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Regulation of energy metabolism by interleukin-1β, but not by interleukin-6, is mediated by nitric oxide in primary cultured rat hepatocytes

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Abstract

The effects of inflammatory cytokines (interleukin-1β, interleukin-6, and tumor necrosis factor-α) on energy metabolism were studied in primary cultured rat hepatocytes. Adenine nucleotide (ATP, ADP, and AMP) content, lactate production, the ketone body ratio (acetoacetate/β-hydroxybutyrate) reflecting the liver mitochondrial redox state (NAD^+/NADH), and nitric oxide formation were measured. Insulin increased ATP content in hepatocytes and had a maximal effect after 8–12 h of culture. Both interleukin-1β and interleukin-6, but not tumor necrosis factor-α, significantly inhibited the ATP increase time- and dose-dependently. Interleukin-1β and interleukin-6 also stimulated lactate production. During the same period, interleukin-1β but not interleukin-6 decreased the ketone body ratio. Furthermore, interleukin-1β markedly stimulated nitric oxide formation in hepatocytes, and this increase was blocked by N^G-monomethyl-L-arginine (a nitric oxide synthase inhibitor) and by interleukin-1 receptor antagonist. N^G-monomethyl-L-arginine reversed inhibition of the ATP increase, decrease in the ketone body ratio, and increase in lactate production, which were induced by interleukin-1β. Interleukin-1 receptor antagonist completely abolished all of the effects induced by interleukin-1β. These results demonstrated that interleukin-1β and interleukin-6 affect the insulin-induced energy metabolism in rat hepatocytes by different mechanisms. Specifically, interleukin-1β inhibits ATP synthesis by causing the mitochondrial dysfunction, a process which may be mediated by nitric oxide.

Keywords: Cytokine; Adenine nucleotide; Ketone body ratio; Nitric oxide; Rat hepatocyte

1. Introduction

Inflammatory cytokines such as interleukin-1 (IL-1), interleukin-6 (IL-6) and tumor necrosis factor-α (TNFα) are detected in the plasma of patients with severe sepsis, and are known to induce a number of metabolic changes [1]. Recent evidence has indicated that these cytokines play an important role in the regulation of energy metabolism in the liver. Infusion of TNFα and IL-1β causes drastic alterations in carbohydrate and energy metabolism in rabbits, and results in the reduction in hepatic mitochondrial pyruvate dehydrogenase activity, indicating the development of liver dysfunction [2]. In fact, TNFα is found to inhibit mitochondrial respiration in cultured rat hepatocytes [3]. Pellicane et al. [4] reported that IL-1 receptor antagonist (IL-1ra) preserves the level of ATP in mouse liver after hemorrhagic shock, suggesting that IL-1 is involved in the reduction in ATP levels during shock. Although ATP plays a critical role in the regulation and integration of cellular metabolism [5,6], few investigators have demonstrated a direct effect of inflammatory cytokines on ATP levels in the liver. Recently, we [7] have established a simple and rapid method for the assay of adenine nucleotides in rat and human cultured hepatocytes,

Abbreviations: IL-1β, interleukin-1β; IL-1ra, interleukin-1 receptor antagonist; IL-6, interleukin-6; KBR, ketone body ratio; L-NMMA, N^G-monomethyl-L-arginine; NO, nitric oxide; TNFα, tumor necrosis factor-α.

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where it is found that the cells maintain a high level of ATP contents during the culture. The ketone body ratio (KBR; acetoacetate/β-hydroxybutyrate) is also a useful parameter to find out the mitochondrial function, because it reflects the mitochondrial redox state (NAD^+/NADH) [8].

In hepatocytes, a mixture of cytokines (IL-1β, TNFα and interferon-γ) and lipopolysaccharide markedly augmented induction of nitric oxide (NO) synthase mRNA and NO formation, but any single cytokine or lipopolysaccharide alone did not cause a significant NO formation [9–11]. NO, a highly reactive free-radical gas, plays a major role in a diverse array of physiological functions such as the regulation of vascular tone, neurotransmission and the mediation of immune responses [12–14]. NO inhibits the mitochondrial electron transport chain (complex I and II) [15,16] and the Krebs cycle enzyme aconitase, in target cells [17].

In this study, we investigated the effect of the inflammatory cytokines, IL-1β, IL-6, and TNFα, on cellular levels of ATP in primary cultured rat hepatocytes. Furthermore, we examined the effects of the cytokines on the KBR, lactate production, and NO formation to elucidate mechanisms involving the regulation of ATP levels by cytokines.

2. Materials and methods

Materials. Human recombinant IL-1β was generously provided by Otsuka Pharmaceutical Co., Tokushima, Japan. IL-1β has a specific activity of 2×10^7 units/mg protein, and contains less than 1 ng of endotoxin per mg protein. Human recombinant IL-6 (5×10^6 units/mg protein) was generously provided by Ajinomoto Co., Yokohama, Japan. Human recombinant TNFα (2×10^6 units/mg protein) was purchased from Amersham International, Bucks, UK. L-NMMA, an NO synthase inhibitor [10], was obtained from Funakoshi Co., Tokyo, Japan. Human recombinant IL-1ra [18], which is a competitive inhibitor of IL-1 activity, was obtained from Pepro Tech Inc., New Jersey, USA. All other chemicals were of reagent grade.

Culture of rat hepatocytes. Male Wistar strain rats (6–8 weeks old, 200–280 g) fed ad libitum were used. All animals received humane care in accordance with the guidelines of the Animal Care Committee of Kansai Medical University. Hepatocytes were isolated by perfusing the liver with collagenase under sterile conditions as described previously [19]. Hepatocytes, showing more than 80% viability by Trypan blue exclusion, were used for culture. Isolated hepatocytes were suspended in culture medium at $5–6 \times 10^5$ viable cells/ml, seeded into plastic dishes (2 ml per dish, 35 × 10 mm, Falcon Plastic, Oxnard, USA) and cultured as monolayers in a CO_2 incubator (under a humidified atmosphere with 5% CO_2 in air) at 37°C. The culture medium used was Williams' medium E (containing 11 mM glucose) supplemented with 10% newborn calf serum, HEPES (5 mM), penicillin (100 units/ml), streptomycin (0.1 mg/ml), dexamethasone (10^{-8} M), glucagon (2×10^{-10} M) and insulin (10^{-8} M). After 2–3 h of culture, the medium was replaced by fresh serum-free medium (1.5 ml/dish), and the cells were cultured for two days, with fresh medium supplied every day, and then used for experiments. The number of cells attached to the dishes was approx. 0.9×10^6 cells/dish, which was calculated from the number of cell nuclei by the method of Horiuti et al. [20]. The nucleus/cell ratio was 1.39 ± 0.03 (mean ± S.E.M., $n = 14$). The purity of the hepatocytes in culture was greater than 98% as determined by microscope observation.

Treatment of hepatocytes with cytokines. After removal of the culture medium, cultured hepatocytes were washed with 1 ml of Williams' medium E to remove hormones, and 1 ml of Williams' medium E supplemented with 5 mM glucose (final concentration is 16 mM glucose) was added. Then, insulin (2.5×10^{-8} M) was added to the medium in the presence or absence of cytokines (10 μl in 0.1% bovine serum albumin-saline) and cells were incubated in a CO_2 incubator. The final concentration of each cytokine in the incubation mixture was 10^{-9} M, if not specified otherwise. IL-1ra (10^{-7} M) and L-NMMA (3.3×10^{-4} M) were added to the medium 15 min before each experiment.

Miscellaneous. Adenine nucleotides (ATP, ADP and AMP) were measured by HPLC as reported previously [7] with minor modifications. Samples were prepared at 4°C or on ice. In brief, immediately after removal of the incubation medium, the cells were washed twice with 1 ml of saline and then 1 ml of 7% perchloric acid was added. Cells were scraped off the dish with a rubber policeman. After adjusting the pH to 6.0–6.5 with 2 M tripotassium phosphate (375 μl), the cell lysate was centrifuged at $10\,000 \times g$ for 10 min. The supernatant was filtered (with a 0.45 μm filter, type 13A Shimadzu Techno Research, Kyoto, Japan) and stored at −80°C until the assays were performed. A reverse-phase column (ODS-M 4.0 × 150 mm, Shimadzu Techno Research) was used at a flow rate of 1 ml/min, with a buffer of 0.1 M ammonium dihydrogen phosphate (pH 6.0), at a column temperature of 50°C. The injection volume was 40 μl, and absorbance was monitored at 254 nm. ATP, ADP, and AMP were eluted with retention times of 2.19 min, 2.48 min, and 4.05 min, respectively.

Ketone bodies (acetoacetate and β-hydroxybutyrate) in the medium were measured enzymatically with a Ketorex kit (Sanwa Chemical, Nagoya, Japan) [21]. The KBR was calculated as acetoacetate/β-hydroxybutyrate. Lactate in the medium was measured enzymatically with a commercial lactate assay kit (Boehringer Mannheim, Mannheim, Germany).

Assay of NO formation. Accumulation of NO_2^- and NO in the medium was considered to represent NO formation.

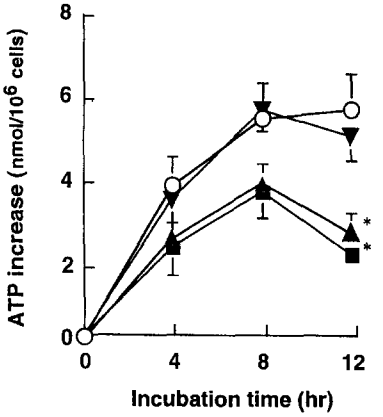


Fig. 1. Effect of inflammatory cytokines on the changes in ATP content in cultured rat hepatocytes. IL-1β (▲), IL-6 (■), or TNFα (▼) were added to the incubation medium (Willams' medium E containing 16 mM glucose and 2.5×10^{-8} M) at a final concentration of 10^{-9} M and incubated for the times indicated. Vehicle (0.1% bovine serum albumin in saline) was added to the control (○). Intracellular ATP content was measured as described in Section 2. The ATP increase was calculated by subtracting basal ATP content at time 0 (16.90 ± 1.92 nmol/10^6 cells) from the value obtained for each experiment at the indicated times. Data are represented as mean ± S.E.M. of 4 separate experiments. * $P < 0.01$ vs. control.

The culture medium (250 μl) was mixed with saturated ascorbic acid solution (2 ml) for 10–15 s in an enclosed tube, to reduce NO_2^- to NO. Levels of NO in the medium were then determined with a redox chemiluminescence analyzer (FES-450, Scholar Tec, Osaka, Japan) [22,23]. Sodium nitrite was used in the generation of a standard curve for the measurement of NO.

Statistical analysis of data. Statistical significance was analyzed by the unpaired Student's *t*-test, and $P < 0.05$ was considered significant. Data are presented as the mean ± S.E.M. Error bars are omitted when they were within the size of the graphic symbol used.

3. Results

Effect of cytokines on intracellular ATP contents in rat hepatocytes. As shown in Fig. 1, insulin augmented intracellular ATP content in hepatocytes time-dependently with the maximal increase occurring at 8–12 h after its addition. The increase in ATP content at 12 h was 5.62 ± 1.28 nmol/10^6 cells (0 time, 16.90 ± 1.92; 12 h, 22.52 ± 2.17 nmol/10^6 cells; mean ± S.E.M., $n = 4$). Basal ATP content in the absence of insulin (with 16 mM glucose) remained unchanged during the incubation period (data not shown). Both IL-1β (10^{-9} M) and IL-6 (10^{-9} M) inhibited the insulin-induced ATP increase in a similar manner and maximal inhibition (50–70%) occurred at 12 h. TNFα (10^{-9} M), however, had no effect on the increase in ATP content. Table 1 shows the intracellular content of ATP, ADP, and AMP, and shows the energy charge in the

Table 1
Effect of cytokines on adenine nucleotide content in cultured rat hepatocytes

Cytokine (10^{-9} M)	Adenine nucleotides (nmol/10^6 cells)			
	ATP	ADP	AMP	Energy charge
Control	21.5 ± 0.7	2.9 ± 0.3	0.5 ± 0.1	0.92 ± 0.01
IL-1β	18.1 ± 0.3 *	2.9 ± 0.6	0.5 ± 0.1	0.91 ± 0.01
IL-6	18.1 ± 0.4 *	2.7 ± 0.4	0.5 ± 0.1	0.91 ± 0.02
TNFα	20.7 ± 1.3	2.9 ± 0.4	0.4 ± 0.0	0.92 ± 0.01

ATP, ADP and AMP levels after 12 h of incubation, energy charge = (ATP + 1/2ADP)/(ATP + ADP + AMP).
Data are represented as mean ± S.E.M. of 4 separate experiments.
* $P < 0.01$ vs. control.

presence and absence of various cytokines at 12 h. IL-1β and IL-6 significantly decreased intracellular ATP content. Neither IL-1β, IL-6, nor TNFα altered the amount of ADP or AMP. Similarly, these cytokines had no effect on the energy charge. Fig. 2 shows the dose-dependent inhibitory effect of IL-1β and IL-6 on the increase in intracellular ATP. Concentrations for the half-maximal and maximal effects of IL-1β were approx. 3×10^{-11} M and 10^{-9} M, respectively. IL-6 showed exactly the same dose-dependency as IL-1β, for the inhibition of the increase in ATP. The effective doses observed for IL-1β and IL-6 are in agreement with previous observations that cytokines act in picomolar concentrations order through specific high-affinity cell surface receptors [1].

Effect of cytokines on the KBR and lactate production. Fig. 3 shows the effect of cytokines on the KBR. The KBR is calculated as acetoacetate/β-hydroxybutyrate, that is directly proportioned to the mitochondrial free NAD^+/NADH. Although the KBR gradually declined with times in all groups, only IL-1β significantly de-

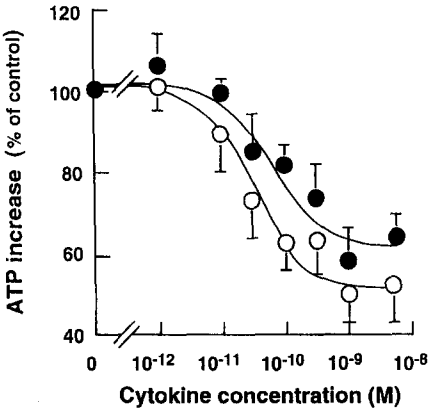


Fig. 2. Dose dependency of the inhibitory effects of IL-1β and IL-6 on the increase in ATP content in cultured rat hepatocytes. Hepatocytes were incubated with various concentrations of IL-1β (●) or IL-6 (○) for 12 h and ATP content was measured as described in Section 2. The ATP increase in the control (without IL-1β) was 5.18 ± 0.64 nmol/10^6 cells (mean ± S.E.M., $n = 4$) and was calculated as 100%. Data are represented as mean ± S.E.M. of 4 separate experiments.

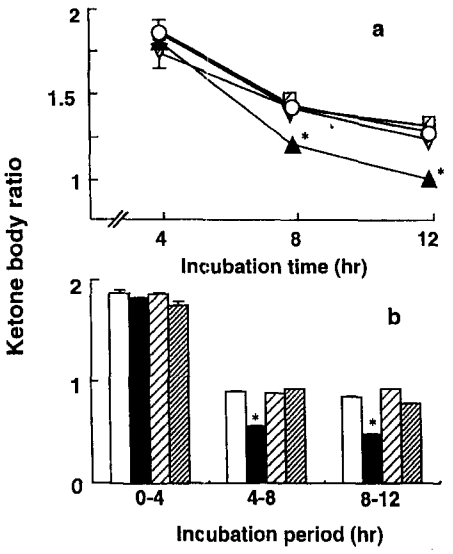


Fig. 3. Effect of inflammatory cytokines on the KBR (KBR is calculated from the number of ketone bodies accumulated in the medium of cultured hepatocytes) (a) over the total incubation time, or (b) over a given period of time during incubation. Hepatocytes were incubated with 10^{-9} M IL-1β (▲), IL-6 (hatched square), TNFα (hatched inverted triangle), or vehicle (○) as described in the legend for Fig. 1. Ketone bodies, acetoacetate and β-hydroxybutyrate, were measured as described in Section 2 and the KBR was calculated as acetoacetate/β-hydroxybutyrate. Data are represented as mean SEM of four dishes in one of the four experiments giving the similar results. * $P < 0.01$ vs. control.

creased the KBR when compared with the control, at 8 and 12 h (Fig. 3a). Hepatocytes cannot utilize ketone bodies as energy substrates, and ketone bodies subsequently accumulate in the medium. Therefore, the KBR was calculated from the quantity of ketone bodies accumulated for a given period of time (0–4, 4–8, and 8–12 h, respectively) to clarify the effect of IL-1β on the KBR. IL-1β had no significant effect on the KBR during the first 4 h of incubation, but the KBR decreased significantly after 4–8 h of incubation (control 0.89 and IL-1β 0.56). This decrease was similar in magnitude to that observed after 8–12 h, suggesting that the IL-1β effect peaked after 4–8 h (Fig. 3b). As shown in Table 2, IL-1β, but not IL-6 or TNFα, increased β-hydroxybutyrate formation. None of

Table 2
Effect of cytokines on ketone body formation in cultured rat hepatocytes

Cytokine (10^{-9} M)	Ketone bodies (nmol/10^6 cells)		
	Acetoacetate	β-Hydroxybutyrate	KBR
Control	160.9±1.4	126.9±1.0	1.28±0.01
IL-1β	155.7±1.2	157.6±1.0 *	0.99±0.01 *
IL-6	175.1±2.7	133.7±2.0	1.31±0.01
TNFα	162.1±1.9	133.0±0.8	1.23±0.02

Acetoacetate and β-hydroxybutyrate levels after 12 h of incubation.
KBR = acetoacetate/β-hydroxybutyrate.
Data are represented as mean±S.E.M. of 4 dishes in one of the 4 experiments giving similar results.
* $P < 0.01$ vs. control.

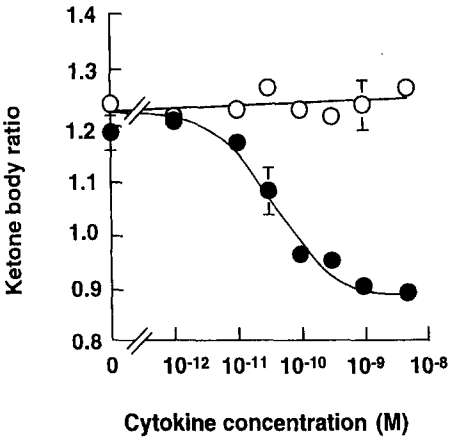


Fig. 4. Dose dependency of the effects of IL-1β and IL-6 on the KBR in cultured rat hepatocytes. Hepatocytes were incubated with various concentrations of IL-1β (●) or IL-6 (○) for 12 h. The KBR was calculated as acetoacetate/β-hydroxybutyrate. Data are represented as mean± S.E.M. of 4 dishes in one of the 4 experiments giving similar results.

these cytokines, however, had any effect on the formation of acetoacetate. Thus, IL-1β increased the concentration of β-hydroxybutyrate (hence the decrease in the KBR). Furthermore, IL-1β decreased the KBR in a dose-dependent manner (Fig. 4). Concentrations of IL-1β which induced half-maximal and maximal effects were 10^{-11} and 10^{-9} M, respectively, which is consistent with those concentrations of IL-1β which had an inhibitory effect on the insulin-induced ATP increase (Fig. 2). Conversely, IL-6 had no effect on the KBR at the concentrations tested (up to 5×10^{-9} M, Fig. 4). Treatment of the cultured hepatocytes with 2-tetradecylglycidic acid (2.5×10^{-5} M), an inhibitor of fatty acid β-oxidation [24], did not affect ketone body formation or the KBR in either controls or IL-1β-treated cells (data not shown), indicating that the IL-1β effect cannot be attributed to the activation of fatty acid β-oxidation in hepatocytes. IL-1β and IL-6 increased

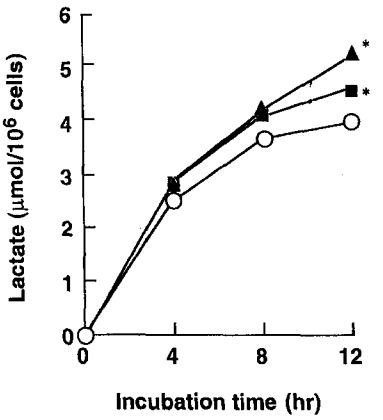
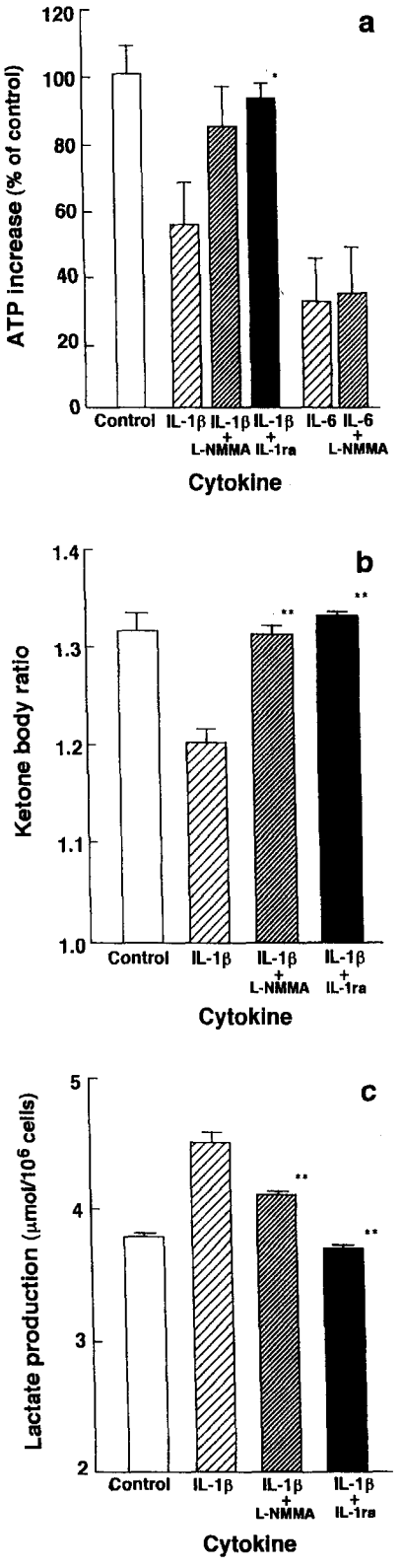


Fig. 5. Effect of inflammatory cytokines on lactate production in cultured rat hepatocytes. Hepatocytes were incubated with IL-1β (▲), IL-6 (■), or vehicle (○) as described in the legend for Fig. 1. Lactate in the medium was measured as described in Section 2. Data are represented as mean S.E.M. of 4 dishes of 4 experiments. * $P < 0.01$ vs. control.

lactate production 1.29-fold and 1.16-fold respectively, when compared to the control at 12 h (Fig. 5). This effect of IL-1β showed the same dose-dependency that effects on ATP content and the KBR demonstrated (data not shown).



Blockage of IL-1β effects on energy metabolism by L-NMMA. Cytokines have been reported to stimulate NO formation in hepatocytes [9–11,25,26] and NO has been shown to inhibit the mitochondrial electron transport chain [15,16]. Because NO is one of the putative mediators of the various effects on energy metabolism which are induced by IL-1β and IL-6, we examined whether L-NMMA (an NO synthase inhibitor) inhibited the above-mentioned effects of IL-1β and IL-6. L-NMMA (3.3×10^{-4} M) reversed the effect of IL-1β on ATP content, the KBR, and lactate production by 67%, 93%, and 58%, respectively (Fig. 6a–c). L-NMMA, however, did not alter the inhibitory effect of IL-6 on ATP content (Fig. 6a). These results suggest that the effects induced by IL-1β may be mediated by NO.

Effect of cytokines on nitric oxide formation. In order to examine whether IL-1β induced NO formation in cultured hepatocytes, we measured NO in the cell culture media. As shown in Fig. 7, IL-1β markedly induced NO formation, but neither IL-6 nor TNFα did. The level of NO formation reached a maximum plateau at 12–16 h and was 50- to 80-fold greater than control, with 10^{-9} M IL-1β (ED_{50} is 3×10^{-11} M, Kitade et al., manuscript in preparation). NO formation by IL-1β was more than 90% inhibited by L-NMMA (3.3×10^{-4} M).

Blockage of IL-1β effects on energy metabolism by IL-1ra. IL-1ra was used to confirm that IL-1β affected energy metabolism through the IL-1 receptor [18]. The IL-1ra (10^{-7} M) completely negated the effects of IL-1β on ATP content, the KBR, lactate formation (Fig. 6a–c), and NO formation (Fig. 7).

4. Discussion

In the present study, IL-1β and IL-6, well-known inflammatory cytokines, were shown to affect energy metabolism in primary cultured rat hepatocytes. IL-1β inhibited the insulin-induced ATP increase (Figs. 1 and 2), decreased the KBR (Figs. 3 and 4), and stimulated lactate production (Fig. 5), in the same dose-dependent manner.

Fig. 6. (a–c): Inhibition by L-NMMA and IL-1ra of IL-1β-induced effects on ATP content, the ketone body ratio and lactate formation. Hepatocytes were incubated with IL-1β, IL-6, and vehicle (control) in the absence or presence of L-NMMA (3.3×10^{-4} M) or IL-1ra (10^{-7} M) for 12 h. L-NMMA and IL-1ra were added to the medium 15 min before the experiment. ATP content, the KBR, and lactate production were measured as described in Section 2. The basal levels for the ATP increase (a), the KBR (b), and for lactate production (c), were 5.85 ± 0.57 nmol/10^6 cells (calculated as 100%), 1.32 ± 0.02, and 3.83 ± 0.02 μmol/10^6 cells, respectively, at 12 h. L-NMMA and IL-1ra did not alter these basal levels in the absence of IL-1β. Data are represented as mean S.E.M. of 4 experiments. * $P < 0.05$ and ** $P < 0.01$ vs. IL-β.

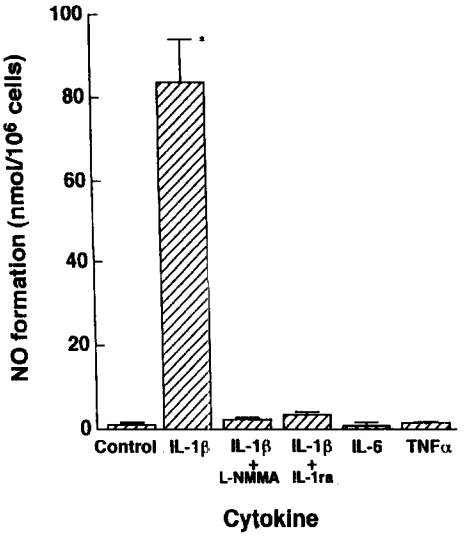


Fig. 7. Effect of inflammatory cytokines on NO formation in cultured hepatocytes. Hepatocytes were incubated with IL-1β, IL-6, TNFα, vehicle (control), or with IL-1β in the presence of L-NMMA and IL-1ra as described in the legend for Fig. 6. NO formation in the medium was measured as described in Section 2. The basal level of NO formation was 0.82×0.20 nmol/10^6 cells at 12 h. Data are represented as mean S.E.M. of 4 experiments. * $P < 0.01$ vs. control.

For reasons mentioned below, the inhibition of ATP synthesis was hypothesized to be mediated by NO via the IL-1 receptor by the following pathway; IL-1β → IL-1 receptor → increase of inducible NO synthase mRNA → NO formation → mitochondrial dysfunction → inhibition of ATP synthesis. This hypothesis was supported by the observation that IL-1ra completely blocked all of the effects induced by IL-1β (Figs. 6 and 7). In support of this observation, Kohira et al. [27] previously identified a biologically functional IL-1 receptor in primary cultures of rat hepatocytes. Furthermore, L-NMMA (an NO synthase inhibitor) not only blocked the induction of NO formation, but simultaneously blocked the decrease in the KBR, the inhibition of the insulin-induced ATP increase, and the stimulation of lactate production (Figs. 6 and 7). IL-1β was also shown to markedly stimulate NO formation (Fig. 7) and to induce NO synthase activity and the expression of NO synthase mRNA (Kitade et al., manuscript in preparation). IL-1β did not induce NO formation in cultured rat Kupffer cells under the same conditions used for hepatocytes (less than 1 nmol/10^6 cells of NO formation at 12 h). These observations eliminated the possibility that the NO formed in the cultured hepatocytes was derived from contaminating Kupffer cells (approx. 1–2% of the cells in our preparations). Finally, mitochondrial dysfunction was confirmed by a decrease in the KBR and an increase in the lactate production. Because the decrease of KBR by IL-1β reflects a higher level of NADH, an initial substrate for oxidative phosphorylation, the inhibition of ATP synthesis by IL-1β can be attributed to mitochondrial dysfunction. NO reportedly inhibits the Krebs cycle as well as the respiratory system at the level of the mitochondria [15–17]. Therefore, the apparent increase in lactate production which was induced by IL-1β may be due to an inhibition of pyruvate entry into the Krebs cycle. However, it is evident that an NO-independent pathway is involved in the effects of IL-1β, since L-NMMA only partially blocked the ATP inhibition (Fig. 6a) and lactate production (Fig. 6c) induced by IL-1β.

In contrast, IL-6 did not affect either the KBR (Fig. 3) or NO formation (Fig. 7), indicating that IL-6 inhibited the insulin-induced ATP increase through a pathway different from that of IL-1β. Hotamisligil et al. [28] demonstrated that, in cultured adipocytes, IL-6, but not IL-1β, decreased insulin-stimulated autophosphorylation of the insulin receptor and the phosphorylation of insulin receptor substrate 1, a major substrate of the insulin receptor in vivo. We found that IL-1β decreased basal levels of ATP but that IL-6 had no effect on the intracellular ATP content in the absence of insulin (unpublished observation). As such, IL-6 might directly interfere with insulin signaling through the insulin receptor, in hepatocytes.

Adamson et al. [29] previously reported that TNFα at concentrations much higher (0.5×10^{-7} M or more) than that employed here (10^{-9} M), drastically decreased intracellular ATP content (25% of control at 16 h) and stimulated NO formation 2- to 3-fold above control levels after isolated mouse hepatocytes were incubated for 24 h. They concluded that this inhibition of energy metabolism was due to the cytotoxicity of TNFα and was not related to NO formation. TNFα, however, affected neither energy metabolism nor NO formation at the lower concentration employed in our experiments (Fig. 1, Fig. 3, and Fig. 7). We have also observed that IL-1α had no significant effects on ATP increase and the KBR (data not shown). Consistent with our in vitro experiments, Evans et al. [30] reported that TNFα and IL-1α did not decrease hepatic ATP and the KBR in rats in vivo.

In conclusion, IL-1β and IL-6 were shown to regulate energy metabolism in the liver. Furthermore, NO formation was shown to be involved in the effects mediated by IL-1β but not those mediated by IL-6. The molecular mechanism for the inhibition of insulin-induced ATP synthesis, including intracellular signaling and the regulation of energy metabolism by IL-1β and IL-6 will be the subjects for further investigation.

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