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Sodium lactate increases LPS-stimulated MMP and cytokine expression in U937 histiocytes by enhancing AP-1 and NF-κB transcriptional activities

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[1]*Ralph H. Johnson Veterans Affairs Medical Center;* [2]*Division of Endocrinology, Diabetes and Medical Genetics, Department of Medicine, and* [3]*Department of Biostatistics, Bioinformatics and Epidemiology,* [4]*College of Dental Medicine, Medical University of South Carolina, Charleston, South Carolina*

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Nareika, Alena, Lin He, Bryan A. Game, Elizabeth H. Slate, John J. Sanders, Steven D. London, Maria F. Lopes-Virella, and Yan Huang. Sodium lactate increases lipopolysaccharide-stimulated MMP and cytokine expression in U937 histiocytes by enhancing AP-1 and NF-κB transcriptional activities. *Am J Physiol Endocrinol Metab* 289: E534–E542, 2005. First published June 7, 2005; doi:10.1152/ajpendo.00462.2004.—The plasma lactate concentration in patients with obesity and type 2 diabetes is often higher than that in nondiabetic individuals. Although it is known that increased lactate concentration is an independent risk factor for developing type 2 diabetes, the underlying mechanisms are not well understood. Because inflammation plays an important role in the development of type 2 diabetes, we postulated that increased lactate level might contribute to the pathogenesis of type 2 diabetes by enhancing inflammation. In the present study, we demonstrated that preexposure of U937 macrophage-like cells to sodium lactate increased LPS-stimulated matrix metalloproteinase (MMP)-1, IL-1β, and IL-6 secretion. Augmentation of LPS-stimulated MMP-1 secretion was diminished when sodium lactate was replaced by lactic acid that reduced pH in the culture medium. Furthermore, quantitative real-time PCR indicated that the increased secretion of MMP-1, IL-1β, and IL-6 was due to increased mRNA expression. To explore the underlying signaling mechanism, blocking studies using specific inhibitors for NF-κB and MAPK cascades were performed. Results showed that blocking of either NF-κB or MAPK pathways led to the inhibition of MMP-1, IL-1β, and IL-6 expression stimulated by sodium lactate, LPS, or both. Finally, electrophoretic mobility shift assays showed a synergy between sodium lactate and LPS on AP-1 and NF-κB transcriptional activities. In conclusion, this study has demonstrated for the first time that sodium lactate and LPS exert synergistic effect on MMP and cytokine expression through NF-κB and MAPK pathways and revealed a novel mechanism potentially involved in the development of type 2 diabetes and its complications.

diabetes mellitus; lactate; lipopolysaccharide; matrix metalloproteinases; nuclear factor-κB; activating protein-1; cytokines

LACTATE IS AN END PRODUCT of nonoxidative glycolysis. Chronic elevation of lactate concentration in the blood occurs in many pathological conditions, such as obesity, hypertension, and type 2 diabetes mellitus (8). Lactate concentration can also be elevated locally in tissues where injury, infection, or ischemia occurs (15). In the fasting state, the brain, skin, erythrocytes, skeletal muscle, and adipose tissue are the main organs of lactate production, which mostly derives from glucose and glycogen breakdown (5, 17).

Early epidemiological data have shown that elevated plasma lactate is an independent risk factor for developing type 2 diabetes (27). To understand this observation, DiGirolamo et al. (8) proposed that increased lactate concentration may play a role in the perpetuation of insulin resistance, a key factor in the pathogenesis of type 2 diabetes. In the following studies in which animals were infused with lactate, it was shown that hyperlactatemia reduced glucose uptake by inhibiting glucose transporter GLUT4 mRNA expression in skeletal muscle (22). It was also shown that lactate infusion suppressed glycolysis and impaired insulin signaling (4). These results suggest that chronic hyperlactatemia induces insulin resistance by controlling gene expression and signaling pathways involved in glucose metabolism.

In recent years, increasing evidence indicates that inflammation plays an important role in the development of type 2 diabetes and diabetic complications (7, 13, 41). It has been reported that the plasma concentration of inflammatory mediators, such as interleukin 6 (IL-6) and sialic acid, is increased in the insulin-resistant states of obesity and type 2 diabetes (6, 30). Furthermore, it has been demonstrated by several studies that an increase in inflammatory mediators and markers, such as sialic acid, IL-6, and C-reactive protein, predicts the future development of obesity and type 2 diabetes (10, 31, 35). It has also been well established now that inflammation contributes to complications of type 2 diabetes (18, 21, 26). Although it is generally accepted that obesity and type 2 diabetes are proinflammatory states (7), the mechanisms by which inflammation is induced and enhanced during the initiation and progression of obesity and type 2 diabetes have not been well understood.

Macrophage activation by bacterial lipopolysaccharide (LPS) is an important event involved in inflammation and inflammation-related diseases (12, 38). LPS-induced macrophage activation is characterized by the induction or upregulation of genes such as matrix metalloproteinases (MMPs) and cytokines (12, 38), which play a crucial role in tissue remodeling and inflammatory process. Although it is known that increased lactate concentration affects gene expression and cell function (14, 34), the role of lactate in LPS-induced inflammation remains unknown. In the present study, we demonstrate that sodium lactate augments LPS-stimulated MMP-1, IL-1β, and IL-6 expression by human U937 histiocytes (resident macrophages). These findings suggest that increased lactate levels, either systematically or locally, may enhance LPS-

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induced inflammation and thus contribute to the development of insulin resistance and type 2 diabetes and its complications.

MATERIALS AND METHODS

Cell culture. U937 histiocytes (37) (American Type Culture Collection, Manassas, VA) were cultured in a 5% CO_2 atmosphere in RPMI 1640 medium (GIBCO, Invitrogen, Carlsbad, CA) containing 10% fetal calf serum (FBS), 1% MEM nonessential amino acid solution, 0.6 g/100 ml HEPES, and 1,000 mg/l D-glucose. The medium was changed every 2–3 days. Histiocytes are also called resident macrophages, and the histiocytic origin of U937 cells was shown by its capacity for lysozyme production and strong esterase activity (37). U937 cells were treated with LPS (Sigma, St. Louis, MO) that was highly purified from *Escherichia coli* by phenol extraction and gel filtration chromatography and was cell culture tested.

Cell DNA assay. Cellular DNA was quantified with a CyQUANT cell proliferation assay kit according to the procedures provided by the manufacturer (Molecular Probes, Eugene, OR).

ELISA. MMP-1 and cytokines secreted into culture medium by U937 cells were quantified using sandwich ELISA kits according to the protocol provided by the manufacturer (R&D Systems, Minneapolis, MN).

Real-time polymerase chain reaction. Total RNA was isolated from cells using the RNeasy minikit (Qiagen, Santa Clarita, CA). First-strand complementary DNA (cDNA) was synthesized with the iScript cDNA synthesis kit (Bio-Rad, Hercules, CA) using 20 μl of reaction mixture containing 0.75 μg of total RNA, 4 μl of 5× iScript reaction mixture, and 1 μl of iScript reverse transcriptase (RT). The complete reaction was then cycled for 5 min at 25°C, 30 min at 42°C, and 5 min at 85°C using a PTC-200 DNA Engine (MJ Research, Waltham, MA). The reverse transcription reaction mixture was then used for polymerase chain reaction (PCR) amplification in the presence of the primers (Table 1). The Beacon Designer software (PREMIER Biosoft International, Palo Alto, CA) was used for primer designing. Primers were synthesized by Integrated DNA Technologies (Coralville, IA). Real-time PCR was carried out in duplicates by use of 25 μl of reaction mixture that contained 1.5 μl of RT mixture, 0.2 μM of both primers, and 12.5 μl of iQ SYBR Green Supermix (Bio-Rad, Hercules, CA). The real-time PCR was performed using the iCycler real-time detection system (Bio-Rad) with a two-step method. The reaction was activated with hot-start enzyme (95°C for 3 min), and cDNA was then amplified for 40 cycles consisting of denaturation (95°C for 10 s) and annealing/extension (56°C for 45 s). A melt-curve was then performed (55°C for 1 min, and then temperature was increased by 0.5°C every 10 s) to detect the formation of primer-derived trimers and dimmers. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) as a control was amplified with the primers (Table 1). Amplicon size and reaction specificity were confirmed by 2.5% agarose gel electrophoresis. Data were analyzed with the iCycler iQ I software. The average C_T (threshold cycle) of fluorescence units was used for analysis. Quantification was calculated using the C_T of MMP or cytokine cDNA relative to that of GAPDH cDNA in the same sample.

Treatment of U937 cells with sodium lactate and LPS. U937 cells were pretreated with different concentrations of sodium lactate (pH 7.4) or lactic acid (pH 7.2–6.8) (Sigma) for 24 h. After the medium was changed, the same concentration of sodium lactate or lactic acid and 100 ng/ml LPS were added to the medium, and the incubation was continued for another 24 h. After the incubation, medium was collected for ELISA assay, and cells were harvested for DNA assay.

Blocking of signal transduction pathways. U937 cells were treated with 12 mM sodium lactate, 100 ng/ml LPS, or both, as described above, in the presence or absence of the specific inhibitors of the NF-κB and MAPK (JNK, ERK, and p38) pathways (12), including BAY 11–7085 (16), SP-600125 (2), PD-98059 (33), and SB-203580 (24, 40). After the incubation, the amount of MMP-1, IL-1β, and IL-6 in the conditioned medium was quantified by ELISA.

Electrophoretic mobility shift assay. U937 cells were seeded at 10×10^6/100-mm dish and incubated with or without sodium lactate for 24 h. After the incubation, the cells were exposed to 100 ng/ml LPS for 0, 1, 2, and 4 h. Nuclear extracts were prepared using the NE-PER Nuclear and Cytoplasmic Extraction Kit (Pierce, Rockford, IL) according to the instructions provided by the manufacturer. Nuclear extracts were quickly frozen at −70°C.

The electrophoretic mobility shift assay (EMSA) was performed using a nonradioactive EMSA kit (Panomics, Redwood, CA) according to the experimental procedures provided by the manufacturer. The sequence for the activating protein-1 (AP-1) motif-containing probe is 5′-CGCTTGATGACTCAGCCGGAA-3′, and the one for the NF-κB motif-containing probe is 5′-AGTTGAGGGGACTTTCCCAGGC-3′.

Statistic analysis. Data are presented as means ± SD. Comparison between treatments was performed using a one-way analysis of variance. A value of $P < 0.05$ was considered significant.

RESULTS

Sodium lactate augments LPS-stimulated MMP-1, IL-1β, and IL-6 secretion. It is known that LPS stimulates MMP-1 expression by monocytes and macrophages (18, 26). To determine whether sodium lactate enhances LPS-stimulated MMP-1 secretion, U937 cells were treated with various concentrations (0–12 mM) of sodium lactate and 100 ng/ml LPS concomitantly for 24 h. DNA assay showed that treatment with sodium lactate and LPS had no significant effect on cellular DNA content (data not shown). ELISA showed a concentration-dependent augmentation of LPS-stimulated MMP-1 secretion by sodium lactate (Fig. 1*A*). Compared with the treatment with LPS alone, treatment with 12 mM sodium lactate and LPS simultaneously had a threefold increase in MMP-1 secretion (133 vs. 43 ng/ml; Fig. 1*A*). Interestingly, when cells were pretreated with 12 mM sodium lactate for 24 h and then exposed to LPS for another 24 h (sodium lactate was still present in the medium), the secretion of MMP-1 was increased sevenfold compared with that by cells treated with LPS alone (226 vs. 33 ng/ml; Fig. 1*B*). Clearly, treatment with sodium lactate before exposure to LPS stimulated more MMP-1 secretion than simultaneous treatment with sodium lactate and LPS (226 vs. 133 ng/ml; Fig. 1, *A* and *B*).

The effect of sodium lactate on LPS-stimulated secretion of cytokines, including IL-1β and IL-6, was also investigated. Intriguingly, results showed that, although sodium lactate had

Table 1. *Primer sequences for real-time PCR*

Genes	5′ Primer Sequence	3′ Primer Sequence
MMP-1	CTGGGAAGCCATCACTTACCTTGC	GTTTCTAGAGTCGCTGGGAAGCTG
IL-1β	CTGTACGATCACTGAACTGC	CACCACTTGTTGCTCCATATC
IL-6	AACAACCTGAACCTTCCAAAGATG	TCAAACTCCAAAAGACCAGTGATG
GAPDH	GAATTTGGCTACAGCAACAGGGTG	TCTCTTCCTCTTGTGCTCTTGCTG

MMP-1, matrix metalloproteinase-1.

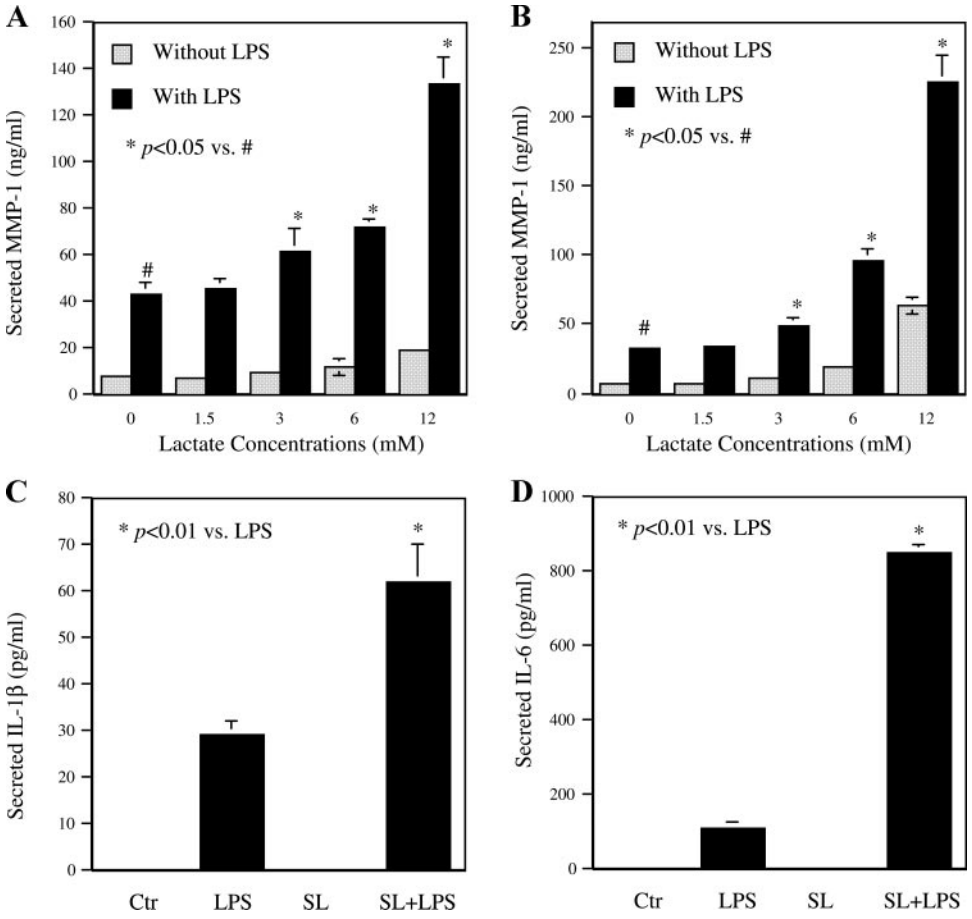

Fig. 1. Augmentation of LPS-stimulated matrix metalloproteinase-1 (MMP-1) secretion by sodium lactate. *A*: U937 cells were treated with different concentrations of sodium lactate in the absence or presence of 100 ng/ml LPS for 24 h. After incubation, culture medium was used for quantification of secreted MMP-1 by ELISA, as described in MATERIALS AND METHODS. *B*: U937 cells were preexposed to different concentrations of sodium lactate for 24 h. After medium was changed, cells were incubated with the previous concentrations of sodium lactate in the absence or presence of 100 ng/ml LPS for another 24 h. Medium was used for quantification of MMP-1 by ELISA, as described in MATERIALS AND METHODS. *C* and *D*: Cell-conditioned medium collected in the experiments shown in *B* was used for quantification of secreted IL-1β and IL-6 by ELISA. Data (means ± SD) presented are representative of 3 independent experiments with similar results.

no effect on IL-1β and IL-6 secretion by itself, pretreatment with sodium lactate led to two- and eightfold increases in LPS-stimulated IL-1β and IL-6 secretion (Fig. 1, *C* and *D*), respectively, compared with the treatment with LPS alone. These data suggest that sodium lactate and LPS have a synergy on the expression of these proinflammatory genes.

Lactic acid is much less effective than sodium lactate in augmentation of LPS-stimulated MMP-1 secretion. In the above-mentioned experiments, we treated cells with sodium lactate that had neutral pH (7.4). To determine whether lactic acid with lower pH also increases MMP-1 secretion in response to LPS stimulation, U937 cells were pretreated with different concentrations (0–12 μM) of lactic acid and then exposed to 100 ng/ml LPS. Results showed that cells pretreated with 3 (pH 7.2), 6 (pH 7.0), and 12 mM (pH 6.8) lactic acid led to only a slight increase (27, 50, and 14%, respectively) in LPS-induced MMP-1 secretion compared with cells treated with LPS alone (Fig. 2*A*). The ineffectiveness of lactic acid on

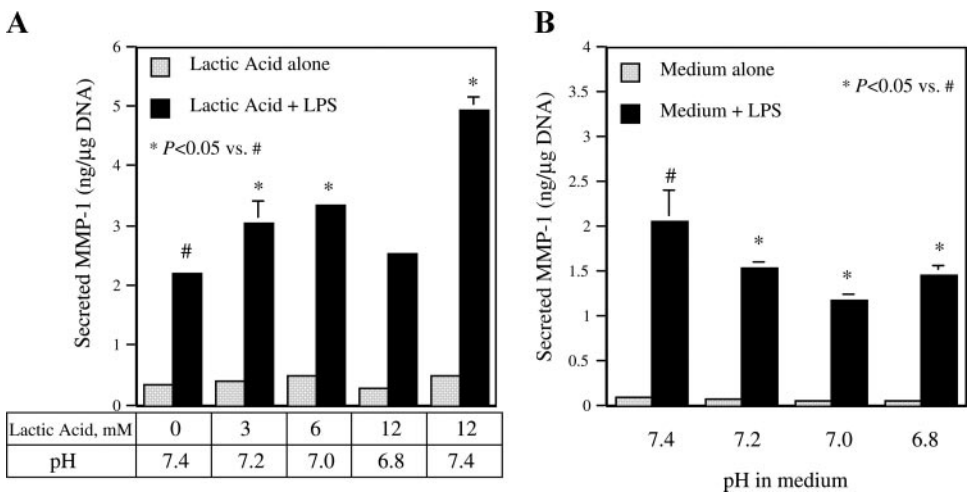

Fig. 2. Effect of lactic acid on MMP-1 secretion. *A*: U937 cells were preexposed to different concentrations of lactic acid for 24 h. Addition of lactic acid into medium resulted in a decrease in pH (7.2–6.8). In additional cell cultures, the pH in the medium containing 12 mM lactic acid was adjusted from 6.8 to 7.4 with NaOH before the experiment. After 24-h incubation, medium was replaced with a fresh one containing the previous concentrations of lactic acid and pH. Cells were incubated in the presence or absence of 100 ng/ml LPS for another 24 h. Medium was then used for quantification of MMP-1. *B*: U937 cells were incubated in medium with different pH (7.4–6.8) for 24 h. After incubation, medium was replaced with a fresh one that had the previous pH. Cells were incubated with or without 100 ng/ml LPS for another 24 h. Medium was then used for quantification of MMP-1.

LPS-stimulated MMP-1 secretion is likely due to the decreased pH, since after the pH was adjusted from 6.8 to 7.4 in medium containing 12 mM lactic acid, preexposure of cells to the medium led to a 130% increase in LPS-induced MMP-1 secretion (Fig. 2*A*). To further validate this notion, U937 cells were incubated with culture medium with different pH (7.4–6.8) prepared with hydrochloric acid for 24 h and then exposed to LPS for another 24 h. Results showed a decrease in MMP-1 secretion by cells exposed to lower pH (Fig. 2*B*).

Enhancement of LPS-stimulated secretion of MMP-1, IL-1β, and IL-6 by sodium lactate is due to increased mRNA expression. To understand how sodium lactate augments LPS-stimulated secretion of MMP-1, IL-1β, and IL-6, the effect of sodium lactate on the mRNA expression of these genes was investigated. Our quantitative real-time PCR showed that pretreatment of cells with sodium lactate increased LPS-stimulated mRNA expression of MMP-1, IL-1β, and IL-6 4.4-, 5.6-, and 18.3-fold, respectively (Figs. 3 and 4), indicating that the increased secretion of LPS-stimulated MMP-1, IL-1β, and IL-6 by sodium lactate is due to a synergistic effect of sodium lactate and LPS on the mRNA expression of these genes.

Sodium lactate and LPS exert synergistic effect on MMP and cytokine expression through NF-κB and MAPK signaling pathways. It has been well established that NF-κB and MAPK (ERK, JNK, and p38) cascades are the major signaling pathways involved in the LPS-induced macrophage activation (12). To determine whether these pathways are also involved in sodium lactate-stimulated gene expression, U937 cells were treated with sodium lactate in the presence or absence of specific inhibitors of these pathways. Figure 5*A* shows that cells treated with 12 mM sodium lactate had a fourfold increase in MMP-1 secretion compared with those without sodium lactate treatment. Ten micromoles of Bay 11-7085 (16), PD-98059 (33), SP-600125 (2), and SB-203580 (24, 40), specific inhibitors of NF-κB, ERK, JNK, and p38 pathways, respectively, inhibited sodium lactate-stimulated MMP-1 expression by 65, 50, 56, and 57%, respectively. Interestingly, Bay 11-7085, PD-98059, SP-600125, and SB-203580 also inhibited LPS-stimulated MMP-1 secretion by 85, 52, 82, and 67%, respectively (Fig. 5*B*). To the cells preexposed to sodium lactate and subsequently treated with LPS, Bay 11-7085, PD-98059, SP-600125, and SB-203580 blocked MMP-1 secretion

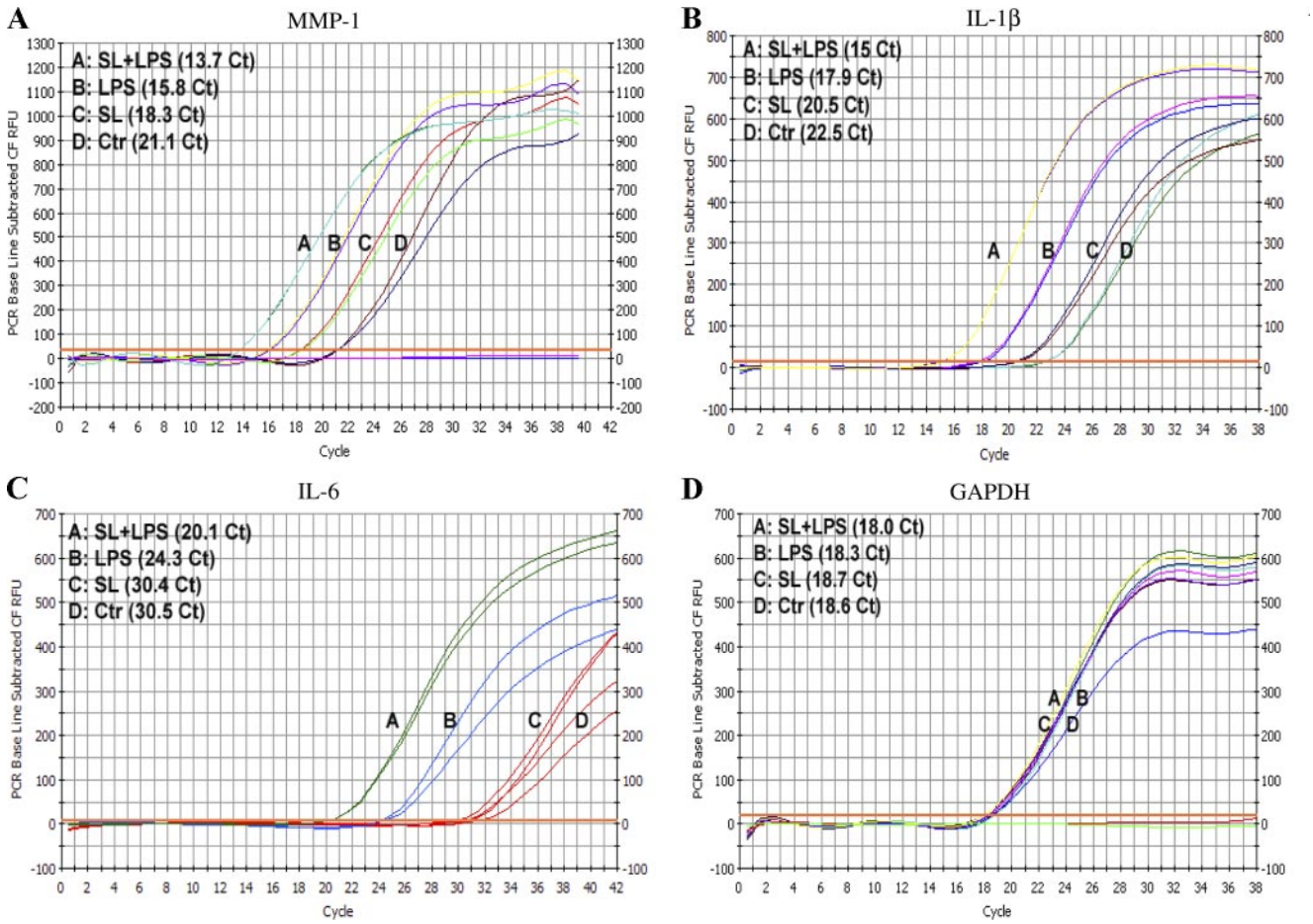

Fig. 3. Real-time PCR analysis of MMP-1, IL-1β, and IL-6 mRNA levels. U937 cells were preexposed to 12 mM sodium lactate for 24 h and then exposed to100 ng/ml LPS and 12 mM sodium lactate for 24 h. Total RNA was isolated, and 5 μg of RNA were converted to cDNA by reverse transcription. Real-time PCR was performed with duplicate cDNA samples. Curves represent the real-time amplification of MMP-1 (*A*), IL-1β (*B*), IL-6 (*C*), and GAPDH cDNA (*D*). No. of cycles before the amount of amplified cDNA reaches to the threshold is designated as C_T (threshold cycles). Amount of PCR products is indicated by relative fluorescence units (RFU, *y*-axis). SL, sodium lactate. Ctr, control. Data (means ± SD) presented are representative of 3 independent experiments with similar results.

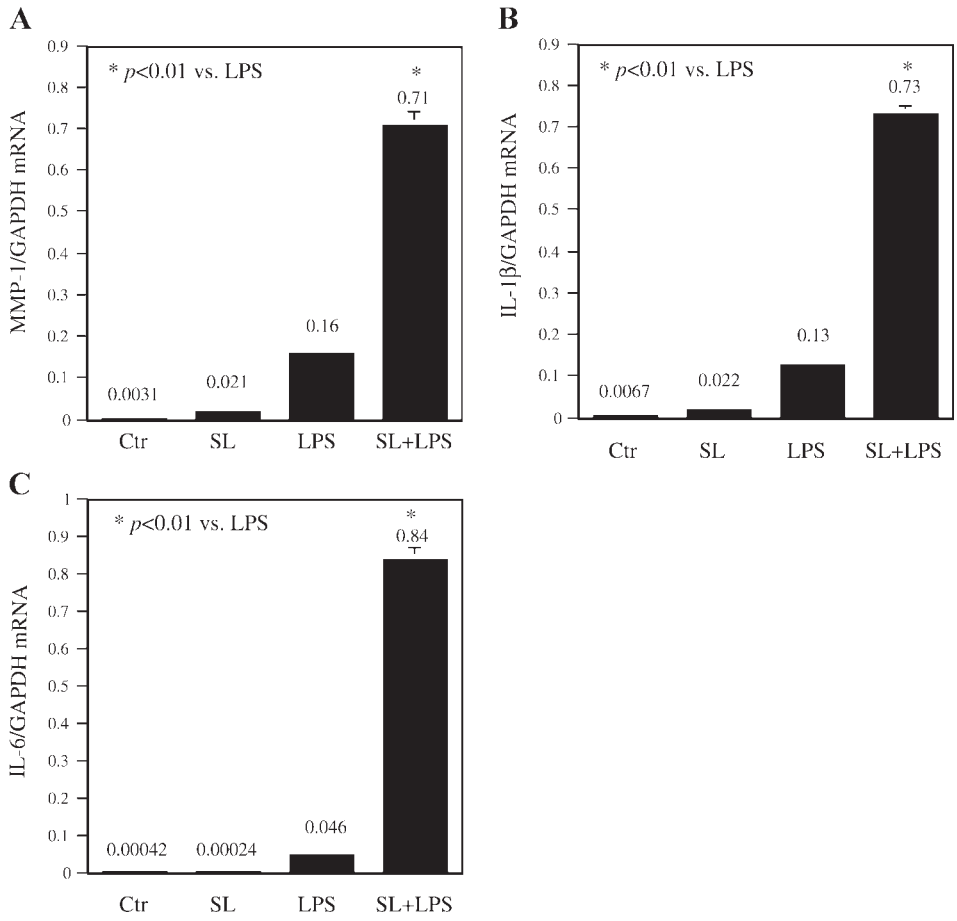

Fig. 4. Quantitative real-time PCR analysis of MMP-1, IL-1β, and IL-6 mRNA levels. The starting quantity of MMP-1, IL-1β, and IL-6 cDNA for each sample was determined from the real-time PCR study (Fig. 3) using iCycler iQ I software and is presented as the ratio of MMP-1, IL-1β, or IL-6 and GAPDH mRNA (nos. above bars). Data (means ± SD) presented are representative of 3 independent experiments with similar results.

by 94, 50, 83, and 66%, respectively (Fig. 5*C*). These data suggest that both sodium lactate and LPS upregulate MMP-1 expression through NF-κB and MAPK cascades. These pathways are also involved in the enhancement of IL-1β and IL-6 expression by sodium lactate and LPS (Fig. 6). The NF-κB pathway seems to be essential for the synergistic effect of sodium lactate and LPS on IL-1β and IL-6 secretion, since blocking of the NF-κB pathway led to the reduction of IL-1β and IL-6 secretion by 97 and 100%, respectively (Fig. 6).

Enhancement of LPS-stimulated AP-1 and NF-κB transcriptional activities by sodium lactate. It is known that the activation of MAPK (ERK, JNK, p38) and NF-κB signaling pathways leads to the activation of AP-1 and NF-κB that are the key proinflammatory transcription factors involved in the regulation of MMP and cytokine expressions (12). To determine whether sodium lactate augments LPS-stimulated MMP-1 and cytokine expression by increasing the transcriptional activities of AP-1 and NFκB, an EMSA was performed. Results showed that exposure of cells to sodium lactate alone increased the amount of shifted AP-1 and NF-κB probes (*time 0*; Fig. 7), suggesting that sodium lactate increases the basal AP-1 and NF-κB activities. Furthermore, preexposure to sodium lactate also enhanced AP-1 and NF-κB activities in cells subsequently treated with LPS (Fig. 7). These results indicate that sodium lactate increases not only the basal but also the LPS-stimulated level of AP-1 and NF-κB activities. The involvement of AP-1 in sodium lactate-augmented MMP-1 expression was further confirmed by the experiments showing that AP-1 inhibitor curcumin (28) inhibited the enhancement of LPS-stimulated MMP-1 secretion by sodium lactate in a concentration-dependent manner (Fig. 8).

DISCUSSION

It has been proposed that hyperglycemia plays an important role in inflammation and inflammation-associated diseases (39). This hypothesis is supported by the studies on advanced glycation end product (AGE), which have demonstrated that AGE is accumulated under the condition of hyperglycemia and causes expression of a range of proinflammatory molecules and activation of NF-κB (39). Furthermore, studies reported by Aljada et al. (1) showed that glucose intake by healthy subjects induced a quick increase in the activities of proinflammatory transcription factors NF-κB and AP-1, elucidating an AGE-independent mechanism that may be also involved in hyperglycemia-promoted inflammation. Recently, a clinical study conducted by the Diabetes Control and Complications Trial Research Group has provided strong evidence that hyperglycemia is essential in inflammation-associated atherosclerosis, as it shows that the stringent control of glucose by intensive therapy in diabetic patients leads to decreased progression of intima-media thickness of carotid atherosclerotic plaques (25). In the present study, we demonstrated for the first time that sodium lactate augmented LPS-stimulated MMP-1 and proinflammatory cytokine expression. Given that a high lactate level is associated with hyperglycemia, our findings have uncovered

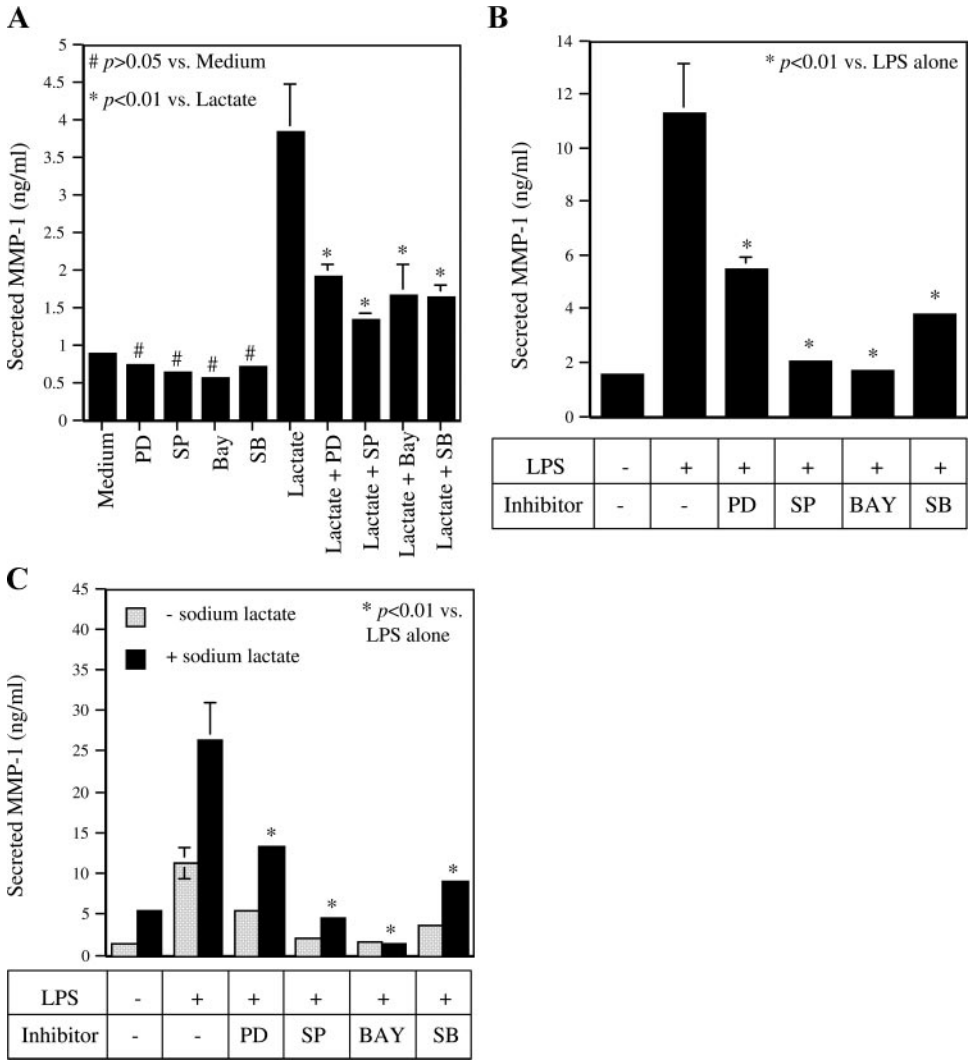

Fig. 5. Inhibition of MMP-1 secretion from U937 cells treated with sodium lactate (*A*), LPS (*B*), or both (*C*) by specific inhibitors. *A*: U937 cells were treated with 12 mM sodium lactate in the presence or absence of 10 μM PD-98059 (PD), SP-600125 (SP), Bay 11-7085 (Bay), or SB-203580 (SB) for 24 h. *B*: U937 cells were treated with 100 ng/ml LPS in the presence or absence of 10 μM PD, SP, Bay, or SB for 24 h. *C*: U937 cells were pretreated with or without 12 mM sodium lactate for 24 h. After medium was changed, cells were treated with 12 mM sodium lactate and 100 ng/ml LPS in the absence or presence of 10 μM PD, SP, Bay, or SB for another 24 h. Conditioned medium was then used for quantification of MMP-1.

a novel mechanism involved in the enhancement of inflammation by hyperglycemia.

High lactate levels have been implicated in many cellular events contributing to inflammation. For example, a high lactate level was reported to stimulate the synthesis of collagen and enzymes important in wound healing and the secretion of factors important for new capillary growth (15). It has been reported that lactate also regulates T lymphocyte functions by affecting the expression of genes such as IL-2 (34). Moreover, lactate was considered an immune activator in vitro, as studies

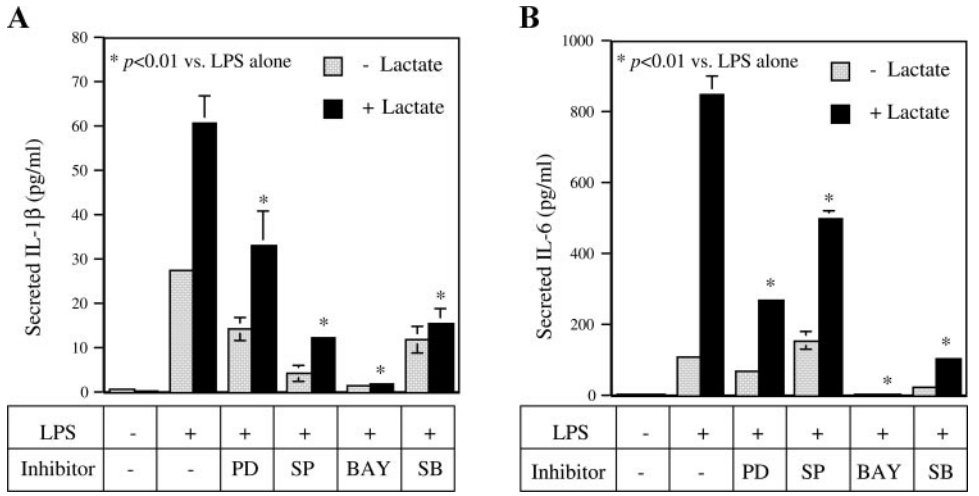

Fig. 6. Inhibition of IL-1β and IL-6 secretion from U937 cells by specific inhibitors. Conditioned medium collected in the experiments described in Fig. 5*C* was used to determine the amount of secreted IL-1β and IL-6.

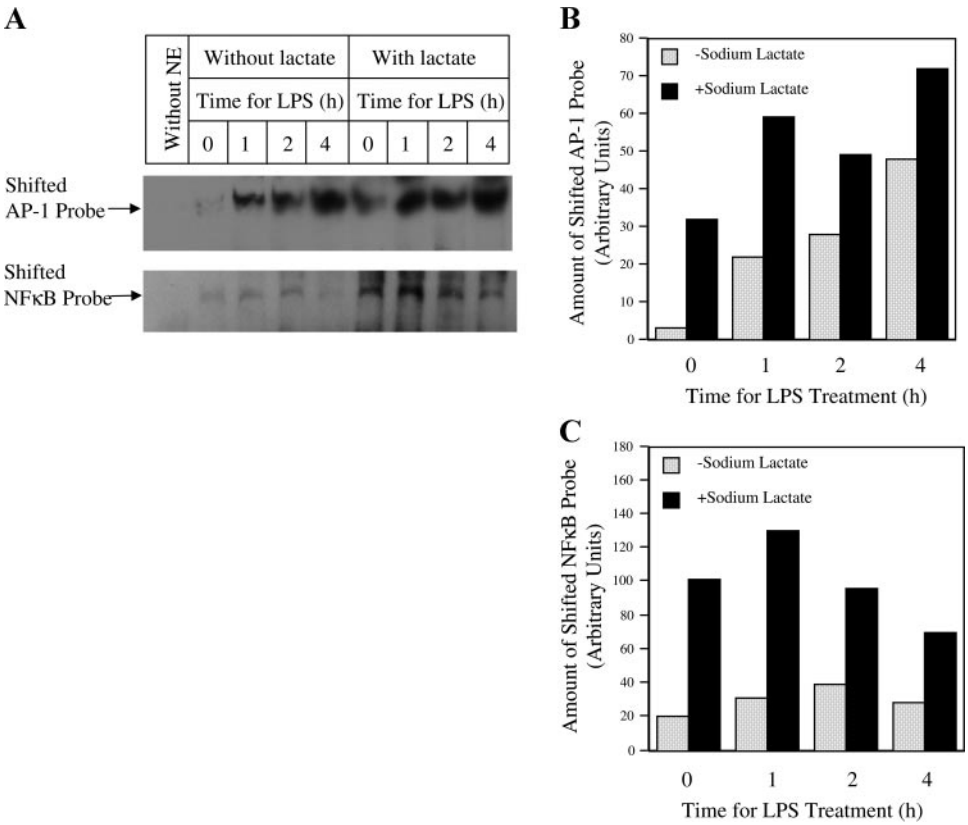

Fig. 7. Preexposure to sodium lactate increases basal and LPS-stimulated AP-1 and NF-κB activities. U937 histiocytes were preexposed to 12 mM sodium lactate for 24 h and then treated with 100 ng/ml LPS for 0, 1, 2, and 4 h. Nuclear proteins were extracted after treatment and used for electrophoretic mobility shift assay (*A*), as described in MATERIALS AND METHODS. Amount of shifted AP-1 and NF-κB probes were semiquantified by scanning densitometry (*B* and *C*).

have demonstrated that lactate can cause macrophage activation from a resting state (15). By showing that sodium lactate augments LPS-stimulated expression of MMP and cytokines, our present study has further stressed the importance of high lactate levels in the regulation of cellular functions related to inflammation.

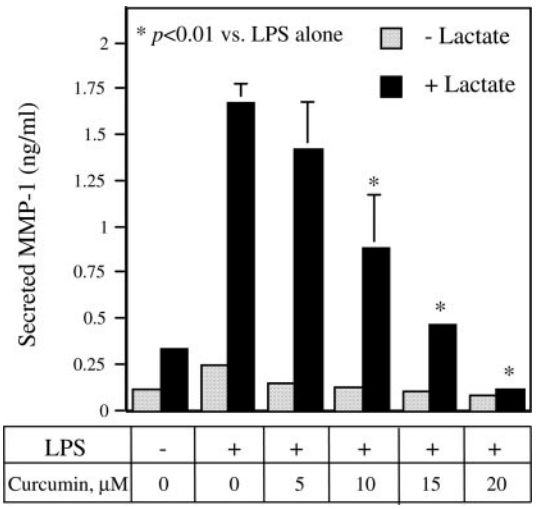

Fig. 8. Concentration-dependent inhibition of MMP-1 secretion by curcumin. U937 histiocytes were pretreated with or without 12 mM sodium lactate for 24 h. Cells were then treated with 100 ng/ml LPS in the presence or absence of different concentrations of curcumin for 1 h. Medium was replaced with a fresh one containing 100 ng/ml LPS, and cells were incubated for 24 h. Secreted MMP-1 in conditioned medium was quantified using ELISA, as described in MATERIALS AND METHODS.

The reported lactate concentrations in patients with type 2 diabetes are higher than those in nondiabetic individuals and range from 1.1 (8) to 3.5 mM (11). Our present study demonstrated that preexposure of U937 cells to sodium lactate at concentrations of 3, 6, and 12 mM increased LPS-stimulated MMP-1 secretion by 47, 190, and 582%, respectively, over the control level of MMP-1 released by cells treated with LPS alone (Fig. 1*B*). Although the concentrations of 6 and 12 mM are higher than the reported concentrations in the blood of type 2 diabetic patients, these concentrations are believed to be present in the local tissues where glycolytically active cells such as macrophages accumulate (9) or where injury, infection, or ischemia occurs (15). Macrophages are highly active in glycolysis, and in vitro studies have shown that macrophages could generate up to 60 mM of lactate in culture medium (9). Thus it is believed that the concentration of lactate is higher in the vicinity of macrophages than that in plasma (9, 34).

It has been reported that increased lactate concentration affects gene expression in macrophages (15). Consistently, our present study also shows that sodium lactate stimulates MMP-1 expression in U937 macrophage-like cells, although the stimulation is less potent than that induced by LPS (Figs. 1*B* and 4*A*). In addition to the stimulation of MMP-1 expression by itself, sodium lactate also augments LPS-stimulated MMP-1 expression. Moreover, it is noteworthy that, in contrast to MMP-1 expression, sodium lactate alone has no apparent effect on IL-1β and IL-6 but enhances LPS-induced secretion of IL-1β and IL-6 two- and eightfold, respectively (Fig. 1). The eightfold enhancement of LPS-induced IL-6 secretion by sodium lactate is remarkable. These observations suggest that sodium lactate not only exerts its regulation directly on mac-

rophage gene expression, but also coordinates with LPS to enhance gene expression.

Another interesting finding from this study is that lactic acid that has a lower pH is much less effective on the augmentation of LPS-stimulated MMP-1 secretion than sodium lactate (Fig. 2*A*). The data presented in Fig. 2*A* show that neutralization of pH in medium containing 12 mM lactic acid leads to a 130% increase in LPS-induced MMP-1 secretion, indicating that the pH reduction by lactic acid may contribute to the ineffectiveness of lactic acid on the augmentation. The results presented in Fig. 2*B* further demonstrate the inhibitory effect of acidic pH on MMP-1 expression. These observations are consistent with previous reports that acidic pH inhibits gene expression and cell functions (19). For examples, Bidani et al. (3) reported that the secretion of TNF-α by macrophages was inhibited by acidic pH. Rabinovitch et al. (32) also showed an inhibition of neutrophil chemotaxis at acidic pH. Indeed, many cellular responses such as cytosolic and membrane-associated enzyme activities, ion transport activity, protein and DNA synthesis, and cAMP and calcium levels are diminished at lowered extracellular pH (3). Thus the present study has underscored the importance of acid-base homeostasis in the maintenance of high cellular responses to inflammatory perturbation.

It is known that the major signaling pathways triggered by LPS for macrophage activation are NF-κB and MAPK (ERK, JNK, and p38) cascades (12). Consistent with this view, our blocking studies using specific inhibitors indicate that these signaling pathways are involved in LPS-stimulated MMP expression. Interestingly, our data also indicate that these signaling cascades are involved in sodium lactate-stimulated MMP-1. Thus it is possible that both sodium lactate and LPS may target same transcription factors through these signaling cascades, resulting in a synergistic effect on MMP-1 transcription. Because it is known that the activation of NF-κB and MAPK (ERK, JNK, and p38) cascades leads to increased AP-1 and NF-κB transcriptional activities that are essential for MMP-1, IL-1β, and IL-6 expression (23, 12), we have determined the effect of sodium lactate on LPS-stimulated AP-1 and NF-κB transcriptional activity using the EMSA technique. The findings from the EMSA validate our hypothesis that sodium lactate and LPS have a synergy on AP-1 and NF-κB transcriptional activities, and this synergy is likely to be responsible for the enhanced expression of MMP-1 and cytokines. Furthermore, in agreement with previous reports (18, 29, 36), our data showed that NF-κB activation by LPS was transient and that maximal stimulation was observed at 1–2 h after LPS stimulation, whereas the effect of LPS on AP-1 activation was sustained longer (Fig. 7).

In summary, we have demonstrated that sodium lactate augments LPS-induced MMP-1, IL-1β, and IL-6 expression by U937 macrophage-like cells through multiple signaling pathways such as NF-κB and MAPK (ERK, JNK, and p38) cascades. We have also shown a synergy between sodium lactate and LPS on the activities of proinflammatory transcription factors AP-1 and NF-κB. Thus these findings have uncovered a link between nonoxidative glycolysis and inflammation that is associated with obesity, type 2 diabetes, and diabetic complications.

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