In vivo stimulation of sympathetic nervous system modulates osteoblastic activity in mouse calvaria

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Kondo, Ayami and Akifumi Togari. In vivo stimulation of sympathetic nervous system modulates osteoblastic activity in mouse calvaria. *Am J Physiol Endocrinol Metab* 285: E661–E667, 2003; 10.1152/ajpendo.00026.2003.—Previously, we demonstrated that epinephrine induced the expression of interleukin (IL)-6 mRNA via β-adrenoceptors in cultured human osteoblastic cells. IL-6 is well known to modulate bone metabolism by regulating the development and function of osteoclasts and osteoblasts. Recently, restraint stress and intracerebroventricular injection of lipopolysaccharide (LPS) have been reported to induce the expression of IL-6 mRNA in peripheral organs in mice in which expression is mediated by the activation of the sympathetic nervous system. To prove the physiological role of sympathetic nerves in bone metabolism in vivo, we examined by RT-PCR analysis the effects of restraint stress and intracerebroventricular injection of LPS on IL-6 mRNA expression in mouse calvaria. The expression of IL-6 mRNA in mouse calvaria was stimulated by either restraint stress (30 min) or intracerebroventricular injection of LPS (50 ng/mouse, 60 min). The treatment of mice with the neurotoxin 6-hydroxydopamine (6-OHDA, 100 mg·kg^{-1}· day^{-1} ip for 3 days) inhibited LPS (icv)-induced expression of IL-6 mRNA in their calvaria. The expression of IL-6 mRNA induced by the restraint stress was not influenced by 6-OHDA, which destroys noradrenergic nerve terminals. Furthermore, pretreatment with a β-blocker, propranolol (15 or 25 mg/kg ip), inhibited both stress- and LPS-induced increases in the level of IL-6 mRNA, but pretreatment with an α-blocker, phentolamine (5 mg/kg sc), did not inhibit them in mouse calvaria. In addition, treatment of calvaria with isoprenaline or norepinephrine increased IL-6 synthesis in the organ culture system. These results indicate that in vivo adrenergic stimulation modulates the osteoblastic activity in mouse calvaria via noradrenergic nerve terminals.

restraint stress; intracerebroventricular injection; interleukin-6; lipopolysaccharide; calvaria; sympathetic activity; osteoblast

HISTOCHEMICAL AND PHARMACOLOGICAL STUDIES indicate the involvement of neural regulation in bone metabolism mediated by osteoblastic and osteoclastic cells. Mammalian bones are widely innervated by sympathetic and sensory nerves, which are particularly abundant in regions of high osteogenic activity, such as the growth plate (4, 15). In heterotropic bone formation induced by demineralized bone matrix, an early ingrowth of noradrenergic nerves has been detected (5).

Moreover, chemical denervation of sympathetic and/or sensory nerves has been demonstrated to modulate the number of bone-resorbing osteoclasts (6, 14). These observations suggest that sympathetic and/or sensory innervation is required for regulating bone metabolism under physiological and pathological conditions. It is well known that β-adrenergic agonists can stimulate bone resorption in the intact mouse calvaria (19). The stimulation may be mediated by the activation of osteoclastic cells and/or the production of osteotrophic factor by osteoblastic cells, which factor is capable of stimulating the development of osteoclasts from their hematopoietic precursors. Recently, we observed that epinephrine increased the expression of osteotrophic factors, such as interleukin (IL)-6, IL-11, PGE$_2$, and receptor activator of NF-κB ligand (RANKL; see Refs. 16 and 29), as well as the formation of osteoclast-like cells from mouse bone marrow cells by activating β-adrenoceptors (29). These in vitro evidences may suggest that osteoblastic-mediated osteoclastogenesis is regulated by sympathetic activity in vivo.

Administration of lipopolysaccharide (LPS) has been shown to increase the norepinephrine (NE) turnover rate in various brain areas and peripheral tissues (1, 12). Recent experiments showed that the intracerebroventricular injection of LPS induced a marked increase in the level of IL-6 in the bloodstream and in IL-6 mRNA expression in the brain and peripheral organs (20, 23). The increases in plasma IL-6 levels by centrally injected LPS were reported to be inhibited by the intraperitoneal administration of adrenergic antagonists, suggesting the involvement of the NE system in the central LPS-induced IL-6 response (11). Immobilization stress also induced an increase in plasma IL-6 levels (27). As in the case of the central LPS-induced IL-6 response, depletion of NE was reported to inhibit the stress-induced increase in plasma IL-6 (27). Thus the peripheral sympathetic nervous system may be involved in the increase of IL-6 in peripheral tissues induced by both centrally injected LPS and immobilization stress.

In the present study, to determine whether sympathetic activity is involved in bone metabolism in vivo, we examined the expression of IL-6 mRNA in calvariae dissected from mice treated intracerebroventricularly

with LPS or subjected to restraint stress to stimulate sympathetic activity, and we pharmacologically characterized the IL-6 expression induced by the central LPS or physiological stress. All experiments were performed in accordance with the guidelines for animal experiments at the School of Dentistry, Aichi-Gakuin University.

MATERIALS AND METHODS

Animals and reagents. Conventional male and pregnant female ICR mice were obtained from SLC (Hamamatsu, Shizuoka, Japan). Mice were caged in plastic tubs covered with stainless-steel tops and containing hardwood-chip bedding under automatically controlled conditions of temperature (23–25°C), humidity (50 ± 10%), and a 12:12-h light-dark cycle and were given ad libitum tap water and rodent chow. LPS from *Escherichia coli* serotype 026:B6 (phenol extracted), propranolol, 6-hydroxydopamine (6-OHDA), phentolamine, NE, isoprenaline (ISP), and phenylephrine were purchased from Sigma (St. Louis, MO). 6-OHDA was dissolved in saline containing 0.1% ascorbic acid. Other reagents were dissolved in saline.

Restraint stress. Five-week-old ICR mice were restrained individually by keeping them in a 50-ml disposable syringe (with volume set for 25–30 ml) with some holes for the desired period. Mice kept unrestrained at room temperature served as controls. After the restraint stress, the calvaria was removed immediately, and the total RNA was extracted by the guanudinium-thiocyanate method (7). In the experiment of chemical denervation, the mice were pretreated with 6-OHDA (100 mg/kg ip) or vehicle for 3 days before being subjected to the restraint stress. In the experiment of receptor blockage, the mice were pretreated with propranolol (15 mg/kg sc), phentolamine (5 mg/kg ip), or saline for 10 min before the restraint stress.

Intracerebroventricular injection of LPS. The intracerebroventricular administration of LPS was performed by following the method of Haley and McCormick (13). Simply stated, ICR mice were injected under ether anesthesia 1 mm lateral and 1 mm anteroposterior to the bregma with a Hamilton syringe (10 μl) fitted with a 27-gauge needle. The intracerebroventricular injection volume was 5 μl, and the injection sites were verified by injection of the same volume of methylene blue in the sites. After the injection of LPS, the calvariae were obtained at various times for analysis of the expression of IL-6 mRNA. Mice were pretreated with 6-OHDA (100 mg/kg ip) or vehicle for 3 days before the injection of LPS. Other mice were pretreated with propranolol (25 mg/kg ip), phentolamine (5 mg/kg ip), or saline for 10 min before the injection of LPS (icv).

Analysis of mRNA levels by RT-PCR. RNA was extracted from mouse calvaria by the guanidinium-thiocyanate method. The total RNA was solubilized in 1 ml guanidinium thiocyanate buffer/calvaria from a mouse and then extracted with phenol and treated with DNase I (Boehringer Mannheim). cDNA was synthesized by using random primer and Moloney murine leukemia virus RT (GIBCO-BRL, Grand Island, NY), and subsequent PCR amplification was done by using synthetic gene primers specific for mouse IL-6 and

Fig. 1. RT-PCR analysis of mRNA obtained from calvariae of mice treated with restraint stress (*A*) or icv lipopolysaccharide (LPS; *B* and *C*). *A*: *a*, RT-PCR analysis of mRNA obtained from mouse calvaria. Mice were subjected to a restraint stress for 0 (no stress), 15, 30, or 60 min. DNA size markers (φX174-*Hae* III digest) are shown on *left* (S). Arrowheads indicate the predicted size of PCR products. Nos. in parentheses indicate cycles for PCR amplification. Data shown are representative of 6 similar experiments. *b*, relative expression of these increases. The mRNA level of interleukin (IL)-6 was calculated by dividing the intensity of the IL-6 band by that of the GAPDH band as determined by fluorescent image analyzer. Values are means ± SE of 6 mice. *$P < 0.05$ vs. nonstressed mice (0 min). *B*: *a*, RT-PCR analysis of mRNA obtained from mouse calvaria. Mice were treated with LPS (50 ng/mouse icv) for 0 (nontreatment), 30, 60, or 120 min. Data shown are representative of 6 similar experiments; *b*, relative expression of these increases. Values are means ± SE of 6 mice. *$P < 0.01$ vs. nontreated mice (0 min). *C*: *a*, mice were treated with 0 (saline), 0.5, 5, or 50 ng/mouse LPS (icv) for 60 min. Data shown are representative of 6 similar experiments; *b*, relative expression of these increases. Values are means ± SE of 6 mice. *$P < 0.01$ vs. saline-treated mice (0 ng/mouse).
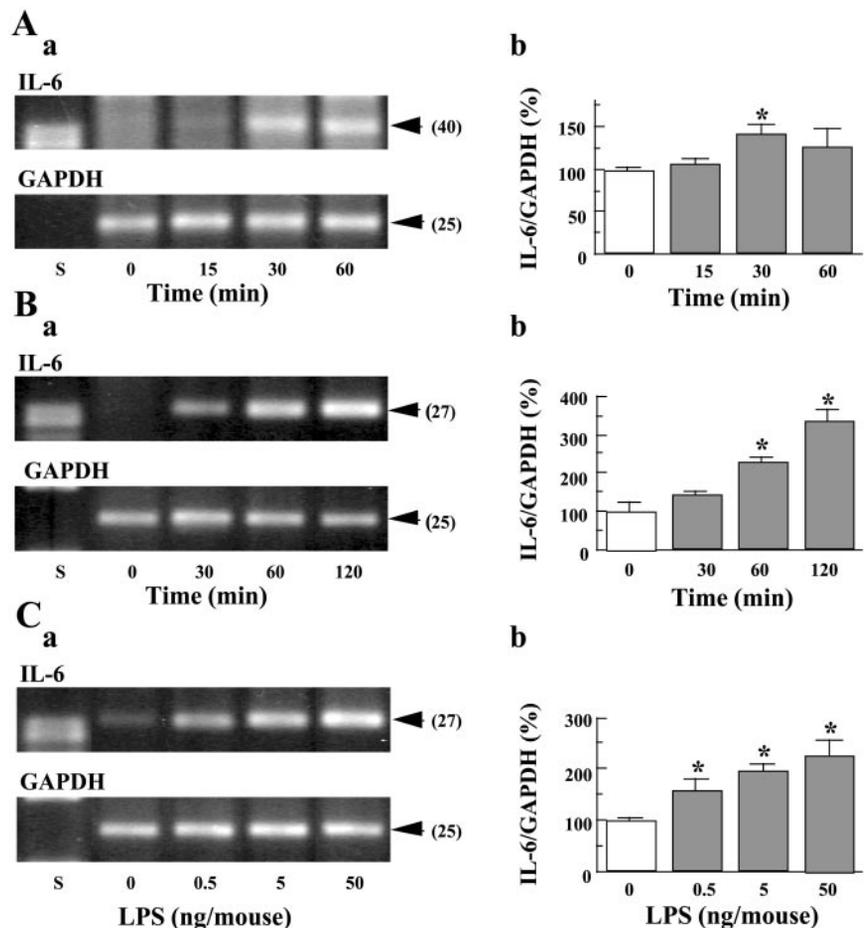
Table 1. *Effect of restraint stress on plasma catecholamine*

	Epi, ng/ml	NE, ng/ml	DA, ng/ml
Control	0.338 ± 0.080	3.388 ± 0.402	0.314 ± 0.036
Stress	$1.656 \pm 0.288*$	3.920 ± 0.379	0.561 ± 0.109

Data are mean ± SE values obtained from mice. Epi, epinephrine; NE, norepinephrine; DA, dopamine. Mice were subjected to restraint stress for 30 min. Next, peripheral blood was collected and subjected to catecholamine analyzer. *$P < 0.05$ vs. control.

Table 2. *Data of ANOVA for the ratio of stress- or LPS-increased IL-6 mRNA in 6-OHDA-treated mice calvaria*

Source	S	DF	V	F	P
Stress	188,745.256	1	188,745.256	13.423	0.0150
6-OHDA	3,965.383	1	3,965.383	0.282	0.6012
Stress 6-OHDA	13.184	1	13.184	0.001	0.9759
e	281,216.78	20			
Total	473,940.603	23			
LPS	57,640.46	1	57,640.46	23.773	<0.001
6-OHDA	71,569.817	1	71,569.817	29.518	<0.001
LPS 6-OHDA	23,050.289	1	23,050.289	9.507	0.0059
e	48,492.873	20			
Total	200,753.439	23			

LPS, lipopolysaccharide; IL-6, interleukin; 6-OHDA, 6-hydroxydopamine; S, Sum of squares; DF, degrees of freedom; V, variance; Stress, mouse subjected without and with restraint stress; LPS, mouse injected without and with LPS; 6-OHDA, mouse pretreated without and with 6-OHDA. F, F value; P, P value; e, error.

mouse glyceraldehyde-3-phosphate dehydrogenase (GAPDH) produced from their respective reported cDNA sequences (2, 12). The oligonucleotide primers were synthesized on a DNA synthesizer (Expedite model 8909; PerSeptiv Biosystem, Cambridge, MA) and purified on a polypropylene filter (Oligo Prep kit; Pharmacia Biotech, Uppsala, Sweden). GAPDH primers (forward primer 5′-ACCACAGTCCATGCCATCAC-3′, reverse primer 5′-TCCACCACCCTTTGCTGTA-3′) were used to amplify a 452-bp cDNA fragment, and mouse IL-6 primers (forward primer 5′-GAAATGAGAAAAGAGTT-GTGC-3′, reverse primer 5′-ATTGGAAATTGGGGTAG-GAAG-3′) were used to amplify a 324-bp cDNA fragment. PCR amplification was performed by using a GeneAmp PCR System (Perkin-Elmer/Cetus, Norwalk, CT) under the following conditions: denaturation at 95°C for 15 s, annealing at 55°C or 30 s, and elongation at 72°C for 30 s for the appropriate number of cycles. PCR products were electrophoresed on a 2% NuSive GTG agarose gel (FMC BioProducts, Rockland, ME), stained with ethidium bromide, and detected on a fluoroimage analyzer (FluorImager 575; Molecular Dynamics, Sunnyvale, CA). All PCR data were obtained from the measurements, which were performed in the linear range of PCR amplification.

Analysis of IL-6 production in calvaria culture system. Calvariae (frontal and parietal) were aseptically removed from 2- or 3-day-old mice. They were preincubated in medium containing 100 U/ml penicillin and 100 µg/ml streptomycin for 18 h at 37°C in air with 5% CO_2. Then calvariae were treated with NE (100 µM), ISP (100 µM), or phenylephrine (100 µM) for 24 h, and condition medium was used for analysis of IL-6 synthesis. IL-6 in condition medium was quantified using an ELISA kit (R&D Systems, Minneapolis, MN).

Statistical analysis. All data were presented as means ± SE. Statistical analysis was carried out by one-way or two-way ANOVA. Fisher's protected least significant difference post hoc test was used when multiple groups were compared (Figs. 2–4), and Student's *t*-test was used when groups were compared with a single control group (Figs. 1 and 5).

RESULTS

The effects of restraint stress and intracerebroventricular LPS on IL-6 mRNA levels in mouse calvaria are shown in Fig. 1. RT-PCR analysis revealed a low level of IL-6 mRNA in the mouse calvaria, which was increased by exposure of the mice to restraint stress for 30 min. The restraint stress for 15 min was not suffi-

Fig. 2. Effect of 6-hydroxydopamine (6-OHDA) on restraint stress-induced (*A*) and LPS-induced (*B*) increases in the IL-6 mRNA levels in mouse calvaria. *a*: RT-PCR analysis of mRNA obtained from mouse calvaria. Mice were injected with either vehicle (−) or 100 mg/kg 6-OHDA (+) for 3 days. Later (3 days), the effects of restraint stress (*A*) for 0 (−) or 30 (+) min and central LPS administration (*B*) at 0 (−) or 50 (+) ng/mouse for 60 min were examined. Arrowheads indicate the predicted sizes of PCR products, and nos. in parentheses indicate cycles for PCR amplification. DNA size markers (φX174-*Hae* III digest) are shown on *left* (S). Data shown are representative of 6 similar experiments. *b*: Relative expression of these increases. Values are means ± SE of 6 mice. *$P < 0.05$ vs. control mice (−−). #$P < 0.05$ vs. control mice with 6-OHDA (−+).

Table 3. *Data of ANOVA for the ratio of stress- or LPS-increased IL-6 mRNA in propranolol-treated mice calvariae*

Source	S	DF	V	F	P
Stress	13,726.821	1	13,726.821	20.046	0.0002
Pro	3,030.206	1	3,030.206	4.425	0.0483
Stress Pro	3,807.896	1	3,807.896	5.561	0.0287
e	13,695.292	20			
Total	34,260.215	23			
LPS	20,334.733	1	20,334.733	25.329	<0.001
Pro	2,944.833	1	2,944.833	3.668	0.0699
LPS Pro	7,866.2	1	7,866.2	9.798	0.0053
e	16,056.171	20			
Total	47,201.937	23			

Pro, mouse pretreated without and with propranolol.

cient to increase the IL-6 mRNA levels (Fig. 1*A*). Restraint stress for 30 min increased the plasma epinephrine level in mice (Table 1). Figure 1*B* shows the effect of LPS (icv) on the IL-6 mRNA levels in mouse calvaria. Treatment of mice with LPS (50 ng/mouse icv) for 60–120 min significantly increased the IL-6 mRNA levels in the calvaria. The increase by treatment with LPS for 30 min was lower than that by treatment for 60 or 120 min (Fig. 1*B*). The dose-dependent effect of LPS on the IL-6 mRNA levels in the calvaria is shown in Fig. 1*C*. By treatment of mice with LPS (icv) for 60 min, the dose-dependent increase in the IL-6 mRNA level was observed at a dose of 0.5–50 ng/mouse.

To determine whether the restraint stress-induced and LPS (icv)-induced increases in the IL-6 mRNA in mouse calvaria required peripheral NE nerve activity, we examined the effect of 6-OHDA (well known to destroy NE nerve terminals) on these increases in IL-6 mRNA levels. Table 1 shows the results of ANOVA for the effects of LPS, stress, and 6-OHDA on IL-6 mRNA in mouse calvaria. In the expression of IL-6 mRNA,

there were significant differences in the LPS group ($P < 0.001$) and stress group ($P < 0.05$) in mouse calvaria. However, there were no significant differences in the interaction of stress and 6-OHDA, but there were significant differences in the interaction of LPS and 6-OHDA ($P < 0.001$). As shown in Fig. 2*A*, the 2.8-fold increase in the IL-6 mRNA levels in calvaria by restraint stress for 30 min was not influenced by the pretreatment of mice with 6-OHDA. Actually, this pretreatment with 6-OHDA caused a slight increase over the level obtained by the stress. On the other hand, the 2.6-fold increase in the IL-6 mRNA levels caused by treatment with LPS (50 ng/mouse icv) for 60 min was inhibited by pretreatment with 6-OHDA (Fig. 2*B*).

To find out whether the restraint stress-induced and LPS (icv)-induced increases in IL-6 mRNA in mouse calvaria were mediated by adrenoceptors in the calvaria, we examined the effect of propranolol, a β-adrenergic antagonist, and phentolamine, an α-adrenergic antagonist, on these increases in the IL-6 mRNA level. Tables 2 and 3 show the results of ANOVA for the effects of LPS, stress, propranolol, and phentolamine on IL-6 mRNA in mouse calvaria. There were significant differences in the interaction of stress and propranolol ($P < 0.05$) but no differences in the interaction of stress and phentolamine. Furthermore, there were significant differences in the interaction of LPS and propranolol ($P < 0.01$) but no significant differences in the interaction of LPS and phentolamine. As shown in Fig. 3*A*, pretreatment with propranolol (15 mg/kg ip) inhibited the restraint stress-induced increase in the IL-6 mRNA level in mouse calvaria. Similarly, pretreatment with propranolol (25 mg/kg ip) inhibited the LPS-induced increase as well (Fig. 3*B*). However, pretreatment with propranolol (15 mg/kg sc) did not inhibit the LPS-induced increase (data not shown). In contrast, pretreatment with phentolamine (5 mg/kg ip) did not affect either the re-

Fig. 3. Effect of propranolol on restraint stress-induced (*A*) and LPS-induced (*B*) increases in the IL-6 mRNA levels in mouse calvaria. *a*: RT-PCR analysis for mRNA obtained from mouse calvaria. Mice were injected with either vehicle (−) or 15 mg/kg propranolol (Pro; +). Later (10 min), the effects of restraint stress (*A*) for 0 (−) or 30 (+) min or central LPS administration (*B*) at 0 (−) or 50 (+) ng/mouse for 60 min were examined. Arrowheads indicate the PCR production of predicted sizes, and nos. in parentheses indicate cycles for PCR amplification. DNA size markers (φX174-*Hae* III digest) are shown on *left* (S). Data shown are representative of 6 similar experiments. *b*, Relative expression of these increases. Values are means ± SE of 6 mice. *$P < 0.01$ vs. control mice (−−).
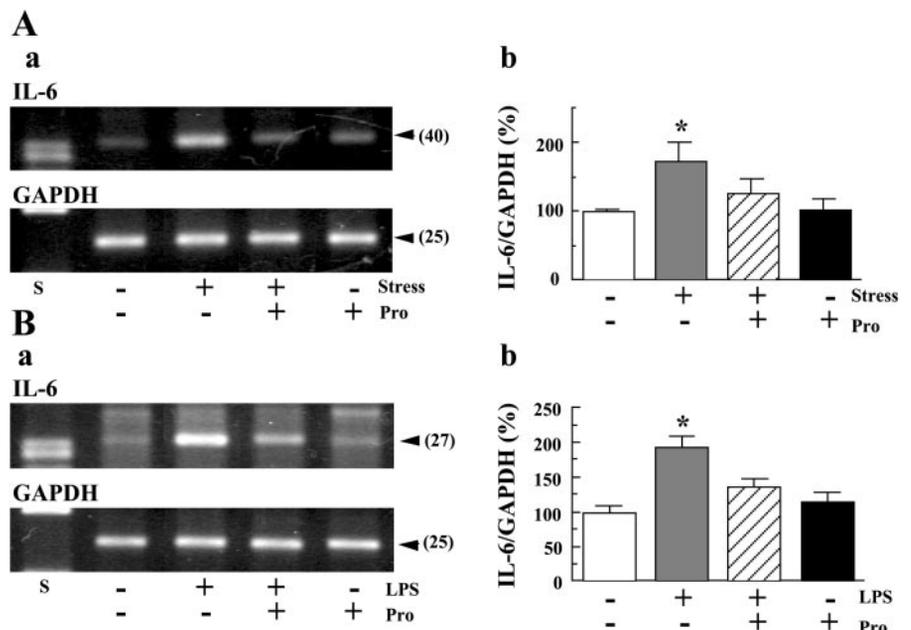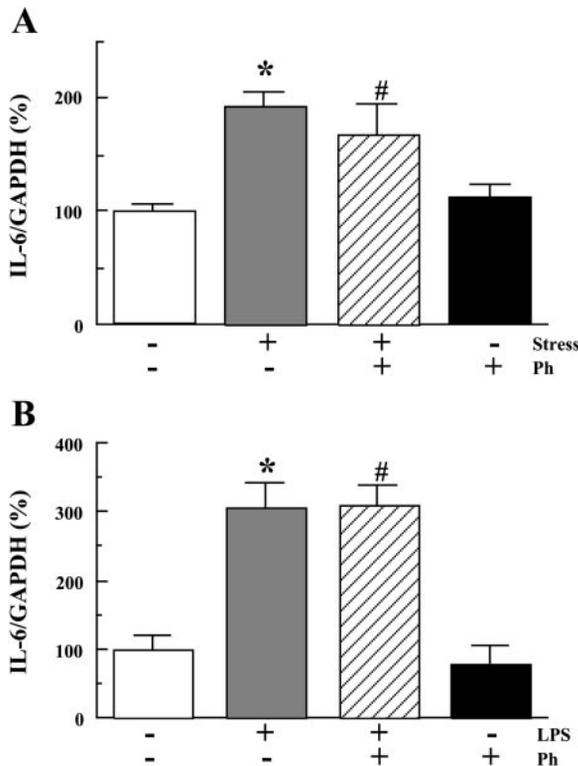
Fig. 4. Effect of phentolamine on restraint stress-induced (*A*) and LPS-induced (*B*) increases in the IL-6 mRNA levels in mouse calvaria. Relative expression of IL-6 was calculated by dividing the intensity of the IL-6 band by that of the GAPDH band as determined with a fluorescent image analyzer. Mice were injected with either vehicle (−) or 5 mg/kg phentolamine (Ph; +). Later (10 min), the effects of restraint stress (*A*) for 0 (−) or 30 (+) min or central LPS administration (*B*) at 0 (−) or 50 (+) ng/mouse for 60 min were examined. Values are means ± SE of 6 mice. *$P < 0.01$ vs. control mice (−−). #$P < 0.05$ vs. control mice with Ph (−+).

straint stress-induced or the LPS-induced increase in IL-6 mRNA levels in mouse calvaria (Fig. 4, *A* and *B*).

As shown in Fig. 5, treatment with 100 μM NE or 100 μM ISP for 24 h increased IL-6 synthesis in mouse calvaria. However, treatment with 100 μM phenylephrine for 24 h did not affect IL-6 synthesis.

DISCUSSION

It has been demonstrated that human osteoblastic and osteoclastic cells are equipped with adrenergic receptors and neuropeptide receptors and that they constitutively express diffusible axon guidance molecules that are known to function as a chemoattractant and/or a chemorepellent for growing never fibers (30–32). These recent findings, in addition to immunohistochemical and pharmacological findings, suggest that the extension of axons of sympathetic and peripheral sensory nerves to osteoblastic and osteoclastic cells is required for the dynamic neural regulation of local bone metabolism. Recently, adrenergic stimulation was shown to increase the expression of osteotrophic factors, such as IL-6, IL-11, PGE_2, or RANKL, which is identical to osteoclast differentiation factor, via β-adrenoceptors in osteoblastic cells (16, 29). Furthermore,
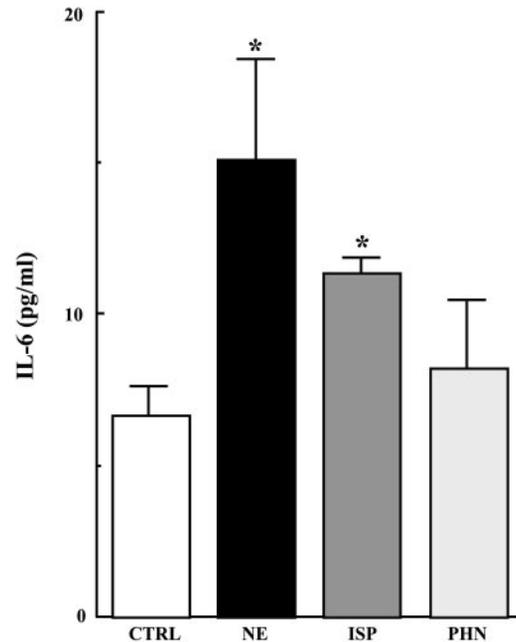

Fig. 5. Changes of IL-6 synthesis in calvaria cultivated with norepinephrine (NE), isoprenalin (ISP), or phenylephrine (PHN). Calvariae dissected from 2- or 3-day old mice were preincubated for 18 h, and then calvariae were treated with norepinephrine (100 μM), isoprenaline (100 μM), or phenylephrine (100 μM). Conditioned media were used for analysis of IL-6 synthesis by the ELISA system. Values are means ± SE of 5 calvariae. *$P < 0.05$ vs. control (CTRL).

both Takeda et al. (28) and Baldock et al. (3) have recently provided pharmacological genetic evidence that hypothalamic autonomic signals proceeding via the β-adrenoceptor regulate bone mass. In association with an increase of sympathetic nerve activity, application of stress (21, 27, 33) and central administration of LPS (8, 9, 18) were shown to increase the IL-6 levels in rodents. These findings led us to evaluate the calvaria expression of IL-6 mRNA under the sympathomimetic condition caused by central LPS injection or restraint stress to assess the physiological significance of sympathetic nerve activity on bone metabolism in vivo.

In the present study, we observed elevated IL-6 mRNA expression in the calvaria of mice subjected to

Table 4. *Data of ANOVA for the ratio of stress- or LPS-increased IL-6 mRNA in phentolamine-treated mice calvariae*

Source	S	DF	V	F	P
Stress	33,442.557	1	33,442.557	22.024	0.0001
Ph	168.441	1	168.441	0.111	0.7426
Stress Ph	2,012.54	1	2,012.54	1.325	0.2632
e	30,368.593	20			
Total	65,992.131	23			
LPS	285,786.547	1	285,786.547	62.19	<0.001
Ph	495.888	1	495.888	0.108	0.7460
LPS Ph	709.685	1	709.685	0.153	0.6989
e	91,908.143	20			
Total	378,900.263	23			

Ph, mouse pretreated without and with phentolamine.

central LPS injection or restraint stress. The significant elevation by central LPS injection was prevented by the destruction of NE nerve terminals by use of 6-OHDA (17), or by blockage of β-adrenoceptors with propranolol, suggesting that the elevation of IL-6 mRNA in the calvaria was mediated by the activation of postganglion sympathetic nerve fibers innervating the calvaria and by β-adrenoceptors in the calvaria. On the other hand, the elevation by restraint stress was prevented by blockage of β-adrenoceptors but not by the destruction of NE nerve terminals, suggesting that the elevation is mediated by elevated secretion of epinephrine from the adrenals. In fact, restraint stress for 30 min caused a significant increase ($P < 0.05$) in plasma epinephrine in comparison with the level in control mice (Table 1). Although it is likely that the effects of propranolol on this expression could be a result of antagonism of β-adrenoceptors in another tissue, with a concomitant decrease of an intermediate factor that is responsible for stimulating the increase in IL-6 mRNA levels, the possibility may be contradicted by a direct increase of IL-6 protein in calvaria treated with β-adrenoceptor activation (Fig. 5). Although several evidences demonstrated the existence of the α-adrenoceptor in human and mouse osteoblasts (26, 30), phenylephrine did not increase IL-6 synthesis in mouse calvaria. These data suggested that peripheral NE increased IL-6 synthesis via β-adrenoceptor activation. This is the first report to demonstrate that physiological and pharmacological stimulation of the sympathetic nervous system modulates bone metabolic activity in vivo, as evaluated by expression of IL-6 mRNA in calvaria.

In the bone microenvironment, there is a dynamic balance between resorption and formation that maintains skeletal homeostasis. Osteoclastic bone resorption consists of multiple steps, such as the differentiation of osteoclast precursor in mononuclear prefusion osteoclasts, the fusion of prefusion osteoclasts to form multinucleate osteoclasts, and the activation of these osteoclasts to resorb bone (22, 25). These steps seem to progress at the site of bone resorption under the control osteotrophic hormones locally produced in the microenvironment (24). Potential paracrine mediators of osteoclast activity include monocyte-macrophage colony-stimulating factor, tumor necrosis factor (TNF)-α, IL-1, IL-6, IL-11, PGE$_2$, and RANKL/osteoprotegerin ligand/TNF-related activation-induced cytokine, all of which are capable of increasing osteoclastogenesis. It is well known that activation of β-adrenoceptors on osteoblastic cells can stimulate bone resorption in intact mouse calvaria (19) and induce the expression of osteotrophic factors, such as IL-6, IL-11, PGE$_2$, or RANKL (16, 29). In the present study, we showed the in vivo sympathomimetic action on the expression of IL-6 mRNA. These evidences suggest that the activity of sympathetic nerves has a significant effect on osteoclastogenesis by modulation of the expression of osteotrophic factors in osteoblastic cells and support the observation that bone resorption in rats was reduced after sympathectomy induced by guanethidine, which specifically destroys sympathetic adrenergic fibers (6). Further studies should clarify the involvement of sympathetic innervation of osteoblastic cells and give insight into the mechanism of sympathetic regulation of bone metabolism.

In conclusion, the findings indicate that restraint stress increased IL-6 mRNA expression via peripheral epinephrine from the adrenal glands and that LPS (icv) increased IL-6 mRNA expression via peripheral NE from sympathetic postganglionic fibers in the mouse calvaria. In consideration of the physiological significance of IL-6 in bone metabolism, we propose that sympathomimetic action on calvaria may be part of the mechanism governing bone resorption.

DISCLOSURES

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