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Sympathetic Activation of Hepatic and Splenic IL-1β mRNA Expression during Oscillation Stress in the Rat

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ABSTRACT. Interleukin (IL)-1β mRNA expression in the liver and spleen was examined after subjection to oscillation stress in the rat. Thirty-minute subjection to oscillation stress increased IL-1β mRNA expression in the both organs. Prior treatment of rats with gadolinium chloride, which eliminates macrophages, prevented the stress-induced IL-1β expression. Either adrenalectomy or treatment of guanethidine, a blocker of norepinephrine release in the sympathetic nerve endings, partially attenuated the stress-induced response, but the combined treatment completely blocked it. Injection of β-adrenergic antagonist (propranolol) also suppressed the stress-induced response. These results suggest that oscillation stress induces IL-1β mRNA expression in the liver and spleen, probably in Kupffer cells and splenic macrophages, and that stress-induced IL-1β expression is elicited by catecholamines released from sympathetic nerve terminals and the adrenal gland.—KEY WORDS: Interleukin-1, Kupffer cell, macrophage, oscillation stress, sympathetic nerve.

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Interleukin (IL)-1 is a pivotal cytokine in the acute phase response to inflammation elicited by infection and/or injury. IL-1 induces lymphocyte proliferation, fever and production of other cytokines such as IL-6, which in turn contribute to host defense [6, 19].

The IL-6 level in blood is increased not only during inflammation, but also during non-invasive stress such as immobilization [3, 5, 8–10, 17, 18]. We have demonstrated in the mouse that immobilization stress elevates the plasma level of IL-6 in association with increased expression of IL-6 mRNA in the liver [8]. The stress-induced IL-6 production has been suggested to be mediated through activation of sympathetic nerves more than adrenal secretions [17]. In fact, very recently we demonstrated that norepinephrine enhances IL-6 mRNA expression in primary cultured rat hepatocytes [7]. Moreover, we found that norepinephrine increases IL-1β production in non-parenchymal liver cells [7]. This latter observation seems compatible to the previous report that the IL-1 secretion in cultured monocytes is increased by physiological concentrations of epinephrine [1]. It has also been confirmed that oscillation stress elevates plasma level of norepinephrine and epinephrine [13]. Thus, we assumed that stress would increase IL-1β expression in the liver by the action of catecholamines from sympathetic nervous system.

In the present study, we determined IL-1β mRNA expression in the liver in rats subjected to oscillation stress to test this hypothesis and compared with that in the spleen.

MATERIALS AND METHODS

Animals: Male Wistar rats (180–250 g) were housed with a 12:12-hr light-dark cycle (light on: 7:00 hr-19:00 hr), and given free access to laboratory chow and water. Handling rats was performed for the first 5 days and left off for the following 2 days before experiments to avoid handling stress during subjection to oscillation stress. The experimental procedure and care of animals were in accordance with the guideline of the Animal Care and Use Committee of Hokkaido University.

Oscillation stress: Oscillation stress was applied according to the method of Nakata *et al.* [13]. Briefly, rats were placed separately in a plastic cage ($30 \times 25 \times 20$ cm) with wood chips, which was shaken on a horizontal shaker with a 3.8 cm amplitude at a frequency of 150 oscillation/min (cycles/min). After 15 to 120 min, rats were sacrificed by cervical dislocation and the liver and spleen were taken, immediately frozen in liquid nitrogen, and stored at -80°C until use for RNA isolation.

Drug treatment: Some rats were given intraperitoneal (i.p.) injection of propranolol (a β-adrenergic receptor antagonist, 10 mg/kg, Nacalai Tesque, Kyoto) 20 min before the oscillation stress. Gadolinium chloride ($GdCl_3$, 7 mg/kg, Wako Pure Chemical Co., Osaka) was given i.p. 2 days before the oscillation stress. Chemical sympathectomy was performed with a single subcutaneous (s.c.) injection of guanethidine (100 mg/kg, Sigma Chemical Co., St. Louis, MO, U.S.A.) 12 hr prior to experiments.

Adrenalectomy: Some rats were subjected to either bilateral adrenalectomy or sham operations under sodium pentobarbital anesthesia (50 mg/kg, i.p.). For bilateral adrenalectomy, two small dorsal incisions caudal to the costal margin were made, and adrenal glands were carefully excised. Sham operation was done with a similar procedure without removing the adrenal glands. Adrenalectomized rats were given 0.9% saline to drink. After 7 days of the recovery period, they were used for experiments.

Isolation of total RNA and Northern blot analysis: Total

RNA was extracted by guanidine isothiocyanate method using TRIzol solution (Gibco BRL, Gaithersberg, MD, U.S.A.). Expression of IL-1β mRNA was determined by Northern blot analysis. In brief, total RNA (50 μg) was electrophoresed in 1% agarose gel, stained with ethidium bromide, and transferred to a nylon membrane (Amersham, Buckinghamshire, UK). cDNA probe corresponding to nucleotides 421 to 798 of the published sequence of rat IL-1β [15] were synthesized by RT-PCR, and labeled with [α-^{32}P] dCTP using a multiprime DNA labeling kit (Amersham). The membrane was hybridized with the labeled probe at 42°C for 20 hr in the presence of 0.2 mg/ml salmon sperm DNA (Sigma), and then washed twice at 42°C for 20 min with 2 × SSC (1 × SSC: 0.15 M NaCl/0.015 M sodium citrate)/0.1% (w/v) SDS, and subsequently washed twice at 52°C for 20 min with 0.1 × SSC/0.1% (w/v) SDS. The radioactivity present on the membrane was analyzed with a bioimage analyzer (BAS1000, Fuji Photo Film, Tokyo). The level of IL-1β mRNA was expressed as relative to those of glyceraldehyde 3-phosphate dehydrogenase (G3PDH) mRNA. cDNA probe for G3PDH was also prepared by PCR using specific primers and a control template (CLONTECH, Palo Alto, CA, U.S.A.).

Statistical analysis: Data was expressed as means ± SEM. Statistical significance was evaluated using the Fisher's protected least significant difference test. *P* values less than 0.05 were considered to be statistically significant.

RESULTS

To determine whether IL-1β expression is increased in the liver during non-invasive stress, rats were subjected to oscillation stress for various periods, and IL-1β mRNA expression was examined by Northern blot analysis. As shown in Fig. 1A, at the basal state before oscillation stress, detectable levels of IL-1β mRNA were expressed in the liver. After subjection to oscillation stress for a short-time (30 min), IL-1β mRNA level increased significantly, but after a little longer oscillation stress (60–120 min) it decreased to the basal or rather lower levels (Fig. 1B). Similar time-dependent effects of oscillation stress were also observed in the spleen, although the IL-1β mRNA level was much higher than in the liver (Fig. 1C and 1D). There was no consistent effect of oscillation stress on the G3PDH mRNA level both in the liver and spleen.

Next, we tested the involvement of non-parenchymal liver cells, especially liver macrophages, in the stress-induced IL-1β expression. For this, rats were given $GdCl_3$, which eliminates macrophages in the liver and spleen [4], 2 days before experiments. As shown in Fig. 2, in both the liver and spleen of $GdCl_3$-treated rats, the IL-1β mRNA level did not increase at all even 30 min after oscillation stress.

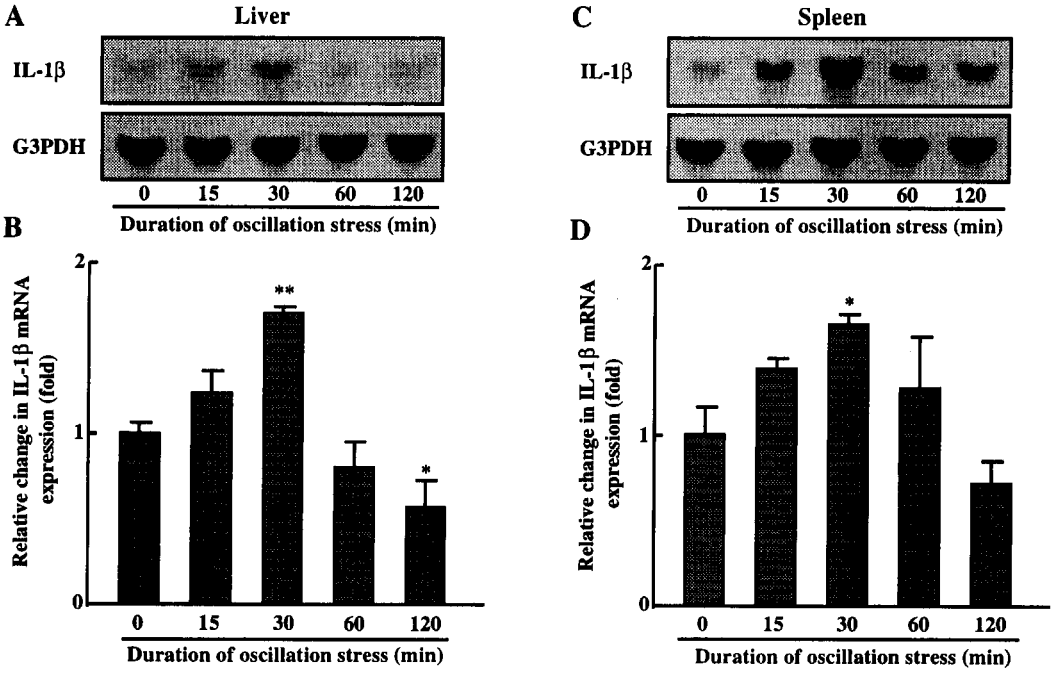

Fig. 1. Effect of oscillation stress on IL-1β mRNA expression in the liver and spleen. Rats were placed in a plastic cage put on a shaker, which was shaken at a frequency of 150 oscillation/min (cycles/min) for the indicated time. They were sacrificed, and the liver and spleen were taken for RNA isolation. Typical autoradiograms of Northern blot in the liver (A) and spleen (C) are shown. The IL-1β mRNA level was normalized by G3PDH mRNA, and expressed as relative to the 0-time control without stress (B and D, n=4). * $p<0.05$, ** $p<0.01$ compared with 0 min.

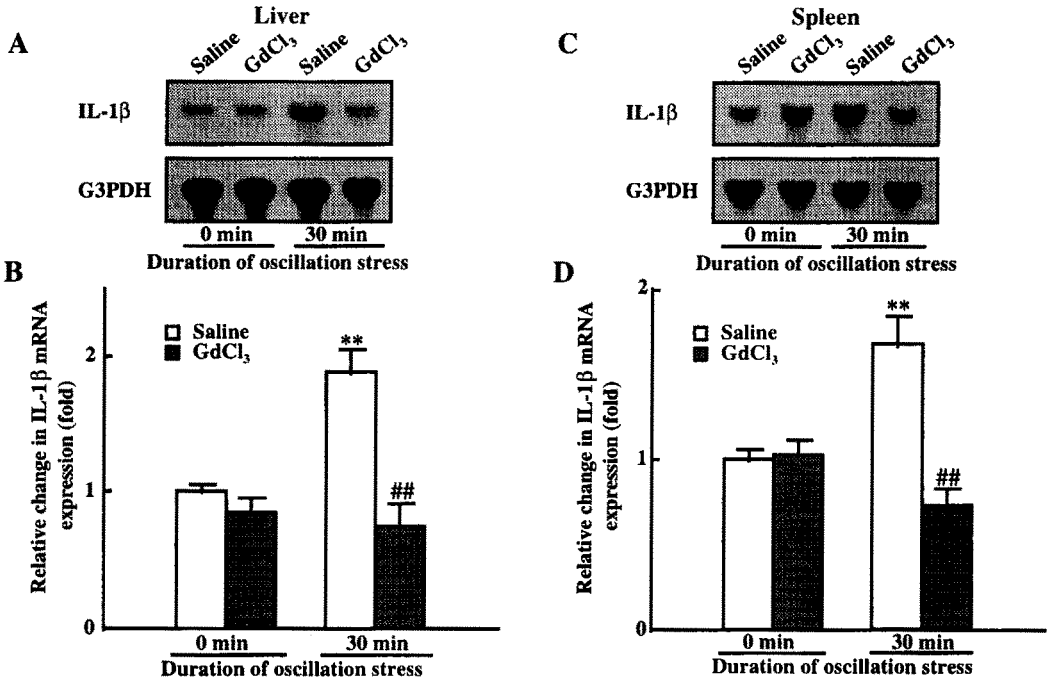

Fig. 2. Effect of $GdCl_3$ on the stress-induced IL-1β mRNA expression. Rats (n=4 in each group) were given $GdCl_3$ (7 mg/kg) or saline i.p., and after 2 days they were subjected to oscillation stress for 30 min. IL-1β mRNA expression was measured, and expressed as in Fig. 1. ** $p<0.01$ compared with 0 min. ## $p<0.01$ compared $GdCl_3$-with saline-treated rats after 30 min oscillation stress.

We also examined the role of catecholamines in the stress-induced IL-1β expression. To assess the role of the sympathetic nervous system, rats were injected with guanethidine, which suppresses norepinephrine release from sympathetic nerve endings [11]. After 30 min-oscillation stress, the IL-1β mRNA level seemed to be low in the guanethidine-treated rats compared to the sham-control rats, although the difference was statistically not significant (Fig. 3). A similar but incomplete attenuation of the effect of oscillation stress was also observed in adrenalectomized rats (Fig. 3). When adrenalectomized rats were treated with guanethidine, the IL-1β mRNA level did not increase at all after 30 min-oscillation stress (Fig. 3). As indicated in Fig. 3, neither guanethidine nor adrenalectomy influenced the basal IL-1β mRNA level without oscillation stress. Finally, the effect of a β-adrenergic antagonist, propranolol, was examined. As shown in Fig. 4, injection of propranolol completely prevented the oscillation stress-induced rise of IL-1β expression in both the liver and spleen, without affecting the basal expression.

DISCUSSION

In the present study we demonstrated that IL-1β mRNA expression is increased in the liver and spleen in response to oscillation stress. As far as we know, this is the first report of IL-1β mRNA expression in peripheral organs induced by non-invasive stress. Elimination of macrophages by the treatment of rats with $GdCl_3$ [4] prevented the rise in IL-1β mRNA expression after the stress. This indicates that hepatic and splenic macrophages are responsible for the stress-induced IL-1β expression. In support of this idea, Kupffer cells and splenic macrophages are known to be capable of producing IL-1 in response to various stimuli [2].

It has been reported that subjection to oscillation stress causes a marked elevation of plasma catecholamine and corticosterone levels [13]. Treatment by propranolol, a β-adrenergic antagonist, prevented the increase in IL-1β expression after oscillation stress. This finding suggests that the stress-induced IL-1β expression is mediated through the β-adrenergic action of catecholamines. In fact, the IL-1β mRNA response was attenuated by either adrenalectomy or the treatment with guanethidine, an inhibitor of catecholamine secretion from sympathetic nerve terminals [11], but not from the adrenal gland [12]. The slight increase in IL-1β mRNA level even after guanethidine-treatment might be due to epinephrine release from the adrenal gland. On the other hand, increased IL-1β expression in the adrenalrectomized animals is likely due to norepinephrine released from sympathetic nerve endings. Thus, the combination of these treatments completely abolished the stress-induced IL-1β mRNA expression.

Among the adrenal hormones, epinephrine more than corticosterone is an inducer of IL-1β mRNA expression, as discussed above. This is quite consistent with the previous

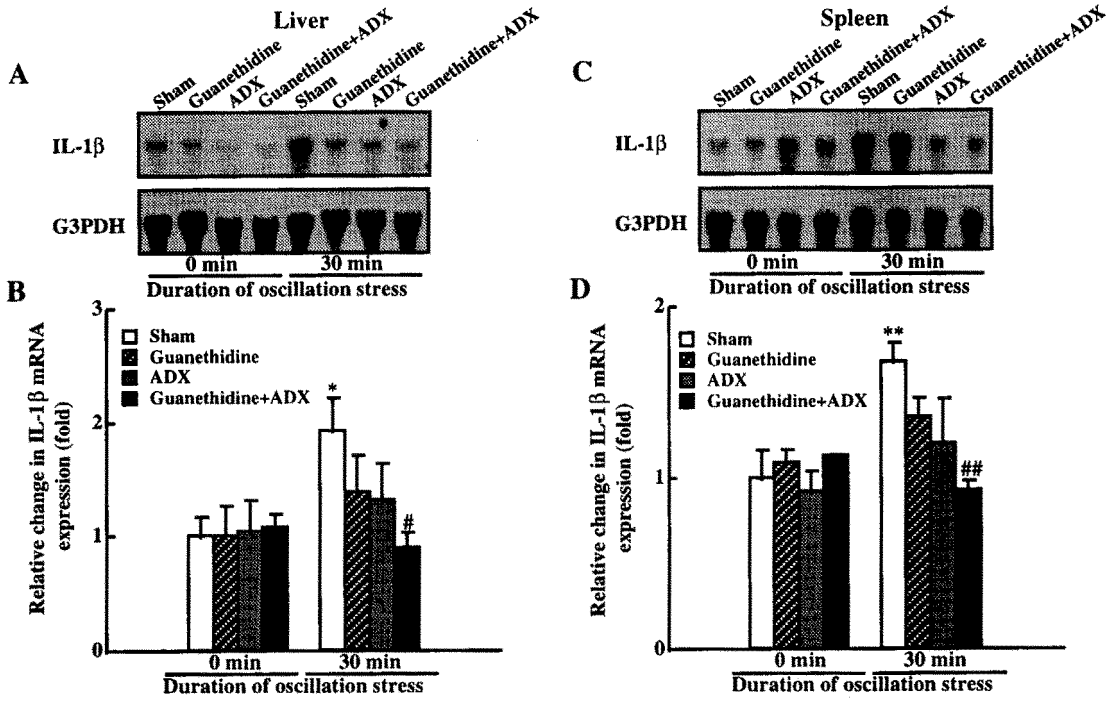

Fig. 3. Effect of chemical sympathectomy and adrenalectomy on the stress-induced IL-1β mRNA expression. Rats (n=4 in each group) were adrenalectomized (ADX) and chemical sympathetomized by guanethidine, and subjected to oscillation stress for 30 min. IL-1β mRNA expression was measured, and expressed as in Fig. 1. $^{*}p<0.05$ and $^{**}p<0.01$, compared with 0 min. $^{\#}p<0.05$, $^{\#\#}p<0.01$, sham operated versus adrenalectomized and chemical sympathectomized rats after 30 min oscillation stress.

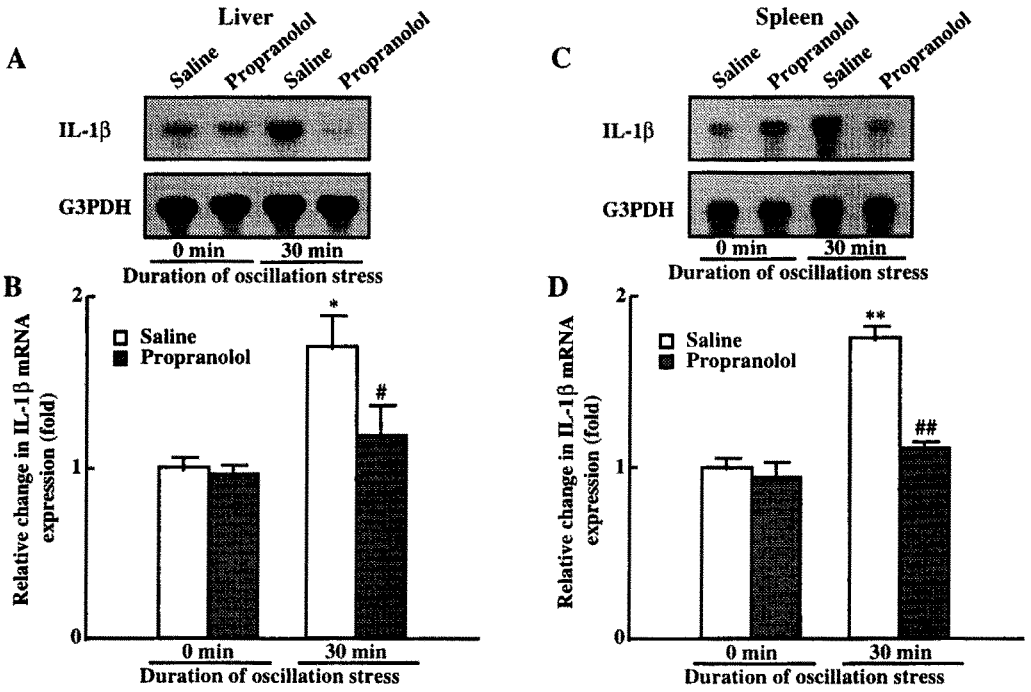

Fig. 4. Effect of β-adrenergic receptor antagonist on the stress-induced IL-1β mRNA expression. Rats (n=5 in each group) were injected i.p. with propranolol (10 mg/kg) 20 min before the oscillation stress. IL-1β mRNA expression in the liver and spleen was determined as in Fig. 1. $^{*}p<0.05$ and $^{**}p<0.01$, compared with 0 min. $^{\#}p<0.05$, $^{\#\#}p<0.01$, saline versus propranolol-treated rats after 30 min oscillation stress.

reports that corticosterone inhibits IL-1 production in macrophages [14, 16]. Thus, corticosterone might be related to the suppression of IL-1β mRNA expression seen after prolonged subjection to oscillation stress (60–120 min). Further work is necessary to clarify this possibility.

Physiological relevance of IL-1β expression in the liver and spleen during oscillation stress is at present unknown. We have recently demonstrated that norepinephrine increase IL-1 production in non-parenchymal liver cells, which in turn induces IL-6 mRNA expression in hepatocytes [7]. Moreover, it has been established that sympathetically mediated IL-6 production in the liver is enhanced by non-invasive stress such as immobilization [3, 8, 17, 18]. Collectively, it may be likely that IL-1β produced during stress might be involved in IL-6 production in the liver.

In summary, oscillation stress induces IL-1β mRNA expression in the liver and spleen, probably in Kupffer cells and macrophages. The stress-induced IL-1β expression is elicited by catecholamines released from sympathetic nerve terminals and also from the adrenal gland.

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