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EXPRESSION OF THE CONNEXIN 43 GENE IS INCREASED IN THE KIDNEYS AND THE LUNGS OF RATS INJECTED WITH BACTERIAL LIPOPOLYSACCHARIDE

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ABSTRACT—At the molecular level, the inflammatory response is characterized by changes in gene expression of various organ systems. One gene by which expression has been observed to be altered in the liver during inflammation is connexin (Cx) 32. Cx genes encode the polypeptide subunits of the hemichannels that comprise gap junctions. In the present study, an increase in the expression of a different Cx gene, Cx43, was observed in the kidney and lung of rats injected with a sublethal dose (1 mg/kg) of bacterial lipolysaccharide (LPS). To elucidate the possible mechanism by which the Cx43 expression is increased during inflammation, the 5′ flanking region of the gene was cloned and coupled to a reporter gene (human growth hormone). This construct was transfected into cells of renal origin (NRK), which express Cx43 constitutively. The Cx43 promoter activity was indeed found in the cloned region, which contained 725 base pairs upstream of the transcriptional initiation site of the Cx43 gene. The Cx43 promoter activity was found to be increased by incubation of the transfected cells with serum obtained from LPS-treated rats. Moreover, direct incubation of the transfected cells with LPS or interleukin 1β, but not with other cytokines, was observed to increase the Cx43 promoter activity. These results suggest the expression of Cx43 after administration of LPS is part of the inflammatory response. Moreover, the expression of this gene seems to be mediated by proinflammatory mediators.

INTRODUCTION

The sequential collapse of different organ systems (lung, kidney, gut, and liver) after severe trauma, sepsis, pancreatitis, or shock, termed multiple organ dysfunction syndrome (MODS), is the most important cause of mortality and morbidity in surgical intensive care units (1, 2). Studies performed during the last decade have suggested that the development of MODS is not caused directly by the primary insult, but rather by the overwhelming host defense system, such as an uncontrolled inflammatory response (3). The inflammatory response is characterized by the coordinated action of several mediators of different natures and origins acting in a variety of organ systems. At the molecular level, the inflammatory response could be described as a change in the pattern of gene expression in different organ systems. For example, the acute phase proteins, which are involved in the systemic protection, are rapidly expressed in the liver during inflammation (4). Metallothionein (MT), which is involved in the cellular defense from oxygen radicals (5) and nitric oxide (NO) toxicity (6), is also induced in the liver during inflammation (7). Simultaneous with the induction of these "protective" genes, there is a decrease in the levels of constitutively expressed genes, such as albumin (4), phosphoenolpyruvate carboxykinase (8, 9), glucose-6-phophatase, palmitoyl transferase II, acetyl coenzyme A-acyltranferase, and ornithine transcarbamylase (10). We have previously postulated that the down-regulation of these nonvital genes during injury is due to the limited cellular capacity for gene expression; this phenomenon has been coined "the adaptive response to stress" (8).

Another important aspect of the inflammatory response is the alteration in the communication between cells and organs. Thus, paracrine and autocrine communication is affected during inflammation by changes in the normal hormonal balance, presence of cytokines, the levels of secondary mediators such as calcium, NO, and arachidonic acid (11, 12). Another form of cellular communication mediated by the direct coupling of adjacent cells, named gap junctional cellular communication, has also been observed to be altered during inflammation (13). Gap junctions are channels that allow the transit of low molecular weight metabolites (e.g., cAMP, inositol 1,4,5-trisphosphate) and ions (e.g., Ca^{+2}, K^{+}, Cl^{-}) between adjacent cells. They play an important role in smooth muscle contraction, propagation of electrical signals, and metabolic regulation (14). Gap junctions are formed by the docking of two hemichannels, termed "connexons," located on the plasma membrane of neighboring cells. Each connexon is composed of six subunits, connexins (Cx), that are transmembrane proteins. There are several Cx genes that have been classified according to the molecular weight of the encoded polypeptide (15). The expression of the Cx genes is tissue specific. For example, Cx43 is the major Cx found in the heart, and Cx37 is predominant in the lungs and kidneys, whereas Cx 32 and Cx26 are present in the liver (15).

We have previously observed a decrease in the expression of connexin 32 (Cx32), the protein subunit of the most abundant hepatic gap junction, in the liver during lipopolysaccharide (LPS) induced inflammation (13). In addition, a down-regulation of the Cx32 expression was observed in both ischemic and

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nonischemic liver lobes following regional hepatic ischemia/reperfusion (16). Consequently, it would be important to determine whether the expression of other Cx genes is affected during stress. In the present study we show a dramatic increase in the expression of Cx43 in the kidney and the lung after administration of LPS. In order to elucidate the possible mechanism underlying the increase in the expression of Cx43 in the kidney and the lung, the promoter region of this gene was cloned. The effect of different proinflammatory mediators on the Cx43 promoter activity was analyzed. It was found that LPS and interleukin 1β (IL-1β) can increase the Cx43 promoter activity in kidney epithelial cells.

MATERIALS AND METHODS

Experimental endotoxemia

Adult male Sprague-Dawley rats (250–300 g) were fasted for 16 h, anesthetized by metophane inhalation and intravenously injected with *Escherichia coli* LPS B 026:B6 (1 mg/kg in saline) or with saline (1 mL/kg) via the dorsal penile vein under aseptic conditions as previously described (8). No food was provided following the administration of LPS. At different time points following the LPS injection, animals were killed, and organ samples were taken. Animal studies were conducted according to protocols reviewed and approved by the Institutional Animal Care and Use Committee and adhered to guidelines promulgated by the National Institute of Health.

Northern blot/hybridization analysis

Total RNA was isolated by the acid guanidinium thiocyanate-phenol-chloroform method (17). RNA (10 μg) was separated in formaldehyde-agarose gels and transferred onto nylon modified membranes (Gene Screen Plus). Blots were hybridized at 42°C with radiolabeled cDNA probes using the random primer method (18), with [α^{32}P]dATP and [α^{32}P]dCTP as previously described (19). Blots were washed with 50 mM Tris, pH 8.6, 1 M NaCl, 2 mM EDTA, 1% SDS at 42°C for at least 1 h with a minimum of four changes. Blots were subsequently washed with 2× standard saline citrate buffer (SSC), .1% SDS at 42°C for 30 min and further with 2× SSC, .1% SDS at 65°C for 10 min, as needed. Blots were exposed to X-ray film (Kodak X-OMAT AR) at −70°C in the presence of intensifying screens. Autoradiograms, in the linear range of detection, were quantitated by laser scanning densitometry (Molecular Dynamics). Signals were normalized to the intensity of 18S rRNA, as determined prior to probing by staining the blots with methylene blue (.03%) in 3 M NaOAc, pH 5.2.

Cloning, transfection, and reporter gene analysis of rat Cx43 promoter

Rat genomic DNA was used as a template for the cloning of the Cx43 promoter region by the polymerase chain reaction (PCR) using Taq polymerase and standard reagents with a $MgCl_2$ concentration of 1.5 mM. The primers used (TCACGCCTTCCCCCCAATG and GGACGTTCACTTCCTCGGAGAC, 5′ and 3′, respectively) were synthesized based on the sequence of the Cx43 gene reported by Yu et al. (20). Reaction conditions were as follows: denaturation, 1 min, 94°C; annealing, 1 min, 55°C; elongation, 2 min, 72°C; 30 cycles and 5 min of final elongation at 72°C. A product of approximately 770 base pairs (bp) was obtained and subcloned into pCRII using the TA cloning kit (Invitrogen). Sequencing of this fragment confirmed that it was indeed a segment of Cx43 gene. The PCR product was subcloned in front of the human growth hormone (hGH) gene (pϕGH). The resulting construct (pCx43/725/hGH) was transfected using Lipofectin (Life Technologies, Inc) into 50% confluent monolayers of normal rat kidney (NRK) cell line maintained in D-MEM supplemented with 5% fetal bovine serum. The levels of hGH in the extracellular medium were measured using a commercial radioimmunoassay (Nichols Institute Diagnostics). In addition to the Cx43 constructs, NRK cells were co-transfected with the vector pOPRSVI-CAT containing the RSV promoter and the chloramphenicol acetyl transferase gene (CAT), which allows the constitutive expression of CAT. Expression of CAT was analyzed by an ELISA (5 prime→3 prime, Inc). Expression of CAT was used to evaluate the efficiency of transfection. Consequently, the activity of the Cx43 promoter (hGH) was normalized by the level of CAT expression and the protein content in the sample. Protein was determined by the BCA assay (Pierce). Data is presented as mean ± SE. Statistical analysis was performed by repeated measures analysis of variance (ANOVA) using Dunnett's methods. To generate a stable cell line transfected with the Cx43 promoter and hGH, cells were co-transfected with pRC/CMV, which expresses an antibiotic resistant gene. Cell were selected with geneticin and cloned by limiting dilution. The resulting clones were tested for the presence of hGH in the extracellular medium.

RESULTS

Expression of Cx43 in kidneys and lungs during inflammation

Fasted rats were injected with a sublethal concentration of *E. coli* LPS (1 mg/kg), which has been previously shown to induce a rapid and reversible acute phase expression in the liver (8). Kidney and lung were harvested at different times after the LPS injection. Total RNA was isolated and analyzed by Northern blot/hybridization using cDNA radiolabeled probes. An increase in mRNA levels of Cx43 and MT was observed in kidneys within 2 h of LPS administration, reaching a maximum after 4 h and returning to basal levels within 12 h (Fig. 1). These two genes are not highly expressed in the kidney of normal rats. Moreover, their expression was not induced by administration of saline (not shown). In addition, the expression of Cx43 was observed in the lungs of rats injected with LPS (Fig. 2*A*). The presence of Cx43 mRNA in the lungs paralleled an increase in the mRNA levels of the inducible form of NO synthase (iNOS) as illustrated in Fig. 2*B*. Expression of iNOS in lungs has been previously associated with the response of this organ to inflammation (21).

Analysis of Cx43 promoter in NRK cells

The sequence of rat Cx43 gene has been previously reported and the promoter activity has been partially characterized (20). To study the mechanism of Cx43 expression in the kidney during inflammation, a region of the Cx43 gene comprising 725 bp upstream of the transcriptional initiation site was cloned by PCR amplification of rat genomic DNA using primers designed on the basis of the reported sequence (20). The PCR product was subcloned upstream of hGH gene used as reporter gene. This construct (pCx43/725/hGH) was transfected into NRK cells, which have been previously shown to express Cx43 constitutively (22, 23). Cells were co-transfected with pOPRSVI-CAT to evaluate the efficiency of transfection. After 48 h of the transfection, the extracellular medium was collected to measure the hGH content using a commercial radioimmunoassay (see Materials and Methods). Cells were also lysed to measure CAT activity. There was a sevenfold increase in the ratio hGH activity/Cat activity/mg protein between cells transfected with pCx43/725/hGH and cells transfected with a promoterless plasmid pϕGH (see Fig. 3). These results indicate that the 725 bp region of the Cx43 gene indeed possesses promoter activity.

Activity of Cx43 promoter in the presence of inflammatory mediators

To study the regulation of the Cx43 gene during inflammation, NRK cells were transfected with pCx43/725/hGH and pRC/CMV. The latter plasmid contains an antibiotic-resistant gene. The transfected cells were selected with geneticin and a

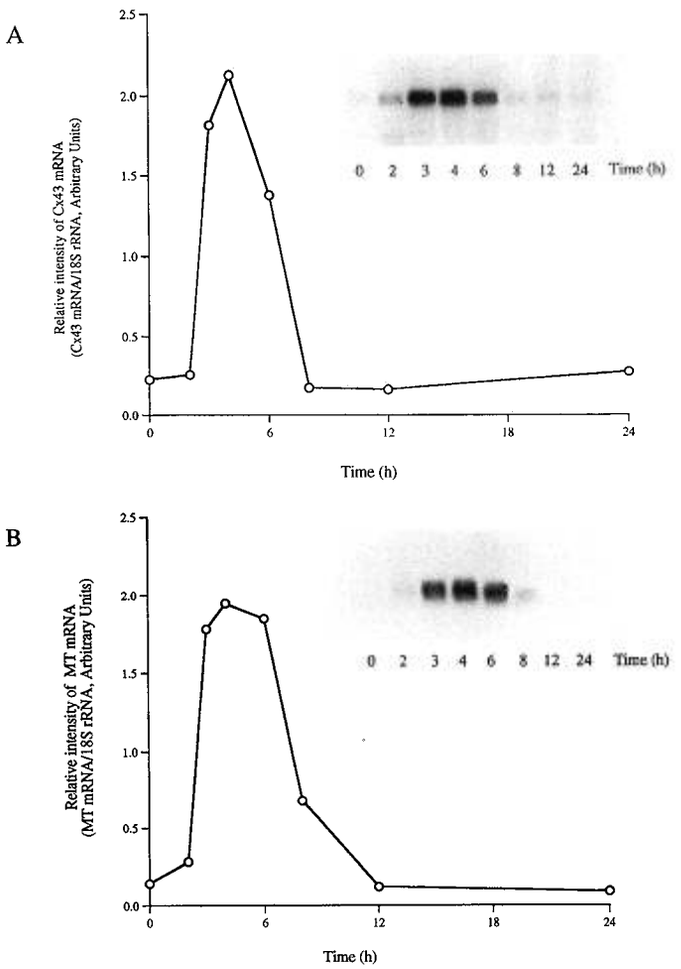

FIG. 1. **Detection of Cx43 and MT mRNA steady-state levels in kidney after administration of LPS.** Total RNA was isolated from rat kidney 0, 2, 3, 4, 6, 8, 12, and 24 h after LPS (1 mg/kg) injection. RNA was separated in a formaldehyde agarose gel and transferred onto nylon modified membranes. Blots were probed with a ^{32}P-labeled cDNA insert coding for rat Cx43 (*A*) or pig MT (*B*) (*insets*). The autoradiogram in the linear range of film sensitivity was quantitated using a laser scanner densitometer (Molecular Dynamics). Differences in RNA loading between lanes were estimated by staining the blots with methylene blue (0.03% in 3 M NaOAc, pH 5.2) before probing. The ratio of signal intensities between Cx43 or MT mRNA (Northern blot) and 18S rRNA (methylene blue staining) was plotted as a function of time.

cell line was obtained (NRK-Cx43/725/hGH). This cell line was incubated for 16 h with serum (1% final concentration) obtained from rats after 0, 1, 2.5, 4, and 24 h of LPS injection. An increase in the content of hGH in the extracellular medium was observed after incubation with the serum obtained after 1 or 2.5 h of LPS treatment. However, serum collected after 4 or 24 h of the LPS injection had no effect on the Cx43 promoter activity (Fig. 4). These findings suggest that there is a component in the serum of LPS-treated rats that stimulated the Cx43 expression, which could be a proinflammatory mediator. To test this possibility, the NRK-Cx43/725/hGH cell line was incubated for 24 h with different concentrations of LPS. The results from this experiment indicate that there is an increase in the Cx43 promoter activity after addition of LPS, which is dose dependent, reaching a maximum value at a concentration of 20 μg/mL (Fig. 5). To confirm this observation, NRK cells were

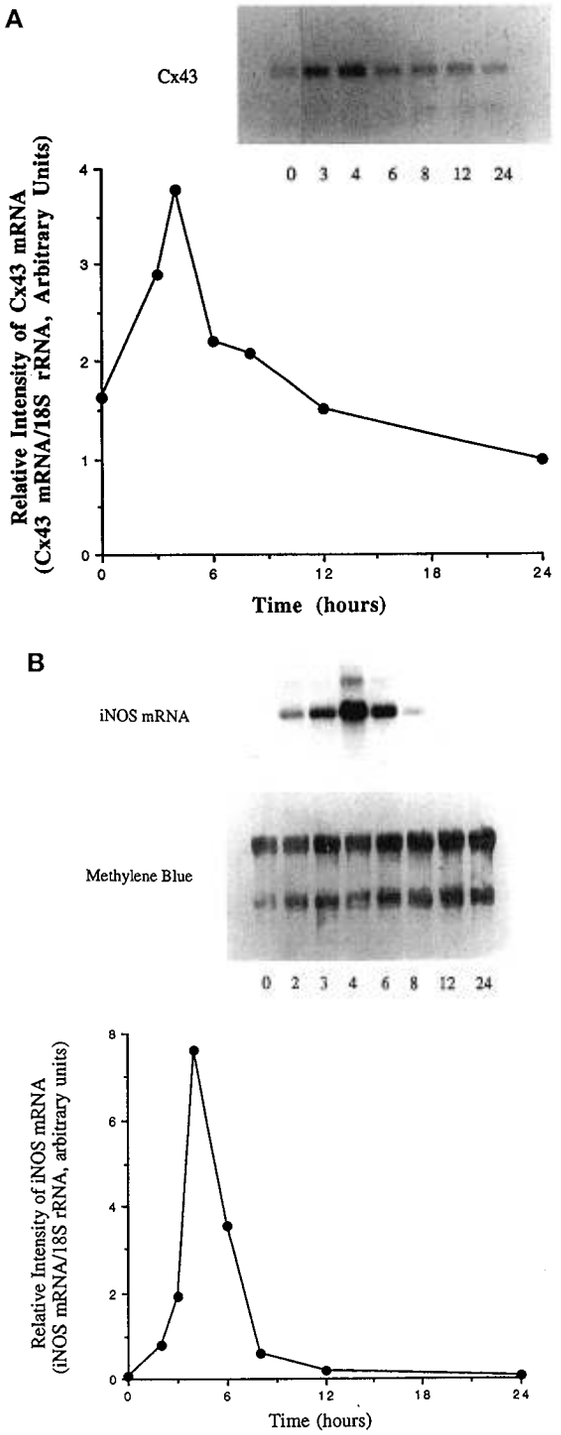

FIG. 2. **Detection of Cx43 and iNOS mRNA steady-state levels in lung after administration of LPS.** Total RNA was isolated from rat kidney 0, 2, 3, 4, 6, 8, 12, and 24 h after LPS (1 mg/kg) injection. RNA was separated in a formaldehyde agarose gel and transferred onto nylon modified membranes. Blots were probed with a ^{32}P-labeled cDNA insert coding for rat Cx43 (A) or human iNOS (B) (*inset*). The autoradiogram in the linear range of film sensitivity was quantitated using a laser scanner densitometer (Molecular Dynamics). Differences in RNA loading between lanes were estimated by staining the blots with methylene blue (0.03% in 3 M NaOAc, pH 5.2) before probing. The ratio of signal intensities between Cx43 or iNOS mRNA (Northern blot) and 18S rRNA (methylene blue staining) was plotted as a function of time.

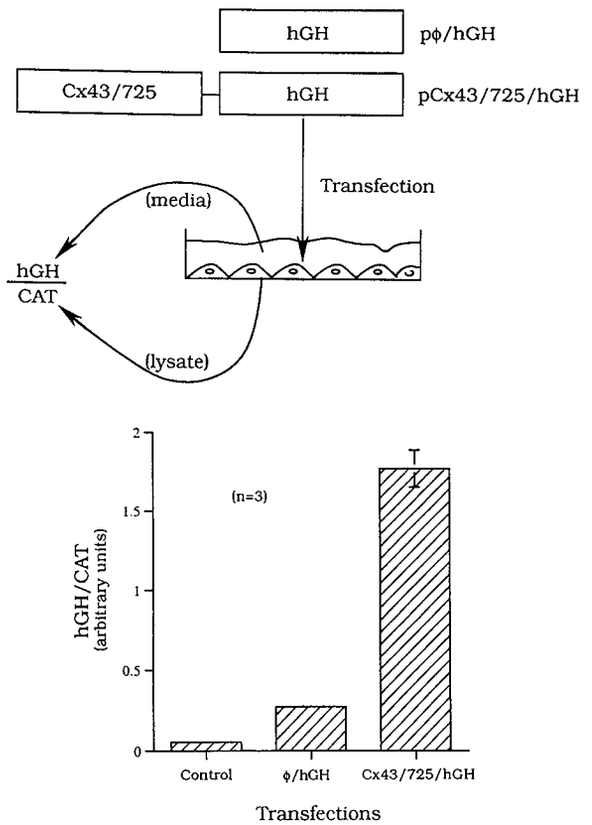

FIG. 3. **Cx43 promoter activity after transfection in NRK cells.** NRK cells (50% confluent monolayers) were transfected with pOPRSVI-CAT and pCx43/725/hGH or pφGH. After 48 h of the transfection, the extracellular medium was collected and cell were lysed. The level hGH in the extracellular medium was measured using a commercial radioimmunoassay as described in "Materials and Methods" and the levels of CAT was analyzed by an ELISA in the cell lysate. Protein levels were also determined in the cell lysate. The Cx43 promoter activity was expressed as the ratio between the specific activity of hGH and CAT. The results correspond to the average of three independent transfections. Both hGH and CAT assays were performed in triplicate.

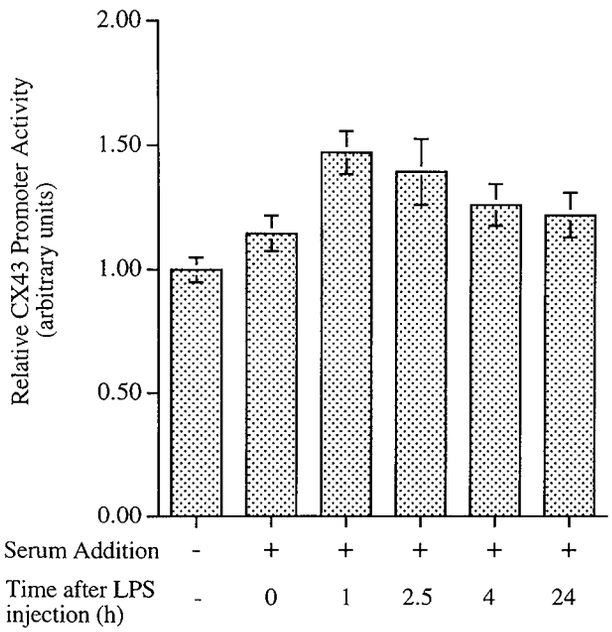

FIG. 4. **Effect of serum obtained from rats that were injected with LPS in the activity of Cx43 promoter in NRK cells.** Rats (triplicate) were injected with LPS (1 mg/kg) and serum was isolated from blood samples obtained at 0, 1, 2.5, 4, and 24 h of the injection. Stably transfected NRK cells expressing hGH driven by the Cx43 promoter were incubated with rat serum (1% final) for 16 h. Expression of hGH was measured in the extracellular medium by a radioimmunoassay. The levels of hGH after addition of serum were normalized to the hGH levels in absence of serum. The results (relative hGH expression) are the average of triplicate determinations carried out with serum obtained from three individual animals for each time point. Each individual serum sample was tested in triplicate with the NRK cell line. Values correspond to mean ± SEM, $p < .05$ for the serum samples of 1 and 2.5 h with respect to the control.

incubated with LPS, and total RNA was isolated and analyzed for the presence of Cx43 mRNA. A 1.5-fold increase in the endogenous levels of the Cx43 mRNA gene was obtained following incubation with LPS (not shown). The NRK-Cx43/725/hGH cell line was also incubated for 24 h with a different concentration of IL-1β, TNF-α, IL-6, or IFN-γ, and the Cx43 promoter activity was determined. An increase in Cx43 promoter activity was observed after incubation of the NRK-Cx43/725/hGH cell line with IL-1β (Fig. 6). There was no significant increase in the Cx43 promoter activity after incubation with TNF-α, IL-6, or IFN-γ (Fig. 6). Co-incubation of the NRK-Cx43/725/hGH cell line with LPS and IL-1β did not increase the Cx43 promoter activity to levels higher than those observed by incubation with the individual mediators. These observations suggest that LPS and IL-1β do not act synergistically. NRK cells incubated with LPS were not found to secrete IL-1β as analyzed by an ELISA (not shown). These result suggest that the effect of LPS on the Cx43 promoter activity was not due to the secretion of IL-1β by NRK cells incubated with LPS.

DISCUSSION

A large body of studies has indicated that the inflammatory response is triggered by various circulating mediators produced by different cell types. For example, macrophages and monocytes secrete cytokines in response to injury that induce the expression of other genes in various organ systems. Thus, acute phase proteins and secondary mediators such as NO and arachidonic acid are all synthesized in response to inflammatory stimuli. These proinflammatory mediators are believed to play an early beneficial role following trauma. However, their uncontrolled expression may be detrimental in the long term. Consequently, it is important to elucidate the molecular and cellular mechanisms underlying the inflammatory response. A profound understanding of these mechanisms may eventually allow us to develop the appropriate strategies to enhance or repress the inflammatory response based on a particular clinical scenario.

We have previously observed the down-regulation of two hepatic genes during inflammation: phosphoenolpyruvate carboxykinase (8, 9), and Cx32 (13, 16). In the present study, we show that the expression of another CX gene, Cx43, was altered during inflammation. In this case, Cx43 expression was increased in kidney and lung following administration of LPS. The expression of Cx43 was observed simultaneously with an increase in kidney and lung mRNA levels of two other genes

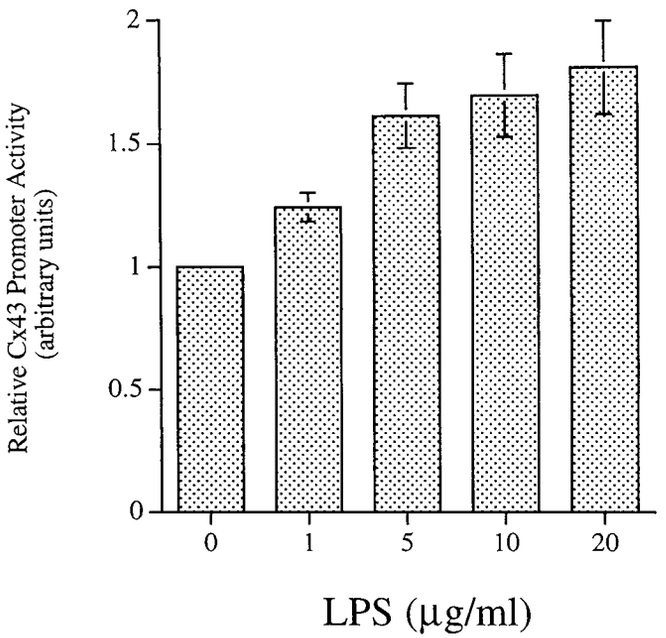

FIG. 5. **Effect of LPS on the activity of Cx43 promoter in NRK cells.** NRK cells were stably transfected with Cx43 promoter/hGH vector. The resulting cell line was incubated for 24 h with LPS (0.5, 1, 5, 10, 20 μg/mL). Expression of hGH was measured in the extracellular medium using a radioimmunoassay. The results were normalized for the protein content in the sample and expressed as a ratio of the samples in which no proinflammatory mediator was added. The data correspond to the average of six independent experiments ± SEM, $p <$ .05 for all LPS determinations with respect to the control.

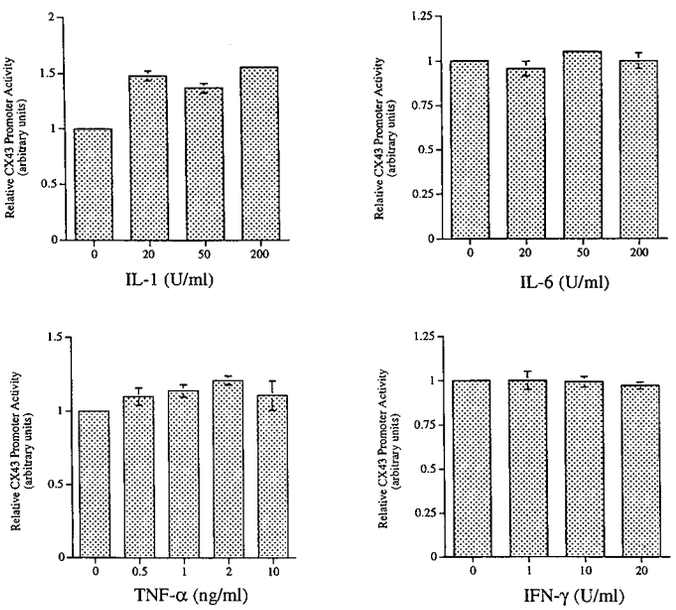

FIG. 6. **Effect of IL1β, IL6, TNFα, and IFN-γ incubation on the activity of Cx43 promoter in NRK cells.** A cell line stably transfected with Cx43 promoter/hGH vector was incubated for 20 h with IL1β (20, 50, 200 U/mL); IL6 (20, 50, 200 U/mL); TNFα (0.5, 1, 2, 10 ng/mL); and IFN-γ (1, 10, 20 U/mL). Expression of hGH was measured in the extracellular medium using a radioimmunoassay. The results were normalized for the protein content in the sample and expressed as a ratio of the samples in which no proinflammatory mediator was added. The data correspond to the average of 3 to 12 independent experiments ± SEM, $p <$ .05 for IL-1 with respect to control and cytokines, respectively.

involved in the inflammatory response: MT and iNOS, respectively. Expression of MT has been observed during inflammatory states induced by various types of injury, such as ischemia/reperfusion (16), endotoxemia (8), and circulatory shock (24). The expression of this gene is regulated by zinc, glucocorticoids, epinephrine, glucagon, IL-1, and IL-6 (25–27). MT is involved in cellular protection from oxygen radicals and NO toxicity (5, 6). There are several studies showing an increase in the expression of iNOS in response to proinflammatory mediators (28, 29). Thus, the expression of Cx43 in the kidney and lung seems to be part of a generalized inflammatory response. Consequently, it would be important to elucidate the possible mechanism of Cx43 expression after administration of LPS and correlate these observations with the expression of other genes such as MT and iNOS. This information will increase our understanding of the different mechanisms of gene regulation involved in the inflammatory response.

In order to study the mechanisms of Cx43 up-regulation during inflammation, the 5′ flanking region of this gene was cloned. This region of the Cx43 gene was coupled to a reporter gene (hGH) and transfected into NRK cells, which are of renal origin. NRK cells were used in this study because these cells have been well characterized for the expression of Cx43 (22, 23). It was found that the Cx43 promoter activity was indeed localized in the cloned region, which contained 725 bp upstream of the transcriptional initiation site. A stable cell line containing the Cx43 promoter region ligated to hGH was obtained to investigate the possible role of proinflammatory mediators. We first tested the effect of serum obtained from LPS-treated rats. It was found that Cx43 promoter activity in this cell line was increased by incubation with serum obtained between 1 and 2.5 h of LPS injection. To determine whether the effect of serum from the LPS-treated rats was due to the presence of proinflammatory mediators, the cell line was individually incubated with several proinflammatory mediators: LPS, IL-1β, TNF-α, IL-6, or IFN-γ. LPS and IL-1β were found to augment the Cx43 promoter activity by 1.5- to 1.7-fold. The other cytokines significantly did not increase Cx43 promoter activity. LPS and IL-1β do not act synergistically, suggesting that their activity may occur through the same signal transduction pathway. Moreover, the effect of LPS was not due to secretion of IL-1β by NRK cells incubated with LPS. The effect of proinflammatory mediators on the expression of Cx43 in the transfected NRK cells was smaller than that observed in rat kidney after administration of LPS. This difference could be explained by several arguments. First, it is possible that the expression of Cx43 in rat kidney requires the presence of several factors that may not be present completely in the serum samples from LPS treated rats. Perhaps, some of these factors are acting in an autocrine fashion. In addition, the basal level of Cx43 expression in NRK cells is higher than that observed in rat kidney. Thus, the relative increase in expression may be lower in NRK cells than *in vivo*. Regardless of the increase in Cx43 expression in the *in vivo* and *ex vivo* systems, our findings support the assumption that the expression of Cx43 is linked to the inflammatory response.

Analysis of the sequence of the Cx43 promoter region re-

vealed several interesting elements that may be involved in the transcriptional regulation of this gene during inflammation. These include AP-1, AP-2, and Sp-1 binding sites within the promoter region (20). Proinflammatory mediators like IL-1 are known to increase the expression of c-fos and c-jun, or activation of c-jun by phosphorylation (30, 31). Since the AP-1 transcriptional factor is formed by the c-fos and c-jun heterodimer, it is tempting to speculate that this may be the potential element involved in the up-regulation of the Cx43 gene in kidney and lungs during inflammation. An increased in Cx43 expression has been observed in the myometrium, which seems to be mediated by estrogen. This increase in Cx43 expression has been correlated with an increase in the levels of c-fos and c-jun (32). In addition to estrogen (20), the expression of Cx43 has been shown to be increased by other mediators, such as basic fibroblast growth factor (33), parathyroid hormone (34), membrane permeable cAMP derivatives (34), and inducers of cellular levels of cAMP, such as forskolin (34, 35).

In summary, the results presented in this study show that the inflammatory response is characterized by changes in the expression of various genes located within different organ systems. The elucidation of the regulatory mechanisms for gene expression during inflammation may provide evidence of common pathways that may be used to modulate the inflammatory response during a disease process.

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REFERENCES

1. Baue AE: Multiple, progressive, or sequential systems failure: A syndrome of the 1970s. *Arch Surg* 110:779–781, 1975.
2. Bone RCCC, et al: American college of chest physicians/society of critical care medicine consensus conference: definitions for sepsis and organ failure and guidelines for the use of innovative therapies in sepsis. *Crit Care Med* 20:864–874, 1992.
3. Livingston DH, Mosenthal AC, Deitch EA: Sepsis and multiple organ dysfunction syndrome: A clinical-mechanistic overview. *New Horizons* 3:257–266, 1995.
4. Kushner I: The phenomenon of the acute phase response. *Ann NY Acad Sci* 389:39–48, 1982.
5. Thornalley PJ, Vasak M: Possible role for metallothionein in protection against radiation-induced oxidative stress: Kinetics and mechanism of its reaction with superoxide and hydroxyl radicals. *Biochem Bioph Acta* 827:36–44, 1985.
6. Schwarz MA, Lazo JS, Yalowich JC, et al: Metallothionein protects against the cytosolic and DNA-damaging effects of nitric oxide. *Proc Natl Acad Sci USA* 92:4452–4456, 1995.
7. Gingalewski C, Theodorakis NG, Yang J, Beck SC, De Maio A: Distinct expression of heat shock and acute phase genes during regional hepatic ischemia/reperfusion. *Am J Physiol* 271:R634–R640, 1996.
8. Wang K, Deutschman CS, Clemens MG, De Maio A: Reciprocal expression of acute phase genes and phosphoenolpyruvate carboxykinase (PEPCK) during acute inflammation. *Shock* 3:204–209, 1995.
9. Deutschman SC, De Maio A, Buchman TG, Clemens MG: Sepsis-induced alterations in levels of mRNA coding for phosphoenolpyruvate carboxykinase: The role of insulin and glucagon. *Circ Shock* 40:295–302, 1993.
10. Andrejko KM, Deutschman CS: Altered hepatic gene expression in fecal peritonitis: Changes in transcription of gluconeogenic. *Shock* 7: 164–169, 1997.
11. Cerra FB, Siegel JB, Border JR, Peters D, McMenamy RH: Correlations between metabolic and cardiopulmonary measurements in patients after trauma, general surgery and sepsis. *J Trauma* 19:621–629, 1979.
12. Nathan C, Sporn M: Cytokines in context. *J Cell Biol* 113:981–986, 1991.
13. Gingalewski C, Wang K, Clemens MG, De Maio A: Posttranscriptional regulation of connexin 32 expression in liver during acute inflammation. *J Cell Physiol* 166:461–467, 1996.
14. Bennett MVL, Barrio LC, Bargiello TA, Spray DC, Hertzberg E, Saez JC: Gap junctions: New tools, new answers, new questions. *Neuron* 6:305–320, 1991.
15. Beyer EC, Goodenough DA, Paul DL: In *Gap junctions* (Hertzberg EL and Johnson RG, eds), pp. 167–175, Alan R. Liss, Inc., New York, NY, 1988.
16. Gingalewski C, De Maio A: Differential decrease in connexin 32 expression in the ischemic and nonischemic regions of rat liver during ischemia/reperfusion. *J Cell Physiol* 171:20–27, 1997.
17. Chomczynski P, Sacchi N: Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. *Anal Biochem* 162:156–159, 1987.
18. Feinberg AP, Vogelstein B: A technique for radiolabeling DNA restriction endonuclease fragments to high specific activity. *Anal Biochem* 132:6–13, 1983.
19. Beck CS, De Maio A: Stabilization of protein synthesis in thermotolerant cells during heat shock. Association of heat shock protein-72 with ribosomal subunits of polysomes. *J Biol Chem* 269:21803–21811, 1994.
20. Yu W, Dahl G, Werner R: The connexin43 gene is responsive to oestrogen. *Proc R Soc Lond B255:* 125–132:1994.
21. Griffiths MJD, Liu S, Curzen NP, Messent M, Evans TW: In vivo treatment with endotoxin induces nitric oxide synthase in rat main pulmonary artery. *Am J Physiol* 268:L509–L518, 1995.
22. Musil LS, Goodenough DA: Biochemical analysis of connexin43 intracellular transport, phosphorylation, and assembly into gap junctional plaques. *J Cell Biol* 115:1357–1374, 1991.
23. Musil LS, Goodenough DA: Multisubunit assembly of an integral plasma membrane channel protein, gap junction connexin43, occurs after exit from the ER. *Cell* 74:1065–1077, 1993.
24. Buchman TG, Cabin DE, Porter J, Bulkley GB: Molecular biology of circulatory shock I. Changes in hepatic gene expression consequent to circulatory shock and resuscitation. *Surgery* 108:559–566, 1989.
25. Cousins RJ, Dunn MA, Leinart AS, Yedinak KC, DiSilvestro RA: Coordinate regulation of zinc metabolism and metallothionein gene expression in rats. *Am J Physiol* 251:E688–E694, 1986.
26. Cousins RJ: Toward a molecular understanding of zinc metabolism. *Clin Physiol Biochem* 4:20–30, 1986.
27. Schroeder JJ, Cousins RJ: Interleukin 6 regulates metallothionein gene expression and zinc metabolism in hepatocyte monolayer cultures. *Proc Natl Acad Sci USA* 87:3137–3141, 1990.
28. Morris SM, Billiar TR: New insights into the regulation of inducible nitric oxide synthesis. *Am J Physiol* 266:E829–E839, 1994.
29. Nathan C, Xie Q-w: Regulation of biosynthesis of nitric oxide. *J Biol Chem* 269:13725–13728, 1994.
30. Camhi SL, Lee P, Choi AMK: The oxidative stress response. *New Horiz* 3:170–182, 1995.
31. Cano E, Mahadevan LC: Parallel signal processing among mammalian MAPKs. *Trends Biochem Sci* 20:117–122, 1995.
32. Piersanti M, Lye SJ: Increase in messenger ribonucleic acid encoding the myometrial gap junction protein, connexin-43, requires protein synthesis and is associated with increased expression of the activator protein-1, c-fos. *Endocrinol* 136:3571–3577, 1995.
33. Doble BW, Kardami E: Basic fibroblast growth factor stimulates connexin-43 expression and intercellular communication of cardiac fibroblasts. *Mol Cell Bioch* 143:81–87, 1995.
34. Schiller PC, Mehta PP, Roos BA, Howard GA: Hormonal regulation of intracellular communication: parathyroid hormone increases connexin 43 gene expression and gap-junctional communication in osteoblastic cells. *Mol Endo* 6:1433–1440, 1992.
35. Wang Y, Rose B: Clustering of Cx43 cell-to-cell channels into gap junction plaques: regulation by cAMP and microfilaments. *J Cell Sci* 108:3501–3508, 1995.