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Transcriptional regulation of α_1-adrenoceptor gene in the rat liver during different phases of sepsis

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Abstract

Changes in α_1-adrenoceptor (α_1AR) gene expression in the rat liver during different phases of sepsis were studied. Sepsis was induced by cecal ligation and puncture (CLP). Septic rats exhibit two metabolically distinct phases: an initial hyperglycemic phase (9 h after CLP, early sepsis) followed by a hypoglycemic phase (18 h after CLP; late sepsis). The [^{3}H]prazosin binding studies show that the density of α_1AR was increased by 30% during the early phase while it was decreased by 24% during the late phase of sepsis. Western blot analyses reveal that α_1AR protein level was elevated by 48% during early sepsis but was decreased by 55% during late sepsis. Northern blot analyses depict that the steady-state level of α_{1b}AR mRNA was enhanced by 21% during the early phase but was declined by 29% during the late phase of sepsis. Nuclear run-off assays show that the transcription rate of α_{1b}AR gene transcript was increased by 76% during early sepsis while it was decreased by 29% during late sepsis. The actinomycin D pulse-chase studies indicate that the half-life of α_{1b}AR mRNA remained unaffected during the early and the late phases of sepsis. These findings demonstrate that during the early phase of sepsis, the increase in the rate of transcription of α_{1b}AR gene paralleled with the elevations in the α_{1b}AR mRNA abundance and α_1AR protein level, while during the late phase of sepsis, the decrease in the rate of transcription of α_{1b}AR gene coincided with the declines in the α_{1b}AR mRNA abundance and the α_1AR protein level in the rat liver. These observations indicate that the altered expression of α_1AR genes in the rat liver during the progression of sepsis was regulated transcriptionally.

Keywords: Septic shock; Glucose dyshomeostasis; Hepatic dysfunction; Gene expression

1. Introduction

Sepsis, septic shock, and ensuing multiple organ system failure continue to be the most common causes of death in surgical intensive care units, despite various major advances in the treatment of sepsis [1,2]. A disturbance in glucose homeostasis has been recognized as one of the major metabolic alterations during sepsis. This disturbance is characterized by a rapid depletion of liver glycogen content, an impaired glycogenesis, an accelerated glycogenolysis, and a depressed gluconeogenesis. The ultimate result of these metabolic alterations is the development of an initial hyperglycemia followed by a progressive hypoglycemia [1–3]. The mechanisms responsible for the altered glucose homeostasis during the progression of sepsis remain unclear. Metabolic pathways such as glycogenesis and gluconeogenesis in the liver are known to be regulated by catechol-

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amines through the Ca^{2+}-linked α-adrenoceptor (αAR) and the cAMP-linked β-adrenoceptor (βAR) mediation [4,5]. αAR consists of two different major subtypes, α_1, and α_2, with α_1AR being the predominant subtype (75–80%) expressed in the rat liver [5,6]. Catecholamine-stimulated glycogenolysis in the rat liver occurs primarily via α_1AR mediation [5,6] and the activation of α_1AR results in an increase in hepatic glucose output into the circulation [6,7]. Previous work from our laboratory has indicated that during the early stage of sepsis, α_1AR was overexpressed in the rat liver and that the overexpression of α_1AR was correlated with the development of hyperglycemia, while during the late stage of sepsis, α_1AR was underexpressed and that the underexpression of α_1AR was associated with the formation of hypoglycemia [8]. It is of interest to note that a similar biphasic alteration in the density of α_1AR was also observed in human livers with intraabdominal sepsis [9]. Since hepatic α_1AR responsiveness has been reported to be regulated through alterations via α_1AR gene expression under a variety of conditions [10–12], the present study was undertaken to examine whether the altered expression of α_1AR is regulated through alterations in its gene transcript in the rat liver during the two different glycemic phases of sepsis.

2. Materials and methods

2.1. Materials

[7-methoxy-^{3}H]Prazosin (85 Ci/mmol) was purchased from DuPont-New England Nuclear (Boston, MA). [α-^{32}P]dCTP (3000 Ci/mmol) and [α-^{32}P]UTP (3000 Ci/mmol) were purchased from ICN Pharmaceuticals (Costa Mesa, CA). Prazosin, Tris and SDS were products of Sigma (St. Louis, MO). Actinomycin D, proteinase K, ribonuclease A, deoxyribonuclease I and *Eco*RI were supplied by Promega (Madison, WI). Full-length hamster α_{1b}AR cDNA cloned into the *Eco*RI site of plasmid pSP65 (the insert was 2 kb) [13] was a generous gift of Dr. Marc G. Caron, Howard Hughes Medical Institute, Duke University Medical Center. 28S rRNA oligonucleotide (40 mer) was obtained from Calbiochem-Novabiochem International (San Diego, CA). Polyclonal (rabbit) anti-α_1AR antibody was obtained from Affinity Bioreagents (Golden, CO). Anti-rabbit IgG, horseradish peroxidase-linked species-specific whole antibody, ECL Western blotting detection reagent, and Hyperfilm-ECL were products of Amersham Life Science (Arlington Heights, IL). Other chemicals and reagents were of analytical grade.

2.2. Animal model

All animal experiments in this study were performed with the approval of the Animal Care Committee of Saint Louis University School of Medicine, and in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals. Male Sprague-Dawley rats weighing from 270 to 320 g were used. All animals were fasted overnight with free access to water. They were divided into three groups: control, early sepsis, and late sepsis. Sepsis was induced by cecal ligation and puncture (CLP) as described by Wichterman et al. [14] with minor modification. Under halothane anesthesia, a laparotomy was performed (the size of the incision was 2.5 cm), and the cecum was ligated with a 3-0 silk ligature and punctured twice with an 18-gauge needle. The cecum was then returned to the peritoneal cavity and the abdomen was closed in two layers. Control rats were sham-operated (a laparotomy was performed and the cecum was manipulated but neither ligated nor punctured). All animals were resuscitated with 4 ml/100 g body weight of normal saline at the completion of surgery and also at 7 h post surgery. Animals were fasted but had free access to water after operative procedures. After anesthetizing the animals with 2% chloralose in 20% urethane (5 ml/kg, i.p.), livers were removed 9 or 18 h post operation and were then used for the biochemical and molecular biological studies. Early and late sepsis refers to those animals sacrificed at 9 and 18 h, respectively, after CLP. The mortality rates were 0% (0/24) for control, 11% (3/27) for early sepsis, and 20% (6/30) for late sepsis. Previous experiments indicate that in septic rats, plasma glucose concentration was elevated by 20% ($P < 0.01$) during the early phase while it was decreased by 69% ($P < 0.01$) during the late phase of sepsis [8].

2.3. [^{3}H]Prazosin binding to liver plasma membranes

α_1AR binding assay was carried out using [^{3}H]prazosin as a radioligand according to a procedure previously described by us [8,15]. Liver plasma membranes were prepared by sucrose gradient centrifugation as described previously [8]. The standard assay mixture in a final volume of 0.2 ml contained 10 mM $MgCl_2$, 50 mM Tris-HCl (pH 7.4), 1 nM [^{3}H]prazosin with a radioactivity of approx. 10 000 cpm, and 0 or 2.5 μM unlabeled prazosin. The mixture was preincubated at 37°C for 2 min. The binding assay was subsequently initiated by the addition of plasma membranes containing 50 μg protein and was allowed to proceed for 20 min at 37°C. At the end of each incubation, the reaction mixture was diluted with 5 ml of ice-cold washing solution (10 mM $MgCl_2$ and 50 mM Tris-HCl, pH 7.4) and filtered immediately through a 0.45 μm glass fiber filter paper (Baxter Healthcare) under suction. The filter paper was washed three times with 5 ml washing solution. The filter paper was then dried and the radioactivity was determined with a liquid scintillation counter. The specific binding was defined as the bound radioactivity displaceable by 2.5 μM of unlabeled prazosin.

2.4. Determination of α_1AR protein level by Western blot analysis

Western blot analysis was performed according to the method of Gallagher et al. [16] with minor modification [17]. Samples of liver plasma membranes containing 90 μg protein were denatured and subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) (7–10% polyacrylamide gradient gel). Using Bio-Rad Trans-Blot cell, proteins separated by SDS-PAGE were transferred to a polyvinylidene fluoride membrane (Bio-Rad) at 8 mA (constant current) for 14–16 h in transfer buffer (25 mM Tris base, pH 8.3, 192 mM glycine, 5% (v/v) methanol). Non-specific binding sites were blocked with 10% (w/v) of non-fat dry milk in Tris-buffered saline (TBS, 20 mM Tris-HCl, pH 7.4, 137 mM NaCl) for 4 h at 4°C. Blots were washed three times (10 min each) with TBST (TBS containing 0.5% Tween-20) followed by incubation with a 1/400 dilution of a polyclonal antibody raised to rabbit α_1AR for 5 h at room temperature. Subsequently, the blots were washed three times (10 min each) with TBS and then incubated with a 1/2000 dilution of an anti-rabbit IgG, horseradish peroxidase-linked species-specific whole antibody for 1 h at room temperature. The blots were then washed four times (10 min each) with TBST, followed by incubation with enhanced chemiluminescent (ECL) Western blotting detection reagent, and finally exposed to Hyperfilm-ECL for 1 h. Autoradiographs were scanned with a Hewlett-Packard ScanJet 4C Scanner, and the relative densities were quantified by a Jandel Scientific Software program (SigmaGel).

2.5. Determination of the steady-state level of α_{1b}AR mRNA by Northern blot analysis

Northern blot analysis was performed as described by Sambrook et al. [18] and Rossby and Cornett [12] with modification [17]. Total cellular RNA was extracted from control and septic rat livers with acid guanidinum thiocyanate-phenol-chloroform mixture using a RNA isolation kit (bulletin 1, TEL-TEST 'B', Friendswood, TX). RNA concentration was determined by absorbance at 260 nm, and the purity was assayed by the 260/280 nm ratio. Samples containing 30 μg of total cellular RNA were denatured, size fractionated on 1% agarose-6.6% formaldehyde denaturing gels, and then transferred to nylon membranes (Micron Separations, Westborough, MA) in 10×SSC (saline-sodium citrate) (1×SSC contained 0.15 M NaCl and 15 mM trisodium citrate, pH 7.0) using a standard method [18]. The membranes were ultraviolet cross-linked, baked at 80°C for 2 h, and then prehybridized at 42°C for 3 h in 50% formamide, 5×SSC, 5×Denhardt's solution (1×Denhardt's solution contained 0.02% each of bovine serum albumin (BSA), polyvinylpyrrolidone, and Ficoll), 0.5% SDS, and 100 μg/ml of denatured fragmented salmon sperm DNA. The prehybridization solution was replaced with fresh solution containing radioactive cDNA probe labeled with [α-^{32}P]dCTP according to the random primed labeling technique [18] and the mixture was hybridized overnight at 42°C. The specific cDNA probe used was full-length hamster α_{1b}AR cDNA cloned into the *Eco*RI site (5′...3′ orientation) of plasmid pSP65 (the insert was 2 kb), a generous gift of Dr. Marc G. Caron

(Howard Hughes Medical Institute, Duke University Medical Center). Insert-containing plasmids were digested with the appropriate restriction enzyme and the inserts were purified by preparative agarose gel electrophoresis. After hybridization, the membranes were washed three times (10 min each) in 1×SSC-0.1% SDS at room temperature and four times (20 min each) in 0.1×SSC-0.1% SDS at 55°C. Washed membranes were exposed to Kodak X-OMAT AR film at −70°C with DuPont Cornex Lightening Plus intensifying screens. Hybridization with ^{32}P-labeled 28S rRNA oligonucleotide served as an internal control. The hybridization signals were quantified by densitometric scanning and all α_{1b}AR mRNA levels were normalized relative to the level of 28S rRNA.

2.6. Measurement of the transcription rate of α_{1b}AR mRNA by nuclear run-off assay

Liver nuclei were isolated by the method of Bush [19]. Liver tissue (approx. 1 g) was homogenized in 10 vols. of a medium containing 2.4 M sucrose, 3.3 mM $CaCl_2$, 2.0 mM $MgCl_2$, 0.1 mM ethylenediaminetetraacetic acid (EDTA), 0.1 mM phenylmethylsulfonyl fluoride, 5 mM dithiothreitol (DTT), and 10 mM Tris-HCl, pH 7.5. The homogenate was filtered through four layers of cheesecloth and centrifuged at 40 000×*g* for 70 min. The pellet was resuspended in 1 M sucrose containing 1 mM $CaCl_2$, rehomogenized, and then recentrifuged at 2600×*g* for 10 min. The resultant nuclei pellet was resuspended in 40% glycerol containing 5 mM $MgCl_2$, 0.1 mM EDTA, 5 mM DTT, and 50 mM Tris-HCl, pH 8.0, stored at −70°C, and then used for nuclear run-off assay. Nuclear run-off assay was performed according to the methods of Greenberg et al. [20] and Deng and Cornett [10] with modification [17]. The reaction mixture in a final volume of 0.5 ml contained 20 mM Tris-HCl, pH 8.0, 150 mM KCl, 5 mM $MgCl_2$, 2 mM DTT, 0.5 mM each of ATP, CTP and GTP, and 0.2 μM of [α-^{32}P]UTP containing a radioactivity of 250 μCi. The reaction was initiated by the addition of 2.8×10^7 nuclei, allowed to proceed for 30 min at 30°C, and then terminated by the addition of 200 units of deoxyribonuclease I in 300 mM NaCl, 5 mM $MgCl_2$, 10 mM Tris-HCl, pH 7.5, and 1.2 mM $CaCl_2$. Subsequently, 150 μg of proteinase K in the presence of 1% SDS and 5 mM EDTA were added, and the mixture was incubated at 37°C for 30 min. ^{32}P-Labeled RNA was extracted and purified as described by Greenberg et al. [20]. The [^{32}P]RNA (1.2×10^7 cpm) was hybridized in 1.4 ml of hybridization solution (10 mM *N*-tris(hydroxymethyl)methyl-2-aminoethanesulfonic acid, pH 7.4, 10 mM EDTA, 0.2% SDS, 300 mM NaCl) with non-labeled α_{1b}AR cDNA probe (a 2 kb full-length hamster α_{1b}AR cDNA cloned into the *Eco*RI site of plasmid pSP65 obtained from Dr. Marc G. Caron) and synthetic 28S rRNA oligonucleotide, which were immobilized on nitrocellulose membranes. Dot-blot hybridization was carried out at 65°C for 48 h. After hybridization the membranes were washed three times in 1×SSC at room temperature, two times in 1×SSC at 65°C for 30 min, and then incubated with ribonuclease A (10 μg/ml) at 37°C for 30 min. The membranes were washed subsequently in 1×SSC-0.1% SDS at 65°C for 30 min, rinsed in 1×SSC. Washed membranes were exposed to Kodak X-OMAT AR film at −70°C with intensifying screens for 7–10 days. Hybridized nuclear [^{32}P]α_{1b}AR and [^{32}P]28S rRNA transcript abundances were scanned and quantified densitometrically. The transcription rate of α_{1b}AR mRNA was calculated from [^{32}P]RNA bound to the specific cDNA fragment for α_{1b}AR and corrected for the amount of input labeled RNA from 28S rRNA oligonucleotide.

2.7. Stability (half-life) assay of liver α_{1b}AR gene transcript

The stability of liver α_{1b}AR mRNA was measured by actinomycin D pulse-chase method [21,22] with modification [17]. Liver tissue slices (20–30 mg each) were incubated at 37°C in Krebs-Henseleit buffer (pH 7.4) containing 2% dialyzed BSA and 5 μg/ml of actinomycin D for various time intervals (0, 2, 4 and 6 h). The reaction mixture was constantly equilibrated with a stream of 95% O_2-5% CO_2. After the designated time of incubation, total RNA was extracted from each sample as described earlier for Northern blot analysis. An aliquot (30 μg) of extracted RNA was dot-blotted on a nylon membrane and hybridized with ^{32}P-labeled cDNA probe (2 kb full-length hamster α_{1b}AR cDNA cloned into the *Eco*RI site of plasmid pSP65) as described for North-

ern blot analysis. Stringent post-hybridization washes of the membranes and the detection of the hybridized signals were performed as described previously. The half-life of α_{1b}AR mRNA was defined as time required for the 50% reduction in mRNA level.

2.8. Protein assays and statistical analysis

The protein content of liver plasma membranes was measured by the method of Lowry et al. [23]. The statistical analysis of the data was performed using one-way analysis of variance followed by Student-Newman-Keuls tests. A P value of less than 0.05 was accepted as statistically significant.

3. Results

Fig. 1 depicts changes in the density of α_1AR in the rat liver during the early and late phases of sepsis based on [^{3}H]prazosin binding studies. The maximal binding capacity (B_{max}) for [^{3}H]prazosin binding was increased by 30% ($P < 0.05$) during early sepsis but

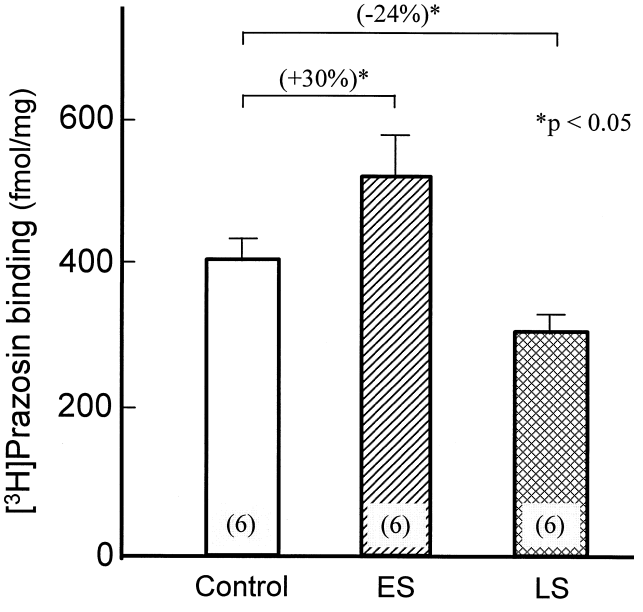

Fig. 1. Changes in the density of α_1-adrenoceptor (α_1AR) in the rat liver during different phases of sepsis. [^{3}H]Prazosin binding was performed as described in Section 2. Early sepsis (ES) and late sepsis (LS) refer to measurements performed at 9 and 18 h, respectively, after cecal ligation and puncture. Vertical bars indicate standard errors of the mean. Number of experiments is shown in the parentheses within each column.

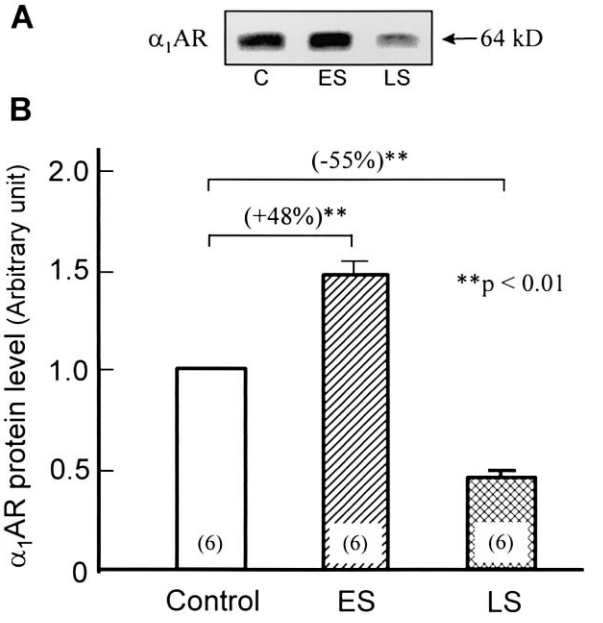

Fig. 2. (A) A representative autoradiograph of Western blot analysis. (B) Western blot analysis of α_1-adrenoceptor (α_1AR) protein levels in the control and septic rat livers. Western blot analysis was carried out as described in Section 2 using polyclonal antibodies raised to rabbit α_1AR. Vertical bars indicate standard errors of the mean. Number of experiments is shown in the parentheses within each column. ES, early sepsis; LS, late sepsis.

was decreased by 24% ($P < 0.05$) during late sepsis (401 ± 25, 520 ± 60 and 305 ± 20 fmol/mg protein for control, early sepsis, and late sepsis, respectively). The affinity (the reciprocal of dissociation constant (K_d)) for [^{3}H]prazosin binding was not significantly affected during sepsis (K_d values in nM: 0.15 ± 0.04, 0.20 ± 0.05, and 0.14 ± 0.02 for control, early sepsis, and late sepsis, respectively). These data indicate that the density of α_1AR in the rat liver was increased during the early phase but was decreased during the late phase of sepsis.

Fig. 2 shows the Western blot analysis of α_1AR protein level in rat liver during different phases of sepsis. α_1AR protein level was elevated by 48% ($P < 0.01$) during early sepsis while it was decreased by 55% ($P < 0.01$) during late sepsis (Fig. 2B). These findings reinforce the data presented in the previous figure that α_1AR was overexpressed in the rat liver during the early phase but it was underexpressed during the late phase of sepsis.

Fig. 3 shows Northern blot analysis of the steady-state level of α_{1b}AR mRNA in the control and septic

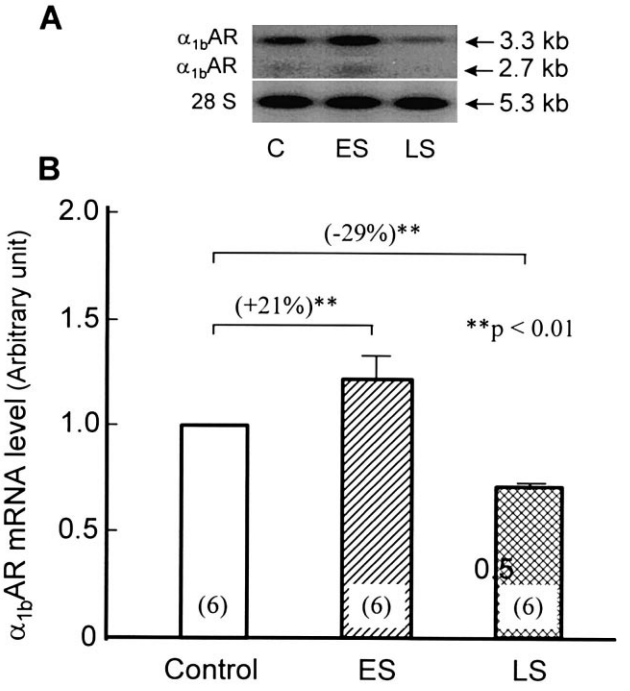

Fig. 3. (A) A representative autoradiograph of Northern blot analysis. (B) Northern blot analysis of the steady-state levels of α_{1b}AR mRNA in the control and septic rat livers. Northern blot analysis was performed as described in Section 2 using total cellular RNA. The specific cDNA probe used was a full-length hamster α_{1b}AR cDNA cloned into the *Eco*RI site of plasmid pSP65. All α_{1b}AR mRNA levels were normalized relative to the level of 28S rRNA to correct for the potential difference in the amount of RNA loaded. ES, early sepsis; LS, late sepsis.

rat livers. Using total cellular RNA, the specific α_{1b}AR cDNA probe, and 28S rRNA oligonucleotide as an internal control to correct for the potential difference in the amount of RNA loaded, the Northern blot analysis reveals two distinct transcripts, an obvious 3.3 kb species and a weak and barely detectable 2.7 kb species. Analyses of the densitometric signals reveal that the steady-state level of 3.3 kb α_{1b}AR mRNA was increased by 21% ($P < 0.01$) during early sepsis but was decreased by 29% ($P < 0.01$) during late sepsis (Fig. 3B). It should be mentioned that the yields and the purities of total RNA remained unaltered among control, early septic, and late septic groups (data not shown), indicating that changes in the steady-state level of 3.3 kb α_{1b}AR mRNA were not a result of alterations in the isolation procedure for RNA. These results demonstrate that α_{1b}AR gene transcript in the rat liver was overexpressed during early sepsis but it was underexpressed during late sepsis.

Figs. 4 and 5 depict changes in the transcription rate and the stability, respectively, of α_{1b}AR mRNA in the control and septic rat livers. Nuclear run-off assays reveal that the transcription rate of α_{1b}AR mRNA was increased by 76% ($P < 0.01$) during early sepsis while it was decreased by 29% ($P < 0.01$) during late sepsis (Fig. 4B). It should be mentioned that the yields (in 10^7 nuclei/g wet tissue: 9.5 ± 0.5, 10.3 ± 0.5, and 10.5 ± 0.6 for control, early sepsis, and late sepsis, respectively) and the viabilities (in %: 96 ± 6, 94 ± 5, and 93 ± 8 for control, early sepsis, and late sepsis, respectively) of hepatic nuclei isolated from control, early, and late septic rats were comparable, indicating that changes in the transcription rate of α_{1b}AR mRNA, as shown in Fig. 4B, were not a result of changes in the isolation procedure for hepatic nuclei. The actinomycin D pulse-chase stability assays demonstrate that the half-life of α_{1b}AR mRNA remained constant during the early

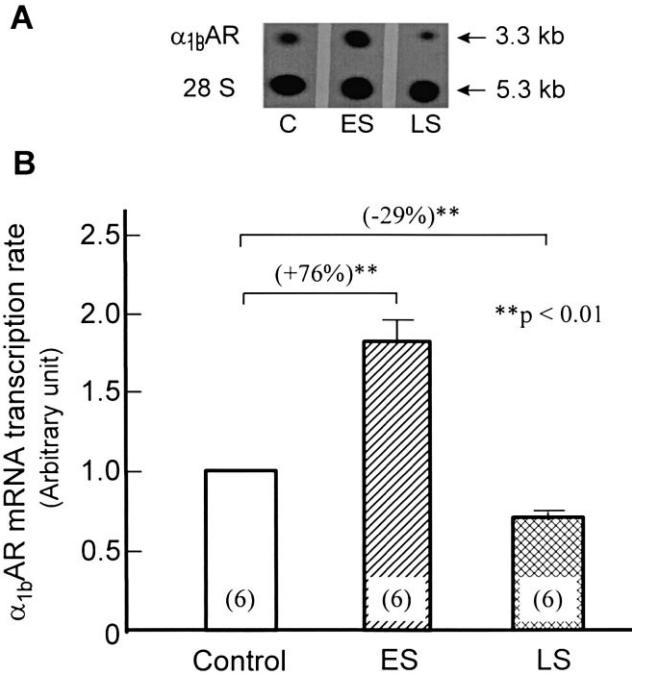

Fig. 4. (A) A representative autoradiograph of nuclear run-off assay. (B) Nuclear run-off assay of the transcription rates of α_{1b}AR mRNA in the control and septic rat livers. Nuclear run-off assay was conducted as described in Section 2 using α_{1b}AR cDNA cloned into the *Eco*RI site of plasmid pSP65 and synthetic 28S rRNA oligonucleotide as specific probes. ES, early sepsis; LS, late sepsis.

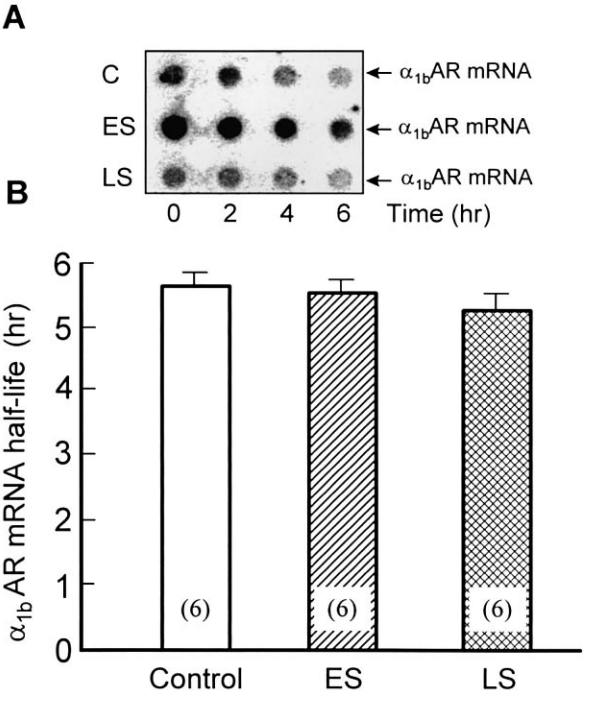

Fig. 5. (A) A representative autoradiograph of half-life assay. (B) Stability (half-life) assay of α_{1b}AR gene transcripts in the control and septic rat livers. The half-life of α_{1b}AR gene transcripts was measured as described in Section 2 using total cellular RNA prepared from liver tissue slices and the specific cDNA probe identical to that of Northern blot analysis. The half-life of α_{1b}AR gene transcript was calculated as time required for the 50% reduction in mRNA level. ES, early sepsis; LS, late sepsis.

and the late phases of sepsis (5.6 ± 0.24, 5.5 ± 0.25, and 5.3 ± 0.31 h for control, early sepsis, and late sepsis, respectively) (Fig. 5B). These data (Figs. 4 and 5) unequivocally demonstrate that the transcription rate of α_{1b}AR mRNA was enhanced during the early stage, followed by attenuation during the late stage of sepsis, without changing the rate of degradation.

4. Discussion

Data presented in the current study demonstrate that the density of α_1AR (Fig. 1) and the α_1AR protein concentration (Fig. 2) were increased in parallel during the early phase while they were decreased in parallel during the late phase of sepsis in the rat liver. These results are consistent with our previously findings that in addition to the altered intracellular translocation between the surface membrane and the intracellular site [8], α_1AR was overexpressed during the initial stage followed by a subsequent underexpression in the rat liver during the progression of sepsis. Since the activation of α_1AR in the liver is known to increase hepatic glucose output into the circulation [6,7], an initial overexpression of α_1AR would lead to the development of the hyperglycemia during the early phase of sepsis while the subsequent underexpression of α_1AR would result in the formation of the hypoglycemia during the late phase of sepsis. Our findings, thus, may have a pathophysiological significance in contributing to the understanding of the altered glucose homeostasis during sepsis.

Recent advances in the studies of molecular biology of α_1AR have indicated that α_1AR is composed of three different genes, α_{1a}, α_{1b}, and α_{1d}, with α_{1b} being the predominant gene expressed in the rat liver [4,6,24]. α_{1b}AR gene has three transcripts of 2.3, 2.7, and 3.3 kb in length. The 3.3 kb species is preferentially expressed in the liver while the 2.7 kb species is highly expressed in the heart. The low abundance 2.3 kb species is difficult to detect in the liver [25–27]. Using a specific α_{1b}AR cDNA probe for Northern blot analysis (Fig. 3), we found that the steady-state level of α_{1b}AR mRNA was increased during the early phase while it was decreased during the late phase of sepsis. Since the increase in the α_{1b}AR mRNA abundance during the early phase of sepsis paralleled with the elevations in the density and the protein concentration of α_1AR, while the decrease in the α_{1b}AR mRNA abundance during the late phase of sepsis coincided with the declines in the density and the protein concentration of α_1AR, it is concluded that changes in the dynamic and the protein level of hepatic α_1AR during the progression of sepsis were a result of the altered expression of α_{1b}AR gene transcript.

Regulation of gene expression is divided into three categories: pre-transcriptional, transcriptional, and post-transcriptional [28,29]. Pre-transcriptional events include signal transduction, second messenger activation, and the activation of transcriptional factors specific to the regulatory region of each specific gene. Transcriptional events include the initiation of RNA synthesis, elongation of the nascent RNA chain, and termination of RNA synthesis. Post-transcriptional events include processing of the RNA

transcript into mRNA, translation of that mRNA into protein, protein modification, and protein export [28–30]. The steady-state levels of mRNA measured by Northern blotting are dependent upon the transcriptional synthesis of mRNA and its post-transcriptional degradation. In the nuclear run-off assay, the isolated nuclei elongate (but not initiate) RNA transcripts and the incorporation of a radioactive RNA precursor is proportional to the transcriptional activity of each gene at the time the nuclei are isolated [20]. Our findings that the rate of transcription of α_{1b}AR mRNA was increased during the early phase while it was decreased during the late phase of sepsis (Fig. 4), thus indicate that the altered expression of α_{1b}AR mRNA during the progression of sepsis was regulated transcriptionally. Although the length of the transcript's poly(A)$^+$ tail and the poly(A)$^+$ binding protein have been associated with the stability of specific mRNAs, and many unstable transcripts have one or more copies of an AU-rich motif in their 3′-untranslated regions, none of these structural elements appear to be present in the rat α_{1b}AR mRNA [10,31]. Our findings that the half-life of α_{1b}AR mRNA remained unaltered during the early and the late phases of sepsis (Fig. 5), thus indicate that the post-transcriptional mechanism did not play a role in the regulation of the altered expression of α_{1b}AR gene transcript during the progression of sepsis.

Structurally, the rat α_{1b}AR gene is composed of two exons and a single large intron. The proximal 5′-flanking region contains three discrete transcription start points, *tsp*1, *tsp*2, and *tsp*3. The α_{1b}AR mRNAs are transcribed from three distinct promoters, P1, P2 and P3, with direct transcription from *tsp*1, *tsp*2, and *tsp*3, respectively [25–27,32]. The proximal promoter, P1, is involved with the generation of the low abundance 2.3 kb mRNA species and contains no homologies with the consensus sequences of known transcription factors. The middle promoter, P2, is responsible for the generation of 2.7 kb mRNA species and contains binding sites for several *trans*-acting factors. The distal promoter, P3, is responsible for the transcription of the 3.3 kb species and contains *cis*-recognition sites for liver-specific transcription factors [25–27,32]. A variety of transcription factors are known to affect the transcription of hepatic α_{1b}AR gene, among which NF1 (nuclear factor 1), HNF5 (hepatocyte nuclear factor 5), and C/EBP (CCAAT/enhancer binding protein) are considered the most important [26,27]. It has been reported that in the remnant liver after partial hepatectomy, the level of NF1 and its binding to P2 were decreased and that the decreases were associated with the declines in the rate of transcription of the α_{1b}AR gene and in the steady-state level of its mRNA [27]. Furthermore, the down-regulation of α_{1b}AR in the rat liver following partial hepatectomy was found to be associated with a marked decrease in the 2.7 kb mRNA species [25], which was thought to be related to the reported decrease in C/EBP expression in the regenerating rat liver [25,33]. In light of the above, it is conceivable that changes in the level and/or the binding affinity of various transcription factors such as NF1, C/EBP, or HNF5 are responsiblez for the altered expression of α_{1b}AR mRNA gene transcripts in the liver during the progression of sepsis. Further investigation of the possible participation of various transcription factors may shed light on the exact mechanism leading to the initial overexpression and the subsequent underexpression of α_{1b}AR gene transcripts in the liver during the progression of sepsis.

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