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Interleukin-1β Suppresses Growth Hormone-Induced Acid-Labile Subunit mRNA Levels and Secretion in Primary Hepatocytes

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Cytokines are thought to mediate the catabolic states induced by infection and trauma. Recent evidence suggests that the cytokine interleukin-1β (IL-1β) directly inhibits the anabolic insulin-like growth factor (IGF)-I:growth hormone (GH) axis. The biological activity of circulating IGF is regulated by the hepatocyte derived, GH-dependent acid-labile subunit (ALS) of the 140-kDa IGF binding protein (IGFBP) complex. ALS buffers the growth and metabolic effects of the insulin-like growth factors by sequestering them in a ternary complex with IGFBP-3. To determine whether IL-1β has a direct effect on hepatic ALS production, we have examined its effect on ALS mRNA levels and secretion in hepatocytes under GH-induced and basal conditions. In the presence of GH (30 ng/mL) half-maximal reduction of ALS mRNA levels and secretion was induced by between 0.3-3 ng/mL rhIL-1β ($P < 0.05$). However, under basal conditions IL-1β had no significant effect on ALS mRNA levels, and only a slight suppression of secretion. Our study suggests that IL-1β regulates ALS gene expression and secretion in a way that is dependent, in part, on interaction with the GH signalling pathway.

***Key Words*: IGF; binding protein; acid-labile subunit; IL-1β.**

Insulin-like growth factors (IGF-I and -II) are related in structure to pro-insulin, and have developmental and growth stimulatory effects as well as insulin-like metabolic actions (1). In the circulation the IGFs are stabilized in a ternary complex with IGF binding protein-3 (IGFBP-3) and the acid labile subunit (ALS) (2, 3). In comparison with other IGF:IGFBP complexes this ternary complex is thought to cross the capillary barrier relatively poorly. This suggests an important role for ALS in regulating the release of IGF from the circulation into the extracellular tissue compartment, thereby modulating their metabolic activities. Recently it has been demonstrated that interleukin-1β has direct effects on the IGF-I:GH axis specifically at the site of IGF-I production, the hepatocyte (4, 5). Cytokines are thought to be mediators of the catabolic conditions induced by sepsis and trauma and their suppression of the IGF-I axis would compound their catabolic effects. Interestingly, IL-1β suppresses hepatocyte GH receptor gene expression which may partly explain the GH resistant state and refractoriness to IGF-I treatment found in these disorders (4). Like IGF-I, circulating ALS is hepatocyte derived and is markedly GH-dependent (6–9). Because of the potential role of ALS in regulating circulating IGF-I biological activity in catabolic states, it is of considerable interest to determine how cytokines might interact with GH in regulating ALS.

The rat ALS gene has recently been cloned (6) and a number of potential cytokine regulatory elements have been identified in its promoter region. The homologous mouse ALS promoter has also been shown to contain interferon-γ activated site (GAS)-like elements which may be involved in the GH-dependent regulation of this gene (7). This contrasts with the IGF-I gene in which the mechanism for GH regulation has not been fully elucidated (8). However, it is currently unknown if this or other related sites are involved in regulation of ALS gene expression by other cytokines.

To begin to elucidate the potential role of cytokines in controlling ALS gene transcription we have examined the regulation of ALS expression in primary hepatocytes by IL-1β.

MATERIALS AND METHODS

Materials. Williams' E medium was obtained from Sigma Chemical Co. (St Louis, MO). Collagenase was obtained from Boehringer Mannheim (Sydney, Australia). Tissue culture plates were from Corning (Trace Biosciences, Sydney, Australia). Zetaprobe GT nylon membranes were obtained from BioRad (Richmond, CA). Na[^{125}I] was

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from ANSTO (Sydney, Australia). Recombinant human GH (rhGH) was provided by Kabi Peptide Hormones (Stockholm, Sweden). Recombinant human IL-1β was obtained from R&D Systems (Minneapolis, MN).

Preparation of rat hepatocytes. Hepatocytes were prepared from 10 week (~250g) female Wistar rats by *in situ* perfusion of livers with collagenase, and plated at 2×10^6 cells/60mm plate in 2mL Williams' E medium containing 300nM insulin, 10% fetal calf serum and antibiotics (0.1 μg/mL streptomycin and 0.06 μg/mL penicillin). The protocol was approved in advance by the institutional Animal Care and Ethics Committee. Medium was removed after 5h and cells were maintained serum free in fresh Williams' E medium containing 0.2% BSA, 300nM insulin and 0.06 μg/mL penicillin. Additions were made 24h after the initial plating, and cells were then maintained for another 24h. IL-1β was diluted into Williams' E medium containing 0.2% BSA. The highest concentration of IL-1β had no apparent effect on the appearance or attachment of the cells to the plates.

RNA extraction and Northern analysis. Total RNA was extracted from duplicate plates of hepatocytes by the guanidine isothiocyanate/acid-phenol technique (9). Total RNA samples (20 μg) were electrophoresed in 1% agarose gels containing 2.2 M formaldehyde. The integrity of the ethidium bromide stained RNA samples was confirmed on a UV light-box. The RNA was then transferred by capillary blotting to Zetaprobe GT membranes, and cross-linked by baking at 80°C in a gel drying apparatus.

A 350 bp rat ALS DNA probe was generated by polymerase chain reaction from a genomic DNA construct containing exon 2 of the rat ALS gene, using oligodeoxynucleotides described previously (10). The cDNA was labeled using a Ready-to-GO random-priming kit (AMRAD-Pharmacia, Australia) and [α^{32}P]dCTP (AMRAD-NEN, Australia). Filters were prehybridized and hybridized (2×10^6 cpm/mL) as described previously, then washed using 0.1× SSC at 42°C. Filters were then quantified using a phosphorimager (Molecular Dynamics, Sunnyvale, CA). Equality of RNA loading was determined by stripping the blots in 0.01× SSC, 0.5% SDS at 80°C, then rescreening with an 18S rRNA cDNA probe (Dr. D Denhardt, Rutgers University, NJ, USA). Data were normalized by expressing the ratio of ALS mRNA to 18S rRNA. Results were expressed as the percentage of the maximum observed ALS/18S ratio in each experiment.

Radioimmunoassays. Conditioned media were collected and stored at −20°C. These media were then thawed once and assayed using a specific rat ALS radioimmunoassay, as previously described (11). Standard curves were constructed with purified rat serum ALS.

Statistics. The data for IL-1β in the presence of rhGH represent the means ±SEM of results from experiments performed with quadruplicate plates of hepatocytes from five independent liver perfusions. The experiments with IL-1β in the absence of rhGH are derived from quadruplicate plates of cells from two independent liver perfusions. Results were analyzed by ANOVA, with P values calculated using Fisher's PLSD (Statview 4.02 for Apple Macintosh, Abacus Concepts, Berkeley, CA).

RESULTS

Effect of IL-1β on Basal ALS Expression by Hepatocytes

In the absence of GH ALS expression was unresponsive to IL-1β over the range of doses used (0.3-30 ng/mL). There was no significant effect on steady state levels of the ~2.2kb ALS transcript (Fig. 1, A and B) and only a minor effect on secretion (Fig. 2). Secretion of ALS appeared to be slightly (not significantly) induced at low (0.3 ng/mL) concentrations. However, at 3 and 30ng/mL IL-1β significantly ($P < 0.05$) suppressed secretion relative to the 0.3ng/mL dose.

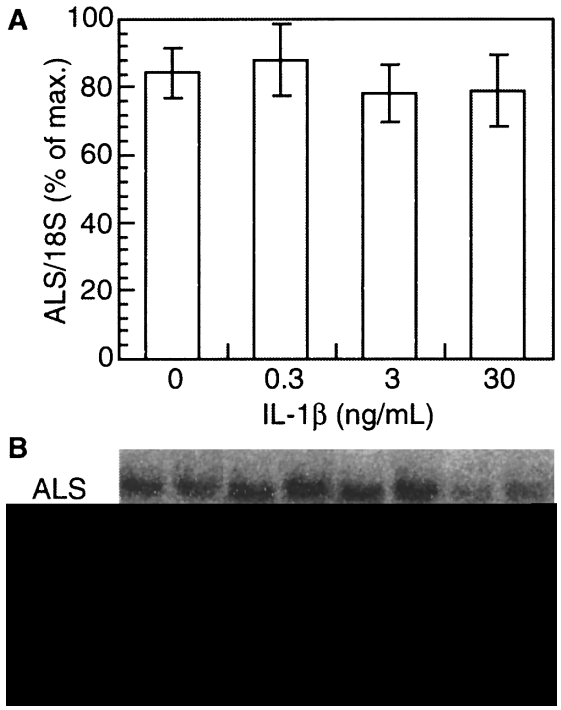

FIG. 1. The effect of IL-1β on steady-state mRNA levels in primary hepatocytes under basal conditions. (A) No significant regulation of ALS mRNA levels by IL-1β was observed under basal conditions. (B) Representative Northern blot of RNA from hepatocytes treated with IL-1β. Each value represents the mean ±SEM of two separate experiments, each IL-1β dose consisting of quadruplicate plates of cells.

Effect of IL-1β on GH Induced ALS Expression

Unlike the basal state, hepatocytes co-incubated with 30 ng/mL rhGH showed parallel dose-dependent suppression of ALS steady-state mRNA levels (Fig. 3, A and B) and secretion (Fig. 4) by IL-1β. The half-maximal inhibition of the ALS response to GH occurred at between 0.3 and 3 ng/mL of IL-1β ($P < 0.05$). Steady state levels of ALS mRNA were suppressed to ~50% of control values by 3ng/mL IL-1β ($P < 0.05$). Similarly, 30 ng/mL IL-1β significantly ($P < 0.05$) reduced ALS secretion to 49% of control levels and significant suppression was also observed at 3 ng/mL.

DISCUSSION

The pleiotropic cytokine IL-1β is involved in the regulation of the immune system, and appears to influence the function of the IGF system. It regulates the circulating levels of IGF-I and the IGFBPs, in particular IGFBP-1 and IGFBP-2 (12). The mechanism by which IL-1β suppresses IGF-I and induces the IGFBPs in this

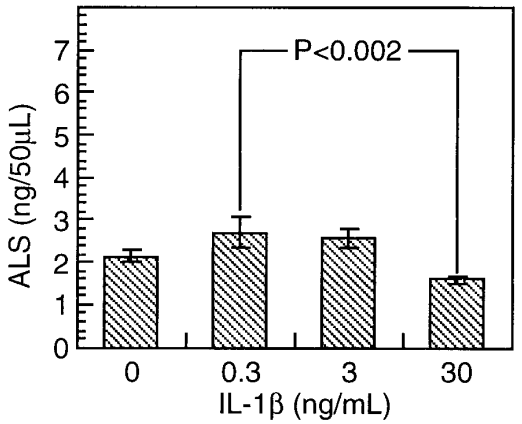

FIG. 2. The effect of IL-1β on ALS secretion by primary hepatocytes under basal conditions. Low concentrations of IL-1β at 0.3 ng/mL caused a slight increase in ALS secretion over untreated controls. At 30 ng/mL IL-1β significantly suppressed ALS expression relative to the 0.3 ng/mL dose, but there was no significant suppression relative to the untreated controls. The Northern was screened twice for ALS mRNA and 18S rRNA. Each value represents the mean ±SEM of two separate experiments, each IL-1β dose consisting of quadruplicate plates of cells.

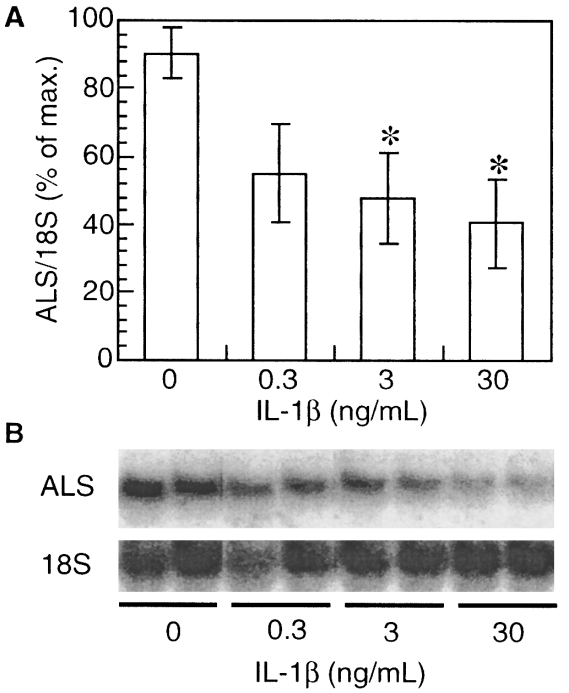

FIG. 3. Effect of IL-1β on ALS gene expression by hepatocytes in the presence of GH. (A) Dose-dependent suppression of mRNA levels is observed under 30ng/mL rhGH induced conditions. Half-maximal suppression was observed at between 0.3 and 3ng/mL IL-1β ($P<0.05$). (B) Representative Northern blots of RNA from GH induced hepatocytes treated with IL-1β. The Northern was screened twice for ALS mRNA and 18S rRNA. Each value represents the mean ±SEM of five separate experiments. *, $P < 0.05$ vs. GH controls not treated with IL-1β.

case is unknown, although recent evidence suggests a direct effect of IL-1b on hepatic IGF-I mRNA levels (4, 5). Defalque *et al* (13), in a preliminary study using a semi-quantitative immuno-blot assay, have identified IL-1β as a potential suppressor of ALS secretion by primary rat hepatocytes. Because we had detected the presence of a number of potential cytokine activated sites, or GAS-like elements, in the 5′ flanking region of the rat ALS gene we were interested in determining the potential role of cytokines in controlling ALS gene expression in the context of its induction by GH.

We have examined, for the first time, the effect of IL-1β on ALS gene expression in relation to its secretion by primary hepatocytes. These parameters were measured by Northern analyis of total RNA and a specific rat ALS radioimmunoassay developed in this laboratory (11). Under basal conditions (in the absence of GH) IL-1β appears to have little effect on ALS expression either at the level of gene expression or of secretion. However, under more "physiological" conditions in the presence of GH, IL-1β had a profound effect on both steady-state mRNA levels and subsequently on secretion. This surprised us as IL-1β has been shown to induce activity of a promoter containing a GAS-like element in the human hepatoma cell-line Hep3B (14) and we had speculated that ALS may be regulated through similar sites via the JAK/Stat pathway (15). The mechanism of suppression of ALS remains speculative. However, the lack of effect of IL-1β in the absence of GH implies that there is some interaction between this cytokine and the GH signalling pathway. In relation to this, the inhibition of ALS correlates well with the IL-1β mediated suppression of GH receptor mRNA levels in primary hepatocytes observed by others (4, 5).

Another influence on the actions of cytokines, includ-

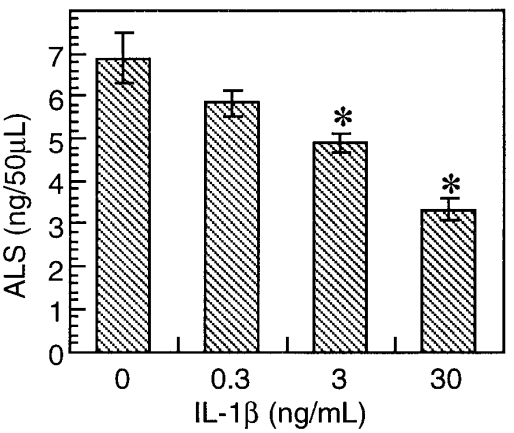

FIG. 4. Effect of IL-1β on ALS secretion by hepatocytes in the presence of 30ng/mL rhGH. Dose-dependent suppression of ALS secretion by primary hepatocytes is observed in the presence of rhGH. Each value represents the mean ±SEM of five separate experiments, each IL-1β dose consisting of quadruplicate plates of cells. *, $P <$ 0.05 vs. GH controls not treated with IL-1β.

ing IL-1β, is the presence of insulin which can abrogate or suppress their effects (16). The primary hepatocytes used in the preceding experiments were grown in the presence of supra-physiological concentrations of insulin to maintain their viability. It is possible that under basal conditions more profound effects of IL-1β might be observed in the absence, or in lower ($<$0.5 ng/mL) concentrations of insulin.

The levels of circulating ALS have been shown to be suppressed in burn patients (17) although this could be related to the dominant effects of changes in GH serum levels or tissue sensitivity rather than specific effects of IL-1β and other cytokines. However, if the suppression of ALS we have observed *in vitro* in hepatocytes can be extrapolated to whole organisms (13), IL-1β would appear to have compound suppressive effects on the IGF axis, particularly in cases of trauma and infection.

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