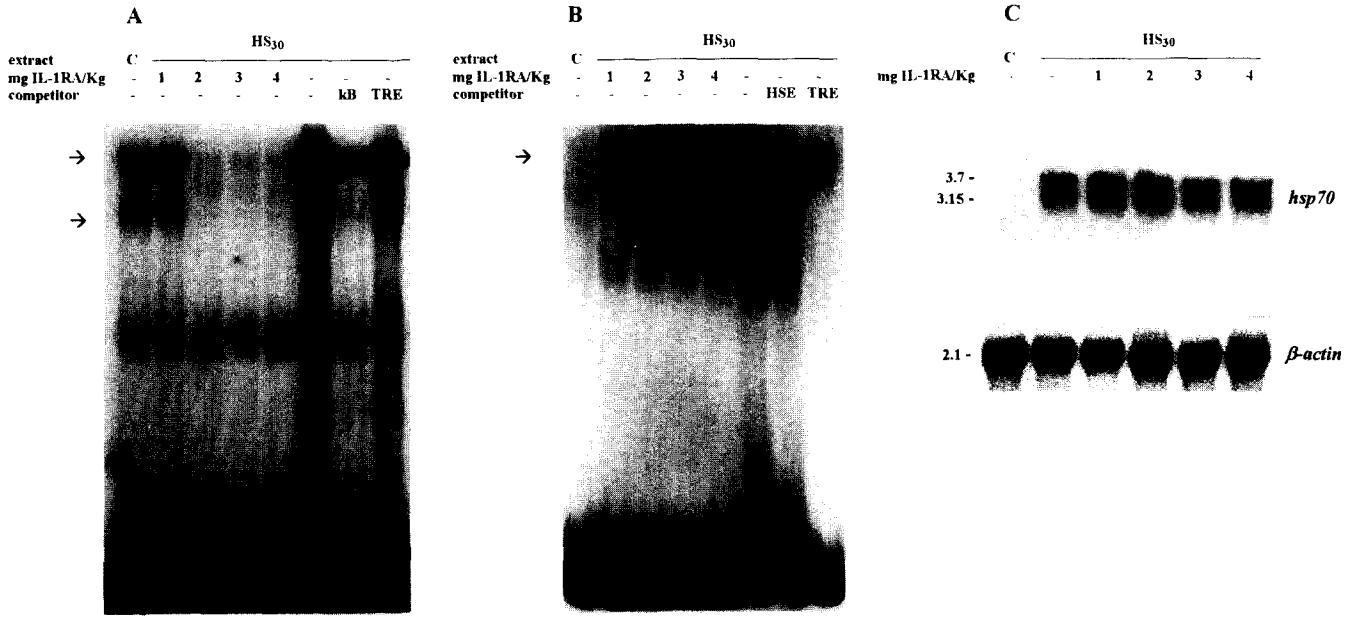

Fig. 3. EMSA of nuclear extracts from rat livers pretreated with IL-1 RA before heat shock: the rats were killed 30 min after the end of heat shock (HS_{30}). Extracts reacted with kB-motif (A) or HSE (B). In (C) Northern blot analysis of the heat shock-inducible hsp 70 mRNA in IL-1 RA pretreated rats; levels of β-actin mRNA shown to demonstrate equal loading. Symbols as in Fig. 1.

3.3. IL-1 activity increases in the blood of hyperthermic rats

The links between IL-1 and NFkB activation are further demonstrated by the presence of IL-1 in the blood-serum of heat shocked rats. IL-1α, but not TNFα, was found to increase consistently in plasma of mice made hyperthermic [7]. In a small-scale side experiment of biological determinations of IL-1 in sera of heat shocked rats the activity, expressed as cpm obtained in thymidine incorporation experiments with 2 rats for each time-point, was of 13,300 and 15,000 in the controls, 97,600 and 117,200 after 30 min of hyperthermia, 267,700 and 209,000 after 50 min of hyperthermia (standard heat shock time) and 100,600 and 145,000 one hour after the end of 50 min of hyperthermia. The growth stimulation index (cpm of the sample/cpm of control) calculated from these data increases from 7.6 at 30 min to 16.8 at 50 min of hyperthermia, but declines to 8.7 one hour after the end of hyperthermia. This real but transitory increase in circulating IL-1 activity during heat shock is consistent with the short half-life of this cytokine and compatible with the transient activation of NFkB caused by exposure to hyperthermia: on the other hand, interference with the association of IL-1 to its receptor would suppress this activation and leave unchanged the response of HSF, which is most probably direct or mediated by different mechanisms.

In conclusion, our results indicate that the activation of the transcription factor NFkB, which occurs in the liver of the living rat subjected to heat shock is largely, if not entirely, mediated by the production and release of IL-1, and can be suppressed by the IL-1 RA. Activation of HSF is not hampered by IL-RA and results in the induction of hsp 70 mRNA, which can occur also under conditions of repressed activation of NFkB.

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